Compendium of Plant Genomes
Series Editor: Chittaranjan Kole

Anil Kumar · Salej Sood · B. Kalyana Babu ·
Sanjay Mohan Gupta · B. Dayakar Rao *Editors*

The Finger Millet Genome

Springer

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Series Editor

Chittaranjan Kole, President, International Climate Resilient Crop Genomics Consortium (ICRCGC), President, International Phytomedomics & Nutriomics Consortium (IPNC) and President, Genome India International (GII), Kolkata, India

Whole-genome sequencing is at the cutting edge of life sciences in the new millennium. Since the first genome sequencing of the model plant *Arabidopsis thaliana* in 2000, whole genomes of about 100 plant species have been sequenced and genome sequences of several other plants are in the pipeline. Research publications on these genome initiatives are scattered on dedicated web sites and in journals with all too brief descriptions. The individual volumes elucidate the background history of the national and international genome initiatives; public and private partners involved; strategies and genomic resources and tools utilized; enumeration on the sequences and their assembly; repetitive sequences; gene annotation and genome duplication. In addition, synteny with other sequences, comparison of gene families and most importantly potential of the genome sequence information for gene pool characterization and genetic improvement of crop plants are described.

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B. Dayakar Rao
Editors

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Editors
Anil Kumar
Rani Lakshmi Bai Central
Agricultural University
Jhansi, India

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

B. Dayakar Rao
ICAR-Indian Institute
of Millet Research
Hyderabad, India

Salej Sood
ICAR-Central Potato
Research Institute
Shimla, India

Sanjay Mohan Gupta
Defence Institute of Bio-Energy
Research (DIBER)
Haldwani, India

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This book series is dedicated to my wife Phullara and our children Sourav and Devleena

Chittaranjan Kole

Preface to the Series

Genome sequencing has emerged as the leading discipline in the plant sciences coinciding with the start of the new century. For much of the twentieth century, plant geneticists were only successful in delineating putative chromosomal location, function, and changes in genes indirectly through the use of a number of "markers" physically linked to them. These included visible or morphological, cytological, protein, and molecular or DNA markers. Among them, the first DNA marker, the RFLPs, introduced a revolutionary change in plant genetics and breeding in the mid-1980s, mainly because of their infinite number and thus potential to cover maximum chromosomal regions, phenotypic neutrality, absence of epistasis, and codominant nature. An array of other hybridization-based markers, PCR-based markers, and markers based on both facilitated construction of genetic linkage maps, mapping of genes controlling simply inherited traits, and even gene clusters (QTLs) controlling polygenic traits in a large number of model and crop plants. During this period, a number of new mapping populations beyond F2 were utilized and a number of computer programs were developed for map construction, mapping of genes, and for mapping of polygenic clusters or QTLs. Molecular markers were also used in the studies of evolution and phylogenetic relationship, genetic diversity, DNA fingerprinting, and map-based cloning. Markers tightly linked to the genes were used in crop improvement employing the so-called marker-assisted selection. These strategies of molecular genetic mapping and molecular breeding made a spectacular impact during the last one and a half decades of the twentieth century. But still they remained "indirect" approaches for elucidation and utilization of plant genomes since much of the chromosomes remained unknown and the complete chemical depiction of them was yet to be unraveled.

Physical mapping of genomes was the obvious consequence that facilitated the development of the "genomic resources" including BAC and YAC libraries to develop physical maps in some plant genomes. Subsequently, integrated genetic–physical maps were also developed in many plants. This led to the concept of structural genomics. Later on, emphasis was laid on EST and transcriptome analysis to decipher the function of the active gene sequences leading to another concept defined as functional genomics. The advent of techniques of bacteriophage gene and DNA sequencing in the 1970s was extended to facilitate sequencing of these genomic resources in the last decade of the twentieth century.

As expected, sequencing of chromosomal regions would have led to too much data to store, characterize, and utilize with the then-available computer software could handle. But the development of information technology made the life of biologists easier by leading to a swift and sweet marriage of biology and informatics, and a new subject was born—bioinformatics.

Thus, the evolution of the concepts, strategies, and tools of sequencing and bioinformatics reinforced the subject of genomics—structural and functional. Today, genome sequencing has traveled much beyond biology and involves biophysics, biochemistry, and bioinformatics!

Thanks to the efforts of both public and private agencies, genome sequencing strategies are evolving very fast, leading to cheaper, quicker, and automated techniques right from clone-by-clone and whole-genome shotgun approaches to a succession of second-generation sequencing methods. The development of software of different generations facilitated this genome sequencing. At the same time, newer concepts and strategies were emerging to handle sequencing of the complex genomes, particularly the polyploids.

It became a reality to chemically—and so directly—define plant genomes, popularly called whole-genome sequencing or simply genome sequencing.

The history of plant genome sequencing will always cite the sequencing of the genome of the model plant Arabidopsis thaliana in 2000 that was followed by sequencing the genome of the crop and model plant rice in 2002. Since then, the number of sequenced genomes of higher plants has been increasing exponentially, mainly due to the development of cheaper and quicker genomic techniques and, most importantly, the development of collaborative platforms such as national and international consortia involving partners from public and/or private agencies.

As I write this preface for the first volume of the new series "Compendium of Plant Genomes," a net search tells me that complete or nearly complete whole-genome sequencing of 45 crop plants, eight crop and model plants, eight model plants, 15 crop progenitors and relatives, and three basal plants is accomplished, the majority of which are in the public domain. This means that we nowadays know many of our model and crop plants chemically, i.e., directly, and we may depict them and utilize them precisely better than ever. Genome sequencing has covered all groups of crop plants. Hence, information on the precise depiction of plant genomes and the scope of their utilization are growing rapidly every day. However, the information is scattered in research articles and review papers in journals and dedicated Web pages of the consortia and databases. There is no compilation of plant genomes and the opportunity of using the information in sequence-assisted breeding or further genomic studies. This is the underlying rationale for starting this book series, with each volume dedicated to a particular plant.

Plant genome science has emerged as an important subject in academia, and the present compendium of plant genomes will be highly useful to both students and teaching faculties. Most importantly, research scientists involved in genomics research will have access to systematic deliberations on the plant genomes of their interest. Elucidation of plant genomes is of interest not only for the geneticists and breeders, but also for practitioners of an array of plant science disciplines, such as taxonomy, evolution, cytology,

physiology, pathology, entomology, nematology, crop production, biochemistry, and obviously bioinformatics. It must be mentioned that information regarding each plant genome is ever-growing. The contents of the volumes of this compendium are, therefore, focusing on the basic aspects of the genomes and their utility. They include information on the academic and/or economic importance of the plants, description of their genomes from a molecular genetic and cytogenetic point of view, and the genomic resources developed. Detailed deliberations focus on the background history of the national and international genome initiatives, public and private partners involved, strategies and genomic resources and tools utilized, enumeration on the sequences and their assembly, repetitive sequences, gene annotation, and genome duplication. In addition, synteny with other sequences, comparison of gene families, and, most importantly, the potential of the genome sequence information for gene pool characterization through genotyping by sequencing (GBS) and genetic improvement of crop plants have been described. As expected, there is a lot of variation of these topics in the volumes based on the information available on the crop, model, or reference plants.

I must confess that as the series editor, it has been a daunting task for me to work on such a huge and broad knowledge base that spans so many diverse plant species. However, pioneering scientists with lifetime experience and expertise on the particular crops did excellent jobs editing the respective volumes. I myself have been a small science worker on plant genomes since the mid-1980s and that provided me the opportunity to personally know several stalwarts of plant genomics from all over the globe. Most, if not all, of the volume editors are my longtime friends and colleagues. It has been highly comfortable and enriching for me to work with them on this book series. To be honest, while working on this series, I have been and will remain a student first, a science worker second, and a series editor last. And I must express my gratitude to the volume editors and the chapter authors for providing me the opportunity to work with them on this compendium.

I also wish to mention here my thanks and gratitude to the Springer staff, particularly Dr. Christina Eckey and Dr. Jutta Lindenborn, for the earlier set of volumes and presently Ing. Zuzana Bernhart for all their timely help and support.

I always had to set aside additional hours to edit books beside my professional and personal commitments—hours I could and should have given to my wife, Phullara, and our kids, Sourav and Devleena. I must mention that they not only allowed me the freedom to take away those hours from them but also offered their support in the editing job itself. I am really not sure whether my dedication of this compendium to them will suffice to do justice to their sacrifices for the interest of science and the science community.

New Delhi, India Chittaranjan Kole

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1 History, Botanical and Taxonomic Description, Domestication, and Spread

Salej Sood, B. Kalyana Babu, and Dinesh Joshi

Abstract

Finger millet (*Eleusine coracana* L. Gaertn) is an annual small-seeded cereal mainly grown in Africa and Asia for both grain and forage. Once considered an orphan crop for subsistence agriculture, it is today's mainstream crop due to its exceptional adaptation qualities and nutritional importance. The name "finger millet" is derived from the shape of its panicles, where spikes look like fingers and thumb. The crop is a domesticated cereal of African origin that spread in pre-history to Asia and is associated with cultural history. Archeological findings suggest Ethiopian highlands as a primary center of origin of the crop, and its domestication happened in western Uganda to the area extending Ethiopian highlands. A long history of cultivation and large variability of finger millet landraces makes India the secondary center of diversity for the crop. The genus Eleusine has about nine species which are found across African, Asian, and South American tropical and subtropical areas. Studies on different species of *Eleusine* suggest that the cultivated gene pool diversity in finger millet in Africa has originated from the weedy progenitor *E. africana*.

Finger millet is an annual, small grain self-pollinated allotetraploid ($2n = 4x = 36$) plant mainly grown in two major continents, Africa and Asia (Sood et al. 2017, 2019). It has wide adaptability and its cultivation extends from sea level to higher elevations in the Himalayas (Gupta et al. 2012). Finger millet has the ability to grow under harsh conditions in diverse environments and has great food value in terms of nutritional profile. It is grown in dry and semi-dry regions for both grains and forage. The crop has exceptional adaptation under low moisture conditions and provides assured harvest under dry spells in marginal areas, suitable for contingency crop planning (Sood et al. 2016). The grains can be stored for years and have many health-promoting benefits besides a very good nutritional profile. The finger millet forage is also highly palatable and nutritious. At the global level among millets, finger millet occupies the fourth place, after major coarse grains, i.e., sorghum, pearl millet, and a minor millet, foxtail millet (Gupta et al. 2012).

Finger millet is a crop under the Poaceae family and Chloridiodeae subfamily. It is the

S. Sood (✉)
ICAR-Central Potato Research Institute, Shimla 171001, HP, India
e-mail: salej.sood@icar.gov.in

B. K. Babu
ICAR-Indian Institute of Oil Palm Research, Pedavegi, Andhra Pradesh, India

D. Joshi
ICAR-Vivekananda Institute of Hill Agriculture, Almora, Uttarakhand, India

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only millet that belongs to the tribe Chlorideae, whereas Piniceae is the tribe for all other millets. The finger millet panicles resemble the shape of the human thumb and fingers, therefore its English name has been given as "finger millet".

Global estimates for precise area and production data on finger millet are not available. However, the literature estimates reveal that 5 million tons of finger millet grains were produced from 4 to 4.5 million ha area globally. The total production of finger millet in Africa was about 2 million tons, which was slightly lower than in India (2.2 million tons) (Sood et al. 2019). In Africa, finger millet is cultivated in eastern and southern African countries mainly Ethiopia, Kenya, Malawi, Tanzania, Uganda, Zaire, Zambia, and Zimbabwe. India and Nepal are the major finger millet producers in Asia, but the crop is also grown to some extent in China, Bhutan, Japan, and Sri Lanka. The latest estimates on area, production, and productivity of the crop in India are 67.2 thousand ha, 61.6 thousand tons, and 1332 kg/ha, respectively, which indicate a considerable decline in area, production, and productivity in comparison to previous years (Directorate of Economics & Statistics, Government of India, 2020, https://eands.dacnet.nic.in/PDF/At%20a%20Glance%202019%20Eng.pdf). Among various finger millet-producing states of India, Karnataka tops the list with >50% area, followed by Maharashtra and Uttarakhand (Chandra et al. 2020).

1.1 Origin and Phylogeny

Earlier botanists argued and suggested the origin of finger millet as India based on historical records and mention of finger millet by Sanskrit writers as ragi or rajika (De Candolle 1886; Dixit et al. 1987). Burkill (1935) proposed that *E. coracana* is the cultivated form ascended through selection in India from wild species *E. indica* (L) Gaertn. It was further stated that finger millet originated in India and Africa independently (Vavilov 1951). More precise studies later explained that *E. coracana* is of African origin, domesticated in Western Uganda and Ethiopian highlands around 5000 years BC. The crop reached the Western Ghats in southern parts of India ~3000 BC (Hilu and De Wet 1976a; Hilu et al. 1979a, b; Fuller 2014).

The archaeologic studies in Ethiopia dating back to the third millennium BC confirm the African origin of finger millet (Hilu et al. 1979a, b). Finger millet has two discrete races, African highland and Afro-asiatic lowland race. The former seems to be a derivative from *Eleusine africana*, which also resulted in the African lowland race. As per Hilu and De Wet (1976b), this African lowland race reached India as an Afro-Asiatic lowland race around 3000 BC. Wide phenotypic variability has been reported in African germplasm collections in comparison to Indian collections in many studies, strengthening the claim that Africa is the primary center of origin of finger millet. During its cultivation for years in the Indian subcontinent, the gene flow has resulted in great diversity in local and primitive crop cultivars, making India the secondary center of origin of the crop (Padulosi et al. 2009; Sood et al. 2016).

The cultivated finger millet species (*E. coracana*) is tetraploid with a basic chromosome number of 9 ($2n = 4x = 36$) (Sood et al. 2019). The species *E. Africana* ($2n = 36$) exhibits great similarity with *E. coracana* in morphological features and gene flow occurs between them (Hilu and de Wet 1976b). First, the cytological studies showed that *E. indica* is one of the genome (AA) contributors to the cultivated species *E. coracana* and later chloroplast genome studies revealed *E. indica* to be the maternal genome donor of *E. africana*. The cytological studies also confirmed that *E. intermedia* and *E. tristachya* also belong to the genomic group "A" along with *E. indica* and these three species have a close genetic grouping (Mallikharjun et al., 2005). The results of genomic in situ hybridization and ribosomal DNA sites comparison on the

chromosomes of diploid and polyploid species inferred *E. indica* and *E. floccifolia* as two progenitors of *E. coracana* and *E. africana* (Bisht and Mukai 2000, 2001). Later, Neves et al. (2005) refuted *E. floccifolia* as a B genome donor based on genome analysis using nuclear internal transcribed spacers and plasmid trnT-trnF sequences. The results of molecular markers of Pepc4 gene inferred that the species *E. coracana, E. africana and E. kigeziensis* are of allopolyploid origin (Liu et al. 2011), and strengthened the claim of two separate allopolyploidization origins for *E. africana-E. coracana* group and *E. kigeziensis*. However in both the cases, the diploid species group, *E. indica-E. tristachya* was recognized as the maternal parent (Liu et al. 2014), the paternal parents could not be traced for both the events as they might not exist now (Zhang et al. 2019). The placement of *Eleusine* in the subfamily Chloridoideae is undisputed.

1.2 Taxonomy and Classification

Eleusine is a small genus with 9–10 species distributed across continents mainly Africa, Asia, and South America in tropical and subtropical habitats (Hilu 1981; Phillips 1972). Out of the nine species, eight species, *E. coracana*, *E. africana*, *E. indica*, *E. kigeziensis*, *E. intermedia*, *E. multiflora*, *E. floccifolia*, and *E. jaegeri* (Phillips 1972) belong to East Africa, which is the center of diversity for the genus, *Eleusine*. The only species which has emerged outside Africa is *E. tristachya* (Neves 2011). This species is native to South America. The three species under the genus Eleusine, *E. coracana*, *E. tristachya,* and *E. indica* has wide adaptation ranging from sea level to high hills, while *E. jaegeri*, *E. floccifolia*, *E. kigeziensis*, *E. intermedia,* and *E. multiflora* are adapted to upland habitats and grow well in areas above 1,000 m amsl. Both diploid and polyploidy species are found in the genus Eleusine with three basic chromosome numbers (x = 8, 9, 10). The species has been classified into two separate groups, annual and perennial based on their growth habit.

The species under the genus Eleusine lack clear separation based on the taxonomic relationships, therefore, the gene pool does not have defined boundaries with respect to the primary, secondary, and tertiary gene pool species. However, phylogenetic studies in the Eleusine genus have categorized the species into three classes. Domesticated and wild forms of finger millet have been placed in the primary gene pool while diploid wild species progenitors constitute the secondary gene pool and all other species belong to the tertiary gene pool (Sood et al. 2019). The primary gene pool includes *E. coracana* subsp. *africana* and *Eleusine coracana* subsp. *coracana*, secondary gene pool comprises *E. indica, E. floccifolia,* and *E. tristachya* and the species *E. intermedia, E. jaegeri, E. kigeziensis, E. multiflora,* and *E. semisterlis (*syn. *E. compressa*) form the tertiary gene pool (Table 1.1).

The cultivated *Eleusine* form can be easily distinguished from wild forms based on its firm spikes and large and ball-shaped grains (Neves 2010). As discussed above, eight species of the Eleusine genus are native to Africa, which also includes the wild species *E. coracana* subspecies *africana* which has moved to America and Asia, particularly in warmer parts. Due to natural interbreeding between cultivated and wild finger millet species, many new hybrid combinations have appeared, most of which are lookalike companion weeds of the crop. This has been studied and demonstrated through scientific evidence using molecular markers that gene flow between subsp. *africana* and subsp. *coracana* has happened in nature (Dida et al. 2008).

1.3 *Eleusine* Germplasm Collections

India holds the largest germplasm collections of 10,507 accessions in the National Bureau of Plant Genetic Resources, New Delhi, under long-term conservation. Although most of these accessions belong to cultivated species and are indigenous, the collection also contains 6 wild species. ICRISAT in India holds about 5,957

Table 1.1 *Eleusine* species, habitat, and salient features

Species	Vernacular name	2n chromosome numbers & genome formula	Gene pool	Geographical distribution	Growth habit and important features
Eleusine coracana subsp. *coracana* (L.) Gaertn	Ragi, *Koracan*, *Coracan*, Kodra, Kodo, Mandal, Nachni, *Wimbi*, *Hawere, Khawke, Mulirubi, Mugumbi*, *Limbi, Lupodo, Malesi, Lipoke, Usanje, Mawe, Koddo*, *Bulo*, *Bule,* etc	36 (x = 9), AABB	Primary	Indian subcontinent (India, Nepal), East Africa (Uganda, Kenya, Ethiopia)	Growth habit—Annual; robust culm; digitate or subdigitate panicle/ inflorescence with 3–10 fingers, thick and firm, fingers straight or incurved and 4–14 cm long, width 9–15 mm Black, brown to reddish, and white globular grains used for food and fermented alcoholic drinks and therapeutic usages, straw is used as fodder
E. coracana subsp. *africana* Kennedy-O'Byrne	-	36 (x = 9), AABB	Primary	Africa, mainly in eastern and southern uplands (Malawi, Kenya, Rhodesia, Tanzania), and Arabia	Growth habit—Annual; moderately robust culm, up to 100 cm length; Glabrous soft leaves digitate or subdigitate panicle with 3–17 fingers, which are 3.5–15.5 cm long and 4–7 mm wide Black to brown ovate-oblong grains, which are about 1.2–1.6 mm long, It is a weed but used as a forage grass
E. indica (L.) Gaertn	Goosegrass, crows foot grass, wiregrass	18 (x = 9), AA	Secondary	Cosmopolitan weed of African origin; mostly tropics and subtropics	Growth habit—Annual; Slender culms; soft, glabrous leaves; digitate or subdigitate panicle with 3–8 narrow fingers, which are mostly straight and around 5–10 cm long, 3–6 mm wide; Elliptic black grains with conspicuous ridges on the surface It is a weed but used as a forage grass and has medicinal value
E. floccifolia (Forssk.) Spreng	*Akirma, akrma, dagoo, garrgorr*	18 (x = 9), BB	Secondary	Ethiopia, Somalia, Kenya, Yemen, Eritrea	Growth habit—Perennial; moderately robust tough culms with an approximate height of 20–70 cm; 8–55 cm long folded leaf blades; subdigitate

(continued)

Table 1.1 (continued)

Species	Vernacular name	2n chromosome numbers & genome formula	Gene pool	Geographical distribution	Growth habit and important features
					panicle with 2–10 fingers, which are mostly straight, 2.5–12 cm long, and 3.5–6 mm wide; Blackish elliptic to oblong grains, 0.9–1.4 mm long
E. tristachya (Lam.) Lam	–	18 (x = 9), AA	Secondary	South America, Brazil	Growth habit—short-lived Annual/Perennial; decumbent 10–45 cm long culms, internodes elliptical in section; 6–25 cm long and 1–4 mm wide leaf blades; digitate panicle with 2–3 straight fingers, fingers 1–4 cm long and 5–16 mm wide; dark brown to blackish oblong to trigonous grains with punctiform hilum; It is a weed but has potential as a fodder crop
E. intermedia (Chiov.) S. M. Phillips	–	18 (x = 9), AB	Tertiary	Kenya, Ethiopia	Growth habit—Perennial; moderately vigorous culms; herbaceous glabrous leaves; sub-digitate to a racemose panicle with 4–15 straight fingers, which are 5–12 cm long and 4–8 mm wide; black elliptic to trigonous grains
E. jaegeri Pilger	Manyata grass, *mafutiana*, *akirma, dagoo, titima*	20 (x = 10), DD	Tertiary	Tanzania, Uganda	Growth habit—Perennial; Vigorous culms; Leather-type leaves with rough margins; subdigitate or racemose panicle with 2–10 straight fingers, which are about 4–17 cm long and 3–7 mm wide; Black elliptic/ oblong to trigonous grains

(continued)

Table 1.1 (continued)

Species	Vernacular name	2n chromosome numbers & genome formula	Gene pool	Geographical distribution	Growth habit and important features
E. kigeziensis S. M. Phillips	–	36 (x = 9), AADD	Tertiary	Uganda, Congo, Burundi, Rwanda, Ethiopia	Growth habit—Perennial; Robust culms; Soft and Glabrous leaves; digitate panicle with 2–7 straight fingers, 7.5–14 cm long and 4.5–5.5 mm wide; Black elliptic to trigonous grains
E. multiflora Hochst. ex A. Rich	–	16 (x = 8), CC	Tertiary	Eritrea, Kenya, Ethiopia, Tanzania	Growth habit—Annual; Slender around 45 cm long culms; Soft, wide, and flat leaves; racemose panicle with 3–8 short wide curved fingers, 1–4 cm long and 8–16 mm wide; black oblong-compressed grains with a ridged surface; It is a weedy species but has potential as a valuable forage grass
E. semisterilis S. M.Phillips	–	Cytologically unknown	–	Kenya (Maybe extinct now)	Growth habit—Perennial; slender, erect, 145 cm high culms; leaf blades linear and loosely folded; subdigitate panicle with around 9 fingers which could be 5–15 cm long, laxly arranged spikelets in the fingers; black obovate grains

Source Phillips 1972; Liu et al. 2011; Sood et al. 2019; http://www.theplantlist.org/browse/A/Poaceae/Eleusine/

global accessions, of which 105 are of wild species. The major collection of wild species of finger millet is conserved and maintained at Agricultural Research Station, Griffin, Georgia, USDA, which has 17 wild species (*E. floccifolia*, *E. indica*, *E. jaegeri*, *E. multiflora,* and *E. tristachya*) out of the total collection of 766 accessions. Eastern Africa, which is the primary center of origin of the crop, Kenya, Zimbabwe, Uganda, and Zambia hold about 1902, 1158, 1155, and 497 accessions. Besides, many other South Asian and African countries hold small germplasm collections (Sood et al. 2019). The global finger millet collection at ICRISAT has been characterized and core, as well as the mini-core set, have been developed for use in breeding and genomics studies (Upadhyaya et al. 2006). Although global diversity of finger millet has been conserved and important accessions have been identified, the wild species particularly, *E. coracana* subsp. *africana* and progenitors also need due attention (Neves 2010).

Based on compactness and shape of inflorescence, finger millet germplasm has been classified into races and subraces. The salient characters of races and subraces under each species are given in Table 1.2 (Prasada Rao et al. 1993; Bharathi 2011).

1.4 Crop Adaptation and Floral Biology

Finger millet has wide adaptation and can be grown in a wide habitat because of its hardy nature and short growing season. Being a C4 crop, it is highly efficient in adapting to environmental fluctuations and climate change. It can be grown from coastal plains to high hills, between 500 and 2,400 m above sea level (Fig. 1a). The genotype response although varies with agro-ecologies. Short-duration varieties are generally adapted to highlands and medium to long-duration cultivars do well in plains and tropical areas. The crop completes its life cycle in 75–160 days. It is generally grown in drylands as a rainfed crop but irrigated crop does well in terms of grain yield and the potential yield under irrigated conditions is around 5–6 t/ha. The crop can tolerate some waterlogging, but water stagnation severely affects crop productivity. Finger millet volunteers, shattering types are common in crop fields and difficult to identify early in the season. They look like normal plants but their seed starts shattering even in the immature stage itself.

The height of finger millet plants varies from 30 to 150 cm and mostly medium height cultivars are grown in India (100–130 cm). Finger millet inflorescence is in the whorl of 2–11 digitate, straight or slightly curved spikes (Fig. 1b and c). The spike is 8–15 cm long and 1.3 cm broad. In each spike, about 50–70 spikelets are arranged alternatively on one side of the rachis (Gupta et al. 2012). Each spikelet contains 3–13 florets. The florets have three stamens and the gynoecium is bi-carpellary, uni-locular with a superior ovary having two styles with feathery branched stigma (Seetharam et al. 2003). The anthers surround the stigma, which ensures self-pollination. Finger millet grains vary in shape from round-oblong/oval, and white -reddish-brown in grain color (Fig. 1.2). The surface of finger millet grains is finely grooved and its pericarp is fused to the surface of the grain. Finger millet wild relatives have seed shattering trait, and at maturity seeds disperse naturally from the panicle, the cultivated species lost the seed shattering trait during domestication but it varies from cultivar to cultivar (de wet et al. 1984). Some cultivars are hard threshers while others still disperse some seed naturally at maturity.

The pollination system studies in finger millet revealed that pollen dust covers the stigma before it comes out of the lemma, leaving no or little chance for cross-pollination (Gupta et al. 2012). The spikelets opening follows the top to the bottom pattern in each spike, and florets in the spikelets open from bottom to top. The studies suggest that one floret in the spikelet opens per day. The flowering completes in around 5–7 days. Anthesis happens early morning between 1.00 and 5.00 a.m., when anthers dehisce to pollinate their stigmas (Gowda 1997). Dodake and Dhonukshe (1998) reported that pollen grains remain viable in finger millet for about 20 min, while stigma receptivity stays for up to 5 h. The estimation of natural crossing does not exceed 1% in finger millet (Seetharam 1998). Inter-varietal hybridization using the contact method (Ayyangar 1934) is the simplest and easiest way for recombination breeding. For successful hybridization, genotypes having dominant character such as pigmentation on nodes are used as the male parent. This helps in the identification of true hybrids in the F_1 generation. However, inducing male sterility through hot water treatment for 5 min at a temperature of 48–52 °C of immature inflorescence on the 3rd to 4th day of emergence was effective in getting few true hybrid seeds (Sood et al. 2019). Genetic male sterility (GMS) and partial GMS source have been identified in the crop but are of little use due to maintenance problem and varying level of sterility/fertility in different locations (Gupta et al. 1997; Gowda et al. 2014; Sood et al. 2019).

Table 1.2 Races and subraces in finger millet germplasm and their features

Species	Subspecies	Race	Subrace(s)	Salient features
E. Coracana	*Coracana*	*Elongata*	*liliaceae, stellata, incurvata,* and *digitata*	This race is commonly found in Africa and Asia. It has long slender 10–24 cm long panicles with digitately arranged spreading fingers, which curve outward on maturity. In the subrace liliacea, the fingers in the panicle are reflexed, while subrace stellata contains twisted fingers. As the name suggests, subrace incurvata have incurved fingers that give a fist-like appearance, and subrace digitata has top curved fingers
		Plana	*seriata, confundere,* and *grandigluma*	This race plana has large 8–15 mm long spikelets, which are arranged on the rachis as even rows of two and look like a flat ribbon The subrace seriata has serially arranged spikelets on the rachis, which gibes ribbon-like appearance, the subrace confundere contains numerous fertile florets which upon grain filling at maturity give a compact look to the panicle, the grandigluma subrace is characterized by very large pointed glumes, which are longer than spikelets
		Compacta		The race compacta members are commonly stated as cockscomb finger millets. The spikelets have 9 or more florets. Fingers incurve at the tip to form a fist-like panicle
		Vulgaris	*laxa, reclusa,* and *sparsa*	The race vulgaris is the most distinct among all the four races of finger millet based on phenotype. It has long slender panicle branches, which are arranged digitately, and spikelets have 4–8 florets. The moderate number of florets in the spikelets give a semi-compact appearance and fingers incurve at maturity As the name suggests, laxa subrace has long open fingers and spikelets arranged in thin rows on the rachis of the panicle fingers. This subrace resembles the wild Africana race. The subrace reclusa is characterized by short open fingers, which do not curve. The sparsa subrace is also characterized by open fingers, however, the spikelets arrangement on the panicle has naked space in between the clusters of spikelets
E. africana	*africana*	*africana*	–	The panicles in the race africana are long and thin, i.e., around 8–17 cm long and about 5 mm wide. The spikelets on the rachis are arranged in two rows on one side with 4–9 flowers. The glumes are short <5 mm, smaller than the spikelet
		spontanea	–	The features of race *spontanea* match with race *africana.* The race *spontanea* contains derivatives of hybridization between *E. coracana* and *E. africana*

Source Bharathi (2011)

Fig. 1.1 Finger millet crop in Uttarakhand hills, India. **a** Crop stand of improved variety VL 376. **b** Immature panicles of finger millet variety VL 376. **c** Mature panicles of finger millet variety VL 376

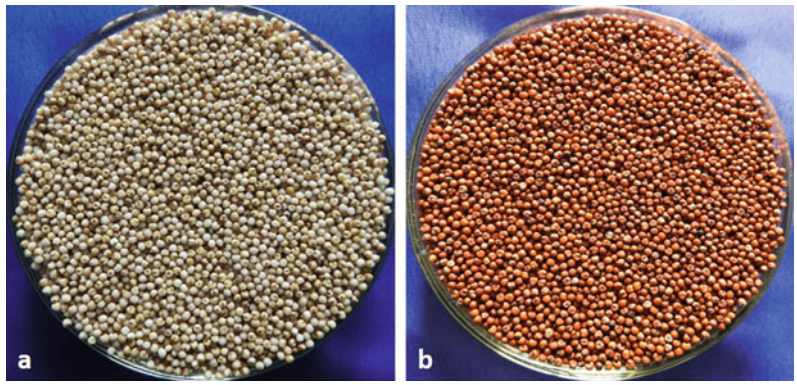

Fig. 1.2 Cleaned finger millet grains after threshing. **a** White grains of finger millet variety VL 382. **b** Reddish-brown grains of finger millet variety VL 376

1.5 Genome Size

Using Feulgen microspectrophotometry, nuclear DNA content of *Eleusine* spp. was first reported by Hiremath and Salimath (1991), which was later substantiated by Mysore and Baird (1997) with more accurate analysis using laser flow cytometry. The 2C DNA content of different *Eleusine* species varied from 1.51 to 3.87 pg. The cultivated species, *E. coracana* was found to have 3.36–3.87 pg 2C DNA followed by *E. coracana* subsp. *africana* (3.34 pg), *E. indica* (1.61–1.76 pg), *E. tristachya* (1.51 pg), *E. floccifolia* (2.0 pg), *E. multiflora* (2.65 pg), and *E. jaegeri* (1.90 pg). A recent study reported 1.20, 1.84, 1.14, 1.21, 2.52 pg 2C DNA content in *E. jaegeri*, *E. multiflora*, *E. tristachya*, *E. indica,* and *E. coracana* subsp. *africana*, respectively (Hittalmani et al. 2017). In their study, the cultivated species *E. coracana* was found to have 3.01 pg 2C DNA content and 1453 Mb genome size. The analysis of genome size of wild species showed a range of 580 Mb in *E. jaegeri* to 1217 Mb in *E. coracana* subsp. *africana*. *E. coracana* subsp. *Coracana,* and *E. coracana* subsp. *africana* were found to have almost similar genome size which was attributed to the domestication of *E. coracana* subsp. *coracana* from *E. coracana* subsp. *africana* (Hittalmani et al. 2017). Although in comparison to many types of grass and other plants, the genome size of the *Eleusine* species is small, still it is too large for genomics studies (Neves 2010).

1.6 Genetic Improvement

This spatial isolation of the crop in India and Africa has led to the appearance of two genetically and morphologically diverse gene pools. However, studies conducted on genetic diversity in African and Indian collections presented much larger variation for inflorescence color in African accessions in comparison to Indian collections. Many studies conducted on the phenotypic evaluation of Indian and African germplasm showed wide variation for inflorescence types in both gene pools. The studies reported that most Indian accessions inflorescence belong to race *vulgaris,* i.e., they have semi-compact to compact ears while varied ear types extending from open to fist-shaped, mostly belonging to two major races *plana* and *compacta* were found in African accessions. Accessions in both the gene pools also vary for many quantitative traits (Naik et al. 1993). More diversity in the African gene pool has been attributed to gene flow from wild species *E. africana* into cultivated finger millet.

Various DNA-based molecular markers have been used to study the genetic diversity of the finger millet gene pool. Both genomic and genic simple sequence repeat (SSR) markers have been used markers for profiling finger millet accessions to study the genetic diversity. Due to the nonavailability of SSRs earlier studies used random amplified polymorphic DNA (RAPD) markers. Most of these studies clustered the finger millet accessions into two major groups, belonging to two distinct gene pools, i.e., African and Indian gene pools. In a study of Indian accessions, DNA markers could clearly classify accessions of North India and southern India. The South Indian accessions were found to be genetically close to African accessions. The results of genetic diversity studies substantiate that accessions of southern India are closer to African genotypes due to their origin from wild species *E. africana*, however, the north and northeast accessions are different and the uniqueness of such gene pool needs to be explored (Panwar et al. 2010a, b).

The introduction and use of African germplasm in India resulted in a higher genetic gain in finger millet breeding. Indo-African crosses in finger millet generated more variability and diverse parents resulted in higher productivity of finger millet, which increased >50% in Karnataka State and around 60 percent in Tamil Nadu State in India (Seetharam 1982; Nagarajan and Raveendran 1983). Blast is the major biotic stress affecting finger millet productivity and the identification of stable sources of resistance is the key to developing resistant genotypes. Screening of diverse germplasm particularly African germplasm has resulted in the identification of stable sources of resistance for the blast in finger millet, which has been used to develop resistant varieties through recombination breeding (Seetharam 1998). To date, more than 30 finger millet varieties have been released in India, where the major breeding objectives were maturity duration, grain yield, fodder yield, and disease resistance. The least emphasis was laid on nutritional quality traits earlier but now the nutritional quality is the integral component of finger millet breeding programs in India and Africa.

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Economic, Nutritional, and Health Importance of Finger Millet

2

Manoj Kumar Tripathi, Anil Kumar, Debabandhya Mohapatra, Rajpal S. Jadam, Shilpa S. Selvan, and C. Nickhil

Abstract

Finger millet (*Eleusine coracana*) is a principal cereal crop in many regions, where low-income people are more. Finger millet grains have predominant differences in color as white, brown, and light brown varieties, great value nutritional compounds, high quantity of phytochemicals molecules, enriched with several amino acids (essential), availability of several crucial minerals compounds, and also gluten-free status. Although the grain is rich in several bioactive and nutritionally valuable compounds, finger millet is enormously ignored and remains extensively underutilized. The biochemical composition of finger millet grains has significant contribution in reducing several human health risks such as metabolic diabetic conditions, high blood pressure, and digestive tract illness. Several traditional processing methods are now available and important for proper utilization of grains, for example, malting, soaking, cooking, fermentation, and popping. The available processing methods play a crucial role in improving the nutritive and organoleptic properties and are also very effective in the reduction of anti-nutritional compounds such ds as phenols, physic acids, and tannins in finger millet. Very few studies are available for finger millet utilization and there is an urgent need for further studies on bioactive compounds, improved processing means, nutraceuticals, and product formulations.

2.1 Introduction

Millets are grown as major and significant crops in several regions of the world. Millet crops are recognized as very healthy grain that can be grown on minimal lands with lower water levels. Short growing season under dry- and high-temperature conditions is favorable for their higher productivity. Majority of millet grows and ripens within 70–90 days of the growing period. Millet crops mainly comprise finger millet *Eleusine coracana* (L.), sorghum (*Sorghum bicolor* (L.), kodo millet (*Paspalum scrobiculatum* L.), little millet (*Panicum sumatrense,)* foxtail millet (*Setaria italica* (L.), pearl millet *Pennisetum glaucum* (L.), proso millet (*Panicum miliaceum* L.), barnyard millet *Echinochloa colona* (L.) and *Echinochloa crusgalli* (L.). In

M. K. Tripathi (✉) · D. Mohapatra · R. S. Jadam · S. S. Selvan · C. Nickhil
ICAR-Central Institute of Agricultural Engineering, Bhopal MP-462038, India
e-mail: manoj.tripathi1@icar.gov.in

A. Kumar
Rani Lakshmi Bai Central Agricultural University, Uttar Pradesh, Jhansi, India

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the developed world, the importance of millets is not very high. At the present condition, the traditional foods are mainly in use for food purposes and major technologies for the purpose of the development of ready-to-cook foods are inadequate. It is found that millet crops have nutritive and health potential comparable to major cereals and many technologies for processing for food development are also available but the use of millet for food is quite limited to major populations in the world and rural populations at the domestic level. The reasons for this are the lack of some more innovative processing technologies, easy to handle, ready to cook, ready to eat, and safety aspects at commercial levels for populations of urban people. In the current scenario, the novel healthy foods products are recognized if their nutritional status is proper and that will also help in convincing the consumer. Major urban consumers prefer breakfast cereals, pasta, noodles, and baked products like bread and biscuits in food.

Increasing demands for healthy foods populations are concentrated on agriculture practices and the production of such types of crops. Consumption of millet grain will increase if process technologies for processing are accessible commercially in millet-consuming countries. Divergence use of food production may play a major role in encouraging production and consumption. The use of whole multigrain food and multigrain substitute are good options for healthy food and healing dietary alteration. Encouraging consumption of millet grain foods is now in practice to decrease the frequency of several diseases in the urban population.

The use of millet grains for food purposes is important in human history, predominantly in Asia and Africa, and has also been cultivated in East Asia for the past 10,000 years. Millet crops are very reliable crops for drought and infertile soils in comparison to other grain crops. The yield of millet on a per hectare basis can improve greatly by suitable irrigation and justifiable soil supplements. The use of advanced breeds of millet can meaningfully increase farm yield production.

2.2 Economic and Nutritional Importance

Keeping up with ideal worldwide public well-being needs promotion of food involved in health and elongation of aging instead of only prevention of chronic illnesses. Presently, there is a greater advancement in medical and health science but we are also facing problems of negative impacts of medicines and their economic burden. In ancient times, it was a well-known dogma that food is the medicine and medicine is the food which specifies that nutrition and its components play a significant role in grand health welfare (Dev et al. 2011). Therefore, the emergent call for healthy food foodstuffs is encouraging revolution and advance in the global scenario. This direction of research and development is mainly concerned with nutrition and health components are designated as nutraceuticals (Chauhan et al. 2013; Keservani et al. 2010; Dev et al. 2011). Several health information advocate the health benefits of nutraceuticals as avoidance and defence against numerous chronic disorders. Therefore, any important change of nutrients due to inopportune nutritive issues affects the health outcome. Presence of health values, whole-grain cereals can be admired as key nutraceutical applicants for human intake and a better lifestyle. This chapter presents a view of finger millet (*Eleusine coracana*), which have several nutritional and nutraceutical qualities to encounter worldwide nutritional disability.

Finger millet comes from the Poaceae family and is usually famous as ragi or madua in India, dagusa in Ethiopia, and rapoko in South Africa (Ignacimuthu and Ceasar 2012). Worldwide, 12–13% of the total millet-producing area is used in finger millet cultivation in more than 25 countries of Africa and Asia and practiced as a major staple food for individuals living with very restricted economic capital. Finger millet can be cultivated on marginal lands, and high altitudes with drought and saline conditions. It needs slight irrigation with little agricultural support but maintains ideal yields.

Finger millet has been reported as the greatest nutritive among all main cereals (National Research Council 1996) and is also thought of as a potential super cereal by the United States National Academies (National Research Council 1996). In view of nutrition, it is extensively rich in minerals which are greater than some key cereal as wheat and rice (Vadivoo et al. 1998; Antony and Chandra 1998). It is reported to be a great source of calcium in comparison to other cereals such as rice, wheat or maize, and milk. Higher contents of iron and fiber, favor this crop as more nutritive. Finger millet has major important amino acids (essential) such as lysine (McDonough et al. 2000) and methionine. Finger millet is also a rich source of some important polyunsaturated fatty acids, for example, linoleic acid and α-linolenic acid (Fernandez et al. 2003), helpful in the brain and nervous system development (Birch et al. 2007; Jacobson et al. 2008). Finger millet is also a good source of vitamins such as thiamine, riboflavin, niacin, and tocopherols (Obilana et al. 2002).

Cultivation and utilization of finger millet are mainly restricted in developing countries with minor farmers having inadequate agricultural resources and referred to as grain of poor people (National Research Council 1996). Even with established health benefits, only a restricted advancement has been made in use as a functional food. Although it has immense potential as nutritional and therapeutic food, it remained underutilized by the common people due to either unfamiliarity or hesitation.

Presently, the commercialization and marketing strategies of finger millet grain increase its opportunities for yield and formulation of several value-added products. Hence, there is an urgent necessity to advance processing technologies such as steaming, soaking, malting, and fermentation for improving the value of the final products. Divergence of value-added foodstuffs would also improve finger millet commercialization aspects. Its exceptional malting value makes it appropriate as a raw substrate for the brewing industry (Taylor et al. 2006). Truly, promoting and demonstrating finger millet and other millets as a healthy food product with greater nutrient excellence shall rise its use in populations who are really aware of their health (Shobana et al. 2013). Numerous reports have emphasized the impact of underutilized grain such as finger millet on revenue generation in both national as well as global markets (Chadha and Oluoch 2007). In case of a developing country like India, by value addition to millet assumed nearly increased two- to threefold farmer incomes and created new employment openings, mainly for females (Vijayalakshmi et al. 2010). This is a great opening to grow markets for non-staple crops which may be a source of income in poor populations (Kahane et al. 2013).

2.3 Finger Millet Grains

Grains of finger millet have low glycemic index and gluten-free crops. (Muthamilarasan et al. 2016; Manjula and Visvanathan 2014). Due to low glycemic index properties, finger millet is found to be a good option for populations with conditions of gluten ingestion and diabetic conditions. Finger millets with the presence of low glycemic index grain may be useful in controlling blood glucose levels (Jideani and Jideani 2011). Finger millet grains are rich in dietary fiber, calcium, and iron minerals in comparison to other cereal grains (Sood et al. 2016). The presence of these minerals is found to be useful in the decline of several diseases such as coronary, cardiovascular, obesity, and diabetic conditions (Kaur et al. 2014; Ramashia et al. 2018). In some reports, it was found that grains of finger millet are very rich in polyphenols and phytates compounds which are familiar to affect the accessibility of some important minerals.

2.4 Morphology of Finger Millet

The grains of the major cultivar of finger millet are brown while a very few with white and red color (Ramashia 2018). Finger millet grains differ from approximately 1.0 to 1.5 mm in diameter (Gull et al. 2014; Siwela 2009). The main

structural portions of the finger millet grains are pericarp, germ, and endosperm. The pericarp is an outer layer and contains three layers as epicarp, mesocarp, and endocarp (Ramashia 2018). The pericarp layer is removed during processing as a nonedible part. (Patel and Verma 2015). The endosperm portion is near the seed coat which is utilized in flour making (Palanisamy et al. 2012).

Finger millet is presently used as a significant primary food in various areas of Asia (Gari 2001) as a major functional food. This grain has tremendous potential for boosting food security and rural development and enhances nutritional value (Oduori 2005). Finger millet may play an important role in improving the nutritive and therapeutic features of formulated foods. Millet grain seed is an edible part and a good source of several biochemical components such as phytochemicals, food fibers, polyphenols, and minerals. The presence of essential amino acid, methionine, and several other micro and macronutrients which are not present in poor people's diets make this grain more valuable (Schaafsma 2000).

2.5 Specialty of Finger Millet

Finger millet is presently used as significant primary food in various areas of Asia (Gari 2001) as a major functional food. This grain has tremendous potential for boosting food security, rural development and enhances nutritional value (Oduori 2005). Finger millet may play important role in improving the nutritive and therapeutic features of formulated foods. Millet grain seed is an edible part and a good source of several biochemical components such as phytochemicals, food fibers, polyphenols, and minerals. The presence of essential amino acid, methionine, and several other micro and macronutrients which are not present in poor people's diets make this grain more valuable (Schaafsma 2000).

The soluble dietary fiber present in enough amounts in grains may support in proper regulation of blood glucose and serum cholesterol level (Anderson 1980). It is particularly suggested as healthy food for diabetic persons. Properly processed and regular use in diets of finger millet is well-known to decrease the threat of diabetic conditions (Gopalan 1981) and gastrointestinal tract illnesses (Tovey 1994). Many health-promoting roles was reported in finger millet which evidences its use as nutraceutical food, nutritive, feed, cultural, and medicinal in industrial and economic prospective. It is also reported as a wonder grain because it has a wide variety of range, abiotic stress tolerant, higher storage, and very rich in nutraceutical compounds.

2.6 Nutritive Value

Finger millet grains contain major nutrients which are essential for human use. Grains are extensively rich in protein, dietary fiber, minerals, and some anti-nutritional compounds such as phytates and phenolic compounds. Several studies suggest that finger millet has a decent quantity of beneficial compounds as dietary fibers which is healthy for digestion. Finger millet grains have a very low amount of fat that is appropriate for dietary sources. Finger millet is the best source of some important minerals like calcium, phosphorus, potassium, sodium, etc., and vitamin B complex. Millets also have significant amounts of essential amino acids specifically rich in sulfur. Finger millet grain is also known for amino acids arginine, histidine, lysine, tryptophan, phenylalanine, tyrosine and methionine.

Finger millet grains are also utilized as a whole which is simply consumable with good flavor (Thapliyal and Singh 2015). Finger millet can be served as good source of vitamins and fatty acids (Rurinda et al. 2014). Important health aid of the grains is low discharge of glucose molecules during digestion into the bloodstream (Chappalwar et al. 2013; Mamatha and Begum 2013), which reduces the demand for glucose frequently and reduces constipation (Vanithasri et al. 2012). Millet grains are also linked with lowering the threat of diabetes, effective in blood pressure, cholesterol control, cancer, and cardiovascular diseases (Pradeep and Sreerama 2015; Subastri et al. 2015) (Table 2.1) (Asharani et al. 2010).

Table 2.1 Some important compounds of finger millet grains associated with health (Chandra et al. 2018; Sarita and Singh 2016; Thilagavathi et al. 2015)

Compounds	Biological functions
Phytic acid	Lowering body cholesterol
Phenolic and Tannin compounds	Role in healing and metabolic aging conditions Useful in several cancer and cardiovascular conditions Effective in blood pressure and diabetes
Ferulic acid	In tissue repair Encourage wound curing
Dietary fiber	Help in hypoglycemic and hypolipidemic conditions Effective in serum cholesterol control Effective in atherosclerosis Anti-cancerous properties
Magnesium	As cofactor for enzyme systems Blood glucose control, Blood pressure regulation
Phosphorus	Formation of bones and teeth Body growth and maintenance Repair of cells and tissues

2.6.1 Mineral, Vitamin, and Fatty Acid Content

Finger millet grains are rich in essential minerals, for example, calcium (Ca) and phosphorus (P) and play significant roles in children's development and pregnancy condition (Jideani 2012; Chappalwar et al. 2013). Their role is also very important in obesity, diabetes, and malnutrition (Jayasinghe et al. 2013; Manjula et al. 2015). Regular consumption of finger millet can be very effective for calcium deficiency (Towo et al. 2006). Phosphorus, whose concentration varies from 130.0 to 283.0 mg/g, is also one of the important minerals found in finger millet grains which contribute to the energy metabolic pathway and tissue repairing in the human body (Vanithasri et al. 2012; Ramashia et al. 2018). Some other important minerals present in finger millet grains are iron (3–20%) (Shukla and Srivastava 2014; Rajiv et al. 2011) and magnesium. Both the minerals play a role in the control of blood pressure, asthma, and heart attack (Saleh et al. 2013; Verma and Patel 2013; Prashantha and Muralikrishna 2014). Several reports indicate that finger millet grains have more nutritive value than other millets (Devi et al. 2014; Dlamini and Siwela 2015) (Table 2.2).

Vitamins are also one of the important micronutrient components essential for proper growth and maintenance of the human body and deficiency of vitamins may cause several

Table 2.2 Nutritional composition (proximate and minerals) of finger millet (Dlamini and Siwela 2015; Devi et al. 2014)

	Proximate composition (%)					
Finger millet	Moisture	Carbohydrates	Dietary fiber	Fat	Protein	Minerals
	7.15–13.1	75.0–83.3	15–22.0	1.8	7.7	2.7
	Common minerals composition (mg/100 g)					
	Phosphorus	Potassium	Magnesium	Calcium	Sodium	Iron
	130–250.0	430–490	78–201	398.0	49.0	3.3–14.89

Table 2.3 Major vitamin content and fatty acids of finger millet (Ramashia 2018; Saleh et al. 2013; Ramashia 2018; Serna-Saldivar 2010)

	Vitamins composition (mg/100 g)				
Finger millet	Vit A (Retinol)	Vit B_1 (Thiamine)	Vit B_2 (Riboflavin)	Niacin	Vit C (Ascorbic acid)
	–	0.2–0.48	0.12	1.0–1.30	0.0–1.0
	Fatty acid compositions (g/100 g of total fats)				
	Palmitic acid	Linoleic acid	Oleic acid	Linolenic acid	
	21.1–24.7	24.2	49.8	1.3–4.40	

deficiencies diseases. Finger millet grains contain a good amount of water-soluble vitamins and fat specifically vitamins A and B complex (Table 2.3) (Devi et al. 2014; Chappalwar et al. 2013).

Grains of finger millet also comprise several major essential fatty acids such as linolenic and palmitic acids, which are critical in the improvement of the brain and nerves (Kunyanga et al. 2013; Muthamilarasan et al. 2016). There are very low contents of fatty acids, which have a better shelf life and are helpful in body weight management (Gunashree et al. 2014; Singh et al. 2012; Verma and Patel 2013). Low content of fat and dietary fiber with higher amounts of carbohydrates in finger millet are important for nutritive and physiological aids (Vanithasri et al. 2012; Banusha and Vasantharuba 2013).

2.6.2 Amino Acids Content

Finger millet is very rich in some important essential amino acids. A good amount of approximately 44% of essential amino acids are found in finger millet grains, which mainly include methionine, cysteine and tryptophan, lysine, isoleucine, leucine and phenylalanine, and threonine (Singh and Raghuvanshi 2012; Ramashia et al. 2018; Sood et al. 2017) These components also work in nutrition as well as health aspects (Thapliyal and Singh 2015). The amount of amino acid methionine was reported to be higher than any millet source (Prashantha and Muralikrishna 2014; Singh et al. 2012).

2.6.3 Anti-nutritional Composition of Finger Millet

Some important anti-nutritional compounds reported in Finger millets are phytate, tannins, trypsin inhibitors, and flavonoids. These anti-nutritional compounds are reported to reduce the nutritional properties of finger millet grains (Palanisamy et al. 2012). The major polyphenolic compounds of finger millet are phenolic acids and tannins and the quantity of flavonoids is reported as miner components. The presence of polyphenolic compounds is responsible to keep a good body's immune system against pathogens and clinical conditions (Udeh et al. 2017; Siwela et al. 2007; Devi et al. 2014). Tannins present in the outer layer of finger millet grains function as a physical barrier against fungal pathogens attack (Devi et al. 2014) and are also reported as an important role in several biological functions. A negative impact was reported in case of some anti-nutritional compounds, which are concentrated in finger millet and this compound reduces the digestibility of some nutrient compounds and the absorption of some important minerals.

Tannin compounds are also reported to affect growth due to their adverse impact on the function of some important body organs such as the pancreas, thyroid gland, and liver. These tannin compounds also influence the color, flavor, and nutritional quality of food products developed from finger millet grains. Tannin compounds were also reported for antioxidant activity and help in aging and the avoidance of some important metabolic diseases (Shibairo et al.

2014). Some current findings have presented that some important processing methods can increase the bioavailability of nutrients such as soaking, steaming malting conditions, fermentation conditions, and decortication process (Sood et al. 2017; Sripriya et al. 1997; Platel et al. 2010; Krishnan et al. 2012).

2.7 Use of Finger Millet Grains

Finger millet grains are principally used for the development and preparation of value-added traditional food, a healthy component in nutraceutical food and beverage. The grains can be used in several ways and form in natural and malted forms. The majority of food products developed from finger millet grain and flour in developing nations are not commercialized and not available commercially in the market. In case of sorghum and wheat, many food products are commercially available in big supermarkets around the world (Siwela 2009). Towo et al. (2006) stated that foods formulated from finger millet are not similar in many countries and their grains are found to be healthy for consumption at any stage however, grains are deserted despite their huge nutritive benefits. Many current studies stress prospects for research, health benefits, and utilization of finger millets in developing nations (Table 2.4) (Amadou et al. 2013). These studies may help in reducing heart illnesses, cancers, obesity, and diabetes in these nations (Kaur et al. 2014).

Being gluten-free and rich in fiber, finger millet can prove to be a boon for people with Celiac disease and can be utilized as preventive drug entity for osteoporosis due to its exceptionally high calcium content. Some important processes and technology are useful in the development of value-added products, minimization of anti-nutritional compounds, and enhancement of several nutritional and nutraceutical compounds (Table 2.5). Six finger millet genotypes differing in grain colors, viz., brown, golden, and white were evaluated for their popping quality to select the appropriate genotype for developing ready-to-eat products

Table 2.4 Current research gaps, scientific investigations for utilization of finger millet (Abraham et al. 2014; Jideani 2012; Saleh et al. 2013; Amadou et al. 2013; Verma and Patel 2013; Shukla and Srivastava 2014)

Earlier studies observations	Investigation gap
Necessities of openings in the area of new research and development Establishment of linkage in the field of finger millet research	Technology for commercial development of value-added and suitable processed food products that fulfil requirement of the urban buyer Promote industries to develop finger-millet-based foods
Promotion for consumption, preparation of value-added products and awareness of health benefits	Boost and commercialization for rise in the intake of finger-millet-based food in urban regions is desirable
Intensive nutritive studies	Upcoming developments must be attention on nutritive food from millet
Processing for nutritious and quality food products, upgrading of finger millet grains and their developed food products	Increase extremely improved millet based food products Novel process and technologies for processing and preparation of food products are required which improve micronutrient bioavailability Improve the value of millet foods in human
Promote development of fortified food	Complementary foods formulations and development to achieve the wide gap of food accessibility and food security
Combined with modern food which control metabolic diseases	Commercialization of finger millet food products for patients suffering from diabetes
Conservation of environmental resources to manage the climatic issues in upcoming time	Educate the community and farmers for conservation of environmental resources

Table 2.5 Process and technology for products development

Procedures	Purposes	Type	Reported work
Soaking process	Enhancement in minerals bioavailability	Soaking grains	Saleh et al. (2013)
Malting process	Enhancement in nutritive value, sensual features, and digestibility	Weaning diets	Verma and Patel (2013)
Milling process	Separation of bran, germ, and endosperm and transforms the grain to flour	Flour	Chandra et al. (2018)
Roasting process	Increases the nutritious value and shelf life	Roasted form	Thapliyal and Singh (2015)
Radiation process	•Increases the nutritive value •Decreases the anti-nutrient compounds •Helpful in preserving the foods for longer time	Canned form	Rodrigues et al. (2014)
Cooking process	Help to make palatable and safe Minimize and inactivate anti-nutritional compounds	Porridge form	Kakade and Hathan (2014)
Popping or puffing process	Increase nutritious value Improves taste, color, and aroma	Breakfast, snack, and ready-to-eat foods	Verma and Patel (2013) Neelofar et al. (2014)
Extrusion process	Dehydrated foods preparation	Baby foods, noodles, macaroni, etc.	Rathore et al. (2016)

and for studying the relationship of their physical, nutritional, and biochemical properties with popping quality. Grain hardness, hydration capacity, and moisture content showed a significant relationship with the popping quality (Neelofar et al. 2014).

2.8 Biochemistry for Grain Stability: Factors Affecting the Shelf Life of Pearl Millet Grains

Finger millet is known for protein, dietary fibers, and mineral compounds (Sripriya et al. 1997; Mathanghi and Sudha 2012). It is significant because of its nutritive value. Its dietary fiber and mineral content is markedly higher than wheat, rice, and fairly well-balanced protein (Ravindran 1991). It reported that FM grains contain polyphenols and phytates which are known to influence the availability of minerals (Kaur et al. 2014; Ramashia et al. 2018; Krishnan et al. 2012).

Since it is gluten free, the grain is ideal for people who have gluten allergy. It is widely recognized as the staple food for poor and small landholder populations, and its biochemical composition also supports nutrients and energy. Due to the presence of lipid and unsaturated fatty acids, its flour suffers from problems of oxidative and hydrolytic rancidity which is responsible for the production of unpleasant taste and off-odor during storage (Carnovale and Quaglia 1973). Due to higher lipase activities and rapid release of fatty acids, which limits its shelf life; so the rapid development of off-flavor (Goyal et al. 2017).

Higher rate of hydrolytic cleavage of lipids, occurrence of several volatile compounds, changes in the composition of lipids and oxidative changes in unsaturated fatty acids, enzyme-catalyzed changes in phenolics, presence of C-glycosylflavones and presence of a high concentration of off-odor-generating volatile 2-acetyl pyrroline have been documented as a possible cause for generation of off-odor (Thiam et al.

1976; Bangar et al. 1999). Higher peroxidase and enzyme activities changes in phenolics (Bangar et al.1999; Reddy et al. 1986; Chugh and Sharma 2012) as other causes for generating off-odor in stored flour of finger millet and pearl millet. Hydrolysis of fats in glycerol and fatty acids by lipase enzyme produces free fatty acid compounds and these unsaturated fatty acid compounds oxidized and produce aldehydes, ketones, and other volatile compounds responsible for off-flavor development in pearl millet. Oxidation of free fatty acids by the activity of lipoxygenase produces free radicals, which are the unstable molecules that damage the cells and develop the odors.

2.8.1 Reason and Mechanism of Rancidity in Millet

The biochemical reaction between fat and oxygen degraded the long-chain fatty acids to form the short-chain compounds. In general, the hydrolytic and oxidative rancidity causes the free fatty acids, bitter, mousy acidic, volatile off-odor compounds. Hydrolytic rancidity occurs due to the action of enzymes called lipases and the presence of high levels of peroxidase, or it is the process of breaking down a lipid and producing glycerol and fatty acids which gives a foul odor and flavor. Whereas in the case of oxidative rancidity, it oxidizes the fatty acid chains or carbon–carbon double bonds in unsaturated fat are half broken, thus making it a carbon–carbon single bond and one bond with an oxygen molecule. This process can be catalyzed by light, enzymes, trace metals, and free radical species.

2.8.2 Lipid Oxidation Mechanism

Lipid oxidation consists of three processes such as initiation, propagation, and termination. In the initiation step, non-reactive fatty acid converts into reactive lipid peroxide which is governed mainly by auto-oxidation or enzymatic oxidation. The resulting lipid–free radicals ($R^{\cdot}$) react with oxygen to form peroxy radicals ($ROO^{\cdot}$). The ROO reacts with more RH to form lipid hydroperoxides (ROOH), which are the fundamental primary products of autoxidation.

Decomposition of lipid hydroperoxides (ROOH) is a very complicated process that creates materials with several biological properties which affect flavor deterioration in fat-containing food (Fig. 2.1).

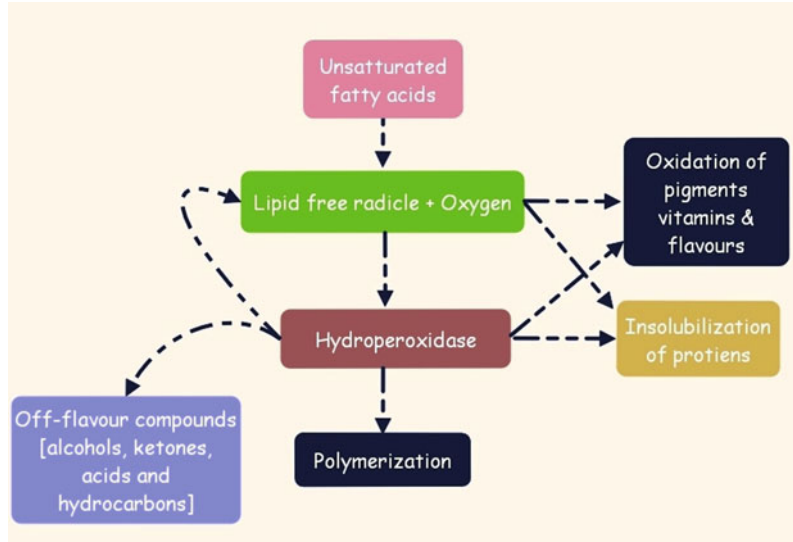

Fig. 2.1 Biochemical mechanism of lipid oxidation in millets

2.9 Factors Affecting the Shelf Life in Millets

The rancid nature of pearl millet grains limits the nutritional benefits of the grains and the off-flavor development restricts the overall acceptability (Goyal et al. 2017). Some of the factors which mainly affect the shelf life of finger and pearl millet or its acceptability are described as follows.

2.9.1 Fat and Fatty Acid Composition

Lipid components is a major factor responsible for the quality deterioration and development of off-odors in millet, especially in the ground one.

2.9.2 Presence of Phenolic Content

Phenolic components are the most significant and secondary products in the majority of grains and have aromatic rings with hydroxyl groups. Several phenolic compounds were also identified and measured in the millets. Usually, catechin was the leading phenolic compound. The mean values reported for the finger millet were 610.4–675.1 mg/kg. Ferulic acid is the major bound phenolic compound in finger millet. In pearl millet, the total polyphenols of flour were reported to be about 228–486 mg/100 g and 175–435 mg/100 g (Chavan and Hash 1998; Kumar et al. 2015). The most common phenolic compound in pearl millet is ferulic acid and its bound form goes through a damaging oxidation response during storage (Ragaee et al. 2014). In the case of pearl millet, polyphenols (Reichert 1979) limit the protein and starch utilization and develops odor during the storage period.

2.9.3 Peroxidase Activity

Peroxidase (POX) is a thermo-stable plant heme-containing enzyme. It catalyzes the oxidation of several unsaturated fatty acids and causes the formation of volatile carbonyl compounds which may contribute to odor (Ashie et al. 1996). Peroxidase enzyme is mainly intense in germ fraction (376 units/g/min) of the grain and found to be responsible for odor generation in case of stored pearl millet meal (Chavan and Hash 1998; Praduman 2006).

2.9.4 Fat Acidity

It was also reported that after milling the fat rancidity increases which is supposedly due to the action of lipase enzyme and causes bitterness and can make millet meal objectionable (Yadav et al. 2012; Arora et al. 2002). Carnovale and Quaglia (1973) in their experiment found that pearl millet flour at 30 °C for 3 months deteriorates in the quality of flour which is mainly by the hydrolysis process rather than the oxidative decay of lipid molecules. Lipase seemed to be more effective in case of defatted millet (Liu et al. 2012; Wang et al. 2014).

2.9.5 Lipoxygenase and Polyphenol Oxidase

The enzyme lipoxygenase catalyzes the oxidation of free fatty acids such as linoleic and linolenic acids. During biochemical reaction, lipoxygenase produces many reactive compounds for example free radicals. These free radicals may react with ascorbic acid, carotenoids, chlorophylls, α-tocopherol, and phenols causing a change of organoleptic and off-odor in products. Banger et al. stated that in several cased polyphenol oxidizing enzymes are a major factor for odor formation in pearl millet (Table 2.6).

2.10 Role of Finger Millet in Nutraceutical Food Development

Several current observations found that nutrition with plant-based foods specifically whole cereals are defensive against numerous illnesses

Table 2.6 Biochemistry for odor generation in pearl millet

Product	Responsible factors	Results	References
Pearl millet	Lipase	Loss of essential amino acids and biological value leads to an off-odor	Kadlag et al. (1995)
Flour	Lipid, Unsaturated fatty acids	Unpleasant taste and off-flavor	Kapoor and Kapoor (1990)
Pearl millet grain	Lipolysis and oxidation of unsaturated fatty acids	Off-flavor	Lai (1980)
Pearl millet flour	Peroxidase activity, Lipids and phenolics	Rancid odor	Goyal et al. (2017)
Grain	lipases, peroxidases and phenolics content	Off-flavor	Chugh and Kumar (2004)
Ground pearl millet	Increase in fat acidity and peroxide value	Rancid odor	Kaced et al. (1984)
Pearl millet	Water-soluble phenolics and peroxidase activity	Odor Generation	Bangar et al. (1999)
Flour	Phenol content and peroxidase	Off-flavor and mousy odor	Yadav (2003)

conditions such as diabetes, cardiovascular, some cancers condition, and metabolic syndrome diseases. Finger millet grain is found to store a house of healthy compounds such as proteins, dietary fibers, micro and macronutrients, and several phytochemicals vital for human well-being (Table 2.7). Developing nutraceutical food using finger millet must be required for biochemical and safety evaluation of ingredients. Polyphenolic compounds of finger millet (about 0.3–3%) are familiar for their health benefits such as hypoglycemic conditions, hypo-cholestrolemic conditions, and many other disease conditions (Pradhan et al. 2010). In common, it is supposed that the polyphenolic compounds of small millets have key valuable roles as antimicrobial, antioxidant, and inhibitory activities against several enzymes (Chethan et al. 2008). Polyphenolic compounds of millets are familiar to inhibit the activity of several digestive enzymes such as amylase, pepsin, trypsin, glucosidase, and lipases (Rohn et al. 2002). They show an important role in the inhibition of amylase enzyme activities and therefore effective to manage type 2 diabetes and contribute to controlling high glucose levels in the blood (Saito et al. 1998). They are also reported to act as inhibitors of amylase and glucosidase and cause a reduction in postprandial hyperglycemia (Bailey 2001). Very little information is available for variations in polyphenolic contents among millet varieties (Chethan and Malleshi 2007a, b). Inhibitory activity against snake venom phospholipases A2 (PLA2) and cataract formation was reported by Chethan et al. (2008). Chethan and Malleshi (2007a, b) also studied several brown and white varieties and stated higher polyphenols in brown varieties in comparison to white varieties. It is also confirmed that the phenolic compounds present in millets are heat stable, pH-sensitive, and in most cases they are highly stable in the acidic pH range (Chethan and Malleshi 2007a, b).

Presence of high fiber content is useful to control constipation, blood cholesterol, and intestinal cancer (Usha 2004). Due to the low glycemic index value, it is useful in the regulation of plasma cholesterol, total serum cholesterol and LDL cholesterol, and triglycerides and plays a significant beneficial effect on the plasma profile (Enas et al. 2003; Mizutani et al. 1999). The leaf juice of finger millet has been used by women during childbirth because of its diaphoretic, diuretic, and vermifuge properties (Watt and Breyer-Brandwijk 1962). Finger millet

Table 2.7 Finger millet and reported nutraceutical properties

S. No	Compounds	Components	Useful role	Nutraceutical role	References
1	Proteins	Albumins, prolamins	Rich in bioactive peptides and essential amino acids	Keeping proper homeostasis, protein energy balance, natural relaxant	Mathanghi and Sudha (2012)
2	Glycoproteins Low fat contents	Biologically important components	Reduction of glycosylation	Aging	Mathanghi and Sudha (2012)
3	Carbohydrates	Free sugars, starch, cellulose, xylose, fructose, glucose, sucrose, oligosaccharides raffinose, resistant starch	Low rate of digestibility	Effective in glucose metabolism	Mathanghi and Sudha (2012)
4	Fibers	Soluble fiber	Plasma cholesterol control, Plasma glucose control, weight management, reduction in blood cholesterol and blood sugar	Effective in glucose metabolism, effective in CVD, effective in gastrointestinal problems, reduces constipation and cancer	Mathanghi and Sudha (2012), Saleh et al. (2013)
5	Vitamins	Vitamins (water- and fat-soluble)	Absorption of minerals	Blood cells production, anemia	Tatala et al. (2007),
6	Minerals	Potassium, sodium, magnesium, calcium, zinc, manganese, iron	Rich in minerals	Effective in osteoporosis, anemia	Platel et al. (2010)
7	Phytochemicals	Tannins, steroids, polyphenols, alkaloids, terpenoids, lignans, phytocyanins	Antioxidants, modulate immune function	Anti-cataractogenic properties, anti-diabetic, cardiovascular diseases, cancer and aging, cholesterol control lowering	Mathanghi and Sudha (2012), Saleh et al. (2013)

(ragi) is a very popular medicine for liver disease, leprosy, measles, pneumonia, and smallpox (Duke and Wain 1981). Several other significant health favorable properties are also linked to finger millet such as antiviral, anticancer, anti-inflammatory, and platelet aggregation inhibitory activity (Chethan and Malleshi 2007a, b).

Antimicrobial activities reported in finger millets are mainly involved in enhancing the body's defence mechanisms and cause inhibition of the angiotensin-I-converting enzyme (ACE) and may be useful for novel treatments for blood pressure patients, heart patients, and diabetes patients (Mizutani et al. 1999). In some reports, it was found that antimicrobial action on the intestinal microflora indicates pharma features. Inhibitory effect on the growth of some common microbes such as *Salmonella typhimurium* and *Escherichia coli* was also reported with fermented finger millet (Usha et al. 1998).

2.11 Nutraceutical Role of Finger Millet

2.11.1 Role in Antioxidant and Antiaging

Numerous phytochemicals are reported as a good source of dietary antioxidants to protect our body against oxidative harm and regularly involved in the maintenance of physiological balance. Several harmful diseases are linked to oxidative processes mediated by reactive oxygen molecules and examples of such diseases are cardiac disease, diabetes, and cancer. The finger millet grains have several phenolic compounds reported to exhibit antioxidant activity (Chandrasekara and Shahidi 2010; Hegde et al. 2005).

At the current time, dietary plant polyphenols receive several considerations from the health and nutrition sector for their role in several health benefits such as the risk of cancer, cardiovascular aging, and diabetes (Tsao 2010; Scalbert et al. 2005; Kaur and Kapoor 2001). Rao and Muralikrishna (2002) reported that proto-catechuic acid is major free phenolic acid found in finger millet grains. In some other research, it was reported that in total phenolic compounds it has 85% benzoic acid derivatives (Chethan and Malleshi 2007a, b). A diet with a higher level of finger millet (55%) enhances the action of some common antioxidant enzymes, for example, glutathione peroxidase, catalase, and glutathione reductase in rats (Hegde et al. 2005).

A different processing condition of finger millet usually decreases the polyphenol contents and reduces the free radical quenching property and examples of this process are thermal or hydrothermal, germination, decortication, and fermentation (Rao and Murali krishna 2007; Shobana and Malleshi 2007). Collagen cross-linking inhibition properties are also reported in finger millet, thus they can be very effective in slowing down aging (Hegde et al. 2002).

2.11.2 Role as Anti-carcinogenic Agent

In the present time, everyone is looking for the prevention of diseases in a natural way. The use of healthy food for prevention and protection against cancer is one of the good and attractive options. The tumor development rate can be minimized by the regular use of anti-carcinogenic food ingredients. Phytochemicals of finger millet are important nutraceutical constituents rich in anti-carcinogenic properties and can be used as terminators for free radical and active oxygen species (Shahidi et al. 1992). As finger millet ensures a range of such types of ingredients that can suppress the cellular oxidation process and protect from different types of cancers (Kawabata et al. 2000; Mori et al. 1999),

A key component of bound phenolic acids is found to be effective as a natural bioactive chemotherapeutic agent against cancer in finger millet (Griffin 1974). Reports indicate that intake of millets in comparison to wheat or maize cause lowers the danger of oesophageal cancer (van Rensburg 1981). Consumption of phenolic components, tannins, and phytate components of finger millet reduces the rate of cancer initiation and progression in several tissues (Chandrasekara and Shahidi 2011). In silico studies with finger and pearl millet phenolic reported by Singh et al. (2015) also indicates strong anti-cancerous evidence.

2.11.3 Role as Anti-diabetic Agent

Diabetes is one of the important health concerns which is rapidly increasing in society in many countries as well as in India. It is an important chronic metabolic disease analyzed by hyperglycemic conditions. It is due to either insufficient insulin production (type-1) or error in action (type-2). Phenolic extracts are found to be

helpful in this condition (American Diabetes Association, 2010; Kim et al. 2011; Shobana et al. 2009). Finger-millet-based food has inferior glycemic index and encourages a lesser glycemic response (Shukla and Srivastava, 2014; Shobana et al. 2007). Abundant dietary calcium and magnesium in finger millet are suggested to reduce type-2 diabetes risk (Pittas et al. 2006; van Dam et al. 2006). Intake of multigrain comprising nearly 30% finger millet fraction was found to significantly drop the level of plasma glucose (Pradhan et al. 2010).

The presence of several anti-nutritional molecules (tannins, polyphenolic, and phytates) in finger millet can very useful in lowering the glycemic response because they decrease the digestibility and absorption of starch (Kumari and Sumathi 2002). Some experiments on rats have positively evidenced that finger millet in diet accelerated the wound healing properties and later the case of generation of cataracts (Shobana et al. 2010). Methanolic extracts of finger millet favor healthy use in the pathogenesis of diabetes mellitus problems (Hegde et al. 2002). These are some reasons why finger millet is categorized as a favorable ingredient of diabetes-related problems.

2.11.4 Role as Cardiac Protective

Cardiac problem is the main cause of mortality worldwide. Cardiac problems are mainly associated with irregular blood pressure, higher cholesterol issues, hypertension, diabetes and obesity issues. Lower incidences of cardiac were reported in the case of finger millet consuming populations (Gopalan 1981). This action of finger millet is supposed due to its role against hyperlipidemia conditions and therefore, reduced levels of triglycerides and cholesterol were reported in blood serum in rats (Lee et al. 2010). Thus, finger-millet-containing diet shows lower lipid peroxidation which cuts down arteriosclerosis and thus plays a role in safeguarding against heart attack. Some other reports also suggested the role in the control of lipid and antioxidant metabolism in high cholesterol intake in rat models (Vasant et al. 2014; Chandrasekara and Shahidi 2012b). Some dietary fiber components decrease the reabsorption of bile acids and also drop the LDL cholesterol (Chandrasekara and Shahidi 2012c). Fermentation of finger millet also increases their functionality and favors against cardiac (Venkateswaran and Vijayalakshmi 2010).

2.11.5 Role as Anti-Bone Illnesses Agents

The WHO has projected osteoporosis as a leading worldwide healthcare fear next to cardiovascular diseases. Intake of high amounts of naturally available calcium helps in the prevention of bone diseases like osteoporosis. Finger millet is one of the rich sources of calcium (with up to 350 mg/100 g) and is reported to be 5–10 times higher than other cereals (Kumar et al. 2013).

2.12 Finger Millet Bioactive Compounds and Their Use

Millet is primarily known for phytochemicals such as polyphenols, dietary fibers, condensed tannin, phytate, and pigments. The total dietary fiber of the millet grain mainly contains both soluble and insoluble fiber. Hemicelluloses and pectin are significant soluble fibers whereas cellulose is a major portion of insoluble dietary fiber (Malleshi et al. 1986). The composition of phytate is found to differ (0.5–2.0%) and is mainly intense in the seed coat (Ravindran 1991). The presence of several polyphenols in millets are supposed for their nutraceutical characteristics.

Polyphenolic compounds are metabolites of plants that usually deal as a defense for plants contrary to pathogens. In the early age, polyphenols were labeled as anti-nutritional compounds, but presently they are categorized as nutraceuticals. The important phenolic compound and flavonoids (Kuhnau 1976) are now used as antidiarrheal, antibiotics, and anti-inflammatory agents (Saito et al. 1998), and are

frequently used in the cure of hypertension, allergies, cholesterol issues, and some other similar disorders (Chung et al. 1998). Naturally present polyphenols in millets differ from the simple structure as phenolic acids to highly polymerized structure compounds such as tannins.

Simple phenols compounds present in different varieties of millets are phenol, cresol, thymol, resorcinol, orcinol, etc., with hydroquinone and their derivatives and phloroglucinol. Phenylpropanoids and more simple phenols are usually covalently linked to cell wall polysaccharides or core lignin (Wallace et al. 1991). Flavonoids signify the most widely scattered group of plant phenolics. Their common structure consists of two aromatic rings connected over three carbons which frequently form an oxygenated heterocycle (Harborne and Mabry 1982). Several studies related to millet and their bioactive compounds indicated that the presence of several biologically active compounds in finger millets is nutritionally very important. These compounds are dietary fibers, minerals, and polyphenols and they also have several possible nutraceutical effects in several biological systems.

Bio-accessibility of several plant-based nutrients is one of the important issues during food formulation and development, which is very important for food nutrients and health (Cardoso et al. 2015). The discharge and action of bioactive compounds in foods are mainly affected by an enzymatic action from the food side or the digestive tract. Fairly few studies have described the bio-accessibility prospective of finger millet bioactive compounds, particularly for some important food compounds such as minerals and phenolics (Tatala et al. 2007; Platel et al. 2010; Chandrasekara and Shahidi 2012a; Hithamani and Srinivasan 2014). The influence of the malting process on the bioavailability of minerals iron, copper, zinc, and calcium in finger millet was assessed by Platel et al. (2010). The malting study found a modest reduction in the mineral contents of millet during the malting process and also an increase in the bioavailability of iron and calcium. In the malting process, it is established to decrease anti-nutritional compounds by activating some endogenous enzymes which result in their break and subsequent decrease in contents. In some other studies, it was stated that the decrease in mineral content is due to the effect of malting on phytate and other anti-nutritional compounds which form complexes with the minerals (Platel et al. 2010).

In some other study, it was found to increase bioavailable iron after the germination process (Tatala et al. 2007). The effect of processing methods was also found on the bio-accessibility of cereal bioactive compounds. These methods cause the release of these compounds by increasing their surface area ratio, bringing the action of some endogenous enzymes and transforming of the bioactive compounds into more active compounds. Some processing methods are sprouting, milling, roasting, fermentation, and enzymatic digestion (Hithamani and Srinivasan 2014). In sprouted methods, the loss of phenolic compounds is found.

In case of sprouting and roasting, a positive impact on the bioavailability of phenolic compounds was found which increased 67% after sprouting. Processing methods were found to increase the activity of the food matrix and change the compounds into more active forms (Hithamani and Srinivasan 2014). Bio-accessibility of phenolic compounds was investigated by Chandrasekara and Shahidi (2012a) and found a great effect of gastrointestinal pH conditions, digestion inside gastric and gastrointestinal conditions on the bio-accessibility of phenolic compounds of finger millet. Protein digestions with the discharge of their grain-bound phenolic are supposed to have high bioavailability. The activity of some endogenous enzymes (such as proteases and esterase) might also involve in the discharge of the phenolic compounds during digestion.

2.13 Conclusion

Health and body well-being is an important issue for all. Finger millet is rich in fiber, minerals, vitamins, and good quality amino acids, which are mainly deficient in the majority of cereals and

also have a very high amount of calcium than other cereals. Finger millet is a principal diet in many countries like Africa and South Asia. The grain is willingly consumable, extremely nutritive, and versatile and it can be used in several food preparations as rice, ground to make porridge or flour, or used to make cakes. These amazing characters make them nutritive and climate change compliant crops. Finger millet can be used as an income crop for agriculturalists and also progress the health of the community as a whole. It is important to raise the production and consumption as it is a very low water consuming crop and in the future it becomes a major alternative for food security. However, this great crop is neglected in our community, science, and policies despite its potential to increase the economy. The use of finger millet in marketable food will boost farmers to grow millets and create several opportunities and revitalize the farmers. The addition of millet diets in state-level, national and international feeding programs will support overcoming the current nutrient shortages of protein, calcium, and iron in emerging countries. There is an immediate need for additional research on finger millet to generate more information on FM utilization. The commercialization of value-added fortified finger millet and other gluten-free products has greater potential as the availability of commercialized finger millet products in developed countries will support mitigating the cases of celiac disease and obesity.

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3 Genetic and Genomic Resources for Crop Improvement in Finger Millet

P. Rajendrakumar and K. N. Ganapathy

Abstract

Finger millet (*Eleusine coracana* L. Gaertn.), a powerhouse of calcium, is a C_4 grass species belonging to the family Poaceae, which is cultivated in a wide range of ecosystems ranging from coastal plains to hilly regions in Asia and Africa. Finger millet is a perfect supplement to major cereals to achieve crop and nutritional diversity due to its wider adaptation in marginal lands, minimal irrigation requirement, nutritional superiority, and inherent tolerance to stresses. Even though many improved varieties have been developed over the years, the genetics of agronomically important traits of finger millet is not clearly understood and demands multidisciplinary efforts to dissect the target traits into effective QTL/genomic regions by taking advantage of the draft genome sequence published recently. The availability of genetic resources with wide diversity, together with advances in genotyping and phenotyping technologies, offer great opportunities in accelerating the genetic improvement of finger millet through marker-assisted breeding. This chapter provides a complete account of germplasm resources, core and mini-core collections, donors for important target traits, mapping populations, genome sequencing, DNA markers and transcriptome sequencing, which constitute a valuable assemblage of resources for the breeders to employ them in finger millet improvement programs toward accelerating the development of high-yielding, nutrition-rich, and climate-resilient cultivars for meeting the future demand of the consumers.

3.1 Introduction

Finger millet (*Eleusine coracana* L. Gaertn.) is a highly self-pollinated annual C_4 grass species belonging to the family Poaceae having regional and nutritional importance, widely cultivated in semiarid regions, particularly in Asia and Africa. It is an allotetraploid ($2n = 4x = 36$) and ranks fourth after sorghum, pearl millet, and foxtail millet based on its importance (Gupta et al. 2012). This crop is grown in more than 25 tropical countries in Africa and Asia (Upadhyaya et al. 2006) and India occupies a prime position in the production (1.98 million tons) from an area of 1.19 million hectares with a productivity of 1661 kg per ha (Sood et al. 2019). It is an important crop in hilly regions, tribal areas, marginal lands, and regions that are prone to

P. Rajendrakumar (✉) · K. N. Ganapathy
ICAR-Indian Institute of Millets Research, Hyderabad, Telangana 500030, India
e-mail: rajendra@millets.res.in

K. N. Ganapathy
e-mail: ganapathy@millets.res.in

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famine (Verma 2009) providing food grain for human consumption and straw for animal feed thereby serving as a subsistence crop and contributing to food security. However, due to the preference of a large section of the consumers toward fine grain with a white or lighter color, this crop has remained a staple mostly in rural areas, lower socio-economic groups, and traditional consumers.

Finger millet is grown in diverse agroclimatic regions ranging from coastal regions of peninsular India to hilly regions of the Himalayas. Even though this crop is adapted to diverse soil types and environmental situations, its phenotypic expression is best in loamy or clay loamy soils that are arable and well-drained. Grains of finger millet is highly nutritious since it is rich in calcium, iron, zinc, dietary fiber, and essential amino acids (Singh et al. 2012; Shobana et al. 2013), endowed with health benefits since it is gluten-free, contains a low glycemic index and abundant in phytochemicals (Chandra et al. 2016; Pandian et al. 2017), making this crop a suitable candidate as a nutri-cereal and functional food. In addition to grains, the stover is a valuable source of nutritive fodder for farm animals and is highly preferred due to its sweet-smelled stalks. Despite the release of many finger millet varieties, there is an immense need for the exploitation of recombination breeding since almost all the varieties are developed either through a selection of germplasm accessions or induction of mutation mainly due to smaller sized florets owing to difficulties in hybridization. Hence, concerted efforts are needed to accelerate the genetic improvement of finger millet by involving genetic resources, hybridization, recombination breeding, and genomic resources for deciphering the genetic factors or quantitative trait loci (QTL) governing complex traits such as grain and fodder yield, nutritional parameters, tolerance to biotic and abiotic stresses, etc. to achieve precise genetic improvement in a short time through marker-assisted breeding.

In the past two decades, rapid advances in the next-generation sequencing (NGS) technologies have greatly contributed to the generation of enormous genomic resources including the whole-genome sequences of the majority of the millets including finger millet. Like the rapid advances in sorghum breeding after the publication of its complete genome, finger millet is poised for accelerated genetic improvement through the dissection of complex traits of consumer preference into effective QTLs by employing genetic and genomic resources and deploying those QTLs through molecular breeding to make it an economically viable, nutritionally superior and climate-resilient crop. Here, in this chapter, we review the genetic and genomic resources encompassing germplasm collection, core and mini-core collection, donors for important traits, wild species and their importance, mapping populations, whole-genome sequencing, and transcriptome sequencing along with future research prospects for genetic improvement of finger millet.

3.2 Genetic Resources

Genetic resources include germplasm accessions, advanced breeding lines, mutant lines, wild species, and mapping populations that collectively are the storehouse of valuable genes of economic importance, which could be employed in the dissection of important target traits as well the genetic improvement of the same. Amid increasing demand for nutritious grains under erratic rainfall patterns due to the impact of climate change, next-generation genomics-assisted breeding for the development of nutrition-rich climate-resilient cultivars in finger millet will rely largely on the identification of adaptation traits from the available diverse gene pool including germplasm collections and wild species and their exploitation in breeding programs.

3.3 Germplasm Collection—Core and Mini-core Collection

Plant breeders have successfully improved the productivity of most crops, resulting in large production increases during the past five decades.

However, yields' reaching a plateau in several crops is a cause for concern and the important reason behind this is that breeders tend to confine themselves to their working collection, consisting largely of highly adapted breeding lines, and rarely use more diverse germplasm sources. Even though a good collection of genetic resources is available for exploitation in crop improvement programs, an extremely limited number of germplasm accessions have been infused into the breeding programs over the years. To promote the effective use of genetic resources/germplasm accessions, a concept of "core collection" for a manageable sampling of the total collection was proposed by Frankel (1984). A core collection is the subset of accessions from the entire germplasm collection of a crop species that represents the maximum diversity of the particular species (Brown 1989a). Information or data generated by extensive evaluation of this core collection for various target traits could be used as a guide for more efficient utilization of the total germplasm collection (Brown 1989b). However, considering a large number of germplasm available in major crops, the size of the core collection becomes unmanageable and therefore demands a further reduction in the size. Such a reduced collection representing the full diversity accounting for about 1% of the entire collection was proposed as a "mini-core" by Upadhyaya et al. (2010).

Finger millet germplasm accessions are conserved in the gene banks at various organizations/institutions nationally as well as globally. At the national level, India holds the largest germplasm collections of finger millet for long-term conservation at ICAR-National Bureau of Plant Genetic Resources, New Delhi, and for medium-term storage for use as working collection at All India Coordinated Research Project on Small Millets (AICRP-SM), Bengaluru. At the global level, a reasonable number of finger millet collections are maintained at the International Crops Research Institute for the SemiArid Tropics (ICRISAT), India; Kenya Agricultural Research Institute (KARI), Kenya; Institute of Biodiversity Conservation (IBC), Ethiopia; USDA Agricultural Research Service (USDA-ARS), United States and Serere Agricultural and Animal Production Research Institute (SAARI), Uganda (Dwivedi et al. 2012; Goron and Raizada 2015; Saha et al. 2016; Gupta et al. 2017).

The National Active Germplasm Collection Site (NAGS) at the Project Coordination Cell, All India Coordinated Research Project on Small Millets (AICRP-SM), Bengaluru, has made extensive efforts in assembling a large collection of finger millet germplasm at the global level. Core and mini-core collections have been constituted to enhance the effective utilization of germplasm accessions in the finger millet improvement programs. A core set of 551 accessions, the majority of them of Indian origin and representing the maximum diversity of the total germplasm collection, evaluated for different morpho-agronomic characters over the years was constituted by Gowda et al. (2007). Character association revealed that associations among yield and yield contributing characters in the entire collection were reflected in the core set. The characters, viz., plant height, culm thickness, number of leaves, flag leaf sheath width, flag leaf blade length and width, leaf blade width, days to 50 percent flowering, and days to maturity could be used as indirect selection for high seed yield. Chandrashekhar et al. (2012) made an effort to develop a core set (77 accessions) from the base germplasm of 1000 diverse accessions of Africa and Asia using the information on geographical origin and data from morpho-agronomic characters analyzed through PowerCore software. Maximum accessions in the base set were from India (595) followed by African countries (388) and the ICRISAT collection (17). The highest number of Indian accessions were from Jharkhand followed by Andhra Pradesh and Tamil Nadu while a greater number of African accessions were from Kenya followed by Malawi, Zambia, and Zimbabwe. The mean diversity observed in the core set (Shannon–Weaver diversity index $H' = 1.65$) was similar to base germplasm ($H' = 1.61$). Similarly, the geographical distribution of germplasm observed in the core set was like base germplasm accessions.

A total of 5,940 germplasm accessions of finger millet belonging to 23 countries are conserved in ICRISAT Genebank at Patancheru, India. A core collection of 622 accessions, comprising predominantly African (58.7%) and Asian (35.8%) accession, representing different geographical regions and biological races of the total collection was established by Upadhyaya et al. (2006) using the data from 14 quantitative traits. Accessions of subsp. *coracana* accounted for 97.4% while that of *africana* accounted for 2.6%. The accessions belong to various races and the subsp. *coracana* was dominated by the race *vulgaris* (62.5%), followed by *plana* (16.8%), *compacta* (12.4%), and *elongata* (8.3%). Since the size of the core collection (622 accessions) is still large for multilocation trials, Upadhyaya et al. (2010) developed a mini-core by utilizing multilocation evaluation of agro-morphological traits of the core collection and selecting ~10% or 1–4 accessions from each of the 40 clusters formed by 10% sampling strategy or by taking a minimum of one accession comprising of 80 accessions. The mini-core represented 12.86% accessions of the core collection and 1.34% accessions of the total collection. The mean differences for various traits in core and mini-core collections were observed to be non-significant for most traits, while variances were found to be homogeneous for most traits. The genetic resources available in finger millet for various genetics and breeding applications are given in Table 3.1.

Being the huge reservoir of valuable alleles for various agronomically important traits, germplasm collections offer enormous promise for the genetic improvement of small millets while core and mini-core collections help in the efficient utilization of the variation available in the germplasm collections. Under the current climate change regime and increased demand for nutritious food grains, genetic gains can be improved by blending new sources of genetic variation from superior donors possessing genes for yield, nutritional and crop adaptation traits.

3.4 Donors for Important Traits

Crop improvement involves the development of new and superior cultivars with high yield, quality, and tolerance to biotic and abiotic stresses as well as the improvement of elite cultivars for specific traits that are lacking in them. Finger millet germplasm collection harbors substantial variation for agro-morphological, quality and stress tolerance traits, and their systematic characterization and evaluation will lead to the identification of promising donors for important target traits of interest for the breeder. In addition to yield and stress tolerance, the focus should be on the development of cultivars suitable for mechanical harvesting, nutrition-rich cultivars fetching premium prices in the market, and those suitable for preparing value-added products such as flour, *vermicelli*, extruded snacks, noodles, ready-to-cook, and ready-to-eat products.

Important donors identified for various agronomic traits (grain yield, biomass yield and its components), stress tolerance traits (blast tolerance, drought tolerance, aluminum toxicity tolerance, nitrogen and phosphorus starvation tolerance), and nutritional traits (iron, zinc, calcium, and protein content in grains) can be used in the genetic improvement programs for the development of nutrient-rich climate-resilient finger millet cultivars to meet the future demands. Molecular breeding approaches are being employed for the genetic improvement of various crop species, which requires the identification of genomic regions/QTLs associated with important target traits. Toward this, the donors may be used for the development of biparental mapping populations that are useful for the identification and mapping of QTLs for the traits of interest. Association mapping population can be constituted by involving diverse germplasm lines along with donors for agro-morphological and stress tolerance traits. These populations along with the genomics resources will help in the identification of QTLs/marker-trait associations for vital traits.

Table 3.1 Genetic resources for exploitation in genetics and breeding applications

Genetic Resources	Size	References
Germplasm accessions		
All India Coordinated Research Project on Small millets (AICRP-SM), India	7,070	Guarino (2012)
Central Plant Breeding and Biotechnology Division (CPBBD), Nepal Agricultural Research Council, Nepal	869	Dwivedi et al. (2012)
National Institute of Agrobiological Sciences (NIAS), Japan	564	Dwivedi et al. (2012)
Institute of Biodiversity Conservation (IBC), Ethiopia	2,156	Dwivedi et al. (2012)
Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Sciences (ICGR-CAAS), China	300	Dwivedi et al. (2012)
International Crop Research Institute for the SemiArid Tropics (ICRISAT), India	6,804	Goron and Raizada (2015)
Mt. Makulu Central Research Station, Chilanga, Zambia	390	Dwivedi et al. (2012)
National Bureau of Plant Genetic Resources (NBPGR), India	10,507	Guarino (2012)
National Center for Genetic Resources Preservation, Fort Collins, USA	702	Dwivedi et al. (2012)
National Gene Bank, Kenya	2,875	Dwivedi et al. (2012)
Plant Genetic Resources Conservation Unit, USDA-ARS, USA	1,452	Goron and Raizada (2015)
SADC Plant Genetic Resources Centre, Zambia	1,037	Dwivedi et al. (2012)
Serere Agricultural and Animal Production Research Institute (SAARI), Uganda	1,231	Dwivedi et al. (2012)
Core and mini-core collection		
Core collection	622	Upadhyaya et al. (2006)
Core collection	551	Gowda et al. (2007)
Core collection	77	Chandrashekhar et al. (2012)
Mini-core collection	80	Upadhyaya et al. (2010)
Composite collection	1,000	Upadhyaya et al. (2005)
Reference set	300	Upadhyaya (2008)
Association panel		
Panel representing global germplasm collection	190	Babu et al. (2014a; b, c, d)
Panel representing global germplasm collection	128	Ramakrishnan et al. (2016)
Panel representing Global Germplasm Collection	238	Yadav et al. (2017)
African Collection	138	Lule et al. (2018)
Genetically Diverse Collection	113	Kumar et al. (2016)

Amid the erratic temperature and rainfall fluctuation due to climate change, genomics-assisted breeding for the development of climate-smart cultivars will depend largely on large-scale genetic variations available in diverse populations involving germplasm collections, landraces, breeding lines, and wild species. Evaluation of available germplasm by various research groups

has led to the identification of useful donors (Table 3.2) for early maturity, high yielding, blast-/drought-/salinity-resistant accessions, which could be employed in breeding programs for the development of climate-resilient finger millet cultivars. In addition, many donors have been identified possessing high calcium, iron, zinc, and protein contents in the grain that can be used for the biofortification of finger millet through conventional or molecular breeding approaches. Thus, the availability of donors for important traits along with the availability of

Table 3.2 Donor sources for agronomic, nutritional, stress tolerance, and special traits

Target Traits	Donor accessions/cultivars	References
Agronomic traits		
Early maturity	IE 49, IE 120, IE 189, IE 196, IE 234, IE 501, IE 509, IE 581, IE 588, IE 600, IE 641, IE 694, IE 847, IE 2030, IE 2093, IE 2158, IE 2275, IE 2293, IE 2322, IE 2323, IE 2957, IE 3104, IE 3537, IE 3543, IE 4425, IE 4431, IE 4432, IE 4442, IE 4711, IE 4734, IE 4755, IE 4759, IE 6013, and IE 6550	Bharathi (2011)
Number of tillers	IE 2296, IE 2034, IE 4711, IE 2293, IE 2299, IE 2608, IE 2619, IE 5145, IE 6553, IE 847, IE 2408, IE 2534, IE 3987, IE 1013, IE 120, IE 2042, IE 2091, IE 2106, IE 2139, IE 2146, IE 2233, IE 2288, IE 2367, IE 2410, IE 2504, IE 2645, IE 2657 and IE 2674	Bharathi (2011)
Number of fingers	IE 6033, IE 3790, IE 4586, IE 6059, IE 3111, IE 4476, IE 3106, IE 2914, IE 4677, IE 5733, IE 5875, IE 5877, IE 4257, IE 5105, IE 5563, IE 6510, IE 4297, IE 2957, IE 5689, IE 5956, IE 4563, IE 3120, IE 2816, IE 6013, IE 2303, IE 2591, IE 6252, IE 6241 and IE 4866	Bharathi (2011)
Finger length	IE 2223, IE 2621, IE 2789, IE 6553, IE 3581, IE 3431, IE 3722, IE 6512, IE 2108, IE 2781, IE 3046, IE 2486, IE 5321, IE 3704, IE 798, IE 3489, IE 5022, IE 2591, IE 2608, IE 4476, IE 2611, IE 3531, IE 2336, IE 4125, IE 4658 and IE 6546	Bharathi (2011)
Grain yield	IE 94, IE 2340, IE 2498, IE 2578, IE 2587, IE 2683, IE 2773, IE 2903, IE 2983, IE 2992, IE 3194, IE 3790, IE 3802, IE 4600, IE 4974, IE 5198, IE 5472, IE 3663, IE 3693, IE 3744, IE 4121, IE 4310, IE 4679, IE 5862, IE 6142, IE 6236, IE 667, IE 1010, IE 2299, IE 2590, IE 2678, IE 2684, IE 2698, IE 2712, IE 2756, IE 2827, IE 2872, IE 3135, IE 3136 and IE 3270	Bharathi (2011)
Forage yield	IE 2117, IE 24, IE 2568, IE 2651, IE 2753, IE 2796, IE 2811, IE 2880, IE 2942, IE 2979, IE 3789, IE 50, IE 672, IE 715, IE 860, IE 908, IE 916, IE 96, and IE 99	Bharathi (2011)
Grain nutritional traits		
Grain iron content (>40 mg kg^{-1})	IE 4708, IE 2921, IE 4709, IE 588, IE 5736, and IE 4476	Upadhyaya et al. (2011)
Grain zinc content (>24 mg kg^{-1})	IE 3120 and IE 7508	Upadhyaya et al. (2011)
Grain calcium content (>450 mg kg^{-1})	IE 4476, IE 2030, IE 6546, IE 4708, and IE 2568	Upadhyaya et al. (2011)

(continued)

Table 3.2 (continued)

Target Traits	Donor accessions/cultivars	References
Grain protein content (10%)	IE 6537, IE 0009, and IE 4709	Upadhyaya et al. (2011)
Stress tolerance traits		
Blast resistance	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), ED 201-5A, ICM 401, PRM 9802, SANJI 1, TNAU 1009, VL 234, VL 324, VL 328, VL 330, VL 332, VL 333, Genotype no. 2400, 4313, 4914, 4915, 4929, 4966, 5102, 5126, 5148, IE 1055, IE 2821, IE 2872, IE 4121, IE 4491, IE 4570, IE 5066, IE 5091, and IE 5537	Mantur et al. (2001); Kumar and Kumar (2009); Babu et al. (2013) and AICSMIP, Bengaluru
Drought tolerance	GE 208, GE 496, GE 596, GE 1855, GE 4434, GE 4730, GE 4976, PR 202, VL 315, PES 400, PRM# 8107, PRM# 8112, and VL 315	Bhatt et al. (2011); Gupta et al. (2014) and AICSMIP, Bengaluru
Aluminum toxicity tolerance	Gute and Degu	Brhane et al. (2017)
Nutrient starvation tolerance		
Nitrogen	GE 3885	Kanwal et al. (2014); Gaur et al. (2018); Gupta et al. (2018)
Phosphorus	GPU 45, IE 5201, IE 2871, IE 7320, GPU 66, Hosur 1, TCUM1, IE 2034, SVK 1, RAU 8, VR 708, and IE 3391	Ramakrishnan et al. (2017)
Special traits		
High Popping types	Co 10, Indaf 3, Karun Kaddi Ragi, PR 202, GN 4, ES 11, and PRM 2	https://www.dhan.org/
White seeded types	IE 3156, IE 3184, IE 2835, IE 2906, Co 9, KMR 340, and OUAT2	Vadivoo et al. 1998; Sonnad et al. (2008)

large-scale genomic resources will help in accelerating the development of nutrient-rich climate-smart finger millet cultivars by enhancing the breeding efficiency.

3.5 Species of *Eleusine* and Their Importance

Finger millet belongs to the genus *Eleusine,* which comprises diploid ($2n = 2x = 16$, 18, and 20) and tetraploid species ($2n = 4x = 36$ or 38; Phillips 1995), displaying considerable morphological and ecological diversity in East Africa and the Americas, except that the cytologically unclear *E. semisterilis* S.M. Phillips, known only through the holotype specimen collected from Kenya (Phillips 1972). East Africa being the primary center of diversity, about eight species are found in this region, including *E. africana*, *E. coracana*, *E. kigeziensis*, *E. indica*, *E. floccifolia*, *E. intermedia*, *E. multiflora*, and *E. jaegeri* (Mehra 1963; Phillips 1972). The details on the species complex of *Eleusine* are given in Table 3.3.

3.5.1 *Eleusine Coracana* ($2n = 4x = 36$; AABB Genome)

Eleusine coracana is highly variable in its centers of origin in Africa and the Indian subcontinent. The species *E. coracana* consists of subspecies *africana* and *coracana*. The subspecies *africana* is a wild type and classified into

Table 3.3 Cultivated and wild species in genus *Eleusine*

Species	Chromosome number	Genome notation	Growth habit
Eleusine coracana (L.) Gaertn	$2n = 4x = 36$	AABB	Annual
E. africana Kennedy-O'Byrne	$2n = 4x = 36$	AABB	Annual
E. kigeziensis S.M. Phillips	$2n = 4x = 38$	AADD	Perennial
E. jaegeri Pilger	$2n = 2x = 20$	CC	Perennial
E. tristachya (Lam.) Lam	$2n = 2x = 18$	AA	Annual
E. indica (L.) Gaertn	$2n = 2x = 18$	AA	Annual
E. floccifolia (Forssk.) Spreng	$2n = 2x = 18$	BB	Perennial
E. multiflora Hochst. ex A. Rich	$2n = 2x = 16$	CC	Annual
E. intermedia (Chiov.) S.M. Phillips	$2n = 2x = 18$	AB	Perennial
E. semisterilis S.M. Phillips	Cytologically unknown		Perennial

Source Phillips (1972); Bisht and Mukai (2002);Liu et al. (2011)

two races, viz., *africana* and *spontanea*. The subspecies *coracana* is a cultivated type and classified into four races, viz., *elongata*, *plana*, *compacta,* and *vulgaris* based on inflorescence morphology (De Wet et al. 1984). The cultivated finger millet (*Eleusine coracana* subsp. *coracana*) is believed to be a selection from a large-grained mutant of *E. coracana* subsp. *africana*, followed by domestication (Dida and Devos 2006; Dida et al. 2008; Fuller et al. 2011; Fuller and Hildebrand 2013). With respect to the evolutionary origin of cultivated finger millet species, it has been clearly established through sequencing of the draft genome of *E. indica* (diploid wild species; AA genome) followed by transcriptome sequencing and phylogenetic analysis that it is the contributor to the A genome, but lack of evidence exists on the contributor of B genome (Devarumath et al. 2005; Liu et al., 2014; Hatakeyama et al. 2018; Zhang et al. 2019a, b). The classification of races and subraces of *E. coracana* is depicted in Fig. 3.1.

3.5.1.1 Subspecies *africana*

This subspecies is a tufted annual generally found along the highlands of East Africa and the grasslands of southern Africa. The plant is characterized by geniculately ascending culms branching at the lower nodes, leaf blades about 36 cm in length and nearly 10 mm in width, 135 cm tall flowering culms, long inflorescence branch ranging between 8 and 17 cm with a width of 5 mm, spikelets arranged in two rows on one side of the rachis, 4–9 flowers per spikelet of 5–8 mm long, shorter glumes, lanceolate-oblong, and narrow winged along the keel (Bharathi 2011).

3.5.1.2 Subspecies *coracana*

This subspecies *coracana* includes all the cultivated finger millet types cultivated for ages by the farmers in arid and semiarid regions in India as well as eastern and southern Africa. It is also cultivated in Nepal, China, and other south-Asian countries. It is an annual crop of herbaceous plant type with robust culms that are soft or glabrous. Inflorescence is typically digitate or subdigitate with 6–10 often incurved spikes. Grains are mostly brown, reddish, or white. The grains are largely non-shattering and exposed grains (Phillips 1972, 1995; Neves 2011). The morphological features of subraces are discussed below (Bharathi 2011).

3.5.1.3 Race *elongata*

It is morphologically the most distinct of the four races (Prasada Rao et al. 1993). This race is characterized by long and slender inflorescence, 10–24 cm long branches, arranged digitately, spikes spreading, and curved outward during maturity. The subrace *laxa* is characterized by long, open fingers and the spikelets arranged in

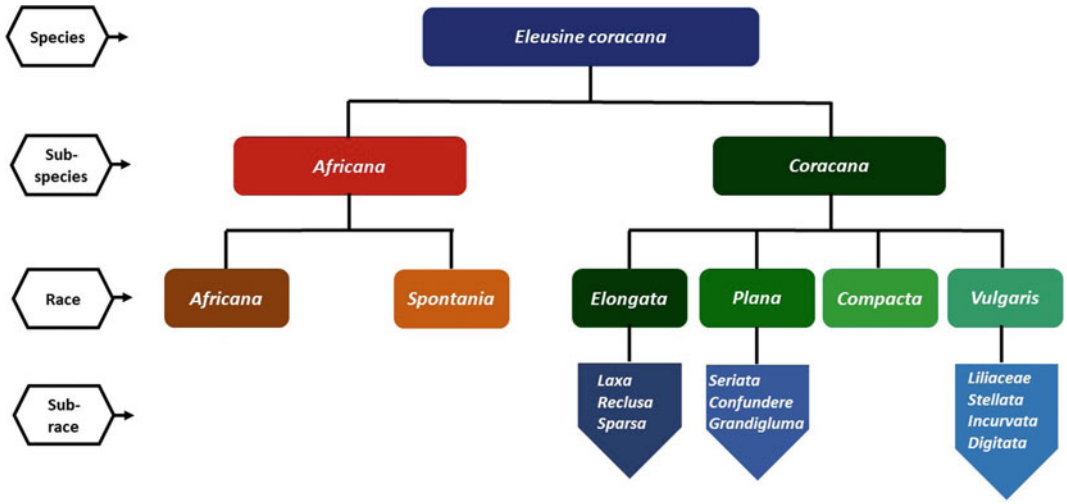

Fig. 3.1 Classification of *Eleusine coracana* species

narrow rows on the inflorescence branches, resembling the wild race *africana*. The subrace *reclusa* is characterized by short, open fingers and the spikelets arranged in clusters on the inflorescence branch with naked space between them.

3.5.1.4 Race *plana*

It is characterized by 8–15 cm large spikelets arranged in two or more rows along the rachis, giving the inflorescence branch a flat-ribbon-like appearance. The subrace *seriata* has serially arranged spikelets, giving a ribbon-like inflorescence while the subrace *confundere* has numerous fertile florets surrounding the rachis at maturity, giving a compact look to the panicle. The third subrace (*grandigluma*) has long pointed glumes that are longer than the spikelets.

3.5.1.5 Race *vulgaris*

It is characterized by 4–8 florets per spikelet, giving a semi-compact appearance and generally tip incurved at maturity (Guarino 2012). Variability is observed for finger arrangement in the subraces, viz., reflexed (subrace *Liliaceae*), twisted (subrace *stellata*), incurved (subrace *incurvata*), and top-curved (subrace *digitata*). This race is commonly found in Africa and Asia.

3.5.1.6 Race *compacta*

The members of this race are popularly known as cockscomb finger millet in Africa as well as Asia. Spikelets are composed of nine or more number of florets and the inflorescence axis is divided at the base, ascending and incurved at the top to give a large fist-like appearance.

The primary gene pool comprises both wild (subspecies *africana*) and cultivated finger millet (subspecies *coracana*), which are important with respect to germplasm collection, conservation, and utilization leading to the genetic improvement of finger millet. Interestingly, the subspecies *africana* occasionally crosses with the subspecies *coracana* to produce fully fertile hybrids and derivatives of such crosses are aggressive colonizers and are grouped under the race *spontanea* (De Wet et al. 1984). The secondary gene pool comprises of diploid wild species, viz., *E. indica*, *E. floccifolia* and *E. tristachya* while the tertiary gene pool comprises of *E. intermedia*, *E. jaegeri*, *E. kigeziensis*, *E. multiflora* and *E. semisterlis* (*E. compressa*) (Guarino 2012). The cultivated finger millet is also cross-compatible with another allotetraploid wild species, *E. kigeziensis* ($2n = 4x = 38$; AADD genome) that is restricted and endemic in eastern Africa (Dramadri 2015).

3.5.2 *Eleusine africana* (2*n* = 4*x* = 36; AABB Genome)

It is an annual allotetraploid grass, which is believed to be the wild progenitor of the cultivated species *E. coracana* due to their genetic similarity (Chennaveeraiah and Hiremath 1974; Hilu and De Wet 1976; Hilu et al. 1978; Hilu 1988; Hilu and Johnson 1997; Bisht and Mukai 2002; Agrawal et al. 2014). Even though a native of East Africa, it is found distributed in tropical and southern Africa mainly in southern and eastern uplands (Neves 2011), also reported to be grown in some parts of India. The plant is characterized by moderately robust culms with glabrous leaves, digitate or subdigitate inflorescence, and ovate to oblong grains with black to brownish color (Neves 2011). This species is often used as a forage grass and is also considered an intolerable weed, if found in fields where cultivated species are grown.

3.5.3 *Eleusine indica* (2*n* = 2*x* = 18; AA Genome)

It is short-lived tufted perennial grass that branches from the base with the plant habit ranging from erect, decumbent, or prostrate. It is a cosmopolitan invasive weed and is commonly known as goosegrass. The species is characterized by slender culms with soft glabrous to sparsely pilose leaves, digitate to subdigitate inflorescence with straight and slender spikes and grains that are elliptic, trigonous, and blackish in color (Neves 2011). *Eleusine indica* is sometimes used as forage and as traditional medicine in parts of Africa and Asia (Neves 2011).

3.5.4 *Eleusine floccifolia* (2*n* = 2*x* = 18; AA Genome)

It is a perennial diploid grass with its distribution in the mid to high altitudes of north-east Africa and Arabia. The presence of small tufts of hair scattered along the margin of leaves is a unique feature for identifying the species (Phillips 1972, 1974, 1995). The plant is characterized by moderately robust culms, subdigitate inflorescence with elliptic to oblong, trigonous, and blackish grains (Neves 2011). The presence of hairs makes it unpalatable and unfit for consumption by the livestock. This species is used for making baskets and other handicraft items, by the locals, particularly in Ethiopia.

3.5.5 *Eleusine tristachya* (2*n* = 2*x* = 18; AA Genome)

It is an annual diploid grass species, which is native to South America. The species is distributed in various parts of the world including North America, Australia, Africa, and Europe (Phillips 1972; Hilu 1980). It is characterized by digitate inflorescence and oblong spikes that are tightly clustered at the top of the axis, and a perpendicular arrangement of spikelets to the axis of the spike (Hilu 1980). The culms are generally slender with soft glabrous leaves. Grains are oblong to globose, trigonous, and blackish in color (Neves 2011). This species is an important forage grass particularly in Argentina (Lovisolo and Galati 2007).

3.5.6 *Eleusine jaegeri* (2*n* = 2*x* = 20; DD Genome)

It is perennial diploid species restricted mainly to the East African highlands (Phillips 1972). The species is believed to be most robust forming dense tussocks of pale green saw-edged leaves. The plant is characterized by robust culms with leathery glabrous leaves having rough margins and short racemose or subdigitate inflorescence with elliptic-oblong, trigonous, and blackish grains (Neves 2011). Like *Eleusine floccifolia,* this species is also unpalatable to the livestock and is usually used by the locals for making baskets.

3.5.7 *Eleusine intermedia* (2*n* = 2*x* = 18; AB Genome)

It is also a perennial diploid grass with extremely limited distribution, especially in the uplands of northern Kenya and southern Ethiopia. This species was first identified as a variety of *E. indica,* but later it was given the status of a species by Phillips (1972) due to several differences. The plant is characterized by moderately robust culms with herbaceous glabrous to pilose leaves and short racemose or subdigitate inflorescence, with elliptic, trigonous, and blackish grain (Neves 2011). *E. intermedia* can be occasionally mistaken with another diploid wild species *E. jaegeri* even though the latter possesses tough, glabrous leaves with rough margins while the former possesses softer, slightly pilose leaves with smooth margins (Phillips 1972). This species may be of some use as a forage grass.

3.6 Genomic and Transcriptomic Resources

Genetic and genomic resources complement each other in the development of superior genotypes with genetic gains for target traits through molecular breeding. Genomic resources help in the dissection of complex quantitative traits and identification of DNA markers linked/associated with important target traits followed by the marker-assisted selection of genotypes in the breeding population toward the development of superior genotypes with precise selection in a short time. Genomic resources include genomic DNA sequences, cDNA, expressed sequence tags (/ESTs), gene sequences, whole-genome sequence, transcriptome sequence, and DNA markers that are employed in the understanding of the molecular mechanisms of important traits, identification of QTLs/genomic regions associated with them and molecular breeding. With the rapid advances in NGS techniques and bioinformatic tools, an ocean of genomic resources is generated on daily basis in various crop species at a rapid pace with considerable automation and less cost. These resources are being deposited and organized in the public databases for their exploitation in QTL mapping, evolutionary studies, comparative genomics, gene expression studies, and molecular breeding programs. In this section, we will discuss in detail the availability of important genomic resources such as genome sequence (nuclear and organellar), DNA markers and transcriptome sequence, their utility, and prospects.

3.6.1 Whole-Genome Sequences

Whole-genome sequence is considered the blueprint of an organism, which contains coding and noncoding DNA sequences that regulate crop growth, developmental process, and response of the crop to changing environmental conditions and also contains DNA sequence variations that were accumulated during its evolution. More importantly, the availability of the genome sequence accelerates the development of genomewide DNA markers such as simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers that could be employed for understanding the genetic diversity among germplasm accessions, population structure, evolutionary relationships, mapping of QTL for target traits and marker-assisted breeding. Advances in NGS technologies and bioinformatics tools in the past decade have not only reduced the cost and time taken for sequencing drastically but also increased the speed of sequencing data analyses encompassing assembly, variant detection, structural and functional annotation as well as understanding of evolutionary relationships. During this golden period, draft genomes of many agricultural crops have been sequenced and among the millets, sorghum was the first genome to be sequenced (Paterson et al. 2009) followed by foxtail millet (Bennetzen et al. 2012; Zhang et al. 2012), pearl millet (Varshney et al. 2017), finger millet (Hittalmani et al. 2017; Hatakeyama et al. 2018), Japanese barnyard millet (Guo et al. 2017), and proso millet (Shi et al. 2019; Zou et al. 2019).

Finger millet is the first polyploid genome sequenced among the millets. Draft genome of finger millet was published by two independent groups within a time span of one year between them (Hittalmani et al. 2017; Hatakeyama et al. 2018). The genome sequence of the cultivar ML-365 (a drought-tolerant and blast disease-resistant) was the first among the finger millet draft genomes sequenced using Illumina (Illumina HiSeq4000 and NextSeq500) and Sequencing by Oligonucleotide Ligation and Detection (SOLiD) sequencing (SOLiD 5500) platforms (Hittalmani et al. 2017) while the draft genome of the cultivar PR 202 was sequenced later using Illumina NextSeq 500, Illumina Miseq, and PacBio RSII sequencing platforms (Hatakeyama et al. 2018) adopting a novel multiple hybrid assembly workflow suitable for polyploid genomes using next-generation sequencing in combination with single-molecule real-time sequencing, followed next-generation optical mapping. The details of the sequencing of these two draft genomes are given in Table 3.4. Even though both draft genomes accounted for ~80% of the finger millet genome, the adequateness of the genome coverage of PR 202 was ascertained by single-molecule real-time sequencing and optical genome mapping (Hatakeyama et al. 2018). Transposable elements account for almost half of the genome, which also contains drought-related transcription factors (TFs) and drought-responsive genes in large numbers.

In addition to the draft nuclear genome of *E. coracana* (Hittalmani et al. 2017; Hatakeyama et al. 2018), a complete chloroplast genome and a draft nuclear genome of *E. indica*, one of the progenitors of the cultivated finger millet, have been reported recently (Zhang et al. 2017, 2019a). With the clarity on the donor of the maternal genome (AA), there is an immense need to identify the donor of the paternal genome (BB). The above-mentioned genome resources of genus *Eleusine* is highly useful for further studies toward understanding evolutionary relationships, gene flow between wild and cultivated species, identification and functional characterization of candidate genes associated with agro-morphological traits, and molecular breeding programs. More importantly, the scope of finger millet improvement programs will be broadened by the genome sequencing of valuable and unexploited wild species leading to the introgression of useful genes through marker-assisted breeding, transfer of genes through genetic engineering, and modification of genomes through genome editing.

Table 3.4 Details of whole-genome sequencing in finger millet

Particulars	Sequencing of ML-365	Sequencing of PR-202
Sequencing platforms used	Illumina and SOLiD	Illumina and PacBio RSII
Assembly type	Contig assembly	Hybrid de novo Assembly
Total assembly size	1.19 Gb	1.19 Gb
Genome coverage	82.31% of estimated size (1.45 Gb)	78.20% of estimated size (1.5 Gb)
Number of contigs/scaffolds	5,25,759	1,897
Contig/scaffold length	0.2 Kb–0.45 Mb	1.24 Kb–13.55 Mb
GC content	44.76%	40.98%
Number of genes predicted	85,243	62,348
N50 of assembly	23.73 Kb	2.68 Mb
NCBI accession no	PRJNA318349	PRJDB5606
Research group	Hittalmani et al. (2017)	Hatakeyama et al. (2018)

3.6.2 DNA Markers

The beginning of the twenty-first century marked the developments in DNA markers and genome sequencing in many millet crops due to the availability of nucleotide sequences in the public databases and advancements in the NGS technologies along with computational algorithms. During the past two decades, there is a continuous accumulation of genomic resources such as nucleotides, ESTs, genome survey sequence (GSS), gene, nuclear genome assembly, and chloroplast reference genome in millets on a daily basis, which formed the main targets for the development of DNA markers (Table 3.5). A large number of DNA markers, especially SSRs and SNPs, were developed quickly in sorghum and foxtail millet from the ESTs as well as whole-genome sequences. Finger millet was lagging in the development of DNA markers due to the presence of a few hundred ESTs as well as the non-availability of the whole-genome sequence. Since, finger millet is grown in different parts around the world and is adapted to diverse climatic conditions, the possibility of the existence of considerable genetic diversity among the finger millet germplasm/population cannot be neglected. Subsequent development of molecular markers has enabled a linkage map of the finger millet genome to be assembled (Dida et al. 2007). With the recent progress, the availability of a published genomic sequence would accelerate the development of markers to assist genotypic classification and breeding practices and the review by Sood et al. (2016) presents the current biotechnological advancements along with research gaps and future perspective of genomic research in finger millet.

Development of SSR markers (82 Nos.) was first reported by Dida et al. (2007) by employing methylation-sensitive restriction enzymes (*Hin*d*III*, *Pst*I, and *Sal*I) following hybridization of colonies obtained from random genomic libraries with a cocktail of di-[$(AG)_{15}$/$(AC)_{15}$] and tri-nucleotide [$(AAG)_{10}$/$(AAC)_{10}$/$(ATC)_{10}$, $(AGC)_{10}$/$(AGG)_{10}$, $(CCG)_{10}$] probes. Subsequently, a set of 92 new genomic SSR markers were developed by employing the next-generation sequencing strategy after enrichment by Musia (2013), of which, 49 markers exhibited polymorphism. SSR markers can also be developed from the mining of ESTs generated from EST projects, and possess an additional advantage of inter-specific transferability since they are developed from relatively conserved regions (genic) within the genome. A set of 31 EST-SSR markers were developed targeting di-, tri-, tetra-, and pentanucleotide repeat motifs by exploiting 1,740 ESTs available in the public domain (Arya et al. 2009). Later, one more set of EST-SSR markers (132 Nos) was developed by adopting in silico mining strategy using 1,927 ESTs of finger millet present in the NCBI database (Reddy et al. 2012). Targeting the development of a greater number of markers, a total of 545 EST-SSR markers were developed by Babu et al. (2014a) by targeting 1956 ESTs of finger millet available in the public domain. At the same time, about 58 new genic SSR markers were developed from the ESTs containing blast disease-related genes along with sequences of blast resistance genes of rice (Babu et al. 2014b). DNA markers developed by various research groups across the globe have been listed in Table 3.6.

In addition to SSRs, SNP discovery in finger millet has been reported through the genotyping-by-sequencing (GbS) approach. About 23,000 SNPs have been reported by Kumar et al. (2016) from the GbS data of 113 diverse finger millet genotypes. During the same period, a set of 23,285 SNPs were generated from the GbS data of two cultivated finger millet genotypes (KNE755 and KNE796) by Gimode et al. (2016), 92 of which were validated for genetic diversity assessment in cultivated and wild species of finger millet. The study also identified about 10,327 genome-wide SSRs. In addition, about 1,14,083 genome-wide SSRs have been identified through de novo sequencing of finger millet cultivar ML-365 (Hittalmani et al. 2017) along with 1,766; 2,866; 146, 56, and 330 R-genes, drought-responsive genes, C_4-pathway genes, transcription factor families, and calcium transport and accumulation-related genes, respectively. Very recently, about 1,69,365 single nucleotide polymorphisms were identified through the genomic

Table 3.5 Comparison of genomic resources available in finger millet with other millets

Resource	Sorghum	Pearl millet	Foxtail millet	Finger millet	Proso millet	Little millet	Kodo millet	Barnyard millet
Nucleotide	3,44,504	80,157	46,862	1,565	1,330	25	31	652
EST	2,10,892	5,265	66,052	1,934	211	–	29	74
Genome survey sequence	8,04,615	4,105	96,975	1	–	–	–	–
Gene	49,167	136	32,192	–	136	132	–	170
Nuclear genome assembly	GCA_000003195.3	GCA_002174835.2	GCA_000263155.2	GCA_002180455.1	GCA_002895445.2	–	–	GCA_015022175.1
Chloroplast reference genome	NC_008602.1	NC_024171.1	NC_022850.1	–	NC_029732.1	NC_032378.1	–	NC_028719.1

Source NCBI, date of collection: 01 January 2021

Table 3.6 SSR markers and SNPs developed by finger millet research groups

Type of DNA marker	No. of markers	References
Genomic SSR	99	Dida et al. (2007)
	27	Reddy et al. (2011)
	49	Musia (2013)
	12	Lee et al. (2017)
	10,327	Gimode et al. (2016)
	10	Reddy and Sivaramakrishnan (2017)
EST-SSR	17	Arya et al. (2009)
	11	Panwar et al. (2011)
	30	Reddy et al. (2012)
	3	Obidiegwu et al. (2014)
	74	Babu et al. (2014a)
	58	Babu et al. (2014b)
	545	Babu et al. (2014c)
	56	Nirgude et al. (2014)
	12	Selvam et al. (2015)
Genomewide SSR	10,327	Gimode et al. (2016)
Genomewide SNP	23,285	Gimode et al. (2016)
	1,14,083	Hittalmani et al. (2017)
	23,000	Kumar et al. (2016)
	1,69,365	Puranik et al. (2020)

data generated by GbS using an assembly of 190 diverse genotypes for identifying the marker-trait association for protein and mineral contents in the finger millet (Puranik et al. 2020). The accumulation of a large number of SSR as well as SNP markers along with the resequencing of diverse genotypes of finger millet will be useful for the development of SNP chip/array for rapid genotyping of large-scale accessions toward the identification of marker-trait associations for important target traits that can be utilized for the genetic improvement of finger millet through molecular breeding approaches.

3.6.3 Transcriptome Sequences

Functional genomics focuses on decoding the functions of genes that are governing the agronomic traits thereby helping in the understanding of the genetic and molecular mechanisms underlying these traits. A handful of technologies such as massively parallel signature sequencing (MPSS), serial analysis of gene expression (SAGE), microarrays, and transcriptome sequencing are useful in understanding the differential gene expression happening at critical developmental stages as well as during biotic and abiotic stresses. The ability of these technologies in generating an ocean of biological information is well known and can also be utilized in understanding gene networks associated with complex agronomic and nutritional traits by employing next-generation hi-throughput platforms. RNA-Seq is considered the most popularly used transcriptomics technology, which facilitates the identification and quantification of differentially expressed transcripts through a high-throughput sequencing platform. Despite its popularity for transcript profiling in various crops, it does not have the ability to accurately identify multiple full-length transcripts from short-read sequences (Steijger et al. 2013; Wang et al. 2016). This limitation is overcome by

innovative sequencing technologies such as Oxford Nanopore and PacBio Single Molecule Sequencing capable of generating full-length cDNA sequences, making it effective in assessing gene regulation and phenotypic diversity (Wang et al. 2016). Transcriptome sequencing was used recently for determining the genetic relationships among six Eleusine species by Zhang et al. (2019b) revealing fewer variants between *E. coracana* and *E. indica* because of the mapping of reads of the former to the chloroplast genes of all other Eleusine species studied. Phylogenetic analysis revealed interesting facts supporting *E. indica* to be the maternal parent of *E. coracana* and *E. africana*, a close relationship between *E. indica* and *E. tristachya*, as well as *E. floccifolia* and *E. multiflora*, and placing E. intermedia as a distinct group. The unexpected grouping of *E. floccifolia* and *E. multiflora* with distinct nuclear genomes (BB and CC) as well as that *E. intermedia* and *E. floccifolia* with similar nuclear genomes (AB and BB) demands a rethinking of the ancestral genomes of these three species.

Inherent climate resilience coupled with calcium-rich grains of finger millet has made the researchers focus mainly on understanding the expression of stress-responsive and calcium transport and accumulation genes through transcriptome profiling. A hypothetical tripartite model was proposed for calcium transport by Kokane et al. (2018) after analyzing the differential gene expression of nutrient-responsive genes. Several differential gene expression studies have been undertaken toward unraveling the molecular mechanisms involved in abiotic stress tolerance (Parvathi et al. 2013, 2019; Gupta et al. 2014; Rahman et al. 2014). Rice gene models have been successfully used for the identification of genes involved in salinity stress through transcriptome analysis involving finger millet genotypes exhibiting the difference in their levels of salinity tolerance (Rahman et al. 2014). Little earlier, higher expression of drought-responsive genes viz., metallothionein, ATFP6, phosphatase 2A, RISBZ4, and farnesyl pyrophosphate synthase were identified in the finger millet variety GPU-28 through e-northern analysis followed by semiquantitative and quantitative RT-PCR (Parvathi et al. 2013). Similarly, the vital role of the abiotic stress-responsive gene, EcGBF3, was revealed through the analysis of differential gene expression using qRT-PCR (Hittalmani et al. 2017; Ramegowda et al. 2017). An important regulatory gene, TBP-Associated Factor6 (EcTAF6), which is involved in drought response in finger millet was identified recently along with the *cis*-element in the promoter region responsible for abiotic stress responses through the screening of the cDNA library (Parvathi and Nataraja 2017). Analysis of 1790 differentially expressed transcripts generated through transcriptome sequencing involving control and drought-stress treatments revealed the stimulation of a cascade of drought-stress signaling genes, while regulatory genes like TATA-binding protein (TBP)-associated factors (TAFs) were activated in response to drought stress, highlighting the importance of regulatory genes in drought situations (Parvathi et al. 2019). Analysis of differentially expressed genes characterized as proteins of unknown functions resulted in the identification of new drought-responsive genes (pentatricopeptide repeat proteins and tetratricopeptide repeat proteins) that play an important role in multi-protein interactions.

Being an important crop with calcium-rich grains, finger millet has attracted a lot of attention among the research groups and has the potential to become a model system for unraveling the genetic and molecular mechanisms operating in the synthesis, transport, and accumulation of calcium. Transcriptome profiling by Mirza et al. (2014) had resulted in the discovery of 82 distinctive calcium sensor genes in finger millet, which were subsequently characterized into two distinct groups comprising eight calcium sensor gene families, viz., (1) Calcium sensor genes like calmodulin (CaM), CaM-dependent protein kinases (CaMK1 and CaMK2), calcium/CaM-dependent protein kinases (CCaMKs), calcium-dependent protein kinases (CDPKs), and Calcineurin-B-like (CBL) protein kinases (CIPKs) and (2) transporter genes like Ca^{2+}-ATPases, 14-3-3, two-

pore channel (TPC1), and Ca^{2+}/H+ antiporter (CAX1) (Singh et al. 2015; Kumar et al. 2015a; Sood et al. 2016). A comparison to rice gene models revealed that 19Ca^{2+} transporter genes discovered from the transcriptome sequencing data generated from developing seed of finger millet exhibited 33–90% sequence variation in amino acid sequence as compared to its counterpart in rice (Kumar et al. 2015b). In addition to calcium metabolism, two transcription factors (EcDof1 and EcDof2) were identified, which act as activator and repressor in the regulation of genes involved in nitrogen as well as carbon metabolism. Higher NUE is achieved by greater activation genes involved in N uptake and assimilation due to a higher EcDof1/EcDof2 ratio in the roots finger millet genotypes with high seed protein (Gupta et al. 2014).

Transcriptomics has undergone a remarkable evolution in the use of technologies starting from northern blotting and hybridization, reverse transcription-polymerase chain reaction (RT-PCR), real-time PCR, and microarrays to the current next-generation sequencing (NGS)-based RNA Sequencing (RNA-Seq). By enhancing the throughput, the NGS technologies will help in improving our understanding of gene regulatory mechanisms and provides deeper insights into biological pathways, gene networks, and molecular mechanisms associated with plant development, nutrition, and stress tolerance.

3.7 Future Prospects

Finger millet is an important crop among the small millets, calcium-rich, and inherently drought-tolerant grown especially by resource-poor farmers under marginal lands with minimal irrigation facilities. The genetic improvement in this crop has been happening at a relatively slower pace as compared to sorghum and pearl millet due to the challenges in hybridization as well as the availability of limited genomic resources. With the growing impact of climate change on crop production, vulnerability to stresses, and prominence of new pests and diseases, it is imperative to develop smart cultivars possessing climate resilience by employing molecular breeding strategies. Plant genetic resources are the reservoirs of valuable genes and alleles for important target traits that could be exploited in any crop improvement programs. Efficient use of germplasm resources in a breeding program is vital for the development of climate-resilient cultivars for achieving food and nutritional security. Characterization and evaluation of the germplasm resources are essential for the identification of trait-specific genotypes, genes, and alleles for further exploitation in the genetic improvement of finger millet. Recent sequencing of the whole genome of finger millet helped in the generation of genomic resources, which could be employed in the characterization of germplasm, dissection of genomic regions/QTL associated with important traits (agronomic, stress tolerance, and nutritional) through GWAS, characterization of functionally important genes, transcriptome analysis for unraveling the gene networks involved in nutritional traits and development of improved cultivars through molecular breeding. The effectiveness of germplasm resources coupled with harmonized use of available genomic resources is the way forward to overcome the challenges associated with the production of nutrient-rich food to meet the demands of the ever-growing population.

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4 Paradigm Shift from Genetics to Genomics: Characterization of Diversity and Prospects of Molecular Markers

Lalit Arya, Monika Singh, Amit Kumar Singh, and Manjusha Verma

Abstract

Food and nutritional security, agrarian sustainability, biotic and abiotic stress tolerance are the key requirements for sustainable agriculture. To attain these goals and to utilize the existing genetic diversity/allelic variation in a particular crop, huge germplasm collections need to be characterized using genomic approaches along with phenotypic and biochemical evaluation. Finger millet is one such crop loaded with massive nutritional and nutraceutical properties and climate resilience. Earlier characterization of diversity was confined to morphological, cytological, biochemical, and molecular markers including random amplified polymorphic DNA (RAPD), inter-simple sequence repeats (ISSRs); simple sequence repeats (SSRs), etc., but now there is a paradigm shift in the way we characterize and utilize the available genetic diversity in finger millet. Genotyping-by-sequencing (GBS) and genome-wide association study (GWAS) have been used to identify candidate genes and marker-trait associations for grain micronutrient, protein and tryptophan content, blast resistance and agro-morphological traits, etc. With the availability of finger millet whole-genome sequence, resequencing of the selected lines is being done to capture the complete and accurate genetic variation, single-nucleotide polymorphisms (SNPs), insertion/deletions (InDels), etc. Further, associative transcriptomics, genomic selection, pan-genome sequencing, and haplotype-based breeding approaches could be applied to dissect complex traits associated with agronomic performance, stress tolerance, and nutritional aspects in finger millet.

4.1 Introduction

Finger millet [(*Eleusine coracana* (L.) Gaertn.); family: Poaceae; subfamily: Chloridoideae; allotetraploid ($2n = 4x = 36$, AABB); A genome donor: *E. indica*; B genome donor: unknown; assembled genome size: 1.2 Gb; genome size based on flow cytometry: 1.5 Gb; self-pollinated] was domesticated from its wild progenitor, *E. coracaca* subsp. *africana*, in Ethiopian highlands and western Uganda more than 5,000 years ago (Hilu 1988; Liu et al. 2011, 2014; Hittalmani et al. 2017; Hatakeyama et al. 2018). Finger millet also known as ragi is cultivated in semi-arid, arid, tribal, and hilly regions of Africa and

L. Arya (✉) · M. Singh · A. K. Singh · M. Verma
Division of Genomic Resources, ICAR-National Bureau of Plant Genetic Resources, New Delhi 110012, India
e-mail: lalit.arya@icar.gov.in

M. Singh
e-mail: monika.singh@icar.gov.in

A. K. Singh
e-mail: amit.singh5@icar.gov.in

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India. India is the secondary center of finger millet diversity, where the crop was introduced in 3000 BC in Western Ghats. Finger millet is a crop of immense importance in view of its climate resilience, massive nutritional and nutraceutical properties (high protein; essential amino acids particularly enriched in lysine and methionine; minerals especially high calcium content, vitamins; dietary fiber; phytochemicals; glycoproteins; gluten free; low glycaemic index) (Saleh et al. 2013; Chandra et al. 2016; Kumar et al. 2016a). The crop is also a storehouse of vital genomic resources because of its excellent adaptability to harsh conditions. Large germplasm collections of finger millet are available in genebanks [ICRISAT genebank: ~6000 accessions (http://exploreit.icrisat.org/profile/Small%20millets/187); National Genebank, ICAR-NBPGR, New Delhi, India: ~11,352 accessions (http://www.nbpgr.ernet.in:8080/PGRPortal/(Shtfofsuczs3o44ns55eh2h45))/SimpleSearch.aspx)], which needs to be comprehensively characterized (high throughput) to know the level of phenotypic, genotypic, and nutritional diversity as well as adaptability under difficult conditions for future food and nutritional security, agrarian sustainability, biotic and abiotic stress tolerance. These large germplasm holdings of genebanks are the repositories of genetic variation and novel alleles/traits, which were earlier characterized through morphological markers to know the level of genetic diversity and develop core/minicore sets (Upadhyaya et al. 2006, 2010). These huge collections are now being characterized through molecular markers and next-generation sequencing (NGS) technologies to develop molecular cores, identify candidate genes and marker-trait associations. So, there is a paradigm shift in the way we characterize and utilize the available genetic diversity/allelic variation in a particular crop.

A number of reports from the genetics era are available wherein characterization of diversity was carried out and variation levels and genetic structure were assessed using different genetic markers, including classical markers such as morphological, cytological, and biochemical markers, and DNA/molecular markers including hybridization-based markers such as restriction fragment length polymorphisms (RFLPs) and polymerase chain reaction (PCR)-based markers such as random amplified polymorphic DNA (RAPDs); amplified fragment length polymorphisms (AFLPs); inter-simple sequence repeats (ISSRs); simple sequence repeats (SSRs); sequence-related amplified polymorphism (SRAP); start codon targeted (SCoT), etc.

But in today's scenario, high throughput, in terms of amount of genetic and genomic data generated and bioinformatic analysis are the backbone of characterization for enhanced utilization of genetic resources. In the era of genomics, information has been generated in finger millet in terms of whole-genome sequence (Hittalmani et al. 2017; Hatakeyama et al. 2018), genome-wide molecular markers/genes discovery, genotyping-by-sequencing (GBS), and genome-wide association study (GWAS), which represents a paradigm shift from genetics to genomics. These genomics approaches need to be augmented by using associative transcriptomics, genomic selection, pan-genome sequencing, etc. to dissect complex traits associated with agronomic performance, stress tolerance, and nutritional aspects and to promote haplotype-based breeding in finger millet.

4.2 Genetics to Genomics: Characterization of Diversity in Finger Millet

Characterization of diversity is the foundation of all crop improvement programs. The factors responsible for creating such variation/diversity are genetic drift and/or recombination, selection, migration, and mutation. Evaluation of genetic diversity of plant genetic resources is very crucial due to bourgeoning population, food insecurity, associated risks with narrowing genetic base of the existing cultivars and climate change. Finger millet is one such crop that can fulfill the criteria of nutritional and future food security as well climate resilience and needs to be thoroughly characterized for its enhanced utilization. A number of reports are available wherein classical markers, viz.

morphological, cytological and biochemical markers; DNA/molecular markers and next-generation sequencing approaches have been used to characterize diversity in finger millet showing a paradigm shift from genetics to genomics.

Morphological markers though monitored easily, are limited in number, and affected by the environment, hence limiting their usefulness. Similar restrictions are put on to the usage of isozyme markers also (Andersen and Lubberstedt 2003). Among morphological, isozyme (biochemical), and molecular markers, the latter give superior power of detection due to recognition of genotypic differences, genomic abundance, level of polymorphism, and non-influence of environmental factors.

Molecular characterization based on DNA molecular markers can be carried out using Random DNA Markers (RDMs-RAPD, RFLP, AFLP, ISSR, SSR, etc.), gene-targeted markers (GTMs), and functional markers (FMs).

In the genomic era, NGS approaches including whole-genome sequencing (WGS) and resequencing, RNA sequencing, metagenomics, high throughput genotyping, pan-genome sequencing, etc., and bioinformatic analysis tools are available to molecularly characterize the germplasm at the sequence level and to best utilize them for crop improvement. The most common complexity reduction high-throughput genotyping technologies are: restriction-site-associated DNA (RAD), GBS, diversity array technology (DArT) and are being frequently used in diversity analysis.

Characterization of all the germplasm holdings of a particular crop in a genebank using genotyping at the sequence level will answer many questions. Duplicates can be identified to mark the redundant germplasm and to decrease the load on genebanks in terms of usage of different resources (financial, manpower, etc.). Diversity level and population structure of the whole germplasm could be assessed and the molecular cores/subsets representing maximum diversity of the whole set could be designed. These cores can then be easily handled for their multilocation morpho-agronomic performance, biotic and abiotic stress resistance evaluation in the field as well as under artificial conditions. These can also be evaluated for quality traits for adding nutritional value to them. Once the whole germplasm set or molecular cores or subsets are characterized for different traits of economic importance, the genotyping along with phenotypic/biochemical data could be used for GWAS to identify SNPs, gene(s), or quantitative trait loci (QTLs) linked to these traits.

One such report (Bharati 2011) is available wherein the global composite collection of finger millet was characterized molecularly as well as for morpho-agronomic traits and a reference set of 300 most genetically diverse accessions capturing 89.2% of the alleles of the whole collection (959 accessions) was constituted using 20 SSR markers. A total of 11.6 alleles per locus along with 121 common alleles and 110 rare alleles at 1% and a mean gene diversity of 0.560 were reported. Wild *spontanea* race accessions showed maximum gene diversity of 0.611. A large number of alleles ranging from 10 to 21 were observed using UGEP81, UGEP10, UGEP102, UGEP26, and UGEP77 SSR loci. UGEP3, UGEP5, UGEP31, and UGEP104 SSR loci detected large numbers of multiple alleles. High PIC values of more than 0.636 were reported using UGEP15, UGEP5, UGEP18, UGEP102, UGEP12, and UGEP77 SSRs. Race and region-specific unique alleles were also reported. QTL UGEP56 in LG6 and UGEP8 in LG3 for days to 50% flowering indicated strong marker-trait associations (MTAs). Accessions were identified for high grain yield, early flowering, more fingers, fodder yield, ear head length, basal tiller number, high iron, and zinc content for finger millet improvement program. More such studies involving entire genebank germplasm and NGS-based genotyping along with morpho-agronomic and biochemical characterization are the need of the hour to characterize and utilize finger millet germplasm.

4.3 Morphological Markers

The establishment of genetic and genomic resources is a crucial step for crop improvement for desired traits (Ceasar et al. 2018). With the

onset of studies on diversity analysis in crops, morphological markers have emerged as potential markers that are dependent on agromorphological characters. Morphological characteristics could facilitate the determination of the agronomic parameters as well as the taxonomic classification of plant species (Ortiz et al. 2008). These markers also play a key role in the maintenance and management of plant genetic resources (PGR), as well as in the Plant Breeders' Rights (PBR) system (Babic et al. 2016).

A number of reports are available wherein morphological markers have been used to characterize genetic diversity in finger millet using either a smaller or larger set of germplasm. Upadhyaya et al. (2006) developed a core subset of 622 accessions representing the diversity of the entire collection of finger millet germplasm based on their geographical origin and data on 14 quantitative traits from the global collection of 5,940 accessions (Africa, Asia, America, Europe, and unknown origin) available at the genebank at ICRISAT, Patancheru, India. All the five races and breeding materials, improved cultivars, landraces, and wild types were represented in the core subset. Upadhyaya et al. (2007) characterized 909 finger millet accessions introduced from the genebank ICRISAT, Bulawayo, Zimbabwe, and grown at ICRISAT, Patancheru. Variability was recorded for plant pigmentation and growth characters (time to 50% flowering, plant height) and inflorescence characters (inflorescence width, length, and exsertion). Dominance of green-type plant pigmentation, erect-type growth habit, light brown grain color was observed. Dwarf plant (up to 75 cm) accessions were mostly from Zimbabwe. Early flowering accessions were reported from Kenya and late flowering from Tanzania and Zaire.

Umar and Kwon-Ndung (2014) characterized 10 finger millet accessions collected from diverse locations in northern Nigeria using morphological characters (plant height, leaf length and diameter, finger length and width, number of fingers, and 1000 seed weights). Significant genetic diversity was observed for the traits studied.

Variability for 19 agromorphological characters between 60 exotic and 89 Indian accessions was reported and flag leaf sheath length, peduncle length, panicle exsertion, ear head width, fingers per head, and 1000-grain weight were found more in the Indian accessions (Babu et al. 2017). Significant and positive correlations were observed between days to 50% flowering and days to maturity as well as for peduncle length and panicle exsertion. The genotypes (IE 7320, IE 4491, GE 1437, VHC 3911, and VHC 3898) and (GE 1437, GE 5192, and IE 5367) were identified as better parents for high photosynthetic efficiency and tryptophan content, respectively.

The germplasm from Plant Genetic Resource Center, Gannoruwa, Sri Lanka, was characterized using morphological markers (Dasanayaka 2016; Kaluthanthri and Dasanayaka 2016; Kumari et al. 2018). Kaluthanthri and Dasanayaka (2016) characterized 20 finger millet accessions and on principal component analysis (PCA), characteristics such as days to flowering, finger number, and yield per plant were found as important traits for variability among the studied genotypes. A set of 24 accessions were characterized using 14 quantitative characters (Dasanayaka 2016). Kumari et al. (2018) morphologically characterized 139 accessions (100 local collections from 15 districts of Nepal, 26 accessions from India, Zimbabwe, and unknown exotic origin, 9 from farmer's fields, 2 standards, and 4 others) using 14 quantitative characters and the highest variability was observed in grain yield, panicle exertion, weight of 20 mature ears, number of productive tillers and length of the longest finger. A significant and positive correlation was observed between grain yield and the number of productive tillers, threshing ratio, weight of 20 mature ears, and panicle exertion. The reported studies could be used for finger millet improvement in Sri Lanka.

Anuradha et al. (2017) evaluated genetic diversity among 25 finger millet genotypes through PCA and cluster analysis. The characteristics, viz., plant height, number of productive tillers, days to 50% flowering, fodder, and grain

yield showed significant variability. Genotypes VR 1101, VR 1098, VR 1112, VR 1113, VR 1111, VR 1115, VR 1116, and VR 1117 were found better for different traits could be further utilized for breeding programs. A set of 27 accessions were morphologically evaluated and a high degree of similarity was observed between IC49979A and IC49974B genotypes, whereas IC204141 and IC49985 showed a low level of similarity (Prabhu et al. 2018).

Mohan et al. (2018) evaluated 38 finger millet genotypes (18 released varieties and 20 landraces from India) using morphological markers (13 qualitative and 14 quantitative traits). Among the qualitative traits studied, diversity was observed for ear shape and size, however, most of the quantitative traits showed significant differences among the genotypes.

Kumar et al. (2019) evaluated 92 accessions for 16 qualitative morphological descriptors at G. B. Pant University of Agriculture and Technology, Pantnagar, India. Erect growth, dark green glume, droopy ears, non-pigmented leaf juncture, nonculm stem branching, lodging susceptibility, non-pubescent leaf sheath, non-branched fingers with multiple whorls, in thumb position branched fingers, and seeds with enclosed glume cover, brown color, round shape, rough surface, unpersistent pericarp with shattering nature were the predominant characters.

Morphology, plant growth, and yield contributing characteristics have been evaluated to characterize 20 accessions of finger millet in the Palghar district of Maharashtra (Patil et al. 2019). Characters such as erect growth habit, light brown seed color, partially enclosed seeds by glumes, and semi-compact ear were found dominant among the studied accessions. Productive tiller number followed by ear head length and finger number were the most varied traits and the lowest variation was shown by a finger width.

4.4 Cytological Markers

Cytological markers are based on variation in chromosomal morphology and have been used to discover progenitors or parents to the cultivated finger millet and also to study relatedness between different species. They can also be used in physical mapping and identification of linkage groups, however, their direct use has been very limited.

Chennaveeraiah and Hiremath (1974) concluded the subspecies *africana* as the direct progenitor of finger millet based on chromosome pairing data and no contribution of *E. indica* toward finger millet genome on the basis of lack of chromosome pairing. The report from this study was contrary to the recent reports wherein *E. indica* is considered one of the genome donors of domesticated finger millet. The reason for this disparity may be due to the fact that they did not mention the number of crosses made and extensive cytogenetical studies are required to conclude phylogenetic affinities (Dewey 1982). Further lack of chromosome pairing does not always show a lack of genomic resemblances (De Wet and Harlan 1972).

Hiremath and Salimath (1992) reported *E. floccifolia* not to be the genome donor to *E. coracana* and *E. multiflora* as a distinct species, with genomic symbol "C" based on mean chromosome pairing. Identification of the "B" genome donor to cultivated *E. coracana* is yet to be identified.

Bisht and Mukai (2000) mapped ribosomal DNA (rDNA) sites of four diploid and two tetraploid species of *Eleusine* by fluorescence in situ hybridization (FISH) and the similarity of the rDNA sites and their location on chromosomes in the studied species showed that diploid species might be the possible genome donors to tetraploid species. *E. multiflora* was differentiated from the rest of the species due to the presence of 18S-5.8S-26S rDNA on the largest pair of the chromosomes, 5S rDNA at four sites on two pairs of chromosomes, and 18S-5.8S-26S and 5S rDNA at the same location on one pair of chromosomes. Tetraploid species, namely, *E. coracana* and *E. africana* were found to possess the same number of 18S-5.8S-26S and 5S rDNA sites located at a similar position on the chromosomes. Diploid species, *E. floccifolia, E. indica,* and *E. tristachya* were found to possess the same 18S-5.8S-26S sites and locations that

showed resemblance with the two pairs of 18S-5.8S-26S rDNA locations in tetraploid species, *E. africana* and *E. coracana*. The 5S rDNA sites on chromosomes of *E. floccifolia* and *E. indica* were found comparable to those of *E. africana* and *E. coracana*.

Bisht and Mukai (2001) revealed *E. indica* and *E. floccifolia* as genome donor/contributor to *E. coracana* (an allotetraploid species) on the basis of in situ hybridization of *E. coracana* genome with the genomic DNA of different diploid species of the same genus. A close genomic relationship was observed between four diploid species, namely, *E. floccifolia*, *E. indica*, *E. intermedia* and *E. tristachya*, and the tetraploid species *E. coracana*. Based on the common genomic in situ hybridization (GISH) signals, it was found that *E. indica* and *E. tristachya* shared close similarities and *E. intermedia* as the intermediate species of *E. indica* and *E. floccifolia.*

Liu et al. (2014) reported *E. indica* as the primary A-genome parent and *E. tristachya* (or its extinct sister or ancestor) as the secondary A-genome donor to finger millet based on multicolor genomic in situ hybridization (McGISH).

4.5 Biochemical Markers

Biochemical markers generally involve the analysis of seed storage proteins and isozymes (enzymes differing in the sequence of amino acids but catalyzing the same biochemical reaction). These markers are based on enzymatic functions and allow the measuring of allele frequencies for specific genes. Studies involving isozymes and seed proteins for characterization of genetic diversity specifically for *E. coracana* are scanty.

Werth et al. (1994) employed 16 isozyme loci coding nine enzymes to analyze genetic variability among seven *Eleusine* species. Genetic variability differed considerably among members of diploid species (*E. indica* and *E. jaegeri).* Both the subspecies of the tetraploid *E. coracana* (subsp. *coracana* and subsp. *africana*) displayed fixed heterozygosity at several loci. Moreover, both the tetraploids also possessed *E. indica* marker alleles at all loci, confirming that they were derived from *E. indica* by hybridization with an unknown diploid.

Chong et al. (2011) studied genetic diversity within and among six glyphosate-resistant (R) and eight glyphosate-susceptible (S) *E. indica* populations from Peninsular Malaysia using isozyme markers encoding acid phosphatase (*Acp*), glutamate dehydrogenase (*Gdh*), glucose-6-phosphate isomerase (*Pgi* or *Gpi)*, glycerate dehydrogenase (*Gly*), isocitrate dehydrogenase (*Idh*), malate dehydrogenase (*Mdh*) phosphoglucomutase (*Pgm*), and uridine diphosphogluconate pyrophosphate (*Ugp*). Genetic variations at 13 enzyme loci from studied enzyme systems were evaluated in a set of 840 accessions. Low levels of isozyme diversity in R and S populations of *E. indica* were observed with a small percentage of polymorphism and the number of alleles per locus. The results inferred that the populations might possess a background of severe or long-lasting population bottlenecks that have shrunken the genetic diversity.

Kumar et al. (2012) studied seed storage proteins profiles of 52 finger millet genotypes from Uttarakhand. Clear and distinct polypeptide bands (15–25) having molecular weights in the range of 10–100 kDa were observed. No major differences in banding pattern among 52 finger millet genotypes were reported based on sodium dodecyl sulfate polyacrylamide gel electrophoresis. However, an additional band of 32 kDa was detected in a few genotypes that need to be studied further from a nutritional point of view.

4.6 Molecular Markers

On the basis of the method of detection, molecular markers are classified into hybridization-based and PCR-based markers. The molecular markers, gene discovery, and advancement of genomic resources were also reviewed by Sood et al. (2016).

4.6.1 Hybridization-Based Markers

RFLP is the first marker system based on hybridization, which relies on polymorphism as a result of insertion/deletions (known as InDels), point mutations, translocations, duplications, and inversions (Nadeem et al. 2018).

Salimath et al. (1995) analyzed genome origins and genetic diversity in 22 accessions belonging to five species of *Eleusine* from Africa and Asia using an eight probes-three enzyme RFLP combination, revealing 14% polymorphism (low level of sequence variability) in 17 accessions of *E. coracana* from Asia and Africa. Along with RFLP, RAPD and ISSR patterns were also studied based on which three species including *E. coracana*, *E. indica,* and *E. tristachya* showed a close genetic assemblage within the genus, whereas *E. floccifolia* and *E. compressa* were found most divergent.

Muza et al. (1995) classified 26 finger millet lines belonging to Africa and India into cytotype groups on the basis of the Southern blot hybridization patterns obtained with maize and sorghum mitochondrial cloned gene probes. Five restriction endonuclease enzymes were used, giving a total of 20 enzyme/probe combinations. A low level of polymorphism was detected with RFLP banding patterns. However, the data based on mitochondrial DNA clone *atp9* hybridization allowed the classification of the lines into three cytotype groups.

Parani et al. (2001) studied 119 accessions belonging to 7 small millet species using the chloroplast *trn*S-*psb*C gene regions to generate PCR–RFLP with 8 restriction enzymes individually as well as in combinations of two enzymes. A combination of two enzymes distinguished all the species. Species-specific differential banding patterns were observed.

4.6.2 PCR-Based Markers

PCR allows amplification of the region of DNA, targeted by the regions of high homology with the primers. PCR-based markers can be categorized as: (i) arbitrary primer-based markers, and (ii) sequence-based markers. As per the published reports, RAPD and SSRs have been the markers of choice for genetic diversity and population structure analyses in finger millet, and to some extent, ISSR and SNP makers have also been used for diversity studies. Very few reports are available on genomic SSR marker development (Dida et al. 2007; Gimode et al. 2016; Hittalmani et al. 2017; Lee et al. 2017) in finger millet. Large-scale genomic SSR marker (18,514) development based on finger millet genome sequence was reported by Hittalmani et al. (2017) and 35 SSRs were validated in 26 *E. coracana* and 14 wild species accessions, the markers identified can be used for diversity and marker-trait association studies to hasten marker-assisted breeding programs in finger millet. Functional/gene-based markers such as expressed sequence tag-simple sequence repeats (EST-SSRs)/transcriptome sequencing-based markers, nucleotide-binding site-leucine rich repeat (*NBS-LRR*) based markers, cytochrome P450 gene-based markers, *Aspartate kinase2* gene based SSRs, calcium transporters and calmodulin based anchored-SSRs, drought stress related genic SSRs, SRAP, SCoT have also been developed and used for molecular characterization of finger millet (Arya et al. 2009; Panwar et al. 2010a, b; Panwar et al. 2011; Naga et al. 2012; Nirgude et al. 2014; Kumar et al. 2015a; Saha et al. 2017; Panda et al. 2020). The different markers employed to study genetic diversity and population structure in finger millet are detailed in Table 4.1.

4.7 Prospects of Molecular Markers in the Post-genomics Era

4.7.1 Genetic Diversity and Population Structure Analysis

Genetic diversity and population structure studies based on RFLP, RAPD, ISSR, and SSR markers, etc. have already been discussed above. Here we will focus on the use of GBS in genotyping followed by genetic diversity and population

Table 4.1 Genetic diversity and population structure analyses in finger millet using PCR-based markers

Reference	Number of genotypes	Markers	Marker characteristics	Key findings
RAPD				
Fakrudin et al. (2004)	12 accessions of African origin (Zambia, Malawi) and Indian (Karnataka)	37 RAPD	Polymorphism: 85.82% Average bands per primer: 6.86	High level of genetic diversity was observed among the accessions studied and the utility of RAPDs in cultivar identification was suggested. Largely African and Indian accessions were clustered separately. African types and landraces were more diverse
Babu et al. (2007)	32 genotypes of Indian origin (Andhra Pradesh, Karnataka, Odisha, Tamil Nadu, Uttar Pradesh, Peninsular India)	50 RAPD	Polymorphism: 91% Average polymorphic bands per primer: 9.6	GEC 182 and CO 12 were found highly distant with contrasting characteristics (GEC 182: early flowering with bold grains, CO 12: late flowering with smaller grains), which could be utilized as parents in breeding programs, to develop improved varieties suitable for peninsular India
Das et al. (2007)	30 genotypes of Indian origin (Andhra Pradesh, Bihar, Dholi, Karnataka, Madhya Pradesh, Odisha, Almora, Pantnagar)	13 RAPD	Polymorphism: 96.77% Distinct bands: 124	The genotypes studied showed a wide genetic base
Das et al. (2009)	15 early duration finger millet genotypes	9 RAPD	Polymorphism: 85% Bands per primer: 2 to 9	Presence or absence of some bands was linked to genotypic adaptation to poorer or rich environments
Kumari and Pande (2010)	12 accessions (Almora, Ranchi)	17 RAPD	Polymorphic bands: 70 Polymorphism: 61.9%	RAPD markers could discriminate different cultivars. Unique markers were identified to discriminate finger millet genotypes
Bezaweletaw (2011)	66 (64 landraces and two improved varieties)	15 RAPD	Polymorphism: 72.35% Polymorphic bands: 89 PIC: 0.0 to 0.50	66 accessions were grouped into 9 clusters not based on their geographic origins and huge variability was observed in the landraces
Arya et al. (2016)	40 Indian (Andhra Pradesh, Bihar, Karnataka, Kerala, Madhya Pradesh, Tamil Nadu, Uttaranchal) and African (Ethiopia, Kenya, Tanzania, Uganda, Zambia, Zimbabwe) genotypes	25 RAPD	Average Nei's gene diversity: 0.18 ± 0.18	African genotypes showed relatively more polymorphism as compared to Indian genotypes Two distinct subgroups, based on the primary and secondary centers of origin of finger millet were revealed based on population structure studies
Ramakrishnan et al. (2015)	128 genotypes from India, Africa, Nepal, Germany, Maldives	25 RAPD	Polymorphism: 76.48% Average PIC: 0.40	All the genotypes were grouped into 12 subclusters and were genetically diverse. Four subpopulations with an admixture of alleles were observed

(continued)

Table 4.1 (continued)

Reference	Number of genotypes	Markers	Marker characteristics	Key findings
Mundada et al. (2019)	12 cultivars	12 RAPD	Polymorphism: 86.08% Average bands per primer: 13.5 Average PIC: 0.30	Significant genetic diversity existed among the cultivars and was placed into three groups
ISSR				
Brhane et al. (2017)	80 accessions from Ethiopia, Zimbabwe and India	6 ISSR	Polymorphism: 77.78% Total genetic diversity: 0.28	High genetic diversity was observed
SSR				
Dida et al. (2008)	79 accessions (*E. coracana* subsp. *coracana*) from 11 African and 5 Asian countries, and 14 wild *E. coracana* subs. *africana* lines from Uganda and Kenya	45 SSR	Average alleles per locus and Average gene diversity: Wild *E. coracana* subsp. *africana*: (2.47; 0.39) African cultivated material: (3.36; 0.33) Asian finger millet germplasm: (2.20; 0.22); *E. coracana* subsp. *coracana*; (4.04; 0.34)	Three distinct subpopulations were generated, representing subsp. *africana* and subsp. *coracana* originating from Africa and subsp. *coracana* from Asia. Lower diversity was observed in the Asian subpopulation
Arya et al. (2013)	67 accessions of African (Ethiopia, Kenya, Malawi, Tanzania, Uganda, Zambia, Zimbabwe) and Indian (Andhra Pradesh, Assam, Bihar, Himachal Pradesh, Karnataka, Madhya Pradesh, Odisha, Punjab, Rajasthan, Tamil Nadu, Uttarakhand) origin	17 SSR (3 genic and 14 genomic SSRs)	Average alleles per locus: 4.0 Average gene diversity: 0.471	19 rare and 9 unique alleles were observed; South Indian accessions clustered with African lowland types and north/highland Indian accessions with that of African highlands; Structure analysis showed the distinctness of Ugandan accessions and five major subpopulations. Higher diversity in African compared to Indian accessions was observed
Manyasa et al. (2015)	340 accessions (301 from Kenya, Tanzania and Uganda) and 15 accessions from the global minicore set and 24 checks (elite and blast-resistant/susceptible lines from the ICRISAT-Nairobi breeding program	23 SSR	Average PIC: 0.606; 57.7% rare alleles, 17.4% private alleles	Highest and lowest genetic diversity was observed in the Kenyan and in the Ugandan accessions, respectively Highest dissimilarity was observed between the accessions from Tanzania and Uganda. Variability between the countries was low as they might have shared the genepool. High diversity within the countries may be due to Farmers' selection for adaptation and end-use; High number of private and rare alleles are due to high diversity in the East African germplasm, as it is the primary center of finger millet diversity

(continued)

Table 4.1 (continued)

Reference	Number of genotypes	Markers	Marker characteristics	Key findings
Ramakrishnan et al. (2016a)	128 genotypes from India, Africa, Nepal, Germany, Maldives	87 SSR	Polymorphism: 59.94% Average PIC: 0.44 Average gene diversity: 0.14	Indian genotypes grouped into a major cluster along with six non-Indian genotypes. Three subpopulations, having an admixture of alleles were reported. All the genotypes found were genetically diverse and grouped based on their geographic regions
Babu et al. (2017)	149 finger millet accessions from Zimbabwe, Kenya, Maldives, Uganda, Malawi, Senegal, Nigeria, Zambia, India, Nepal, and Germany	46 SSRs	Average alleles per locus: 2.9 Average PIC: 0.44	Accessions were grouped into Indian and exotic groups. Morphological and molecular clustering pattern was similar to some extent Highly polymorphic markers: UGEP65, UGEP24, UGEP60, and UGEP78
Lee et al. (2017)	76 accessions (Asia, Africa, and unknown origins)	12 SSR	Average number of alleles: 3.3 Average PIC: 0.301	These accessions were grouped into two subpopulations having an admixture of alleles
Lule et al. (2018)	138 accessions from Ethiopia (96), Eritrea (8), Kenya (7), Zambia (9), Zimbabwe (13)	20 SSR	Total alleles: 222 Alleles per locus: 11.1 Average PIC: 0.61	Cluster analyses based on molecular and phenotypic data grouped these accessions into four major clusters (not entirely based on their geographical origins)
Manjappa et al. (2018)	47 elite genotypes of African (Ethiopia, Kenya, Uganda, Zambia, Zimbabwe) and Asian (Andhra Pradesh, Gujarat, Jharkhand, Karnataka, Maharashtra, Odisha, Tamil Nadu, Uttarakhand) origin (18 germplasm and 29 varieties)	16 SSR	Average PIC: 0.22 1 to 14 alleles (Average number of alleles: 4.69)	Germplasm showed slightly less genetic variation compared to varieties, which may be due to the smaller number of germplasm used Significant genetic variation among 47 genotypes, clustering of genotypes concordant with their geographical origin
Functional/gene-based markers				
Panwar et al. (2011)	73 blast resistant and susceptible genotypes from Maharashtra, Karnataka, Uganda, Kenya, Malawi, Uttarakhand, Andhra Pradesh, Uttar Pradesh, Bihar, Tamil Nadu	9 NBS-LRR 11 EST-SSRs	Polymorphism: NBS-LRR: 75.6% EST-SSRs: 73.5% Average PIC: NBS-LRR- 0.417; EST-SSRs-0.709	Markers for the blast resistance: NBS-05_{504}, NBS-09_{711}, NBS-07_{688}, NBS-03_{509}, and EST-SSR-04_{241} NBS-5, NBS-9, NBS-3, and EST-SSR-04 clearly differentiated resistance from susceptible genotypes
Babu et al. (2014a)	190 genotypes from Africa, India, Nepal, Germany, and America	46 SSRs	Average alleles per locus: 3.1 Average PIC: 0.442 Average gene diversity: 0.528	Phylogenetic and population structure analysis revealed two major clusters based on their geographical origin

(continued)

Table 4.1 (continued)

Reference	Number of genotypes	Markers	Marker characteristics	Key findings
Babu et al. (2014b)	190 genotypes from Africa, India, Nepal, Germany, and America	74 genic SSRs	Scorable alleles: 133 Average PIC: 0.408 Average gene diversity: 0.501	*Aspartate kinase2* gene based SSRs were more polymorphic compared to other candidate genes studied. Three major clusters based on tryptophan content were observed
Babu et al. (2014c)	190 genotypes from Africa, India, Nepal, Germany, and America	58 genic SSRs	Scorable alleles: 95 PIC: 0.385 Gene diversity: 0.487	Four population groups, largely corresponding to their blast disease response were observed
Nirgude et al. (2014)	103 genotypes	36 EST-SSR (*opaque2* modifiers) 20 anchored-SSR (calcium transporters and calmodulin)	Polymorphism: 68.23% Average gene diversity: 0.29 Average PIC: 0.17 Average gene diversity: 0.21 Average PIC: 0.18	Primers, viz., FMO2E30, FMO2E33, FMO2-18, and FMO2-14 were identified as highly polymorphic; Finger millet genotypes could be classified into high, medium, and low protein groups, but were broadly grouped based on calcium content using respective candidate gene-based EST-SSR markers. A significant negative correlation was observed between calcium and protein content
Gimode et al. (2016)	30 wild accessions 59 cultivated genotypes	80 SNPs	Average PIC: Wild accessions: 0.30 Cultivated genotypes: 0.15 All accessions: 0.29 Average gene diversity in all accessions: 0.38	STRUCTURE and UPGMA analysis revealed two major groups/subpopulations (cultivated and wild) SNP markers abundance: (1/3.3 kbp) New germplasm collections were correctly identified
Pandian et al. (2018a)	90 Indian genotypes from Africa, India, Nepal, USA, Germany, Maldives	43 genic SSRs (drought stress related)	PIC range: 0.41–0.79 Average gene diversity: 0.176	Considerable diversity among the genotypes. Three subpopulations consistent with their ecogeographical origins were observed
Panda et al. (2020)	15 indigenous genotypes including 12 from Koraput and 3 from Bhubaneswar (Odisha)	36 SCoT	Moderately high level of genetic diversity	Highest genetic dissimilarity was observed among (*Jhana, Lala, Kurkuti, Ladu, Bhadi,* and *Taya*) and modern high yielding genotypes, and was marked as the potential genetic resources
Combination of markers				
Panwar et al. (2010a)	52 genotypes belonging to different districts of Uttarakhand	18 RAPD 10 SSR 10 CytP450	Polymorphism: 49.4% 50.2% 58.7% Average PIC: RAPD-0.351; SSR-0.505; cytochrome P450 gene-based markers-0.406	Finger millet genotypes from Uttarakhand have a wide genetic base and were clustered separately based on low, medium, and high calcium contents

(continued)

Table 4.1 (continued)

Reference	Number of genotypes	Markers	Marker characteristics	Key findings
Panwar et al. (2010b)	83 accessions belonging to various geographical regions of Africa and India	18 RAPD 10 SSR 10 CytP450	56.17% 70.19% 54.29% Average PIC: RAPD-0.280; SSR-0.89; cytochrome P450 gene-based markers-0.327	SSR markers were stated as highly effective in defining polymorphism. Genotypes from different geographical regions were grouped separately. SSRs and cytochrome P450 gene-based markers were designated as useful markers for genetic diversity studies
Kumar et al. (2012)	52 genotypes from Uttarakhand	20 RAPD 21 SSR	Average PIC RAPD-0.351 SSR-0.557	RAPD profiles discriminated a few of the genotypes based on their protein content also SSR profiles clustered all the genotypes in different groups showing high diversity in their profiles
Rajendran et al. (2016)	42 genotypes from different geographical regions of southern India (Andhra Pradesh, Karnataka, Tamil Nadu, Telangana)	10 RAPD 5 ISSR 36 SSR	Polymorphism: RAPD-71.3% ISSR-37.4%, SSR-46.6% Average PIC: RAPD-0.44 ISSR-0.28 SSR-0.14	Three subpopulations (based on RAPD and SSR data) Four subpopulations (based on ISSR data)
Saha et al. (2017)	67 genotypes (resistant or susceptible to fungal blast disease)	12 SRAP 12 SSR	Polymorphism: SRAP-95.1% SSR-93.1%	High genetic diversity within the resistant and susceptible genotypes Two of the genotypes, IE 4709 (blast resistant) and INDAF 7 (susceptible) were identified as most diverse to be used as parents; Several genotype-specific bands were detected with SSR primers
Pandian et al. (2018b)	90 genotypes (including mini-core of ICRISAT) from India, Uganda, Nepal, Kenya, USA, Zambia, Germany, Malawi, Zimbabwe, Burundi, Maldives, Senegal, Nigeria	14 ISSR 10 RAPD 8 DAMD (SPAR markers)	Polymorphism and average PIC: ISSR-84.15%; 0.79 RAPD-83.49%; 0.81 DAMD-84.31%; 0.62	Geographic origin-based clustering was observed, genotypes studied are genetically diverse
Prabhu et al. (2018)	27 accessions from different parts of India	SSR RAPD	Polymorphism: SSR-87.50% RAPD-77.20% Amplicons: SSR: 64 RAPD: 301	Molecular markers-based clustering was consistent with their morphological and cytological data
Mohan et al. (2018)	38 finger millet genotypes (18 released varieties and 20 landraces from India)	RAPD ISSR	Polymorphism: RAPD-61.62% ISSR-57.00%	Landraces and varieties were clustered separately with some exceptions. Higher variation in landraces compared to varieties

(continued)

Table 4.1 (continued)

Reference	Number of genotypes	Markers	Marker characteristics	Key findings
Joshi et al. (2020)	40 landraces from Kaski and Dhading districts of Nepal	9 RAPD 5 SSR	Average PIC: RAPD-0.314 SSR-0.37	SSR markers revealed higher genetic distance compared to RAPD markers. Intermediate diversity was observed among the landraces studied Purbeli landrace was marked as unique landrace

SRAP: Sequence-related amplified polymorphism
SCoT: Start codon targeted polymorphism
PIC: polymorphism information content
SPAR: Single primer amplification reaction
DAMD: Directed amplification of minisatellite region DNA

structure analysis. The technique is highly cost-effective, robust, and was developed by Elshire et al. (2011). GBS is an NGS-based approach employed for discovering and genotyping SNPs in crop genomes and populations (He et al. 2014). SNP markers generated through GBS possess outstanding genetic attributes such as high reproducibility, wide genome coverage, codominant mode of inheritance, and chromosome-specific location, and thus extensive genotyping followed by the selection of diverse parents/alleles for breeding programs is achievable. GBS has been employed in finger millet for diversity and population structure analysis for extensive characterization at the sequence level.

Kumar et al. (2016b) studied 113 accessions from Africa, India, Nepal, Maldives, and Germany through GBS and generated 33 GB of data (160 million raw reads). Genome-wide set of 23,000 SNPs segregating across the entire collection and several thousand SNPs segregating within each accession were observed. Based on phylogenetic analysis and model-based STRUCTURE program three groups/subpopulations: Subpopulation 1 (southern Asia (India, Nepal, and the Maldives), followed by eastern Africa, Europe, unknown origin); Subpopulation 2 (southern Asia, followed by eastern Africa, western Africa); Subpopulation 3 (southern Asia, followed by eastern Africa, unknown origin) consistent with geographical distribution with some exceptions were inferred. The results also confirmed the hypothesis of African domestication of finger millet followed by its introduction to India.

Nyongesa et al. (2018) genotyped 95 genotypes from Kenya, India, Uganda, Malawi, Zambia, Zimbabwe, Nigeria, Nepal, and Germany using GBS. The genotypes were divided into three subpopulations (A, B, and C) and all three showed an admixture of alleles based on 117,542 SNPs. Cluster B comprised of genotypes showing high resistance to *Striga*, clusters A and C contained the most susceptible genotypes. Existing genetic variation can be used for marker-assisted breeding for *Striga* resistance. The highly diverse nature of the composite collection was revealed based on racial and regional diversity. Structure analysis closely corresponded with the phylogenetic analysis.

In another study by Puranik et al. (2020), 190 genotypes of Asian (India, Nepal, Pakistan, Sri Lanka, and the Maldives); east African (Burundi, Ethiopia, Kenya, Tanzania, and Uganda); south African (Malawi, Zambia, and Zimbabwe) and European or American origin were characterized by GBS (169,365 SNPs including 16,000 putative SNPs in the stringent and 73,419 putative

SNPs in the relaxed parameter). The less diverse genetic background between the East and South African accessions indicated their common evolutionary lineage and evolution from the same natural population. Three subpopulations: Subpopulation 1 (East African origin); Subpopulation 3 (the South African origin) and Subpopulation 2 belonging to Asian origin (India, Nepal, Pakistan, Sri Lanka, and the Maldives) were identified. European or American origin genotypes were not clustered with any particular group. This kind of clear geographic distinctions was also reported based on random markers systems.

4.7.2 Phylogenetic Relationships

Chloroplast DNA sequence analysis is a reliable tool for concluding phylogenetic relationships in polyploid species as compared to cytogenetic studies. Hilu (1988) revealed *E. indica* as the maternal genome donor of finger millet based on chloroplast DNA sequence analysis as chloroplast and its genome are predominantly maternally inherited.

Hilu (1995) used RAPD markers and reported the close genetic affinity of *E. tristachya* to the *E. coracana-E. indica* group and the distinctness of *E. multiflora*. A loose correlation between geographic distribution and pattern of genetic variability was observed. The allotetraploid nature of the finger millet was also confirmed.

In another study by Neves et al. (2005), *E. indica* was suggested as the A-genome (maternal) donor of *E. coracana* based on nuclear ITS and plastid *trnT-trnF* sequences. And also reported that *E. floccifolia* is not the second genome donor and the B genome donor is unidentified or extinct.

Liu et al. (2011) reported *E. indica-E. tristachya* clade as possible A-genome progenitors to *E. coracana* based on a biparentally inherited nuclear *Pepc4* gene tree and a maternally inherited plastid 6-gene tree.

Liu et al. (2014) suggested a single allotetraploid origin for the *E. africana-E. coracana* subclade based on the low-copy nuclear gene (waxy). *E. indica* and *E. tristachya* were found as the A-genome donors, with a differential degree of relatedness to *E. coracana*.

Zhang et al. (2019) also concluded *E. indica* as the maternal parent of *E. coracana* and *E. africana* group, based on transcriptome analysis of *E. multiflora*, *E. floccifolia*, *E. tristachya*, *E. intermedia*, *E. africana*, *E. coracana*, and *E. indica*. The study also supported a close relationship between *E. indica* and *E. tristachya*. The close relationship between *E. multiflora* and *E. floccifolia* was unexpected since they have distinct nuclear genomes, CC and BB, respectively.

4.7.3 Generation of Linkage Maps

The genetic maps provide important information for genetic analysis and crop improvement. Large numbers of highly variable markers are required to generate maps useful for trait analysis and eventually plant breeding. The first linkage map of finger millet based on 332 loci and F_2 population developed by crossing between Okhale-1 (a cultivated accession) and MD-20 (a wild accession), was generated by Dida et al. (2007). This group used RFLP, AFLP, EST, and SSR markers to generate the map of the tetraploid finger millet. The map spans A genome: 721 cM and B genome: 787 cM and covers almost all 18 finger millet chromosomes. The map was also used for a comparative study between rice and finger millet genomes.

The first high-density genetic map (4,453 SNP markers/18 linkage groups) of finger millet was developed by Qi et al. (2018) using the same population as discussed above. Paired-end GBS reads (278,880,767) and a new pipeline (UGbS-Flex) was used to generate the map. This pipeline can be used in species having different breeding systems, ploidy, and polymorphism levels, and even in the absence of a reference genome sequence.

4.7.4 Genetic Purity Testing of Hybrids

RAPD, ISSR, and SSR markers were used to find polymorphism between two parental lines, viz. PR 202 and IE 2606 of finger millet (Ajeesh Krishna et al. 2020). Twelve RAPD, 4 ISSR, and 21 SSR markers showed polymorphism and were used for genetic purity testing of the F_1 hybrids. Molecular markers were found useful in the identification of true hybrids and comparing the efficacy of hybridization methods. Hot-water-based emasculation was found much better compared to the hand-emasculation method of hybridization in finger millet.

4.7.5 Whole-Genome Sequencing and High-Throughput Genotyping by Resequencing

A paradigm shift from marker-based to genomics-based high-throughput sequencing approaches is generating sequencing data by several orders of magnitude with the advantage of dense marker coverage, less time requirement, and cost-effectiveness. Availability of whole-genome sequence in a particular crop is a boon since it provides a reference for resequencing of genotypes constituting core/minicore/trait-specific reference sets/mapping populations, etc. in a particular crop. This will deliver extensive and quality information at the sequence level for estimation of diversity, marker-trait associations, identification of candidate genes, taxonomic and evolutionary studies, high mapping accuracy and resolution, comparative mapping, etc. The data generated will further aid in functional genomics, forward and reverse genetics, and proteomic studies.

Developments of NGS tools have progressed the WGS and transcriptome sequencing in several crop species (Ceasar et al. 2018). The trend and timeline of genome sequencing in millets have been summarized in Fig. 4.1. Presently there are 2 reports available on WGS in finger millet, 1 using ML 365 yielded 1,196 Mb (~82% of the total estimated genome size) and predicted 85,243 genes and 1,14,083 SSRs using Illumina and SOLiD platforms (Hittalmani et al. 2017). Another group, Hatakeyama et al. (2018) reported 1.2 Gb as against 1.5 Gb genome size estimated based on flow cytometry and 62,348

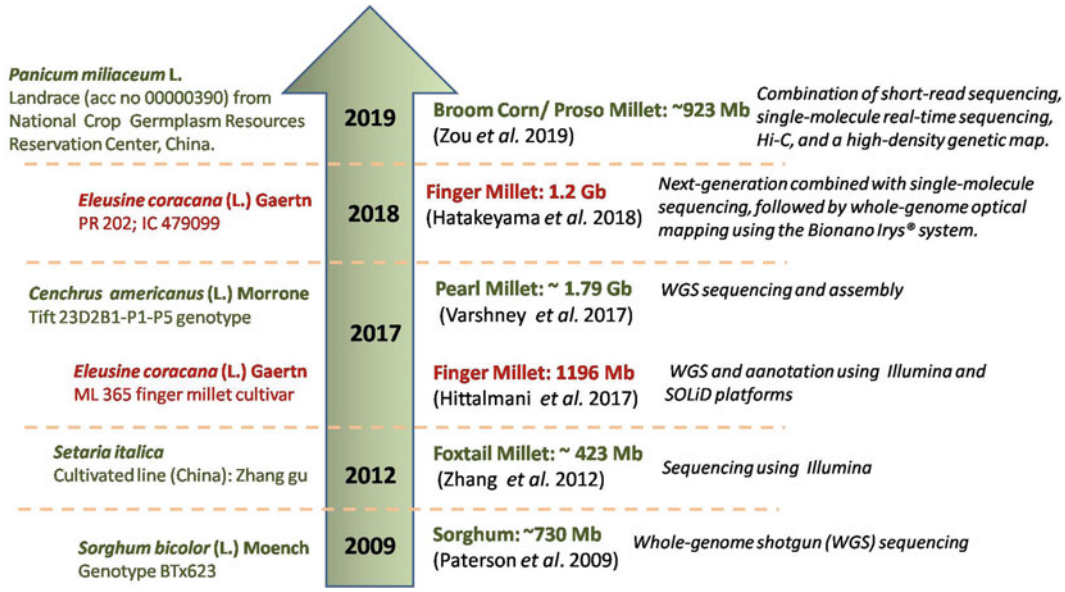

Fig. 4.1 Whole-genome sequencing trend and timeline in millets

genes in PR 202 based on NGS combined with single-molecule sequencing and by whole-genome optical mapping with Bionano Irys® system.

This whole-genome sequence data in finger millet has opened up the door for resequencing and utilization of huge variability available in genebanks. Resequencing data of 88 finger millet genotypes accomplished by GoK-funded Finger Millet Genomics Project is available at NCBI. This is being used for the development of superior finger millet varieties for Karnataka using Integrated genomics-assisted breeding approaches. But the whole germplasm of finger millet available in national genebanks needs to be characterized by NGS-based genotyping followed by the development of molecular cores/minicores and then these cores can be resequenced along with phenotypic and biochemical characterization for genome-wide marker-trait associations studies and identification of candidate genes.

4.7.6 Trait Mapping

Finger millet germplasm collections available with national and international genebanks are the repertoire of useful variations important for crop improvement. Previous studies on finger millet germplasm/core sets have identified genotypes for desirable traits such as high grain mineral content (Fe, Zn, and Ca content), protein content, resistance to biotic and abiotic stresses, etc. (Upadhyaya et al. 2011; Babu et al. 2013; Krishnamurthy et al. 2014). However, the true potential of finger millet germplasm collections has not been realized due to the lack of information about genes/alleles associated with these traits. In plants, the linkage mapping approach has been widely used to identify QTL(s)/gene(s), however, this method was not much successful in millets due to the difficulty involved in the generation of mapping populations. Recent advancements in genomics particularly the availability of high throughput genotyping technologies (GBS and resequencing), have made it possible to map diverse traits using a complementary gene mapping technique, association mapping (AM). AM approach exploits linkage disequilibrium at adjacent loci in diverse sets of genotypes, mostly natural populations to establish the correlation between genotype and phenotype (Gupta et al. 2014). This approach is highly efficient for genetic dissection of complex traits and has been used widely for mapping traits in a wide range of crops and would also be useful in the case of finger millet. There are different variants of association mapping, which include candidate gene association mapping, genome-wide association mapping, and associative transcriptomics. Potential applications of these approaches in finger millet are described below.

4.7.6.1 Candidate Gene-Based Association Mapping

Candidate gene association approach involves sequencing of candidate genes from diverse genotypes in order to discover variants which are then tested for their association with the target trait using statistical models. The application of this approach has allowed the identification of allelic variants for many useful traits in many crops including rice (Mishra et al. 2016; Abbai et al. 2019), wheat (Sukumaran et al. 2015; Ma et al. 2016), maize (Cook et al. 2012), soybean (Ikram et al. 2020). The approach can be very well applied in finger millet to identify novel allelic variants of candidate genes associated with various desirable traits. Since, some studies in finger millet have already identified some putative candidate genes for economically important traits including grain protein, micronutrient, tryptophan content, and blast resistance (Babu et al. 2014b, c; Puranik et al. 2020; Tiwari et al. 2020), candidate association mapping studies can be conducted for these genes using genebank collections/core sets. For nutritional traits, the important genes that can be potentially targeted for association analysis work are: gene encoding for no apical meristem-associated (NAM) protein, a member of the *NAC* family associated with iron content (Puranik et al. 2020), gene encoding for aspartyl protease exhibiting strong association with grain protein content (GPC) (Tiwari et al. 2020) and

genes involved in lysine and tryptophane biosynthesis (Babu et al. 2014b). Similarly, candidate gene association analysis can also be attempted for orthologues of *NBS-LRR* family blast resistance genes of rice such as *PiKh* and *Pita* as the pathogen *Magnaporthae grisea* causes blast disease of rice and finger millet (Babu et al. 2014d). Besides, the above-described traits, drought tolerance is another desirable trait in finger millet as it is mainly grown as a rainfed crop. Candidate gene association mapping can play important role in the identification of novel alleles for abiotic stress tolerance genes. To date, a host of abiotic tolerance genes and transcription factors (*DREB*, *MYB*, *NAC*) have been identified in other cereals such as rice, wheat, foxtail millet, etc. (Lata et al. 2011). Orthologues of these genes can be annotated from the finger millet genome and targeted for candidate gene-based association mapping. Importantly, there is a need to identify more number of functionally validated candidate genes in order to unravel superior alleles/haplotypes for various traits from germplasm collections using candidate gene based association mapping approach.

4.7.6.2 Genome-Wide Association Study

Genome-wide association study (GWAS) is a very powerful technique and does not require mapping populations' generation step which is a laborious, time-consuming, and technically demanding exercise. Moreover, since GWAS uses a diverse set of lines that may capture many historical recombinant events, linkage disequilibrium (LD) mapping can enable mapping resolution up to 100–200 Kb as compared to 10–20 cM in the case of biparental mapping, and sometimes it may even identify causative genes associated with the target trait. GWAS has been widely used in many plant species including, arabidopsis (Togninalli et al. 2018), rice (Lekklar et al. 2019), wheat (Chaurasia et al. 2020; Kumar et al. 2020), foxtail millet (Jaiswal et al. 2019), soybean (Fang et al. 2017), maize (Mazaheri et al. 2019), pearl millet (Srivastava et al. 2019), etc. for identification of genomic regions/genes associated with economically important traits. Recently, with the availability of finger millet genome sequence and high throughput genotyping methods, it is possible to conduct large-scale genome-wide association studies in this crop for diverse traits. As of now, GWAS studies in finger millet have been reported for various traits such as agro-morphological characteristics, blast resistance, nutritional traits, and low phosphorus tolerance (Babu et al. 2014a, b, c; Kumar et al. 2015b; Ramakrishnan et al. 2016b, 2017; Tiwari et al. 2020). Babu et al. (2014a) conducted AM on a panel of 190 diverse lines and identified markers associated with agromorphological traits including flag leaf width, plant height, basal tiller number, and 50% flowering. Grain protein and tryptophan content associated genomic regions have also been identified using GWAS (Babu et al. 2014b; Tiwari et al. 2020). Moreover, integration of AM with comparative mapping has proved very effective in the identification of genomic region for tryptophan and grain protein content. Using this approach, Babu et al. (2014b) have identified 2 QTLs for tryptophan and 1 QTL for grain protein content (GPC) in a global collection of finger millet. Of the 2 QTLs controlling tryptophan, 1 was major, explained 11% of phenotypic variance and was found associated with SSR marker OM5 designed from 27-kD *γ-zein* gene of OPM (opaque-2 modifiers). It was a very significant finding as OPM influences tryptophan content to a large extent (Babu et al. 2014b). GWAS has also been conducted for mapping of blast disease which is caused by *Magnaporthe grisea* and is considered to be one of the major limiting factors in finger millet production. A total of four QTLs for finger blast and one QTL for neck blast have been mapped in global finger millet collection using SSR markers (Babu et al. 2014c). The marker FMBLEST32 and RM262 explained 8% and 10% of the phenotypic variance, respectively, for blast resistance. UGEP81 and UGEP18 were associated with finger and neck blast and explained 7.5% and 11% of phenotypic variance, respectively. The above-described studies indicate the great potential of GWAS in the genetic dissection of important traits in finger millet. GWAS was also done to find the association of SNPs with *Striga*

reaction based on field *Striga* resistance and GBS using 95 finger millet genotypes and markers TP 85,424 and TP 88,244 were identified for *Striga* resistance (Nyongesa et al. 2018). In another study, 14 agromorphological traits were mapped in a panel of 113 finger millet accessions using GBS-derived SNP markers and three different GWAS models, viz., SLST, MLMM, and MTMM. A total of 109 novel associations were identified for 14 different agomorphological traits. Further, among these 109 novel MTAs, 9 were common across 3 different models (Sharma et al. 2018). Recently, Tiwari et al. (2020) applied association mapping on 113 diverse finger millet lines to dissect complex genetic regulation of GPC and uncovered 5 reliable genomic regions for GPC. Out of these five regions, one contained gene encoding for aspartyl protease, which was considered a major promising candidate gene contributing to variation in GPC content. In a recent study (Puranik et al. 2020), the application of a combination of GBS and GWAS on a panel of 190 finger millet genotypes revealed genomic regions underlying putative candidate genes associated with grain micronutrient content (iron, zinc, calcium, magnesium, potassium, and sodium). A total of 34 highly reliable MTAs were identified, out of which 18 markers showed homology with the candidate genes and suggested to have putative functions in remobilization, binding, and transport of metals.

4.7.6.3 Associative Transcriptomics

Associative transcriptomics is a variant of GWAS which involves the analysis of transcripts across association panels to discover genomic regions controlling complex traits (Harper et al. 2012). This approach has allowed the identification of both transcript-level sequence variation (SNPs/InDels) and changes in the expression (Gene expression markers; GEM) that could be associated with diverse traits. Initially established in *Brassica*, this approach has also been used in other species such as wheat (Miller et al. 2016; Wang et al. 2017) maize (Azodi et al. 2020), and chestnut (Kang et al. 2019) for mapping complex traits. Indeed, associative transcriptomics has opened a way to identify expression-level variations in genomic regions/genes critical for the development of important traits. In the case of finger millet, this approach can be used to identify differentially expressed genomic regions associated with variations in grain mineral nutrients such as Ca, Fe, Zn, etc. as for these traits it is the changes in the expression of individual genes that could have more role than sequence-level variation (Kumar et al. 2016a).

4.7.6.4 Genomic Selection

The main factors limiting the utilization of large finger millet germplasm resources conserved in genebanks are the requirement of huge financial resources and technical expertise to identify potential lines for various desirable traits. In recent years, with the availability of cost-effective high throughput genotyping methods, genomic selection (GS) holds great promise for the selection of superior germplasm lines from the gene bank collections. (Muleta et al. 2017). The GS approach involves estimation of genomic estimated breeding values (GEBV) of traits in a reference population using genome-wide markers and phenotypic data, and subsequently, these GEBVs are used to predict the performance of respective traits in a related genotype sets exclusively based on the genomic data. Since the GS approach does away with multilocation/multiyear evaluation of germplasm required for trait identification, it can facilitate early selection of useful germplasm from genebank collections and accelerate genetic gain in the breeding program. In the beginning, the GS approach was mainly considered for the selection of lines in the breeding population, however, recently a few studies have reported the potential of GS in the identification of desirable traits in a germplasm collection of crops such as soybean, wheat, rice, etc. (Muleta et al. 2017; Kehel et al. 2020). In soybean, a GS model for white mold disease developed using a reference population of a diverse set of lines, could reliably identify white mold resistance genotypes from the United States Department of Agriculture (USDA) soybean germplasm collection using their genotyping data. In the case of finger millet, GS has not been reported so far as there was very limited

genomic resources information available in this crop. However, with the availability of finger millet genome sequence and advanced genotyping methods, this approach can be potentially considered for selecting potential lines for yield contributing traits, blast resistance, and rich in micronutrient contents from genebank collection. The development of GS prediction models in finger millet would require constituting diverse finger millet germplasm lines with variations for various traits. The panel can be densely genotyped and phenotyped for various traits under various environments and the GEBV is calculated. The GEBV values for traits are used to select useful lines from the genebanks exclusively based on genomic information.

4.8 Concluding Remark and Future Prospects

4.8.1 Pan-Genome Sequencing: Constitution of Pan and Super Pan Genomes of Finger Millet and Its Wild Relatives

The actual assessment of the extent and pattern of genetic diversity in finger millet germplasm collection is critical for its conservation and efficient utilization in the breeding program. However, so far, the genetic diversity studies in finger millet have used traditional PCR-based markers and a limited number of genotypes (Vetriventhan et al. 2020), which have failed to provide a comprehensive picture of the actual genomic diversity in genepool of finger millet at the level of total genes. Recently, with a decrease in sequencing costs, NGS-based genotyping approaches such as GBS/resequencing are becoming popular for characterizing genetic diversity in crop genebank collections (Milner et al. 2019). However, both these approaches have some limitations. The GBS, approach which involves only partial sequencing of individual genomes, may not be able to capture genomic diversity/sequence variation at a large portion of the genome across the studied set of genotypes/genebank collection. Similarly, in the resequencing approach, SNP/InDel variants are identified by aligning sequencing reads of each accession against the single reference genome so the genomic regions/genes that are present in one or some individuals but absent in the reference genome cannot be analyzed/compared using this approach. Therefore, currently, the emphasis is on pan-genome sequencing in which individuals of the targeted species are sequenced, de novo assembled, and compared to unravel the total genes present in the genepool of that species (Bayer et al. 2020). The pan-genome represents total genomic diversity available in a species collection and is comprised of a core set of genes that are present in all the individuals as well as variable genes which are absent in some individuals. The first pan genome in plants was constituted using seven wild individuals, which revealed a variable number of genes for seed composition, organ size and biomass, flowering and maturity time genes, etc. in soybean (Li et al. 2014). Thereafter pan genomes have been constituted in many plant species including rice (Stein et al. 2018), sunflower (Hübner et al. 2019), and sesame (Yu et al. 2019). Likewise, pan-genome sequence of finger millet collections could be constituted that would unravel novel genes/alleles for different traits and accelerate their utilization in breeding programs. Pan-genome sequencing can enable the comparison of genomes of ancestral and cultivated species and tracking of gene frequency during the domestication process and breeding.

4.8.2 Haplotype-Based Breeding

Recently, haplotype-based breeding is emerging as a potential strategy for designing tailor-made crops. The approach involves mining of superior haplotypes of genes controlling agronomically useful traits from germplasm collection and their deployment in the best combinations for developing high-yielding superior varieties (Sinha et al. 2020). Some studies in this direction have already been initiated in rice and pigeon pea. In

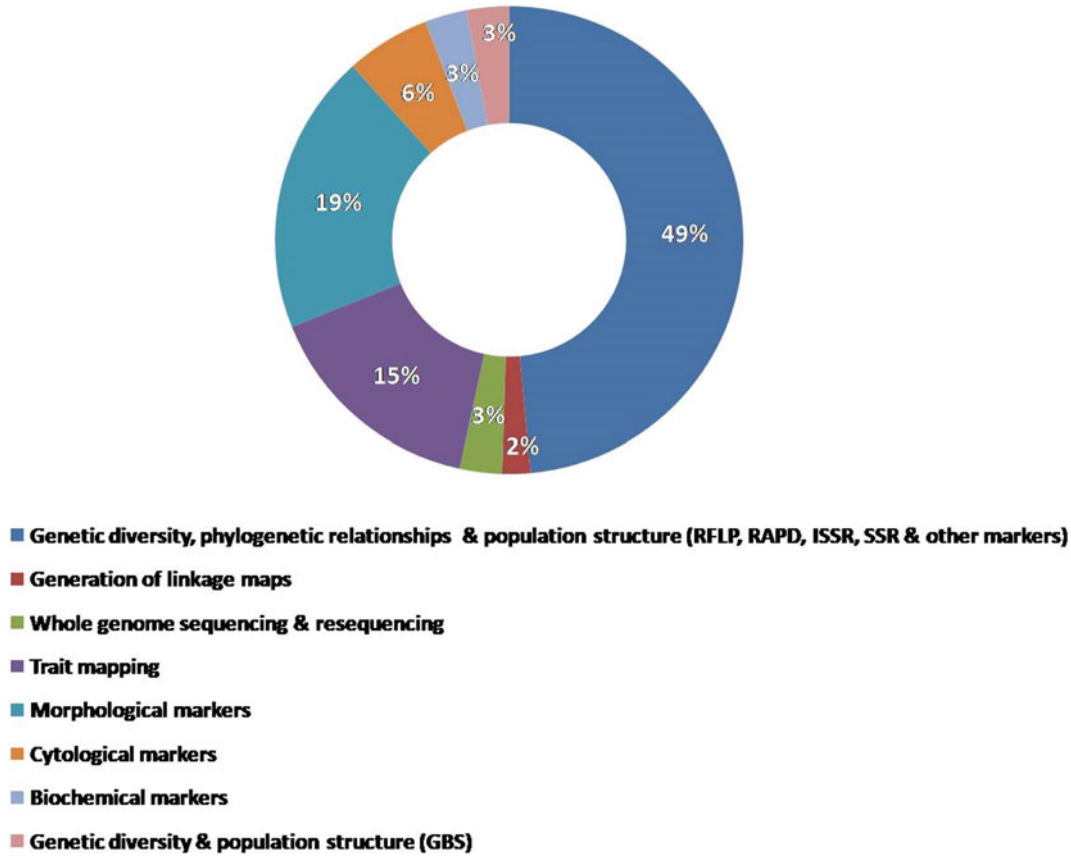

Fig. 4.2 Overview of genetics and genomic developments in terms of characterization of diversity and prospects of molecular makers in finger millet

the coming years, resequencing of finger millet germplasm resources/core set is expected to unravel allelic diversity and thus would aid in harnessing genetic diversity. This would facilitate the identification of genotypes with novel superior alleles for agronomical and yield traits that could in turn be used in finger millet improvement. For finger millet, low productivity is one of the major factors limiting its wide-scale cultivation, haplotype-based breeding may enable mobilization of superior alleles and would pave the way for the development of tailor-made finger millet varieties.

The developments like GWAS, genome sequencing, transcriptome analysis, trait mapping, etc. have ensued in finger millet but not to the extent as required (Fig. 4.2). Enhanced usage of genomic tools and approaches is the need of the hour to efficiently utilize the huge amount of diversity available in the genebanks for food and nutritional security, biotic and abiotic stress resilience, and overall sustainability. Further genomic selection, associative transcriptomics, pan-genome sequencing, and haplotype-based breeding approaches should be employed to accelerate finger millet crop improvement.

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Molecular Mapping in Finger Millet

5

K. V. Vijaya Kumar, Laavanya Rayaprolu, M. V. C. Gowda, Rajeev Gupta, and Santosh Deshpande

Abstract

Finger millet is an important staple in the semi-arid and tropical regions of the world, with an incredible ability to adapt to adverse agroecological conditions. Finger millet grain is gluten-free and exceptionally rich in micronutrients, including calcium, folic acid, and iron. There is relatively less research on finger millet due to a lack of genetic resources. There have been studies on the potential use of the secondary and tertiary gene pools to broaden the narrow genetic base that the inbreeding nature of the crop has created. The availability of the draft genome and linkage maps recently developed will pave the way forward to execute large-scale genomics-assisted breeding for crop improvement. With the increasing demand for finger millet, genomic resources need to be utilized in germplasm characterizations for efficient breeding. This chapter discusses the genetic and genomic resources available for finger millet and ways for their exploitation to enhance its adaptability to climate change.

5.1 Introduction

Finger millet (*Eleusine coracana* L. Gaertn.) ($2n = 4x = 36$) belongs to the family Poaceae, genus *Eleusine* in the tribe Eragrostideae. The genome size of the finger millet is 1,593 Mb and is a self-pollinated crop (Goron et al. 2015). Finger millet is nutritionally comparable to rice and wheat and ranks fourth in importance among millets in the world after sorghum, pearl millet, and foxtail millet (Upadhyaya et al. 2007). Finger millet, a C_4 photosynthesis cereal, is adaptable to a harsh environment, has unique nutritional status viz., better mineral content (Ca, Fe, Zn, Mg, Cu, and Cr), dietary fiber, and quality protein. It also has nutraceutical properties with many health benefits, including antioxidant property, blood glucose-lowering effect, cholesterol-lowering effect, improved hemoglobin status in children, and anti-ulcerative property (Shobana et al. 2013).

With the advent of molecular markers, it is common to trace valuable alleles in segregating populations and map them. The generation of framework maps opens up avenues to use a large number of markers derived from various

K. V. Vijaya Kumar · L. Rayaprolu · R. Gupta · S. Deshpande (✉)
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)-HQ, Patancheru 502324, India
e-mail: santosh.ds1p2@gmail.com

K. V. Vijaya Kumar · M. V. C. Gowda
All India Coordinated Research Projects On Small Millets, ICAR, GKVK, Bengaluru, India

R. Gupta
US Department of Agriculture (USDA) Agricultural Research Service (ARS), Fargo, ND, USA

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techniques to saturate the maps as much as possible. Once mapped, these markers are efficiently employed in tagging the desirable traits and use them in marker-assisted selection (MAS). This will eliminate the need for biochemical analysis, screening for individual traits phenotypically in the early generation breeding program, and minimizing the time required to develop new genotypes with favorable traits adoptable across environments.

In finger millet, crop improvement through the transfer of most desirable traits from germplasms to elite background adaptable to different agro-climatic situations has been unsuccessful. Several factors affect genomics-assisted molecular breeding approaches in finger millet, including allotetraploid (AA and BB sub-genomes) nature; high level of inbreeding; low polymorphism levels; enormous homeologous single nucleotide polymorphisms (SNPs); less genomic resources (<100 simple sequence repeats, SSRs); and non-availability of suitable mapping population for understanding genetics. Little progress in research work in deciphering genomic regions associated with the phenotype expression was made to date. Finger millet still lacks the required basic genomic resources for efficient breeding.

5.2 Molecular Marker Developments in Finger Millet

5.2.1 Diversity Studies

The development and use of molecular studies in finger millet were meager till recently. In one of a kind, the first study on finger millet, Salimath et al. (1995) studied molecular diversity of 20 finger millet accessions using isozyme, restriction fragment length polymorphism (RFLP), and random amplified polymorphic DNA (RAPD) markers. This study had given an initial indication of lesser genetic diversity in finger millet accessions, with 16 isozyme loci and 15 RFLP loci exhibiting uniformity among the accessions.

Weising et al. (2005) attempted to identify the transferability of 210 simple sequence repeat (SSR) markers from major cereal crops (wheat, rice, maize, and sorghum) to *E. coracana*. More than half (57%) of the SSR primers generated reproducible cross-species or cross-genus amplicons. The transfer rate of SSR markers was correlated with the phylogenetic relationship or genetic relatedness. Kalyana Babu et al. (2007) studied the diversity of 32 finger millet genotypes using 50 RAPD markers. A total of 529 loci were amplified, of which 479 loci (91%) were polymorphic and informative to differentiate the accessions. Dida et al. (2008) reported the population structure of 79 finger millet accessions with 45 SSR markers and identified a significant difference in plant architecture and yield in Asian and African subpopulations. The details of the research studies related to marker discovery and marker applications in finger millet are described in Table 5.1. Most of the recent studies in finger millet have focused on using the information on the candidate genes associated/regulating the traits related to the major stress, both biotic and abiotic stress (Kalyana Babu et al. 2014a, b, c, d, e; Nirgude et al. 2014), and agronomic performance (Ramadoss 2014; Kumar et al. 2015) from other model species. These syntenic studies added valuable information about the major trait-linked markers for finger millet breeding application and their possible use in developing new cultivars with improved and/or better performance for the target trait.

5.2.2 EST-Based SSR Markers for Finger Millet Crop Improvement

Microsatellites or SSRs have become the markers of choice over the past two decades for many crops. Functional markers-based molecular characterization of resistance gene analogs encoding nucleotide binding site-leucine-rich repeat (NBS-LRR) disease resistance proteins in finger millet by Panwar et al. (2011) described the association of NBS sequences with the blast disease resistance in large accession. Reddy et al. (2011) showed five amplification of resistance gene homologs in finger millet. A BLAST search of cloned finger millet DNA fragments showed

Table 5.1 Detail of marker development and marker applications in finger millet

References	Study area	Markers used	Significant results
Dida et al. (2007a, b, c)	A genetic linkage map with 131 markers mapped to 16 LGs spanning 721 cM on the A genome, and 196 markers mapped to 9LGs covering 787 cM on the B Genome	82 SSR markers	First genetic map in finger millet
Dida et al. (2007c)	Synteny between rice and finger millet 332 loci from 266 primer pairs mapped to 26 LG highly conserved gene orders between rice and finger millet		
Arya et al. (2009)	New SSRs from EST sequences of finger millet	31 EST SSRs	9 polymorphic markers
Panwar et al. (2010)	Genetic diversity concerning calcium content in finger millet	10 genomic SSRs	5 polymorphicSSRs
Panwar et al. (2011)	Finger and neck blast disease	20 EST SSRs (9NBS-LRRand11EST SSRs)	5 markers identified to be associated with blast resistance in finger millet
Reddy et al. (2011)	Finger and neck blast disease–Conserved region of resistance gene homologs in finger millet using degenerate primers of previous studies	6 RGH specific SSRs	6 markers for blast resistance developed
Kumar et al. (2012)	Diversity analysis for protein content	24 genomic SSRs	21 polymorphic markers
Reddy et al. (2012)	New SSRs from EST sequences of finger millet	30 EST SSRs	20 polymorphic markers
Arya et al. (2013)	Development of EST SSRs	3 genic SSRs-	
Musia (2013)	New genomic SSRs using in-silico tools from Roche 454 GS-FLX Titanium sequence data	92 SSRs	49 polymorphic genomic SSRs
Kalyana Babu et al. (2014a, b, c, d, e)	Finger and neck blast disease- SSR markers designed from finger millet, rice NBS-LRR region; *M.griseae* genes of rice; cloned rice blast genes	58 SSRs (43 genic SSRs and 15 rice genomic SSRs tightly linked to blast QTLs)	28 polymorphic markers
Kalyana Babu et al. (2014c)	*Opaque2* modifiers-from EST sequences of rice, maize, sorghum; Candidate genes of lysine and tryptophan metabolic pathways	67 EST SSRs(33 EST SSRs, and 34 candidate genes based SSRs) and 7 maize genomic SSRs tightly linked to *opaque2* modifier genes	35 polymorphic markers
Kalyana Babu et al. (2014e)	Finger and neck blast disease-SSRmarkers designed from rice NBS-LRR region showing similarity with finger millet EST sequences	13 EST SSRs	8 polymorphic markers
Nirgude et al. (2014)	Opaque2 modifiers-from EST sequences of rice, maize, sorghum	36 EST SSRs	15 polymorphic markers

(continued)

Table 5.1 (continued)

References	Study area	Markers used	Significant results
Nirgude et al. (2014)	Calcium content-from calmodulin candidate genes viz., rice, maize, wheat, and barley	20 anchored SSRs	5 polymorphic markers
Obidiegwu et al. (2014)	New SSRs from EST sequences of finger millet	45 EST SSRs	3 polymorphic markers
Ramadoss (2014)	Transcriptome analysis of drought tolerance	288 genomic SSRs	32 polymorphic markers
Yadav et al. (2014)	Calcium content-designed from calcium transporters and sensors of rice and sorghum	146 EST SSRs	No polymorphism
Kumar et al. (2015)	Calcium content-designed from calcium transporters and sensors of rice and sorghum	3 anchored EST SSRs	14 polymorphic markers

strong homology to NBS-LRR type R-genes of other crop species. Of the 107 clones sequenced, 41 showed homology to known R-genes and are denoted as EcRGHs (*Eleusine coracana* (L) Gaertn resistance gene homologs). Kalyana Babu et al. (2014a, b, c, d, e) utilized association mapping for blast resistance with 104 SSR markers. The study reported four and one quantitative trait loci (QTLs), respectively, for finger blast and neck blast resistance. The genomic marker RM262 and genic marker FMBLEST32 were linked to finger blast disease at a p value of 0.007 and explained, respectively phenotypic variance (R^2) of 10% and 8%. The genomic marker UGEP81 was associated with finger blast at a P value of 0.009 and explained 7.5% of R^2. The QTLs for neck blast were associated with the genomic SSR marker UGEP18 at a *p* value of 0.01, which explained 11% of R^2. The resistant alleles were present mostly in the exotic genotypes. Saha et al. (2017) characterized genetic polymorphism among and between 45 resistant and 22 susceptible finger millet genotypes. A total of 154 sequence-related amplified polymorphism (SRAP) and 33 SSR markers were utilized to assess genetic variations and the selection of diverse parents. Twelve SRAP and SSR primers produced 95.1 and 93.1% polymorphic bands. Finger millet accessions, IE 4709 (blast resistant) and INDAF 7(susceptible) were distinguished as the most diverse genotypes as parents. The study reported a high genetic diversity within the resistant and susceptible genotypes using Nei's gene diversity (*h*) index and AMOVA. The finding helps us understand the extent of genetic polymorphism between blast disease resistant and susceptible finger millet genotypes to exploit in resistance breeding programs.

However, finger millet needs to focus on large-scale development of genomic information for efficient breeding and saturation of high-density genetic linkage map. The application of next-generation sequencing (NGS) technologies made remarkable advances in genome sequencing non-model organisms, which provides ultra-throughput sequences to revolutionize plant genotyping and breeding. The advent of markers assisted breeding (MAB) increases the efficiency and speed of selection. Few markers were reported based on comparative analyses revealing colinearity between the finger millet and rice gene spaces/regions (Dida et al. 2007c), characterization of resistant gene analogs (RGAs) encoding NBS-LRR proteins for blast resistance (Panwaret al. 2011, Reddy et al. 2011 and Kalyana Babu et al. 2014a, b, c, d, e). Only a few genomic studies based on genotyping by sequencing (GBS) approach were reported in the generation of genomic resources (Kumar et al. 2016a, b, c and Gimode et al. 2016). Limited information on molecular markers linked to blast resistance was reported but need extensive validation for applied use in breeding via marker-assisted selection (MAS). However, with the

advancement of NGS technologies, it has become more rapid and cost-effective in characterizing huge samples (Edwards et al. 2013), which is currently getting used in several ongoing finger millet research projects.

5.2.3 Linkage Mapping Studies in Finger Millet

Dida et al. (2007a, b, c) constructed a genetic map of the tetraploid finger millet genome by using RFLP, AFLP, EST, and SSR markers. The map spans 721 cM on the A genome and 787 cM on the B genome and covers all 18 finger millet chromosomes (at least partially). A total of 18 major linkage groups with seven or more markers were formed (with a high threshold of LOD 11). Dida et al. (2007c) studied the comparative genomic analysis of finger millet and rice genomes through molecular markers and observed 85% synteny.

5.3 Trait Mapping Efforts in Finger Millet

Molecular markers are important tools employed for identifying and improving target traits in the crop breeding scheme for varietal development. The molecular marker applications include QTL mapping, association mapping, and transcriptomics studies; a few of these were reported for finger millet improvement. These studies targeted identifying markers for agronomically important traits such as grain yield, blast resistance, drought resistance, and finger millet nutrition. Details of these studies in finger millet are elaborated in Table 5.2.

Kalyana Babu et al. (2014a) identified 46 genomic SSR markers for four agro-morphological traits such as basal tiller number, days to 50% flowering, flag leaf blade width, and plant height in a set of 190 finger millet genotypes. Another group (Sharma et al. 2018) evaluated 14 agro-morphological traits viz., days to 50% flowering, days to maturity, basal tiller number, plant height (cm), culm thickness (cm), flag leaf blade length (cm), flag leaf blade width (cm), peduncle length (cm), ear length (cm), ear width (cm), length of the longest finger (cm), the width of the longest finger (cm), fingers number per ear and grain yield (g/plot) in a set of 113 diverse finger millet accessions. This study extensively used different genetic models viz., single locus single trait (SLST), multi-locus mixed model (MLMM), and multi-trait mixed model (MTMM) for detecting marker-trait associations. The SLST model identified 20 marker-trait associations (MTAs) for five traits at a p value of < 0.01 and < 0.001. Models MLMM identified 36 MTAs and MTMM resulted in 53 MTAs. Out of a total of 109 associations, nine MTAs were common in all three mapping approaches (SLST, MLMM, and MTMM). Due to the unavailability of finger millet reference genome, cross-species validation of SNP markers was done to see sequence similarity using Basic Local Alignment Search Tool (BLAST) with rice, wheat, maize, foxtail millet, sorghum, and switchgrass. Among the nine SNPs identified, five SNP sequences had homology to candidate genes of rice and foxtail millet. These genes play an important role in flowering, maturity, and grain yield.

In another study, two QTLs (OM5 and FM8) for tryptophan content, and one QTL (FMO2-EST1) for protein content were identified in 190 genotypes of finger millet using 120 SSR markers. These QTLs were linked to opaque2 modifiers (*Opm*) gene (Kalyana Babu et al. 2014b). Seven QTLs were reported in 128 finger millet genotypes with 87 genomic SSR markers for seven agronomic traits; productive tillers, seed yield, leaf blast resistance, and the number of tillers (Ramakrishnan et al. 2016a, b). Four QTLs (*qLRDW.1, qLRDW.2, qHSDW.1,* and *qHRL.1*) associated with root dry weight, shoot dry weight, and root length, respectively were identified in finger millet by association mapping under P-deficient and P-sufficient conditions. In the seedling stage, shoot and root growths were severely affected by P-deficiency. Hence, the P-deficiency tolerance in the seedling stage is an essential trait that needs to be used in finger millet cultivars (Ramakrishnan et al. 2017). A research by Rahman et al. (2014) on salinity

Table 5.2 Mapping studies for different trait components in finger millet

Sl. No	References	Trait	Trait component (s)	Marker system used	Method	Chromosome/Map length	No of QTLs	PVE (%)	Genes
1	Kalyana Babu et al. (2014a)	Blast	Finger blast	104 Genic and genomic SSRs	Association Mapping	2A(72 cM), 6B (20 cM), 3B (115.3 cM)	4 QTLs	RM262(10%) FMBLEST32 (8%), UGEP81 (7.5%) UGEP24 (8%) and UGEP53 (10.5%)	Pi-d(t), Pi5 blast genes of rice
			Neck blast			1B(70 cM)	1 QTLs	UGEP18 (11%)	–
			Both			–	3 QTLs	–	–
			Leaf blast			4B(7.0 cM), 6B (3.5 cM), 4B (6.0 cM)		FMBLEST35 (10%), RM23842(11%), FMBLEST15(8%)	Pi21, NBS-LRR
2	Ramakrishnan et al. (2016a, b)	Blast	Leaf blast	87 Genomic SSR	Association Mapping		2 QTLS	UGEP101(21.05%) and UGEP95(8.95%)	
		Agronomic traits	Number of tillers				3 QTLs	UGEP98 (9.97%),	
			Root length				2 QTLs	UGEP9(8.12%) and UGEP57(6.28%)	
			Seed yield				1 QTLs	UGEP9 (7.57%)	
			Plant height					UGEP50	
3	Kalyana Babu et al. (2014b)	Agronomic traits	Basal tiller number	46 genomic SSRs	Association mapping	3B(65.2 cM)		UGEP81(10.8%)	
			Days to 50% flowering			3B(4.8 cM), 4B (23.3 cM), 6B (65.2 cM)		UGEP77 (10%) and UGEP90 (8.7%)	
			Flag leaf width and plant height					FM9 (14.1%) and (11.2%)	

(continued)

Table 5.2 (continued)

Sl. No	References	Trait	Trait component (s)	Marker system used	Method	Chromosome/Map length	No of QTLs	PVE (%)	Genes
4	Kumar et al. (2015)	Ca content		23 anchored SSR markers			9 QTLs	M16(41%), M36(19.1%), M6(18.7%), M65(13.4%), M26(12.6%), M2(10.6%)	Calcium exchangers, calcium channels, calcium ATPase and calcium sensors like calmodulin of cereals (finger millet, rice, maize, wheat, and barley)
5	Kalyana Babu et al. (2014c)	Tryptophan content		opaque2 modifiers specific	Association mapping			OM5(9%) and FM8(11%)	27-kDac-zein gene of opaque2 modifiers of maize
		Protein content		EST-SSRs				FMO2EST1 (9%)	RISBZ1 gene of rice
6	Mirza et al. (2014)	Calcium content			Gene expression			CAX1, TPC1, ATPase, CaM, CaMK1, CaMK2, and 14–3-3 genes are involved in calcium sequestrations in root, stem, and leaf at different reproductive stages	Calcium exchangers and calcium sensor gene family
7	Bharathi (2011)	Agronomic traits	Basal tiller number	SSRs		6B(2.9 cM), 5Ab (25.9 cM),		UGEP81 (10.4%), UGEP1 (10.8%),	
			Flag leaf blade length			3A(75.8 cM), 3B (64 cM)		UGEP31(4.3–95.2%)	
			Finger number			3B(65.2 cM)		UGEP8(3.7%)	
8	Dida et al. (2007a, b, c)	Inter-subspecific cross		RFLP, AFLP, EST, SSR		131 markers covering 721 cM on the A genome and 196 markers covering 787 cM on the B genome			

(continued)

Table 5.2 (continued)

Sl. No	References	Trait	Trait component (s)	Marker system used	Method	Chromosome/Map length	No of QTLs	PVE (%)	Genes
9	Yadav et al. (2017)	Grain calcium content		85 genic and non-genic SSR	Association analysis	1B	1 QTL	UGEP78(6.4%) and UGEP60(13.8%)	
10	Ramakrishnan et al. (2017)	Root dry weight under P-deficiency level		SSR and QTL			qLRDW.1, qLRDW.2	UGEP19, UGEP68	
		shoot dry weight under P-sufficient level					qHSDW.1	UGEP13	
		root length under P-sufficient level					qHRL.1	UGEP90	
11	Sharma et al. (2018)	Agronomic traits	Days to 50% flowering	SNPs	Association mapping	3 SNPs			
			Days to maturity			7 SNPs			
			Ear length (cm)			1 SNP			
			Flag leaf blade width (cm)			1 SNP			
			Grain yield			8 SNPs			
12	Tiwari et al. (2019)		Seed coat protein	SNPs	Association mapping (GAPIT)	12 SNPs			
			Days to maturity			12 SNPs			
			Grain yield			11 SNPs			

responsiveness in finger millet using transcriptome analysis is also reported.

The behavior of transcription factors *Dof1* and *Dof2* was analyzed to investigate the high nitrogen utilization efficiency (NUE) in finger millet. The EcDof1/EcDof2 ratio in the roots of a high-protein variety was greater than that of a low-protein variety. This indicated a higher activation of N uptake and assimilation genes (Gupta et al. 2014). The authors suggest that this ratio may in the future be utilized to screen other genotypes for high NUE (Ceasare et al. 2018).

The analysis of key genes involved in N transport in finger millet was reported in a few studies. In two consecutive studies, Gupta et al. (2011, 2013) analyzed the expression profile of key genes, including – (i) *Eleusine coracana high-affinity nitrate transporter* (*EcHNRT2*); (ii) *Ec low-affinity nitrate transporter* (*EcLNRT1*); (iii) *Ec nitrate reductase* (*EcNADH-NR*); (iv) *Ec glutamine synthetase* (EcGS); (v) *Ec glutamine oxoglutarate aminotransferase* (*EcFd-GOGAT*); and (vi) *Ec DNA binding with one finger 1* (*EcDof1*), involved in N uptake and assimilation were analyzed in two genotypes with contrasting (GE-1437, low-protein, and GE-3885, high-protein) grain protein content. Pudake et al. (2017) analyzed the expression of three genotypes (RagiKorchara, Khairna, and VHC 3611) of finger millet accessions and identified four phosphate transporter 1 (*EcPT1* to *EcPT4*) genes. A drought response regulatory gene of finger millet, *TBP Associated Factor6* (*EcTAF6*), was identified by screening the cDNA library of finger millet, and its expression in response to various stresses was analyzed in finger millet genotype GPU-28 (Parvathi and Nataraja 2017). In a salinity tolerance expression study (in leaf tissues) involving two contrasting finger millet genotypes viz., Co-12 (susceptible) and Trichy 1 (tolerant), Rahman et al. (2014) identified several salinity stress-responsive genes using RNAseq approach.

A total of 138 finger millet accessions were evaluated (Lule et al. 2018) for 10 agronomic traits, viz., days to 50% heading, days to maturity, productive tiller number, plant height (cm), finger length (cm), finger number per ear, ear weight (g), number of grains per spike, thousand-grain weight (g) and grain yield per plant (g) followed by genetic variation, population structure, and association mapping. This study used genome-wide association study (GWAS) approach with a mixed linear model and reported a significant ($p < 0.01$) association of 16 alleles of 13 markers for six agronomic traits, viz., days to maturity, finger number, grain yield per plant, grain number per spikelet, productive tiller number and thousand-grain weight. In two cases, different alleles of the same marker were found to be associated with similar traits; such as grain yield per plant and thousand-grain weight (Lule et al. 2018). A study by Tiwari et al. (2019) reported association mapping results on seed protein content, days to maturity, and grain yield in 113 diverse finger millet genotypes with 2,977 SNPs. The study used the TASSEL pipeline with the GLM model and identified 7, 10, and 9 MTAs, respectively, for seed protein content, days to maturity, and grain yield. The MLM model identified 4, 9, and 7 MTAs. Among GLM and MLM, 4, 7, and 6 MTAs were common for seed protein content, days to maturity, and grain yield, respectively. Further, this study by Tiwari et al. (2019) also used GAPIT pipeline, reported 12 MTAs each for seed coat protein and days to maturity; and 11 MTAs for grain yield. BLAST results for common SNPs for seed coat protein resulted in five different genes with significant identity in plants such as *Arabidopsis*, *Oryza, Setaria*, *Beta vulgaris*.

5.3.1 First-Generation Genetic Maps

To study the inheritance of qualitative and quantitative traits, developing markers for molecular breeding and comparative genomic studies constructing genetic maps is a primary requirement. A densely populated map provides a better choice in the quality of markers, increasing the probability of polymorphic markers (Mace et al. 2009).

The first two attempts to construct genetic maps were across genomes of tomato and potato and three diploid genomes of hexaploid wheat

that did not give useful information. Genetic maps have been created in related genomes using RFLP probes, maize probes to map sorghum, and wheat probes to map rye (Gale and Devos 1998). Shortly, a consensus grass map was reported between maize, rice, and wheat along with diploid oat. There have been reports of consensus map of foxtail millet with rice (Devos et al. 1993); complex polyploid sugarcane mapped alongside maize and sorghum (Grivet et al. 1994, Dufour 1996). Further, maps using RFLP markers constructed on pearl millet (Liu et al. 1994), finger millet (Dida et al. 2007a, b, c), and ryegrass (Armstead et al. 2004) were studied.

Finger millet is an important cereal in East Africa and Southern India, serving as a food security crop because of its high nutritional value and excellent storage qualities. It is a tetraploid with genome composition AABB and a basic chromosome number of 9 ($2n = 4x = 36$), and with genome size of 1593 Mb (Ceasare et al. 2018), relatively large compared to other crop species. Breeding has always been difficult in finger millet due to its high self-pollinating nature and small flower size. The development of new hybrid varieties was possible due to contact pollination and hot water emasculation. The genome size of cultivated finger millet (ML-365) was estimated to be 1453 Mb (Pandian and Ramesh 2019). Okhale-1 was used to generate a mapping population of 151 F_{2s}. Dida et al. (2007a, b, c) used RFLP, AFLP, EST, and SSR markers to generate the first genetic map of finger millet. Di- and tri-nucleotide SSRs were extracted from random genomic *Hind*III, *Pst*I, and *Sal*I libraries of finger millet accession PI 321,125 following hybridization of 18,432 double-gridded colonies with mixtures of nucleotides (Sood et al. 2016).

Because of low variations in finger millet, a cross between *E. coracana* subsp. *africana*a ccession MD-20 and *E. coracana* subsp. *coracana* cv. was developed. Linkage groups carrying a minimum of two duplicated loci were used to identify homoeologous chromosome segments. Hybridization patterns of probes Okhale-1, MD20, and *Eleusine indica* acc. MD-36 were compared to assign linkage groups to the A and B genome. All homologous and heterologous markers (RFLP, AFLP, EST, and SSRs) were utilized in the analysis, and a genetic map consisting of 22 linkage groups arranged in nine homoeologous groups and with four unknown alignment. The variation between subsp. *coracana* and *africana* were limited to only one genome for RFLP probe combinations. Seventy-five percent of probes assessed with one restriction enzyme showed variation between subsp. *coracana* and *africana* in one genome or the other. All nine homology groups have A- and B-genome linkage groups corresponding to the 18 finger millet chromosomes. The structure of homoeologous A- and B-genome chromosomes were highly similar, but chromosomes 3A and 3B were inconsistent. The presence of loci Xlfo112.1 and Xlfo112.2 in different genome locations suggests rearrangement in 3A compared to 3B, which could be due to the large chromosome segment. The marker distribution was even across all the maps except for a few that corresponded to low recombination. The A genome is 721 cM in 16 linkage groups, whereas the B genome had 787 cM in 9 linkage groups. Further, 45 SSR markers were used to study the genotypic variation in 79 accessions (*E. coracana* subsp. *coracana*) from Africa and Asia. Diversity analysis and population structure revealed *E. coracana* germplasm to have formed three distinct subpopulations. This confirmed the origin of subsp. *Africana* and subsp. *coracana* originating from Africa and subsp. *coracana* originating from Asia. Intercrossing showed a mix of African and Asian germplasm, and this was confirmed by phenotypic evaluation, where significant differences in plant architecture and yield were recorded. The diversity in the Asian subpopulation was low, confirming its ancestry from a smaller number of plants (Dida et al. 2007a, b, c). Another report by Arya et al. (2013) revealed three major clusters by the UPGMA method of diversity analysis of 67 diverse finger millet accessions of African and Indian origin. Diversity analysis from STRUCTURE showed five genetically distinct subpopulations. A relationship between rice and finger millet was studied for a better understanding of the linkage

group and breeding applications. Six of the nine-finger millet homoeologous groups corresponded to a single rice chromosome. The remaining three groups were orthologous to two rice chromosomes. Gene orders between rice and finger millet are limited largely to single marker transpositions and small putative inversions encompassing at most three markers (Dida et al. 2007c).

Synteny study of finger millet with other Poaceae species (rice, foxtail millet, sorghum, maize, and Brachypodium) identified 10,291 core orthologous groups (COGs) gene families shared across the species. The COGs gene families consisted of 35.22% of rice, 28.66% of finger millet, 47.37% of sorghum, 43.33% of maize, 41.05% of foxtail millet and 57.65% of Brachypodium genes. Phylogeny analysis using single-copy orthologous gene (766 genes across six species) among six species revealed that finger millet is closer to rice followed by foxtail millet. Highly conserved genomic regions were observed in intergenomic collinear analyses between finger millet, Brachypodium, foxtail millet, sorghum, maize, and rice, signifying a close evolutionary relationship among these grass species. Collinearity block studies revealed 98% similarity between finger millet and foxtail millet followed by rice of 97%, 96% with maize, and 95% with sorghum (Hittalmani et al. 2017).

The recent advancement in the NGS technologies has provided opportunities to generate extensive genomics resources, including the development of genome assemblies for many of the 'orphan' crop species, including finger millet. The availability of whole genome sequencing of ML-365 and PR-202 will further support constructing a linkage map followed by identifying genes for important traits (Ceasare et al. 2018). The genetic map of finger millet proved to be an important tool for genetic analysis and crop improvement using marker-assisted breeding.

5.3.2 Comparative Genetic Maps

With limited genome information available for finger millet, comparative genomics plays an important role in identifying the genes involved in the trait. Kalyana Babu et al. (2014a, b, c, d, e) evaluated 58 genic SSRs on a population of 190 finger millet genotypes from Africa, South Asia, and Germany. The AMOVA estimates and F_{ST} estimates informed that the finger millet genotypes were grouped according to their blast disease response. AMOVA showed significant differences among various genotypes evaluated with a greater variance (72%) by individuals within a population, while between the populations, it was less (28%). The phylogenetic analysis using a panel of 104 SSR markers revealed four population groups with ΔK value K = 4, while the principal component analysis (PCA) resulted in grouping into three clusters. Comparative genomic analysis with rice for genetic diversity, population structure, and association mapping was studied to identify SSRs/QTLs for blast resistance. Association mapping by GLM model with 104 SSRs resulted in identifying five MTAs for blast resistance (four for finger blast and one for neck blast). However, seven markers were detected with the leaf, neck, and finger blast by the MLM approach. The three markers RM262, FMBLEST32, and UGEP18 were linked to blast disease by GLM and MLM approaches. Association mapping revealed that finger millet's 2nd and 6th chromosomes are the major hub of finger blast and neck blast-resistant genes. Indian genotypes VHC3997, VHC3996, and VHC3930 are highly resistant, which can be further used as parents for developing blast-resistant cultivars. The identified QTLs will further lead to fine mapping, gene cloning, and marker-assisted breeding of finger millet.

Multi omics study of the iron and zinc homeostasis was studied in 179 metal homeostasis candidate genes were retrieved from the NCBI database in finger millet and cereals; rice (*Oryza sativa*), wheat (*Triticum aestivum*), maize (*Zea mays*), barley (*Hordeum vulgare*), and foxtail millet (*Setaria italica*). A total of 65 genes from *O. sativa* and 32 genes from *S. italica* had homology with finger millet transcriptome. Transcriptome-wide expression analysis showed genes involved in uptake and translocation of

iron and zinc in the GP-1 genotype, while those involved in the bioavailability of iron and zinc are expressed more in the GP-45 genotype of the finger millet (Chandra et al. 2020).

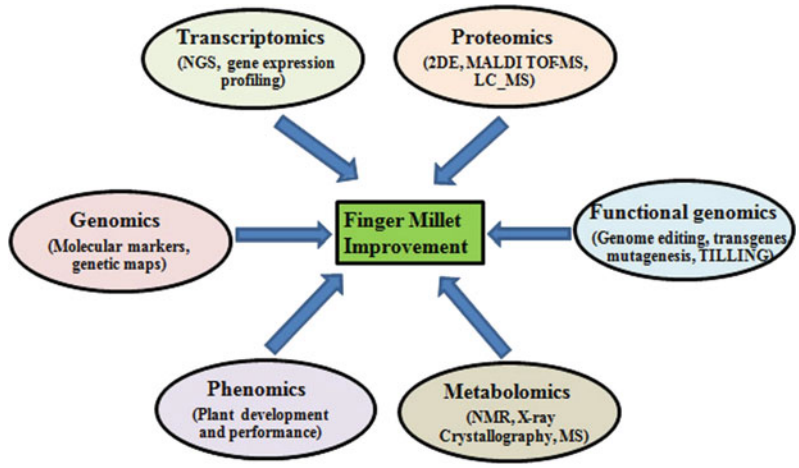
Extensive conservation of gene content and gene order in wheat, maize, and rice, and other grass species over the 60 million years of radiation of Poaceae using common DNA probes was reported. The highly conversed 25 rice linkage blocks account for linear organization of genes in some nine different genomes differing in basic chromosome number from 5 to 12 and nuclear DNA amount from 400 to 6,000 Mb. The confounding of the extent at the micro-level by gene duplication and micro-rearrangements in intergenomic colinearity is a still open question. The sequence analysis of smaller genomes such as rice (with 400 Mb genome) will help predict the organization of the larger genomes in grass family such as maize (4500 Mg genome). In the same context, as the genomics and trait information in finger millet (with larger genome of 1.9 Gbp) will leverage on the characterization of other grass species such as rice and sorghum for further genome characterization. The process of the gene characterization can further be fast-tracked by extending the studies from Arabidopsis on the basic biology research on the candidate gene/pathway. These comparative genetics approaches will provide the key to unlock the genomic secrets of crop plants with bigger genomes.

The linking of genes between genomes is still sparsely understood to find the candidate homoeologous gene(s). The time is fast approaching when the grasses, including all of the major cereals, can be considered a single entity. All of the information available accumulated over the past century in the different species can be pooled. Data on gene structure, gene action, metabolism, physiology, and phenotype for all the cereals/grasses should be pooled and reached to breeders for exploitable variation. Conserved genome relationships have been developed in legumes and crucifiers, including *Brassica* species. Synteny studies describe the independent evolution of dicots and monocots, defining the conserved regions. The discovery of these conserved regions has led to several genomic studies in major cereals. The genomes of few staple crops like wheat and maize are larger than the human genome but there has been a breakthrough in sequencing and genomics. Arabidopsis and rice genomic sequences have been used in mapping, identification of QTLs with a further focus on omic studies (Gale and Devos 1998).

5.4 Future Scope of Work

Finger millet is an important crop in the semi-arid tropics, coming up as a potential solution for malnutrition worldwide and is gaining more importance with the availability of genomic resources. Finger millet is highly tolerant to abiotic stresses and can be used as a model to explore other complex traits, including genomics approaches. The current conventional breeding approaches are not sufficient to fully utilize the potential of the crop. Therefore, molecular biology and biotechnology tools can be used to efficiently improve the produce and better tolerance to biotic/abiotic stresses. Integration of various advanced high throughput omics strategies will revolutionize finger millet research with the large-scale identification of stress-responsive genes/proteins/metabolites and potentially used for crop improvement. Both secondary and tertiary gene pools will be of great value as sources of novel genes and for broadening the genetic base of the crop.

With the availability of a draft whole genome sequence, finger millet research will need to focus on characterizing important traits to develop an integrated finger millet breeding scheme, providing anchor points for identifying functional variants of target traits identified through several *omics* approaches for the efficient release of superior varieties.



Dedication This book chapter is dedicated to memories of Dr Vijay Kumar KV (the first author), who lost his life to the battle of COVID-19 infection, toward the end of this draft manuscript preparation.

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The Complete Genome Sequence of Finger Millet

6

H. B. Mahesh, K. G. Manasa, N. R. Raghavendra, Meghana Deepak Shirke, and Shailaja Hittalmani

Abstract

Finger millet (*Eleusine coracana* subsp. *coracana*) is a cereal food crop for millions of resource-poor people of Africa and Indian subcontinent. It is climate-resilient nutri-cereal which survives under drought and marginal fertile soil. Owing to its ability to grow under diverse agroclimatic conditions, it is a model crop to study the genomic factors responsible for tolerance to abiotic stresses and the same knowledge can be transferred to improve other cereal crops. Finger millet can give nutritional security since it is rich in iron, methionine, and calcium. It is a recommended diet for diabetic patients because of its low glycemic index, high fiber content and lowers cholesterol level. In spite of this, genomic resources developed for this crop were very meager. Fortunately, two genomes of finger millet varieties namely ML365 and PR202 have been sequenced and made available to the scientific community. These genome sequencing efforts will strengthen millet scientists for allele discovery, development of DNA markers, genetic mapping, and identification of candidate genes for disease resistance, drought tolerance and other agronomically important traits, which will boost the finger millet production in near future.

6.1 Introduction

Finger millet (*Eleusine coracana* subsp. *coracana*) belongs to the family Poaceae and is one of the neglected and underutilized cereal crops. The grain is nutritious but can be further improved into a nutritious "super cereal" that alleviates malnutrition, especially in women and pre-school children in most countries of southeast Asia and Africa. Finger millet is a more nutritious food grain crop than other cereals (Bhandari et al. 2004; Dida and Devos 2006) in terms of protein (7.3g/100 g) (Malleshi and Klopfenstein 1998), dietary fiber (15–20%) (Chethan and Malleshi 2007) and calcium content (344 mg/100 g) (Gopalan et al. 1971). Furthermore, finger millet is cultivated in semi-arid areas. There are predictions indicating its production will be adversely affected by climate change and it will become a risk to livelihoods of

H. B. Mahesh (✉) · K. G. Manasa · N. R. Raghavendra
Department of Genetics and Plant Breeding, College of Agriculture, Mandya, University of Agricultural Sciences, Bangalore 560065, India
e-mail: maheshhbg@gmail.com

M. D. Shirke
Department of Biotechnology, KTHM College, Nashik 422002, India

S. Hittalmani
Department of Genetics and Plant Breeding, College of Agriculture, GKVK, University of Agricultural Sciences, Bengaluru 560065, India

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millions of people who are depending on this crop. Hence, development of climate-smart finger millet varieties through innovative technologies is very essential to sustain finger millet cultivation. Initially, finger millet improvement was carried out by conventional breeding methodologies. Such breeding efforts across the globe have led to release of many varieties possessing improved yield and other desirable traits. However, the speed of crop improvement strategy in this crop is very slow as compared to other major cereal crops. This arduous nature could be attributed to slow release of variability, due to polyploidy and difficulty in hybridization owing to small florets in finger millet. The polyploid nature of the crop demands greater shuffling of genes for reaching higher genetic potential. However, these bottlenecks could be delimited with the availability of versatile genetic and genomic resources.

6.2 Complexity of Finger Millet Genome

The cultivated Finger millet (*Eleusine coracana* subsp. *coracana*) is an allotetraploid with "AABB" genomes with estimated genome size of ~1.5 billion bases (Dida et al. 2007; Hiremath and Salimath 1991; Mysore and Baird 1997). The *E. coracana* ($2n = 4x = 36$) exhibits morphological similarity to both *E. indica* ($2n = 18$) and *E. africana* ($2n = 36$). Based on the cytological, biochemical and molecular evidences *E. indica* has been "AA" genome progenitor of cultivated *E. coracana* and *E. africana* (Hilu 1988, 1995; Mallikharjun et al. 2005; Werth et al. 1994) species. Both the tetraploid species of finger millet viz., *E. africana* and *E. coracana* are genetically related and had a greater advantage of gene flow between them, which is indicating the origin of *E. coracana* from *E. africana* through selections and with mutations toward larger grain type (Chennaveeraiah and Hiremath 1974; Hilu and Wet 1976). Further, the detailed study of diploid (*E. indica* and *E. floccifolia*) species with ribosomal DNA (rDNA) in comparison with tetraploid species (*E. africana*) suggested that, the two diploid species might be the donors of two genomes to *E. africana* (Bisht and Mukai 2001, 2000). But the possibility of *E. floccifolia* to be as B genome donor was disproved based on nuclear internal transcribed spacers (ITS) and plasmid *trn*T–*trn*F sequences (Neves et al. 2005). Kinship was estimated between *E. indica* and *E. tristachya*, and between *E. floccifolia* and *E. jaegeri* based on biochemical and genetic evidences between them (Hilu and Johnson 1992; Hiremath and Chennaveeraiah 1982; Hiremath and Salimath 1991; Liu et al. 2011). Genetic characterization of *E. africana*, *E. coracana* and *E. kigeziensis*, based on plastid phylogeny indicated the presence of a common ancestor with *E. indica—E. tristachya* clade, which represents a source for the maternal parents. However, with the recent whole genome and transcriptome sequencing of *Eleusine* species confirmed that *E. indica* is the maternal parent of *Eleusine coracana* and *Eleusine africana* (Zhang et al. 2019a, b).The exploration studies in defining the source of paternal parent (contributor of BB genome) of three tetraploids indicated the extinction of the actual/probable donor of BB genome and they are not available in any of the germplasm resources available across the globe (Liu et al. 2014).

One more complexity of finger millet genome is transposable elements. Though transposable elements are the major components of eukaryotic genomes but their integration in the genome plays a vital role in genome evolution and duplication. Based on ML365 genome study (Hittalmani et al. 2017), ~49.92% of finger millet genome is repetitive comprising of various retroelements (35.56%), unclassified repeats (9.73%) and DNA transposons (4.48%). Previous study prior to whole genome publication also indicated the abundance of repeats in finger millet genome based on DNA reassociation kinetics (Gupta and Ranjekar 1981). This repetitive nature of the finger millet genome is attributed to larger lengths of interspersed DNA repeats as reported in pearl millet (Gupta and Ranjekar 1981; Wimpee and Rawson 1979). This was one of the major problems in assembling genome to chromosome/pseudomolecule level.

Next-generation sequencing (NGS) technology particularly second-generation (Illumina and Ion Proton sequencers) can provide genome sequence in a short period of time (weeks to months) with reduced cost per genome. Although, assembly and annotation are technically challenging due to short read nature of second-generation sequencing. Many crop plants with polyploidy genomes with high repetitive DNA have seen a thrust in their crop improvement programs after the inception of genomic studies providing reference genome assemblies. The later developments in third-generation sequencers like Oxford Nanopore and Pacific Biosystems SMRT sequencing have immensely helped to resolve the repetitive DNA structures of several genomes including finger millet. One such effort was attempted in finger millet by Hatakeyama and coworkers to develop a better assembly of PR202 variety using diverse sequencing technologies. They have used novel multiple hybrid genome assembly workflow coupled with whole genome optical mapping using the BionanoIrys system (Hatakeyama et al. 2018).

Taking into consideration of the facts of genome sequencing and its challenges, attempt in sequencing finger millet genome has been done by two individual groups (Hittalmani et al. 2017; Hatakeyama et al. 2018), which was a leap in developing genomic resources for finger millet. Further, very recent reports of high throughput genotyping and marker trait studies by several researchers will make finger millet a resource-rich crop for further genomics-assisted breeding programs. The current chapter focuses on discussing the available genome assemblies of finger millet.

6.3 ML365 and PR202 Genomes

Recent advances made in NGS technologies facilitated whole genome sequencing of many orphan crops and largescale sequence-based genotyping. Much awaited whole genome sequence of ML365, a drought-tolerant finger millet variety was released in 2017 (Hittalmani et al. 2017), followed by whole genome hybrid assembly release for PR202 variety (Hatakeyama et al. 2018).

ML365 is developed through recombination breeding of Indaf-5 × IE1012 and released in 2008 as a short duration, drought-tolerant and neck blast resistant variety (Gandhi et al. 2012). The PR202(Godavari) is a pureline selection from a landrace of Mettachodi ragi of Araku valley released in 1976 as a drought tolerant and blast susceptible variety (https://www.dhan.org/smallmillets/docs/report/Compendium_of_Released_Varieties_in_Small_millets.pdf), where as PR202 is also used as national check by All India Co-ordinated Research Project (AICRP) on Small Millets for multilocation yield-evaluation trials.

Comparative studies of these genomes is a large-scale approach to understand the similarities and difference of the crop at genome level in multiple perspectives. The important highlights of comparative studies are enumerated in this chapter.

6.3.1 Sequencing Platforms, Data Pre-processing and Assembly

The ML365 whole genome sequencing (WGS) was performed by Illumina and SOLiD sequencing chemistries. Whereas, PR202 genome was sequenced by combination of both second and third generation sequencing technologies like Illumina and PacBio, respectively. In addition, genome optical mapping was carried out for PR202 on a BionanoIrys® system (Bionano Genomics). The WGS of ML365 and PR202 assemblies used both pair-end and mate-pair library preparation workflow. In paired-end sequencing, sequencing will be done from both the 5′ and 3′ ends producing both forward and reverse orientation of sequence reads. Mate pair sequencing involves long insert paired-end libraries, which are later circularized, fragmented and ligated to another set of adapters and sequencing them in pair-end sequencing chemistry. Once the raw sequence is generated from

the sequencer, analysis starts with quality check and pre-processing of raw sequence reads, which are scanned for low base quality, adapter contamination, and duplicate reads. Quality scores measures the probability of incorrect base calling. There are many tools which have been developed for read quality control and pre-processing including FastQC, PRINSEQ, Trimmomatic, Cutadapt, FastX and many more (Table 6.1). Currently, for pre-processing of PR202 raw sequence Trimmomatic was used (Bolger et al. 2014). Trimmomatic is a java based tool that facilitate to remove adapters and trim reads based on quality. FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) is another popular tool used for pre-processing of raw fastq/fasta files of ML365.

6.3.2 Genome Assembly, Scaffolding and Hybrid Assembly

Genome assembly refers to aligning DNA sequence to reconstruct the correct order of original sequence. The reference-based and de novo-based are the two methodologies opted for assembling high-quality sequence reads into contigs. ML365 genome assembly is the first de novo assembly published for finger millet and SOAPdenovo assembler was used for the same, where it is specially designed for Illumina sequences and optimized for large genomes like plant and animal.

Assembling a genome has many computational limits one among them is long tandem repeats, resolving them using short reads is highly difficult which can be accomplished using long reads generated from third-generation sequencing technology, the main challenge of using this technique alone is limitation in accuracy and higher error rates comparatively to short read sequencing. Hybrid assembly is a method that uses data from different sequencing technologies to achieve accuracy. PR202 was assembled using hybrid assembly technique. Platanus is a massive parallel shotgun de novo assembler used for assembling Illumina data and DBG2OLC is a hybrid assembler used for assembling PacBio CLR long reads along with Illumina contigs as anchor points. Pilon is a widely used assembly polish software, used in improving the accuracy of PR202 through an internal reassembly process by correcting sequence errors, mis-assemblies and filling gaps.

Scaffolding is a reconstruction of genome sequence from contigs. SSPACE is a stand-alone scaffolding program used in both ML365 and PR202 assemblies, where contigs were assessed for order, distance and orientation to find a link and combine into scaffolds. Contigs are always of different length, if part of the terminal sequence of one contig fragment overlaps with other reads of contigs it will be combined to make single scaffold and gaps are included as 'N' (any nucleotide base) where varied sequence ends are present, so contigs are continuous genomic sequences whereas scaffold contains contigs and gaps (represented as N). As gaps are very commonly generated through scaffolding process, gap filling is a part of assembly process. The GapCloser and Gmclose tools were used in ML365 and PR202, respectively for closing the gaps. GapCloser is a SOAPdenovo module designed to close the gaps using pair relationship of short reads. Gmcloser uses pre-assembled contigs and measures likelihood ratios to improve accuracy and efficiency. Gmcloser was used on super scaffolds of PR202. Super scaffold of PR202 includes assembly from BNG contigs generated from BioNanoIrys®System through optical mapping. Optical mapping is a comprehensive method to understand the genomic structure and structural variation through genome-wide restriction map, it has been widely used to improve the de novo assemblies of eukaryotic as well as prokaryotic genomes. BioNanoIrys®System was used for optical mapping of PR202. Optical mapping was followed by IrysView for de novo assembly for the same. IrysSolve was used for generating contigs from BNG contigs and hybrid scaffolding using BNG contigs as well as hybrid assembly from DBG2OLC assembler.

Table 6.1 Tools/softwares used in ML365 and PR202 genome assemblies

Tools used	ML365	PR202
Pre-processing of raw reads	Fastx-toolkit	Trimmomatic
Contig assembler	SOAPdenovo2	Platanus
Hybrid assembler	–	DBG2OLC
Assembly polish	–	Pilon
Scaffolding	SSPACE	SSPACE
Optical mapping	–	BionanoIrysView and IrysSolve
Gap filling	GapCloser	Gmclose
Assembly assessment	CEGMA	BUSCO
Annotation	AUGUSTUS	MAKER
Annotation reference species	*Zea mays*	*Oryza sativa*

6.3.3 Comparison of Genome Statistics

Total genome size was estimated in M365 and PR202 finger millet varieties. Propidium iodide was used in staining the nuclei and suspension of stained nuclei were analyzed using a flow cytometer using *Pisum sativum* and *Lycopersicon esculentum* as an internal standard for ML365 and PR202, respectively. The assembly generated for ML365 includes Illumina and SOLiD sequencing technologies with mate pair and pair-end reads. Total assembly generated for ML365 is 1196 Mb covering 82.31% of the estimated genome size, which includes 525,759 scaffolds/contigs with average length of 2.2 Kb representing N50 of 23.73 Kb (Table 6.2). PR202 assembly was generated from combination of short reads from

Table 6.2 Assembly statistics of ML365 and PR202 genomes

Genome statistics	ML365	PR202	
Estimated genome size (Gb)	1.4	1.5	
Sequence coverage of estimated genome size (%)	82.31	78.20	
Internal standard used for flow cytometry	*Pisum sativum*	*Lycopersicon esculentum*	
Staining solution	Propidium iodide	Propidium iodide	
Measurement technique	BD FACS	Flow cytometer	
Hybrid assembly	–	Available	
		Contig	Hybrid scaffold
No. contigs/scaffolds	525,759	2,812,919	1897
Minimum length of contigs/scaffolds (bp)	200	115	1244
Maximum length of contigs/scaffolds (bp)	454,778	27,802	13,553,037
Average length of contigs/scaffolds (bp)	2274.92	464.72	626,665.76
N50 (bp)	23,732	1410	2,683,090
GC content (%)	44.76	43.15	40.98
No. of genes predicted	85,243	62,348	

Illumina with mate pair and pair-end reads and PacBio long reads. Total assembled genome size is 1189 Mb covering 78.20% representing 28,22,919 contigs in 1,897 hybrid scaffolds with average contig length of 464.72 bp and average hybrid scaffold length of 626.66 Kb representing N50 of 1.4 Kb for contig and 2.6 Mb for hybrid scaffold. The highest GC content of land plants have been found in grasses (Poaceae). GC content of monocots varies between 33.6 and 48.9% (Šmarda et al. 2014), finger millet being monocot belonging to Poaceae family, the GC content of the ML365 assembly was 40.98% and GC content of PR202 assembly estimated to be 44.76% (Table 6.2).

6.3.4 Validation of Genome Completeness and Comparison of Gene Annotation

Assessing the completeness of the assembly for its quality, contiguity and correctness is most important. CEGMA (Core Eukaryotic Genes Mapping Approach) was used in ML365. CEGMA uses highly conserved orthologous genes across eukaryotic species. As per the analysis, around 94.35% of core eukaryotic genes (CEG) were present in the ML365 genome. Whereas BUSCO (Benchmarking Universal Single-Copy Orthologs) which assesses the assembly through single-copy ortholog from OrthoDB database was used in PR202. Around 96.5% of the universal single-copy genes were identified indicating that quality of the PR202 genome was good. BUSCO is an improved version of CEGMA. Both the assemblies possessed good quality for further downstream annotation. Annotation refers to identification of relevant features of genome sequence. It is divided into two types structural and functional annotation; structural annotation identifies the gene locations which includes exons, introns, UTRs, etc., whereas functional annotation refers to assigning the function to genes which it encodes like physiological function, cellular function, biochemical and metabolic activity so on. Structural gene annotation of ML365 genome was carried out by AUGUSTUS (tool widely used in eukaryotic gene prediction based on generalized hidden Markov model) with RNA-seq evidence using *Zea mays* as a reference model. This enabled prediction of 85,243 genes in ML365 genes and gene ontology (GO) annotation was done with Viridiplantae protein sequences retrieved from UniProt database. The PR202 hybrid assembly was used for gene prediction using MAKER tool using *Oryza sativa* as a model species and 62,348 genes were identified. RNA-seq data generated from young leaves were mapped to the hybrid assembly of PR202 using STAR aligner showed 95.9% mapping, which strongly supported assembly may contain ~95% predicted gene set through MAKER.

Owing to finger millet's ability to grow under diverse agro-climatic conditions, it is a model crop to study the genomics of drought tolerance as compared to other crops like rice, sorghum, maize, foxtail millet. In ML365, 2,866 drought tolerant genes have been identified based on RNA sequencing experiment followed by gene annotation. In addition, 11,125 genes in ML365 genome are known to harbor transcription factor (TFs) distributed across 56 various families. The most widely distributed TFs are bHLH, MYB, FAR1, WRKY, NAC, MYB related, B3, ERF, bZIP, HD-ZIP, C2H2, C3H, G2- like, TALE, GRAS, ARF, M-type, Trihelix, GATA, WOX, LBD, HSF, MIKC, S1Fa-like, HB other, CPP, and YABBY. This strongly validates the inherent nature of drought tolerance character of the finger millet.

6.3.5 Comparative Analysis of Functional Classification of Proteins

Total proteins predicted from both the assembly were subjected for their functional classification using reverse position specific BLAST (RPS-BLAST) program on KOG (EuKaryotic Orthologous Groups) database with e-value 0.001 to obtain specific hit using WebMGA. The RPS-BLAST searches protein query against the

conserved domain database (CDD) collected from many source databases either in standalone or using web server. Functional domains were classified into three main classes viz.., information storage and processing, cellular process and signaling, and metabolism. There is one more class which is poorly characterized which includes 2 sub class falls on general and unknown function classification, remaining 23 classes out of 25 falls on main class functional groups (Fig. 6.1a). All predicted genes in ML365 (85,243) were subjected to RPS-BLAST to predict the domain, out of them only 36,866 were predicted to have specific hit and found in 3,519 conserved protein domain family (Pfam). In case of PR202, total number of genes predicted is 62,348 and all of them were used in RPS-BLAST. 37,711 were found to have specific hit into 3677 Conserved Protein Domain Family. Comparative Pfam domain analysis between ML365 and PR202 showed that 30,460 ORFs (open reading frames) were common, 7,251 ORFs were unique to PR202 and 6,406 ORFs were unique to ML365 (Fig. 6.1b).

6.3.6 Clustering of Gene Families

Predicted genes of ML365 (85,243) and PR202 (62,348) were clustered using OrthoVenn2 tool. Gene repertoire of these varieties formed a 24,445 core homologous clusters consisting of 75,459 genes (41,906 from ML365 and 33,553 from PR202). The gene ontology (GO) annotation of these core homologous genes showed that majority of genes belonging to response to osmotic stress, oxidative stress and lipid catabolic process (Fig. 6.2a). Remaining 43,337genes of ML365 were unique which formed 3,113 clusters (18,651 genes are 2 copies—paralogs) and 24,686 single-copy genes. Tricarbolic acid cycle, glycolytic process, DNA integration, RNA-mediated transposition and fatty acid beta-oxidation were the major gene ontology annotations of paralogs genes in ML365 (Fig. 6.2b). Similarly, 4,115 paralog clusters comprising of 11,026 genes and 17,769 single-copy genes were identified in PR202 genome. Majority of these paralogs have a gene ontology function in plasma membrane, protein glycosylation response to oxidative stress and osmotic stress (Fig. 6.2c).

6.4 Current Status of Finger Millet Production

Over the last two decades area and production of finger millet is in declining phase due to replacement of finger millet by other competitive crops. Finger millet varieties have been developed mostly through selection or hybridization followed by selection. Attempts have been made in realizing high yield potential using the worldwide genetic resources. The maximum yield potential of 5000–5500 kg/ha have been achieved and is almost reaching stagnation. In three

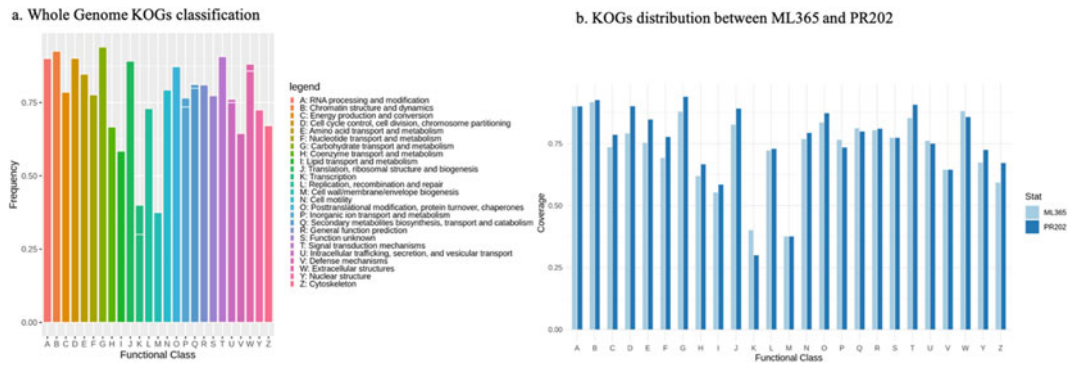

Fig. 6.1 Comparison of functional classification of KOGs (Eukaryotic Orthologous Groups) between ML365 and PR202

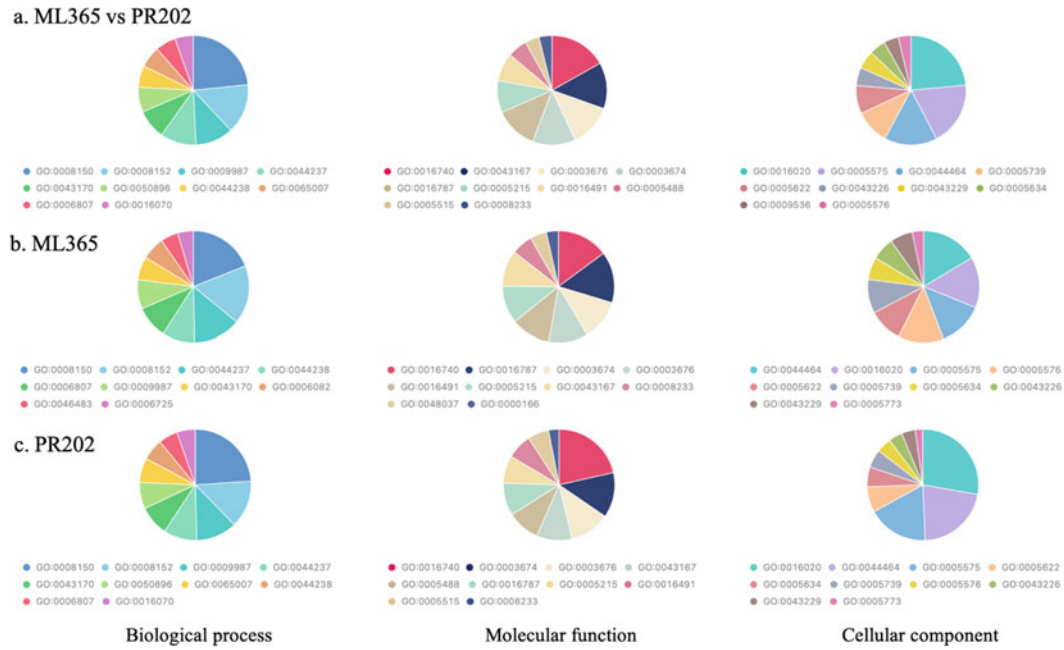

Fig. 6.2 Comparative gene family analysis of ML365 and PR202

decades, finger millet productivity in India has increased considerably and is the highest (1500 kg/ha) among all the millets including sorghum and pearl millet. This is largely due to the development and spread of high yielding and blast-resistant varieties by exploiting African germplasm. But newly developed and currently cultivated varieties are relatively constrained by biotic and abiotic stresses. Therefore, there is an urgent need to enhance tolerance to biotic and abiotic stresses in order to stabilize productivity, extend their adaptation and tailor them to suit changing climate. In the coming years climate change, water scarcity, increasing world population, rising food prices, and other socio-economic impacts are expected to generate a great threat to agriculture and food security worldwide, especially for the poorest people who live in arid and sub-arid regions. The crisis can be challenged by sustainable food production through development of high-yielding cultivars in finger millet. Breeding of finger millet with genetic and genomic studies aided by recent high throughput genotyping platforms may be helpful to develop cultivars/varieties with desired features/traits of interest.

6.5 Production Constraints in Finger Millet

Finger millet production is severely affected by both biotic and abiotic stresses (Saha et al. 2016). Fungal blast is a major disease caused by *Magnaporthe grisea* (anamorph *Pyricularia grisea*), affecting growth and yield of finger millet. The fungus mostly infects young leaf and causes leaf blast, whereas under highly favorable conditions, neck and finger blasts are also formed at flowering (Babu et al. 2013). This disease has been identified as the highest priority constraint to finger millet production in Eastern Africa, and India since most of the genotypes are highly susceptible. The disease affects the crop at all growth stages however, neck blast and finger blast are the most destructive forms of disease.

The blast fungus enters and causes the breakdown of parenchymatous, sclerenchymatous, and vascular tissues of the neck region, thereby inhibiting the flow of nutrients into the grains (Rath and Mishra 1975). Subsequently, grain formation is partially or totally inhibited and the infected spikelets will be shorter than healthy spikelets, which affects the grain formation (Ekwamu 1991; Rath and Mishra 1975). Till now there are no reports on the molecular characterization and mapping of blast resistant genes in finger millet. So, there is a need to identify the molecular markers linked to the blast resistance for their further introgression into locally well-adapted germplasm.

Major abiotic stresses such as deficiencies of nutrients [nitrogen (N), phosphorus (P), and zinc (Zn)], drought, and salinity also seem to affect the growth and yield of finger millet. According to a recent study, N deficiency decreased the tiller number in finger millet (Goron and Raizada 2015). Low P stress also affected the growth and biomass of finger millet seedlings in glass house conditions (Ramakrishnan et al. 2017). Zinc deficiency resulted in stunted growth, delayed seed maturity, appearance of chlorosis, shortened internodes and petioles, and malformed leaves (Yamunarani et al. 2016). Drought is also one of the major abiotic constraints of finger millet production. Drought stress caused wilting and leaf rolling and resulted in the reduction of leaf solute potential and chlorophyll content with the induction of many drought stress-responsive genes when compared to control condition (Parvathi et al. 2013). Salinity also reduced the water content, plant height, leaf expansion, finger length and width, grain weight, and delayed the flowering (Anjaneyulu et al. 2014). In addition to these, the other concerns are poor understanding of the Finger millet genome biology, non-availability of microsatellite markers and single nucleotide polymorphisms (SNPs). Non-availability of an appropriate bi-parental mapping population for traits of interest leading to non-availability of genetic linkage maps, limiting the application of translational genomics and marker-assisted selection (MAS). Similarly, the non-availability of physical maps till date has limited the deployment of genome-wide association study (GWAS) and genomic selection (GS) strategy in crop improvement programs and limited knowledge about sequence diversity between cultivated and wild species in finger millet has stalled the prospective of genomic assisted breeding.

6.6 Future Perspectives

Indian agriculture is always a gambling with monsoon and this uncertainty could be minimized by growing drought-tolerant crops like finger millet and other millet crops. Finger millet is right now occupying an important position as a 'Nutri-cereal' rather than as a coarse cereal due to its potential use in combating malnutrition and hidden hunger worldwide. Finger millet is rich in iron, methionine, and calcium and can give a nutritional security to mitigate malnourishment in the country. Finger millet grain contains ten times more calcium than any other cereals, such calcium transport and accumulation-related genes have been identified through ML365 genome sequencing study. With profound nutritional significance now a day's finger millet is popularizing as a trendy food among diet-conscious people to maintain healthy lifestyle and to prevent lifestyle disorders, chronic and non-communicable diseases. Finger millet is a recommended food for diabetic patients because of its low glycemic index (slow releasing of sugar to the blood), high fiber and cholesterol-lowering ability. These inherent properties make finger millet an ideal model for studying genomics and a plausible source for gene mining for complex traits. Molecular breeding has witnessed its importance as a promising tool for imparting stress tolerance in economically important plants, however, until now the progress is limited in finger millet mainly due to lack of appropriate genomic resources. High throughput sequencing platforms have enabled to generate more of genomic resources is less possible time in neglected/overlooked crops like finger millet. The recent release of draft genomes may aid to develop high-resolution studies, namely, forward

and reverse genetics, functional genomics, and proteomics studies in finger millet. With the availability of the high-quality genome sequence of *E. coracana*, it will be possible to identify new targets of selection and its use in genomic selection coupled with prediction approaches. In addition, these advances will not only enable us to overcome the challenges of understanding large and complex finger millet genome but will also help to understand the regulation of genes at transcriptional, post-transcriptional, epigenetic level. This will speed up the breeding process and will allow cumulative improvement for yield, disease resistance and nutritional quality. Development of 'ideotype breeding' in finger millet may also be possible in the future by incorporating various agronomically important traits into the genome of a single finger millet genotype/cultivar. Thus, utilization of current advances in molecular breeding and with advanced well-defined genome assemblies will have an impact in improving the present scenario of research in finger millet. The new genomic resource is expected to enrich the finger millet research in many domains including dissection of key traits involved in nutrient enrichment and drought tolerance using GWAS, genetic diversity analysis based on SNP and functional genomics studies. Overall, the recently released WGS of finger millet is expected to augment the finger millet research for its breeding and improvement.

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Comparative Genomics of Finger Millet

7

B. Kalyana Babu, Salej Sood,
Vikram Singh Gaur, and Anil Kumar

Abstract

High synteny existing among the closely related genomes of cereals with millets paved the way for comparative genomics approaches in unraveling the genetic mechanisms and identification of orthologs and paralogs in the related species like finger millet. Recent publication of the finger millet draft genome helped in comparative genomics studies aiming at bridging the yield gaps in finger millet cultivation by finding the genes of important economic traits of interest. The present chapter describes the comparative genomic approaches for biotic and abiotic stress resistance as well as for quality traits. Also discussed is the comparison among the genomes of closely related species like pearl millet, foxtail millet and also with other crops like rice and maize.

7.1 Introduction

Ragi is a highly nutritious crop having 18 chromosomes with tetraploid in nature, an important nutraceutical millet crop under the family Poaceae and the Eragrostideaetribe. It is normally called as ragi in India, and with other names in different places (Sood et al. 2017). It is a crucial food crop grown to some extent in dry parts of the world, especially in Asian and East African countries viz., India, Sri Lanka and China (Fakrudin et al. 2004). It is observed that the finger millet was evolved tentatively dated to the third millennium BC as per the oldest archaeological record, suggesting that domestication could have occurred in East Africa approximately around 5,000 years back. The finger millet crop is cultivated mainly by marginal and poor farmers which serves as a crop with more nutritional value crop due to its high calcium and fiber values and drought resistance nature (Dida et al. 2007; Kumar et al. 2012; Sood et al. 2019). Though the crop is neglected to a large extent, the production and productivity of finger millet crop is stagnating between 400 and 2,000 kg/ha (Dida et al. 2007). Earlier days conventionally the genetic changes in crops are characterized by

B. Kalyana Babu (✉)
Biotechnology Department, ICAR-Indian Institute of Oil Palm Research, Pedavegi, Eluru, Andhra Pradesh 534450, India
e-mail: kalyan_biotek@yahoo.co.in

S. Sood
ICAR-Central Potato Research Institute, Shimla, Himachal Pradesh 171001, India
e-mail: salejlplp@gmail.com; salej.sood@icar.gov.in

V. S. Gaur
JNKVV, Jabalpur, Madhya Pradesh, India

A. Kumar
Rani Lakshmi Bai Central Agricultural University, Jhansi, Uttar Pradesh, India

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variation in the morphological parameters in plants. Several studies were also proved by cytological analysis for depicting the genetic variation. With the advancement of molecular marker technology, considerable amount of growth in research of marker-assisted selection (MAS) was occurred in major crops except in minor millets, like finger millet. In crops like rice, maize and wheat these molecular tools are in wide use for analysis of molecular characterization, mining of quantitative trait loci (QTLs), association studies and MAS studies (Babu et al. 2018a; Kumar et al. 2016a, b).

7.2 Comparative Genomics

Finger millet has genome coverage of 1,593 Mb (Goron and Raizada 2015) and is monocot crop, cultivated by low income people of the world. But due to its nutritive properties, it is being considered recently as a high value crop by most of the rich people and is widely recommended for consumption by diabetic patients. It has wealthy nutrient composition of in comparison to rice, wheat and sorghum (Sharma et al. 2017; Gupta et al. 2017). Ragi is well recognized for remarkable more calcium (Ca) content (0.34%) of total seeds as against with cereals (0.01–0.06%) (Kumar et al. 2016a; Panwar et al. 2011; Gupta et al. 2017). The ragi is having rich amount of amino acids, fiber content, iron and trypsin inhibitory factors (Ceasar et al. 2018; Babu et al. 2014c; Sood et al. 2016; Chandra et al. 2016). In comparison to the major cereals like rice, the finger millet genome sequence has been postponed and was sequenced second after the foxtail millet genome, leaving it at back among cereals. The first draft genome for rice was released in the year 2005 (International Rice Genome Sequencing 2005) with the complete annotation in 2013 (Kawahara et al. 2013). Till 2013 foxtail millet is the one having whole genome sequence (WGS) among the millet crops. In the year 2012 only two genotypes of foxtail millet whole genome sequence were released (Bennetzen et al. 2012; Zhang et al. 2012). However, a big gap has taken place to release the second draft WGS of any millet that is finger millet, where the prime draft sequnce of finger millet was out in 2017 (Hittalmani et al. 2017), which is almost over a decade after release of rice genome and half a decade after foxtail millet genome published. Because of that very few genomic studies were conducted and in-depth high resolution analysis is yet to carry out after the sequencing in finger millet.

Comparative genomics studies will help to identify key genes of abiotic and biotic stress tolerance genes and nutrient strengthening into other crops. Prediction and identification of genes and pathway analysis using KEGG automatic annotation server shown that the carbohydrate and amino acid metabolism-related genes are more expressed in finger millet (Ceasar et al. 2018; Subramani and Manikandan 2019). This drought-tolerant crop can grow well even under harsh climatic conditions, due to its efficient carbon assimilating mechanism through the C4 pathway. The KEGG metabolic analysis combined with bioinformatics on ragi genome may help to be acquainted with key characters like abiotic traits and nutrient parameters, which can be applied to crops like rice and wheat (Subramani and Manikandan 2019). High synteny was revealed by genome colinearity among the cereals like rice and finger millet followed by foxtail millet by the workers (Subramani and Manikandan 2019). However, less synteny was observed between finger millet and maize (Subramani and Manikandan 2019). Though 60 million years of evolution have taken place for major grass families, it was shown that comparative genome analysis resulted in high synteny between grass genomes belonging to Pooideae, Panicoideae, and Ehrhartoideae (Subramani and Manikandan 2019).

7.3 Comparative Genomics with Other Species

Due to high colinearity and similarity in the grass genome sequences belonging to cereals, comparative genomics has been playing a key role in utilizing the crucial information present in the

high value crops like millets especially, finger millet (Gale and Devos 1998; Moore et al. 1995). The impact of comparative genomics analysis is also important in major cereals as well as in minor cereals which paves the way for identifying the important metabolic pathways for yield and other characters. Proof for analogous genome sequences are already well established in wheat (Roder et al. 1995) and rice (Zhao and Kochert 1993), legumes (Weeden et al. 1992) and crucifers (Lagercrantz et al. 1996). These results are road maps for identification of major QTLs or genes of significant economic and morphological traits in minor cereal or millets like finger millet. The first report on syntenic regions between finger millet and rice was observed (Srinivasachary et al. 2007) using genic and genome-wide microsatellite markers. It was found that on an average 85% resemblance existed between these two genomes by using 218 markers. High syntenic relation observed among rice, finger millet and foxtail millet might be due to similarity in the gene sequences of these crops. It was also found that genome of finger millet remains greatly preserved though it diverged from a familiar predecessor of grass genomes like rice long ago 60 million years. The circus maps representing comparative analysis of finger millet and rice showed that chromosome number differences between these two genomes and rearrangements had taken place (Subramani and Manikandan 2019).

The similarity analyses between millets like finger millet, sorghum, foxtail millet, Brachypodium, and cereals showed maximum similarity, which has evidence of more closeness during evolution of these crops. Nearly 1592 similar regions were identified among rice and ragi; whereas among foxtail millet and finger millet it is 1709 and 436 between finger millet and Brachypodium, representing 97, 98, and 82% of genome of ragi (Hittalmani et al. 2017). These syntenic relations will help in mapping the orthologous genes/QTLs of interest. These QTLs will be further used for MAS in transfer of desirable traits into most desirable genotypes. Babu et al. (2007) studied the cross transferability of 345 rice genomic simple sequence repeat (SSR) markers into 12 finger millet accessions for their applicability in genomics studies. Among them, 58.6% of SSR nearly 202 showed its applicability. They observed higher synteny or similarity for yield-related traits followed by leaf and root traits (Fig. 7.1). Among the amplified microsatellites, polymorphism was observed with thirteen microsatellite markers among GPU48 and VR708, however five were found polymorphic among PRM801 and GE86. They also studied similar kind of analysis for cross transferability, identification of polymorphic markers and genetic diversity of finger millet between maize and finger millet using 64 maize SSRs. Out of 64 microsatellites, 43 (67%) were present in the finger millet cultivars. Similarities were observed between genetic diversity analysis in differentiation of finger millet geno types using markers of maize and ragi. Likewise, few finger millet and maize microsatellites at the genomic regions were used for their application in other millets like barnyard millet for genetic diversity, population studies and for other genomic studies. It was observed by the researchers that thirty-nine SSRs were generated reproducible amplicons in the barnyard millet cultivars.

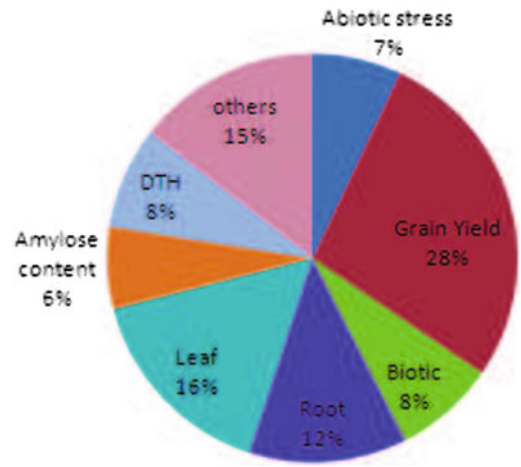

Fig. 7.1 Syntenic regions between finger millet and rice using transferability (Babu et al. 2017)

7.4 Comparative Genomics for Biotic Stress Resistance

Biotic stress conditions like pest and disease incidence play major role in influencing the yield levels of any crop including finger millet. In general *AVIRULENCE(Avr)* and *RESISTANCE (R)* genes govern the disease resistance in most of the crop species which codes for certain recognition factors. These hypersensitive responses are responsible for gene action combined by the restriction of development of pathogen colonization (Carine et al. 2008). They cloned nearly R-genes from many plant species using different molecular biology approaches (Okuyama et al. 2011). The nucleotide binding site—leusine rich repeat (NBS–LRR) also plays significant task in the disease resistance mechanism. These genes belong to major group of gene families and they are highly varied, depends on distinctive N-terminal features. These are used to isolate and classify motifs related to different amino acids which encode important genes. The ascomycetes, *Magnaporthegrisea*is the causal organism for finger millet blast which is a devastating disease. The approximate yield loss due to this disease is around 28%, but under severe conditions, it may be up to 80–90% (Babu et al. 2018b). The most common stages for pathogen attack are neck, finger and seedling stages. Till now, in case of finger millet very less or no R-genes were identified or mapped. Sequencing of NBS-LRR alleles specific to the resistant and susceptible genotypes was done by Babu et al. (2012) using the ABI 3130XL genetic analyzer. It was found kinase-2 and kinase 3a motifs of R-genes might be playing important role in finger millet defence mechanism (Fig. 7.2). So, these identified motif sequences of NBS region may be effectively used in molecular biology applications to trace out the defence-related genes.

7.5 Comparative Genomic Analysis for Blast Resistance Genes

Very few genomic studies were conducted in Finger millet and very much ignored crop. Data available on expressed sequence tags (ESTs) is very scarce in finger millet. In such cases the comparative genomics aids to compare with fully sequenced rice or maize genome to identify the similar genes related to economically important. Preliminary studies on molecular aspects on blast resistance were studied by Babu et al. (2014a) where they used few microsatellites representing different QTLs or genes responsible for biotic stress like blast resistance in finger millet. They were identified in the coding regions, untranslated regions of 5′ and 3′ end in the expressed sequences of finger millet and rice. These expressed sequence were found to belong to NBS-LRR region of disease resistance like blast in finger millet such as *Pi2, Pi16, Pi21* etc. They found that SSR marker FMBLEST32 in finger millet and RM262 loci in rice were strongly

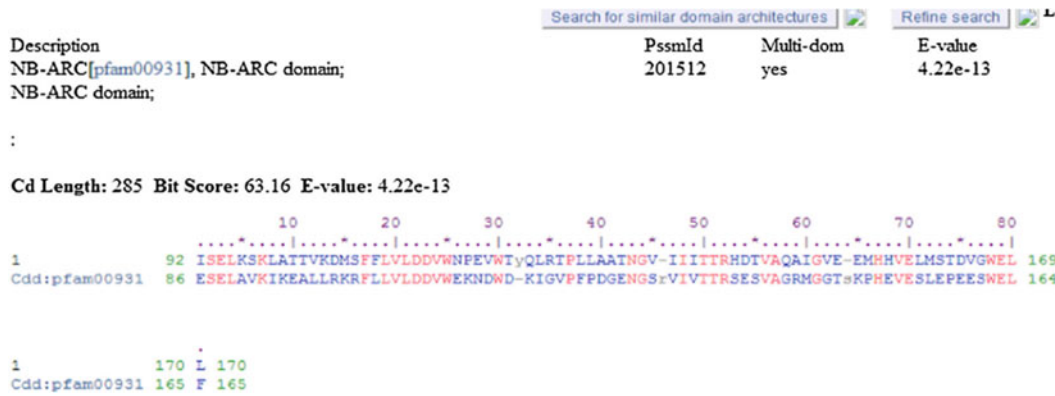

Fig. 7.2 The NBS-LRR domains in finger millet as revealed by in silico analysis (Babu et al. 2014d)

associated for blast resistance QTLs at a *P*-value of less than 0.007. This marker was developed from the key candidate gene *Pi5* which is reported to be responsible for resistance to *M. griseae* in rice (Wang et al. 1994). It was found that *Pi5* gene is a dominantly inherited gene which shows resistance to minimum of six races belonging to four lineages. The results also found that *Pi5* gene displayed resistance to diverse isolates and found to have broad-spectrum resistance. The results from the comparative studies showed that similar types of pathogen races may be responsible in causing the disease. So, based on these findings, the *Pi5* gene can be a potential or key candidate which may be playing important role in finger blast disease resistance. This gene in general codes for LRR motif, N-terminal coiled coil (CC), and NB domain.

7.6 Comparative Genomics for Abiotic Stress Management

Abiotic stress is a complex trait which is influenced by several parameters. Finger millet is a sturdy crop with tolerance to abiotic stress conditions like drought conditions which will act as a model crop in unraveling the genes responsible for drought tolerance. Several works identified that Pfam-dependent regulatory genes for drought stress discovered that many genes were found in more than nineteen Pfam domains. Even now, insufficient amount of researches were accomplished on the classification of key genes in ragi for key agronomic and economic traits. For instance, in case of tobacco, it was found that heterologous expression of *NAC1* gene of finger millet resulted in drought and salinity tolerance. It was also found that *PIN1a* plays major role in improvement of root hairs and lateral roots in finger millet. For acquisition of phosphorous from soil it was found that four phosphate transporter1 (PHT1) genes, such as *EcPHT1 1, 1;2, 1;3,* and *1;4* were involved. Different candidate genes are involved in several pathways like protein kinases (PF00069), BTB/POZ (PF00651), protein tyrosine kinases (PF07714), U-box (PF04564), universal stress protein family (PF00582), NAD-dependent epimerase/dehydratase family (PF01370), were mainly spread in ML-365 genome (Hittalmani et al. 2017). These linked quantitative loci were mostly associated with drought-tolerant genes like MYB, MYC, AREB and NF-Y transcription factors, (Singh et al. 2002; Vinocur and Altman 2005). Application of these transcription factors (TFs) is to study the association of transcription regulators and studying cis-acting elements for perceptive study on drought tolerance. So, characterization and prophecy of factors involving cis elements by promoter examination is an important aspect in finding the signaling networks and functional properties. Now-a-days novel technique like clustered regulatory interspaced short palindromic repeats (CRISPRs), RNAi, overexpression, transcription activator-like effector nucleases (TALENs), and zinc finger nucleases (ZFNs), are available for understanding the function of regulatory factors. The technical tools will have chief blow in creating resistant or tolerant varieties for abiotic stress breeding (Gaj et al. 2013; Rabara et al. 2014). Among the minor cereals and other millet crops, finger millet has rising quantity of calcium, tryptophan, sulphur, methionine, and fiber-containing amino acids. Adding up, it has mechanism of C4 carbon assimilation photosynthesis, exploits water and nitrogen resourcefully under arid regions with no harshly upsetting production (Hittalmani et al. 2017). They did sequencing and annotation of whole genome procedure of ML-365 finger millet at a size of 1196 Mb covering just about 82% of total genome. The finger millet genome was thought to be more similar or collinear with rice and foxtail millet compared to other crop plants (Hittalmani et al. 2017).

7.7 Comparative Genomics for Quality Trait Improvement

The major breakthrough happened in the finger millet improvement after the availability of finger millet rough draft genome sequence, published by Hittalmani et al. (2017). In their report, they made comprehensive description and identification of genes for different functions and

prediction of the molecular mechanism of finger millet. This paved the way for validating the calcium and other nutrient transport and regulatory genes which are involved in grain filling for future research activities. In other words, in total the draft genome sequence will be a great source to meet the food and nutritional security especially in the developing and developed countries. Genes for nutritional parameters like Calmodulin gene (CaM) were cloned by molecular techniques from ESTs of *Eleusine coracana* which are freely accessible in dbEST database (www.ncbi.nih.com). It was found that 613 bp obtained from PCR reactions were consistently present in most of the cereals and millets except *Setaria-italica*. Researchers like Manoj et al. (2010) eluted the amplified PCR products from gel, cloned, sequenced and did similarity search through BLASTn analysis. They also did multiple sequence alignment, motif prediction and analysis, and phylogenetic construction of tree. From these phylogenesis results, it was found that CaM genes were similar to all cereals except *Triticum aestivum*. The translation of CaM amino acid sequences confirmed that they also have conserved amino acids of 110 which were consistently pragmatic across many poaceace crops except sorghum. The CaM genes protein motif revealed a very close evolutionary relationship between different cultivars or varieties of finger millet like PRM1, PRM801 and PRM 701. They also showed relationship with *Hordeum vulgare* for their higher amount of seed calcium than rice and other millets. These works showed that dissimilarity at structure level in CaM genes has direct influential role in the calcium build up pathway. The in silico 3D-structural patterns identified revealed comparable model and high degree of preservation in CaM in terms of structure and relations with calcium ions, thus reflecting to additional inspection into the role of CaM same forms, with transport mechanism drawn in calcium assimilation (Manoj et al. 2010; Yadav et al. 2014, 2020).Yadav et al. (2014) developed SSR markers from the coding and non-coding sequences of for factors related to calcium and transport genes viz. calcium-binding proteins, and calcium-regulated protein kinases in sorghum and rice. They found a conserved behavior from corner to corner of the finger millet genotypes representing the mineral transport which remains preserved in plants and even microsatellite differences in them remain hidden all through the track of development.

It was also evident that structural closeness among the CaM genes in their probable functional activities and accumulation of nutrients like calcium. The similarity was also observed with reference to the finger millet varieties viz. brown, golden and white. Researchers amplified identical set of CaM genes through in silico analysis of PCR amplified products and cloned sequences (Nirgude et al. 2014). They were also checked with finger millet EST primers along with other EST-designed primers of sorghum, rice, maize and millets. All the cloned EST sequences were deposited to GenBank database and obtained the accession numbers.

The protein content of cereals is in general deficient in lysine and tryptophan amino acids which are highly essential. Generally, most of the cereals consisted of 1.5–2% lysine and 0.25–0.5% tryptophan which need more amount for healthier human metabolic activities. Young et al. (1998) opined that 5% lysine and 1.1% tryptophan are required for optimal human nutrition. The seeds of cereals and millets contain zein proteins which are resulted from mutation in the *opaque2* gene which resulted in reduced synthesis of certain seed storage proteins which belong to multigene family. Fascinatingly, a rice cDNA encoding a similar bZIP transcriptional activator, RISBZ1, was obtained from tissues of seeds (Onodera et al. 2001), demonstrating that the *o2* gene arise much early than the splitting of maize and rice evolution. The finger millet quality parameters like high tryptophan and lysine influenced by *opaque2* modifier genes will be improved by using molecular tools. Some researchers found SSR markers linked to *o2* genes in maize and rice. These were designed from 3'UTR, 5'UTR, CDS and intron regions of the sequences (Babu et al. 2014b). These EST sequences were selected from *opaque2* modifier genes of maize, and rice RISBZ sequences factors and sorghum *opaque2* modifier genes. The

results of this study linked SSR locus FMO2-EST1 to protein QTLs at a *P*-value of 0.002. This marker was obtained from the rice gene, RISBZ1 which was found to regulate tryptophan content to maximum extent. The tryptophan and protein are inversely related and this marker may be down regulating the *o2* modifier genes (Babu et al. 2014b). RISBZ1 is a bZIP transcriptional activator sourced from seeds tissues of rice, and given a hint that orthologous genomic regions of *o2* modifiers may be present in the ancestors of cereals and millets before they evolute (Babu et al. 2014b).

7.8 Future Strategies

Though the finger millet genome has been sequenced recently, it led to many questions on the effective analysis of the whole genome, and its comparison with relative species at genome level. There is need to focus on identifying the specific genes for drought tolerance, important nutritional traits, and biotic stress resistance traits for their use in breeding better cultivars with more yield. This also will pave the way for enriching the genomics-assisted selection for drought tolerance in the related species. At the same time, researchers need to focus on improving the yield levels of finger millet, since the yield levels are very low and there is need to produce high-yielding cultivars.

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Finger Millet Transcriptome Analysis Using High Throughput Sequencing Technologies

8

Rajesh Kumar Pathak, Dev Bukhsh Singh, Dinesh Pandey, Vikram Singh Gaur, and Anil Kumar

Abstract

During the past two decades, high-throughput transcriptome sequencing has developed into a well-established and widely used research method. Transcriptomics experiments allow researchers to classify transcriptional behavior, concentrate on a subset of specific target genes and transcripts, or simultaneously profile thousands of genes to create a global image of cell function. Besides, the simultaneous maturation of bioinformatics tools are playing a crucial role in the analysis of generated transcriptomic data for novel discovery. Finger millet *(Eleusine coracana L.)*, an essential species of C4 which is known for its resistance to stress and dietary significance is a nutritional and health security crop as well due to extraordinarily high levels of calcium (Ca). Ca being considered the most important macronutrient in the diet, is needed in relatively large quantities to maintain a healthy body. Therefore, analysis of transcriptome/RNA-seq data generated via different high-throughput sequencing platform and their integration through systems biology offered new opportunities to decode the molecular mechanisms of entire finger millet systems in different conditions for identification of key genes involved in drought stress, growth and development, disease resistance, synthesis of bioactive compounds, proteins, carbohydrate and, Ca accumulation and so on. This book chapter highlights the principles of finger millet transcriptome analysis from data generation to data analysis.

R. K. Pathak
Department of Biotechnology, Govind Ballabh Pant Institute of Engineering and Technology, Pauri Garhwal, Uttarakhand, India

D. B. Singh (✉)
Department of Biotechnology, Siddharth University, Kapilvastu, Siddharth Nagar, Uttar Pradesh, India
e-mail: answer.dev@gmail.com

D. Pandey
Department of Molecular Biology and Genetic Engineering, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

V. S. Gaur
College of Agriculture, Balaghat, Madhya Pradesh, India

JNKVV, Jabalpur, Madhya Pradesh, India

A. Kumar
Rani Lakshmi Bai Central Agricultural University, Jhansi, Uttar Pradesh, India

8.1 Introduction

Finger millet *(Eleusine coracana L.)* a nutritionally important crop has recently gained a lot of attention due to its nutritional components. The finger millet (FM; also known as ragi) ranks fourth after the Sorghum (*Sorghum bicolor*), pearl millet (*Pennisetum glaucum*), and foxtail

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millet (*Setaria italica*) worldwide (Kumar et al. 2016). It is an important food crop, which is widely cultivated in arid and semi-arid regions of the world, particularly in eastern Africa; India and the rest of Asia including Sri Lanka and China (Belton and Taylor 2002; Kumar et al. 2016).

Despite being an excellent source of nutrients and minerals with the well-documented health benefits FM, also possess some anti-nutrients (commonly referred to as phytochemicals). The slow digestibility of FM is supplying energy during the day. The plant itself is diaphoretic, diuretic and vermifuge, and its leaf juice was offered to women during childbirth (Antony and Chandra 1998; Vadivoo et al. 1998; Subba Rao and Muralikrishna 2002; Fernandez et al. 2003; Ćujić et al. 2016; Kumar et al. 2018). It has also been used for various diseases, including leprosy, liver disease, pleurisy, pneumonia and smallpox, as a folk remedy. Finger millet has a high fiber content that tends to reduce constipation, high cholesterol, diabetes and intestinal cancer. Besides, it is also suggested that the risk of diabetes and gastrointestinal tract disorders may be effectively minimized with daily intake of FM in diet (Gibson and Helme 2001; Mooser and Carr 2001; Scalbert et al. 2005; Lattimer and Haub 2010; Schatzkin et al. 2007; Fardet et al. 2008).

Despite the fact that many essential nutritional features are available in finger millet grains, research work has largely been ignored to exploit the tremendous potential of this crop for solving the problems associated with protein-energy malnutrition and nutritional deficiencies (Murtaza et al. 2014; Kumar et al. 2018). This may be attributed to the fact that before and during the green revolution, staple crops such as wheat, rice and maize gained a great deal of research attention in order to improve food production. Moreover, the availability of very limited genomic knowledge in past years has further limited finger millet crops improvement programs. However, but, the first time the transcriptome (Rahman et al. 2014; Kumar et al. 2015a, b) and genome data (Hittalmani et al. 2017; Antony et al. 2018) of finger millet is now available due to advances in omics science and technology. After that many papers are now available addressing the complexity of transcriptome data and reported some essential genes/proteins associated with different biological processes and its nutritional potential (Lanham-New 2008; Singh et al. 2014; Chinchole et al. 2017; Kokane et al. 2018; Gupta et al. 2018; Avashthi et al. 2018, 2020; Parvathi et al. 2019). These available data hold enormous information related to millet breeding program. There is a need of time to explore these data and generate new transcriptome data for comparative analysis and mining of novel information through computational and experimental approaches. The chapter highlights the pipeline of finger millet transcriptome analysis from data generation to analysis, integration and deposition of data in public repository for the benefit of the scientific society (Fig. 8.1).

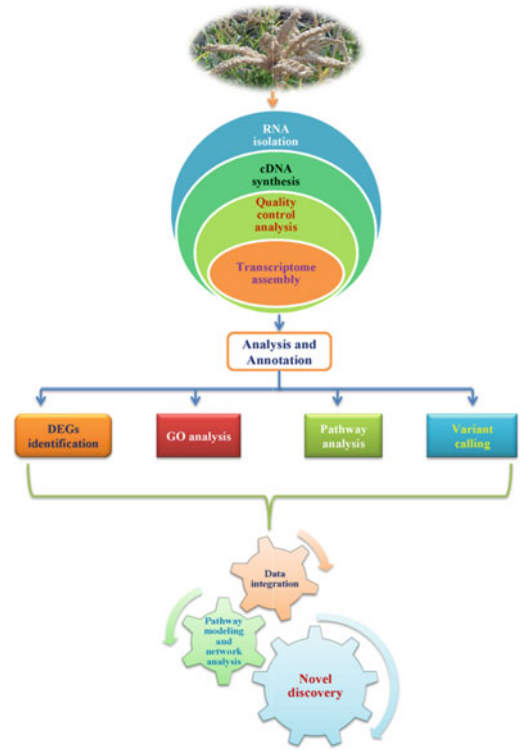

Fig. 8.1 Integrated approach for decoding the finger millet transcriptome for novel discovery

8.2 Overview of High-Throughput Transcriptome Sequencing Platform

The pyrosequencing-based 454 system by Roche, the sequencing-by-synthesis-based GA/HiSeq/MiSeq devices from Illumina and the sequencing-by-ligation SOLiD system, Pacific Biosciences and Oxford Nanopore are currently the most widely used sequencing platform, and some others are under development (Wolf 2013; Klepikova et al. 2016; Pathak et al. 2018b). These sequencing platforms will be utilized for finger millet transcriptome sequencing as per objective of the research projects (Kumar et al. 2015a, b). The sequencing technology and its estimated experimental cost are mentioned in Table 8.1 (Pathak et al. 2018a, b).

8.3 Experimental Set-Up for Data Generation

As we know that finger millet is one of the agriculturally important crop which holds immense potential for improving human life and prevent from several diseases due to its nutraceuticals properties. Therefore, transcriptome sequencing and its analysis is necessary for identifying key components involved in different biological processes for several purposes. Before, going to the experimental setup for data

Table 8.1 Sequencing technology and its estimated experimental cost in India; the prices mentioned are for sequencing a single sample with a single library

S. N	Technology/methods	Read length	Output per lane	Output per run	Per lane cost (INR)	Per run cost (INR)
Second-generation sequencing technology						
1	454 sequencing	Up to 1 Kb	500 MB	–	–	500,000 Outdated/discontinued
2	Illumina HiSeq1000	100 bp	15 GB	–	300,000	2,100,000 Outdated/discontinued
3	ABI SOLiD system	50–100 bp	–	–	–	Outdated/discontinued
4	Polonator G.007	26 bases per amplicon	–	–	–	Outdated/discontinued
5	Ion Torrent Sequencing	Up to 200 bp	1 GB	1 GB	–	100,000
Third-generation sequencing technology						
6	PacBio	800–1000 bp	–	–	–	100,000
7	HeliscopeTM Sequencer	33-nt read length	–	–	–	Outdated/discontinued
Fourth-generation sequencing technology						
8	Oxford Nanopore	62–70 bases sequences per DNA nanoball (DNB)	–	–	–	110,000
9	Illumina HiSeq3000	150 bp	50 GB	400 GB	275,000	2,000,000
10	ABI SOLID 550XL system	75 bp	120 GB	120 GB	–	200,000 Outdated/discontinued
11	Ion Proton	200 bp	6 GB	6 GB	–	150,000
12	Pac-Bio RSII	1–30 kb	750 MB	750 MB	–	125,000

generation, several points' need to be addressed which are discussed in the following sections.

8.3.1 Purpose of Transcriptome Sequencing

The starting point for each experiment is to identify its basic aims and determine its viability with respect to the budget and the technique available. For a transcriptome sequencing experiment, questions like, why we need transcriptome data, what type of genes will be characterized or identified and why, what are the future applications of identified genes, etc., need to be addressed.

8.3.2 Statistical Design

It is one of the important steps before going for transcriptome sequencing. Here, we will focus on biological replication and statistical model selection. It is a common practice in many biological investigations. In order to integrate biological replication, the statistical treatment of transcriptome data has moved from single sample studies to more complex statistical designs, such as generalized linear regression models. In case of transcriptome analysis, it will help in sample collection and analysis of data for comparative analysis. For example collections of samples in different time interval as compared to control plants followed by sequencing and analysis. This will help in data analysis for fruitful results.

8.3.3 Choice of Tissue and Time

The abundance of transcript and isoform identity is radically different across tissues, and they change drastically not only throughout embryological development, but also over the entire life of an organism. Therefore, it is important to understand, which tissue and at which, physiological level is most likely to observe difference related to the problem at hand.

8.3.4 Collection of Sample

Sample collections will be done carefully because RNA degrades rapidly. Therefore, after cutting put sample immediately in ice box, generally it a good job for preserving RNA for some time at room temperature, then stored at −80 °C for further utilization and their isolation.

8.3.5 RNA Extraction and Their Quality Assessments

RNA extraction has to be suited to the focal RNA species: during normal mRNA extraction, tiny RNA molecules (<200 bp) will be destroyed following traditional LiCl precipitation or commercially available kits. For small transcripts, such as micro-RNAs, different extraction protocols will be needed. The RNA integrity assessment is a crucial first step for obtaining significant measurements of gene expression.

8.3.6 cDNA Synthesis

Until sequencing, most sequencing platforms usually require RNA to be converted to cDNA. The reverse transcriptase enzymatic reaction may either be prepared by hybridizing an oligo-dT primer onto the mRNA template of poly-A tail or by use of random hexamer primers. It can easily be done by following standard lab protocol or protocol provided through kit.

8.3.7 Library Preparation

Library preparation is an important step and it is platform specific. Library can be prepared as per objective of the research project. During library preparation, cDNA is broken into smaller pieces, which then act as a sequencing template. The cDNA pieces are partly sequenced from one end while a single-end approach is used, where paired-end sequencing read short sequences from both ends. For initial transcriptome assembly and

isoform identification, paired-end sequencing may be helpful, but one should be careful that the insert size should not be too big (usually <300 bp), else the small size fraction of transcripts would be lost. On the other hand, too short sizes of insert can give adapter contamination, which may need trimming or removal of reads, leading to complicated analysis.

8.3.8 Sequencing Strategy and Platform Selection

Illumina HiSeq, IonTorrent, Pacific Biosciences are the most commonly used sequencing platform right now and others are under development. For platform selection, cost per base pair, error rate and error profiles, total output and read length are the relevant parameters to note. Where there is a trade-off among read length and total output, for transcriptome data, the latter seems more significant. In *de novo* assembly, longer reads are very helpful and paired-end reads also work equally well. In the end, what matters is the number of appropriately aligned reads per gene, which defines the precision of the calculation of gene expression.

8.4 Bioinformatics for Data Analysis

Bioinformatics is an interdisciplinary science because it is made from combination of several scientific disciplines, i.e. plant science, animal science, chemical science, physical science, pharmaceutical science, mathematical and statistical science supported by computer science and information technology as support system. We can't think about transcriptome analysis without bioinformatics. It is playing vital role in dissecting the complexity of transcriptome data for identification of key genes/proteins involved in various biological processes and their expression with respect to time in different tissues. A strong computational skill is required for handling of the transcriptome data and softwares. Several important tools used for transcriptome analysis are highlighted in Table 8.2.

8.4.1 Computational Resources and Programming Skills

During transcriptome sequencing, a big amount of data will be generated. Therefore, we need good computational resources in terms of data storage and analysis, because during data analysis other files such as assembled file, ban, sam, etc., will be generated in the form of results, which also take big space in the computer. Besides, good programming skills are necessary for fetching of key sequences, their annotation and analysis. R, Perl and python are the most demanding language right now in this area. Good command in UNIX/Linux operating systems is must for transcriptome analysis.

8.4.1.1 Understanding of File Format

At the time of transcriptome sequencing and analysis, various files will be generated. The knowledge of these files formats such as fastq, fasta, sam, bam, vcf, etc., is necessary to understand data and results.

8.4.2 Quality Analysis of Generated Data

After transcriptome sequencing, the generated data will be analyzed using bioinformatics tools such as fastqc to determine the quality of generated data. If some error has occurred in data, the software like trimmomatic, cut-adapter, etc., will be run to remove low quality reads from the data. Further, fastqc will be run to evaluate the quality of data after trimming.

8.4.3 Method for Data Assembly

It is a computational method to reconstruct longer sequence (e.g. a transcript) from sort sequence reads. Basically, two methods are available for the assembly of transcriptome data, i.e. de novo assembly and reference-based assembly. In finger millet, the first de novo assembled transcriptome was published in the year 2013.

Table 8.2 A list of software used for analysis and integration of high-throughput transcriptome sequencing data along with their application and availability

S. N	Software	Utility	Availability	References
1	FastQC	It is a platform for checking the quality of high-throughput sequencing data	https://www.bioinformatics.babraham.ac.uk/projects/fastqc/	Andrews (2010)
2	Cutadapt	It detects and removes adapter sequences, primers, poly-A tails and other forms of unnecessary sequence	https://cutadapt.readthedocs.io/en/stable/	Martin (2011)
3	Trimmomatic	It is utilized for performing a number of useful trimming tasks on paired-end and single ended data generated from illumina	http://www.usadellab.org/cms/?page=trimmomatic	Bolger et al. (2014)
4	Trinity	It is used for de novo assembly of transcriptome data	https://github.com/trinityrnaseq/trinityrnaseq/wiki	Grabherr et al. (2011)
5	Bowtie2	It is used for aligning sequencing reads with long reference sequences	http://bowtie-bio.sourceforge.net/bowtie2/index.shtml	Langmead and Salzberg (2012)
6	Cufflinks	It is used for assembly of transcriptome data and their differential expression analysis	http://cole-trapnell-lab.github.io/cufflinks/	Trapnell et al. (2012)
7	edgeR	It is used for differential expression analysis of transcriptome data	http://bioconductor.org/packages/release/bioc/html/edgeR.html	McCarthy et al. (2012)
8	Blast2GO	It is a tool used for functional annotation of transcriptome data	https://www.blast2go.com/	Conesa and Götz (2008)
9	MISA	It is a tool used for SSR identification	https://www.ipk-gatersleben.de/en/bioinformatics-tools/marker-data/misa/	Thiel et al. (2003), Beier et al. (2017)
10	VCFtools	It is used for variant calling	https://vcftools.github.io/man_latest.html	Danecek et al. (2011)
11	CellDesigner	It is used for pathway building/modeling and their simulation analysis	http://www.celldesigner.org/	Funahashi et al. (2003)
12	Cytoscape	It is used for visualization and analysis of biological network	https://cytoscape.org/	Shannon et al. (2003)

8.4.3.1 De novo Assembly

De novo assembly uses more computational resources as compared to reference-based assembly. A computer may contain at least 8 cores and 256 GB of RAM to allow assembly within a suitable time frame. An assembled transcript facilitates gene expression studies and annotations for novel discovery. Usually, de novo assembly is more challenging and less accurate than reference-based assembly.

8.4.3.2 Reference Based or Genome-Guided Assembly

Here, the genome of a target organism (if available) or closely related organism will be taken as reference. The transcriptome/RNA-seq reads will be mapped on the reference genome to construct the longer leads from small reads for further investigation. This is considered as more accurate method for assembly of transcriptome data.

8.4.4 Annotation of Assembled Transcript

Annotation is a very common term in Bioinformatics. It is a process to find out biologically important regions in sequences, its expression with respect to particular condition and time, and their involvement/role in different biological processes. The obtained information will be further utilized for validation and other research program.

8.4.4.1 Identification of Differentially Expressed Genes (DEGs)

Identification of differentially expressed genes (DEGs) from transcriptome data is one of the key steps in transcriptome assembly and annotation. If an observed difference or expression level among two experimental conditions is statistically important, a gene is declared as differentially expressed. Bioinformatics analysis of transcriptome data play vital role in investigation of differentially expressed genes. These genes will be further annotated and validated for various uses including crop improvement program through molecular breeding and genetic engineering approaches.

8.4.4.2 Gene Name Assignment

The identified DEGs, i.e. up-regulated and down-regulated sequences will be subjected for gene name assignment based on the available information in databases through computational prediction. Designation of gene name on identified sequences is one of the key steps in characterization of genes and construction of their relationship with known sequences of the related organisms through multiple sequence alignment and building of phylogenetic tree.

8.4.4.3 Gene Set Enrichment Analysis

A set of up-regulated and down-regulated sequences will be subjected to enrichment analysis in terms of gene ontology, i.e. molecular function, biological process and cellular components. Based on gene set enrichment analysis, we will annotate the function of genes identified via transcriptome analysis.

8.4.4.4 Pathway Analysis

Pathway analysis is now being done with bioinformatics applications or web resources that accept and interpret various data from omics (Cirillo et al. 2017). It has become an essential tool for determination of candidate genes involved in different pathways. These studies have promoted an interactive assessment of the genes, their role, regulation or association.

8.4.5 Variant Calling

Development of molecular markers within the putatively functional genomic elements of transcribed DNA is one of the key applications of transcriptomic data. Many tools are available for variant calling, such as the extremely versatile GATK pipeline. Generally, partially overlapping sets of variants will call, as they take various statistical approaches and vary in which parts of the data are used.

8.4.6 Systems Biology for Data Integration and Novel Discovery

Systems biology has emerged in recent years as an effective approach for understanding crop plant systems to improve food production and how their dietary components support our health and avoid diseases, as well as for studying the bioactive molecules that are involved in these impacts (Pathak and Singh 2020). Computational methods and statistical models allow for a broad study of the response of key genes and their role in improving the nutritional content and influence of plant products on human health. This has contributed to the recognition of several essential genes and proteins involved in the growth and nutrition of plants, as well as the discovery of bioactive molecules linked with human, animal and plant health (Kumar et al. 2015a, b, 2018; Pathak et al. 2017a, b; Pathak et al. 2018a; Rana et al. 2020; Pathak and Singh 2020).

8.4.6.1 Pathway Modeling and Simulation Analysis

It is a powerful approach to study the behavior of different genes involved in pathway at different amount/concentration with respect to time. The obtained information from transcriptome analysis and information already available in literatures will be used to build model pathway using systems biology graphical notation (SBGN). The different types of SBGN symbols are available for representing gene, protein, transcription factor, metabolite, etc. (Pathak et al. 2013). We can use these symbols to model pathway. Further, kinetic rate equation will be generated for each species in the model to simulate its dynamic behavior for identification of key components involved in regulation of different molecular mechanisms in the biological systems. A pathway of molecular interactions between calcium exchangers and sensors in different tissues involved in the regulation of calcium transport and their accumulation in seeds of finger millet has been proposed through transcriptomics and systems biology approaches (Fig. 8.2) (Kokane et al. 2018).

8.4.6.2 Network Generation and Analysis

The key genes or proteins obtained from transcriptome analysis will be utilized for network generation and analysis for identification of hubs from the large set of genes. It is a powerful

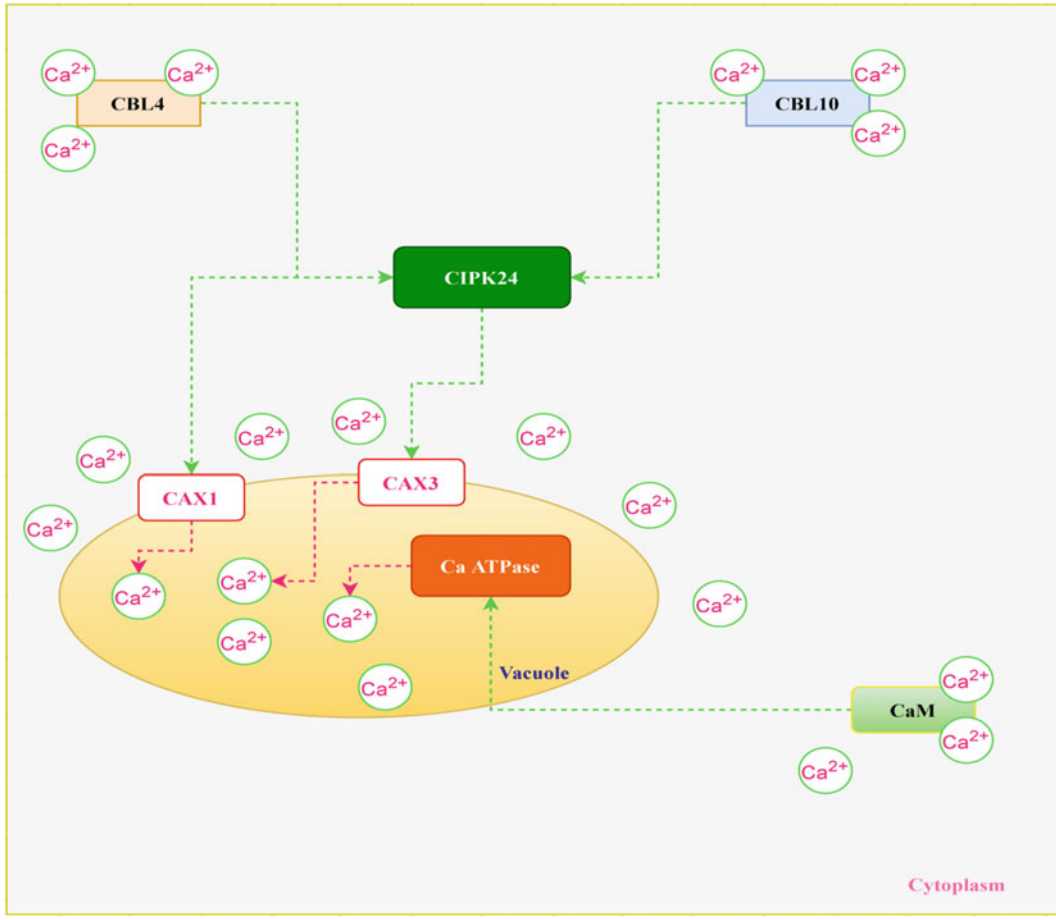

Fig. 8.2 Figure depicted the molecular mechanism of transport and accumulation of calcium in finger millet seeds

approach that emerged in recent years in the area of biological sciences and made a new discipline called network biology. With the help of network biology approaches, we can sort key genes as hub from large set of genes based on topological parameters for further validation and implementation in the crop improvement program. The modeled pathway will be imported as network via network visualization tools for their analysis. Dehydroascorbate reductase (DHAR) was identified as a key gene regulating different biological processes in Finger millet through network analysis (Fig. 8.3) (Avashthi et al. 2020).

8.4.7 Validation

The results generated from above analysis, i.e. transcriptome sequencing, analysis and their integration with systems biology will be validated through experimental approaches for further implementation in finger millet research program with respect to resistance to abiotic and biotic stresses, food and nutritional security.

8.4.8 Submission of Generated Data in International Data Repository

Advances in sequencing platforms generated huge amount of sequencing data in daily basis right now. Storage and management of these data is a challenging task for bioinformatician. Bioinformatics has tremendous potential in management and analysis of omics data. A lot of database, i.e. primary, composite, secondary, structural and specialized databases are available on internet for management of biological data. Right now Sequence Read Archive, commonly known as SRA (https://www.ncbi.nlm.nih.gov/sra) hosted at National Center for Biotechnology Information (NCBI) is a major resource for submission of transcriptome data. Data related to finger millet transcriptome are also available at SRA for further analysis and their integration with newly sequenced data for novel discovery. A list of finger millet transcriptome sequencing data available in public domains is highlighted in Table 8.3.

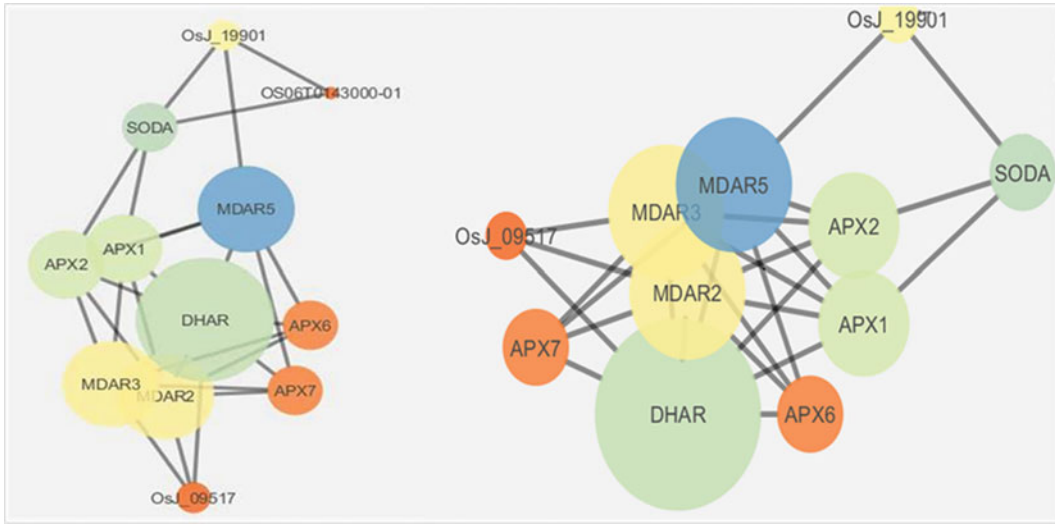

Fig. 8.3 Identification of hub genes involved in different biological processes in finger millet through integrated transcriptome data analysis

Table 8.3 List of finger millet transcriptome sequencing data available in public domain for their further analysis and integration

S. N	Tissues/sample	Date of submission	SRA accession of different samples	Submitted by
1	Shoot base including young leaves and meristem	2013-11-24	SRX383838 SRX383848 SRX383849 SRX383850 SRX383851 SRX383852 SRX383853 SRX383854	University of Alberta
2	Developing spike	2014-01-30	SRX456024 SRX456025	G. B. Pant University of Agriculture and Technology
3	Leaf	2014-02-28	SRX478150 SRX478151 SRX478152 SRX478153	Tamil Nadu Agricultural University
4	Leaves	2014-11-26	SRX768581 SRX768590	National Bureau of Plant Genetic Resources
5	Leaf	2015-04-28	SRX1012247 SRX1013389	University of Agricultural Sciences
6	Leaf	2016-02-05	SRX1562181 SRX1562183 SRX1567494 SRX1567497	National Research Centre on Plant Biotechnology
7	Seedlings	2017-04-25	SRX2753924	Auburn University

8.5 Application of Transcriptome Sequencing and Data Analysis in Breeding and Improvement of Finger Millet

The availability of finger millet transcriptome sequencing data, re-sequencing approaches and computational resources has led to a new era of breeding, as they make it easier to research the genotype and its relationship to the phenotype, particularly for complex traits. It aids in the discovery of new genes and regulatory sequences, as well as discovery of molecular markers. Besides, breeders will learn about the molecular basis of complex traits via expression studies. They also make it possible to find markers that are related to genes and QTLs. The generated information from the data analysis will be further utilized in the development of smart finger millet crops through molecular breeding or biotechnological approaches for ensuring nutritional security. The identified key candidate's genes will be also useful in improving nutritional quality, drought resistance, heat resistance, etc., to the other cereal crops, i.e. wheat, rice, maize, etc., via genetic engineering method.

8.6 Conclusion

As per our traditional knowledge finger millet seeds is considered a powerhouse of nutritionally important compound as biochemical factory for ensuring nutritional security. The present chapter highlights the key information related to finger millet transcriptome data analysis for identification of key genes/proteins involved in different biological processes, i.e. calcium accumulation, synthesis of secondary metabolites useful in

human health and other information. This will be helpful for the readers for understanding the methodology of transcriptome data analysis and their outcomes, which will be further utilized in crop improvement program for food and nutritional security.

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9 Seed Biology and Packaging of Finger Millet Using Omics Approaches for Nutritional Security

Anil Kumar, Rajesh Kumar Pathak, Sanjay Mohan Gupta, and Salej Sood

Abstract

Seeds act as storage organs for nutrition, bio-energy, processing, and other essential bio-molecules, as well as a distribution mechanism for genetic information to be passed on to future generations. Research into deciphering the complex system of gene regulation and pathways related to seed biology and nutrient partitioning is still in its early stages. There has been a renewed interest in the cultivation and consumption of nutricereals in recent years, especially small millets such as *Eleusine coracana* (L.) Gaertn (finger millet; FM). It could be an ideal model system for studying nutraceutical properties using multi-pronged molecular approaches to decode seed biology, processes of nutrient partitioning, and biologically relevant molecule packaging. To comprehend the complexities of seed biology and packaging, it is essential to understand the genes and pathway(s) involved in biomolecule homeostasis and accumulation at the time of seed development. Multi-layered "Omics" methods and high throughput platforms have recently been used to distinguish the genes and proteins involved in different metabolic and signaling pathways and their regulations for understanding the molecular genetics of biosynthesis and homeostasis of bio-molecules in many model organisms such as Arabidopsis and rice. However, there is very little information is existing in scientific domain about the molecular biology of seed development in FM. This can be accomplished by combining multi-omics data with systems biology to better understand the complexities of seed developmental biology and nutrient partitioning. Via pathway engineering and biotechnology, this information can be used to improve biologically essential chemicals for large-scale development of nutrients in seeds and nutraceuticals.

9.1 Introduction

In India, the govt. is setting in situ Associate in Nursing Initiative for biological process Security through Intensive Millet Promotion (INSIMP),

A. Kumar (✉)
Rani Lakshmi Bai Central Agricultural University, Jhansi, Uttar Pradesh, India
e-mail: anilkumar.mbge@gmail.com

R. K. Pathak
Department of Biotechnology, Govind Ballabh Pant Institute of Engineering & Technology, Pauri Garhwal, India

S. M. Gupta
Min of Defence, Defence Institute of Bio-Energy Research (DIBER), DRDO, Haldwani, India

S. Sood
ICAR-Central Potato Research Institute, Shimla, HP 171001, India
e-mail: salej.sood@icar.gov.in

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whereas within the developed world millets and alternative ancient grains have appeared on the shelves of specialized outlets. Vocation them as "climate-resilient crops" and "powerhouse of nutrients" government of India has declared eight millet crops (sorghum, pearl millet, finger millet, barnyard millet, foxtail millet, proso millet, kodo millet and little millet) and 2 pseudocereals (amaranth and buckwheat) as "nutricereals" (The Gazette of India, thirteenth April 2018). Finger millet may be a tiny cereal grain big within the semi-arid sub-tropical and tropical regions of Africa and Asia wherever it's one among the cereal staples (ICRISAT/FAO 1996; Obilana and Manyasa 2002). The America National analysis Council (1996) states, "Despite its importance, FM is grossly neglected each scientifically and internationally". FM is sort of entirely a subsistence crop and in Africa, it's used primarily for the assembly of ancient foods, virtually none of them square measure commercial (ICRISAT/FAO 1996), whereas the use of sorghum, another less common cereal, is being inflated by victimization to supply novel business merchandise like food, bread, cookies, and snack foods (Taylor et al. 2006), identical is extremely restricted with FM. Wheat is the ideal cereal for manufacturing food as a result of it contains protein proteins, that square measure essential for the standard of the merchandise, however it grows well in cooler climates and therefore countries in hotter regions import half or all of the wheat victimization the scarce exchange. Partial substitution of wheat with FM in bakeshop merchandise like cookies could have several benefits together with a high biological process worth, saving exchange, and health-promoting (due to the phenolic resin antioxidants).

Seeds square measure one among the key materials of nutraceutical and prescription drugs resources consisting of all attainable sorts of nutritionally necessary and bioactive molecules that embrace varied soluble carbohydrates, storage proteins, starch chemical compound and lipids required for our daily diet. Aside from this, they conjointly operate as necessary delivery system of genetic info from generation to generation. Seeds square measure one among the key materials of nutraceutical and prescription drugs resources consisting of all attainable sorts of nutritionally necessary and bioactive molecules that embrace varied soluble carbohydrates, storage proteins, starch chemical compound and lipids required for our daily diet. Aside from this, they conjointly operate as necessary delivery system of genetic info from generation to generation. This chapter describes grain structure and composition, seed development, factors poignant seed development, nutrient partitioning and completely different 'omics' tools and alternative branches that square measure united to make the foremost engaging space of analysis toward establishing the seeds as organic chemistry factories for human health and nutrition.

9.2 Seed Development: Transition from Vegetative Stem to Developing Spikes to Seed Development

On the basis of morphology and development stage of ovary and anthers, four different developmental stages of the spike, inflorescence immergence or booting, anthesis, grain filling, and grain maturation or ripening, were identified and designated as (a) S1 stage, i.e. booting or inflorescence immergence when florets are compact, androecium and gynoecium are very small, closely arranged, and (c) S3 stage, i.e. grain filling, occurs when there is a noticeable increase in particles in the liquid endosperm, and the caryopsis is crushed between fingers (d) The S4 stage, which occurs after 50% of the spikelets have ripened and the caryopsis has hardened to the point where it is difficult to divide by thumb-nail, was critical for determining the expression of putative transporters and their regulatory genes (Fig. 9.1).

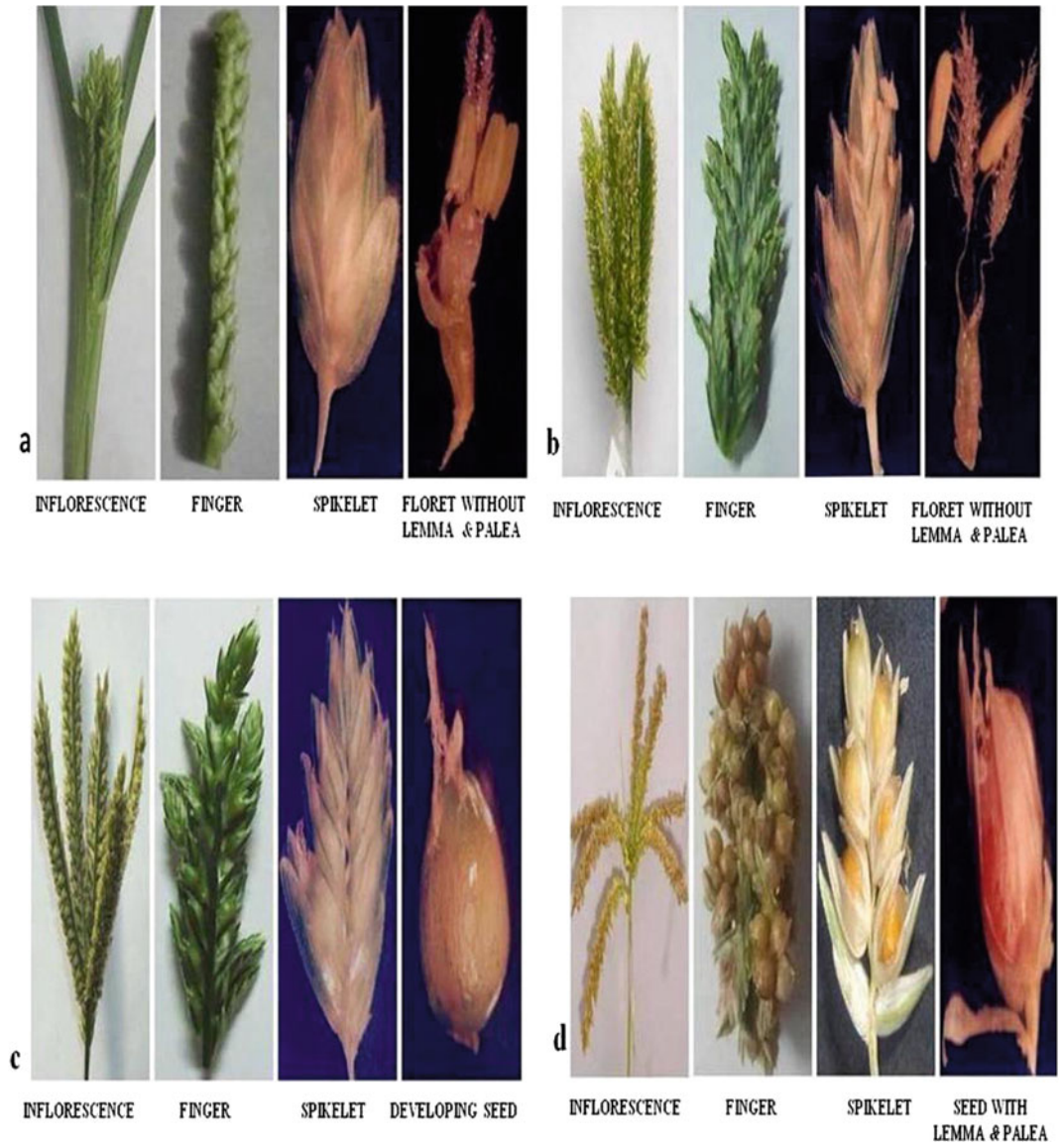

Fig. 9.1 Developmental stages of finger millet spike namely, spike, booting or inflorescence immergence, anthesis, grain filling and grain ripening or maturation were identified on the basis of morphology and development stage of ovary and anthers and were designated as S1, S2, S3 and S4, respectively. (Adapted from Mirza et al. 2014)

9.2.1 Factors Influencing Seed Developmental Machinery

9.2.1.1 Environment

Herbivores or a lack of water can alter maternal influences on seed development. It has the potential to reduce the resources available for seed development and to impact the critical regulatory event that occurs during and after pollination (Gehring and Delph 2006; Diggle et al. 2010). During seed development, resource lamination might induce mother plants to produce smaller seeds. However, the molecular mechanisms of maternal control are poorly understood. The paternal part also influences the

developmental mechanisms of seeds in two ways: in the first, paternal alleles contribute to embryo and endosperm vigor, resulting in variation in seed size and mature embryo; in the second, paternal alleles contribute to embryo and endosperm vigor, resulting in variation in seed size and mature embryo (Kigel 1995).

9.2.1.2 Nutrition

Nutrient translocation during seed development into the seed coat, endosperm, or embryo is still unknown. Only a few plant species have been thoroughly examined so far. Plants use atypical transport systems for embryo feeding, according to previous research, because there is no vascular connection between the embryo and the vascular bundle. Nutrients can be transported via two different pathways: apoplast and symplast. CO2 and O2 can be transferred through the middle layer of the parenchyma's intercellular spaces (Kigel 1995). Glycolysis, gluconeogenesis, amino acid biosynthetic pathways, energy, and pyruvate metabolism are among the routes involved with seed biology and their involvement in nutrition. Seeds are also linked to hormone signaling pathways such as IAA/ethylene, jasmonate, and gibberellin production (Sreenivasulu and Wobus 2013; Li et al. 2015). Through a systems biology approach, progress has recently been achieved in identifying the main components of seeds that control nutrient loading during their maturation process (Zhang et al. 2007; Kumar et al. 2015a, b).

9.2.1.3 Physiology

A better understanding of plant physiological characteristics is one of the most important concepts in building a biology background for predicting seed growth. Single fusion pairings are observed using micromanipulation and video microscopy. In synergids, genes involved in pollen discharge or pollen tube guidance, as well as genes with differential expression in sperm, central cells, and eggs before and after fertilization, have been identified (Raghavan 2000). The continual perception and transmission of signals by endosperm, seed coat, and the embryo is essential for the coordination of development, maturation, and differentiation processes. Furthermore, the ratio of phytohormones in certain signals controls specific stages of seed development (Locascio et al. 2014). In addition, numerous methods have been developed, such as infrared thermography, to decode biophysical and biochemical changes associated with imbibition, germination, and overall seed vitality (Kranner et al. 2010; Macovei et al. 2017). Reactive chemical element species (ROS) are thought to play an important role in seed development as both a signaling and a harmful molecule (Kumar et al. 2015a, b). A tight link between phytohormones, reactive oxygen species (ROS), and DNA repair have been predicted at all stages of seed development, from embryogenesis to germination (El-Maarouf-Bouteau et al. 2013; Kumar et al. 2015a, b). The seed's hydration state, on the other hand, is a significant element that influences ROS signaling and DNA repairing (Bewley et al. 2012).

During seed germination and imbibition, ROS as signaling molecules is found in a variety of signaling pathways, including the pentose phosphate pathway and mitogen-activated protein kinases (Diaz-Vivancos et al. 2013; El-Maarouf-Bouteau et al. 2013). Several antioxidative enzymes are either activated or inhibited during seed imbibitions, in addition to these (Balestrazzi et al. 2011). On the other hand, several phytohormones have been found to influence ROS production during germination and seed development, such as ABA, which reduced ROS accumulation in rice and sunflower (Zhang et al. 2013), barley (Ishibashi et al. 2012), and gibberellic acid, which increased ROS generation in Arabidopsis (Lariguet et al. 2013). Given this critical information, it is imperative that omics approaches be used efficiently to unravel several complex mechanisms involved in seed development.

9.2.1.4 Epigenetic Modifications

In flowering plants and mammals, genomic imprinting is an independent and self-contained phenomena. Imprinted genes are determined to be responsible for the regulation of nutrition transfer to the developing offspring at the site of embryo-nourishing organs, such as the placenta

and endosperm. The antagonistic activities of Polycomb group-mediated histone methylation and DNA methylation have a significant impact on the regulation of many imprinted plant genes (Jiang and Köhler 2012). Long-term selection can preserve imprinted expression at some loci because genes with imprinted expression are conserved between monocots and dicots (Ohnishi et al. 2014). A harmonization of the genetic route, HAIKU (IKU), with epigenetic regulators limits genome dosage in Arabidopsis. Actually, the seed size is determined by DNA methylation and the deposition of trimethylated lysine 27 on histone H3 (H3K27me3) (Li et al. 2013). The regulatory implications of DNA methylation in rice seed maternal integument development have recently been investigated, with a whole genome bisulfite deep sequencing technique utilized to identify differentially methylated areas and genes driving this important process (Wang et al. 2017). According to another study, a seed-specific transcription factor gene LEAFY COTYLEDON 1 reverses the silent state inherited from gametes by encouraging the early development of an active chromatin state and activating its expression de novo at FLOWERING LOCUS C (Tao et al. 2017).

9.2.1.5 Small Regulatory RNA

Small non-coding RNAs (sncRNAs), together with microRNAs (miRNAs) and short meddlesome RNAs (siRNAs), are vital monitors of organic phenomena at the transcriptional and post-transcriptional stages, and have also been found to play critical roles in seed biological processes and germination (Rodrigues and Miguel 2017). Because studies have focused solely on miRNAs, which are typically 21-nt length, the biological significance of the presence of different siRNA profiles in seeds is not yet understood (Rodrigues and Miguel 2017). MiRNA serves a critical function in seed growth and germination by down-regulating target genes (Rogers et al. 2013). In Arabidopsis mutants, differential expression of miRNAs in tissues has been proven (Zhai et al. 2014). Artificial miRNA (ami-RNA) was created and expressed to target a specific factor, resulting in abnormal embryogenesis. The mechanical device Like-1 (DCL-1) factor is in charge of catalyzing the creation of the right structure from the secondary precursor (Nodine and Bartel 2010). Similarly, emb76, a DCL-1 deficient mutant, caused embryo arrest and did not prevent the development of a kind of cell known as a suspensor cell (Bewley et al. 2013). More research into the dcl1-5 and dcl1-10 mutants revealed that DCL1 mutations have an early influence on the 8-cell embryonic stage, most likely due to up-regulation of miR156-targeted SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) ten and eleven (Xu et al. 2016). The involvement of alkyl enzyme has been identified for the aim of reading miRNA biogenesis and maturation (Pluskota et al. 2011). The temporal order of seed maturation is likewise controlled by miRNAs (Willmann et al. 2011). In dcl15 mutants, up-regulation of FUS3, LEC2, L1L, NF-YB6, MYBs, and bZIPs was observed, while down-regulation of Arabidopsis 6B-INTERACTING PROTEIN1-LIKE1 (ASIL1) and ASIL2 and simple protein deacetylase HDA6/SIL1 and simple protein deacetylase HDA6/SIL1 was observed. Furthermore, miRNAs have been implicated in seed dormancy and germination transition. MiR156 and miR172 are both involved in seed germination, with miR156 inhibiting germination in lettuce and Arabidopsis and miR172 promoting it (Huo et al. 2016). In Arabidopsis, miR159, which targets MYB33 and MYB101, is known to fully regulate ABA responses required for the shift from seed dormancy to germination (Nonogaki 2017). Furthermore, a recent study on rice plants demonstrated the presence of different junction events of secret writing and long non-coding ribonucleic acid (lncRNA) throughout the biological process stage of seed via comparison of immature seed with embryo as well as reproductive structure of mature seed during the biological process stage of seed. It was projected that developing seeds would have more different junction events, i.e. 5.8–57 times more than root, leaves, buds, flowers, and procreative meristems. As a result, additional research is needed to understand IncRNA's labyrinthine process during seed development (Kiegle et al. 2018).

9.3 Finger Millet Grain Structure and Composition

A fruit coat or pericarp covers the seed in most cereal grains, which comprises of an embryo or germ and an endosperm wrapped by a nucellar epidermis and testa or seed coat (Hoseney 1994). A wheat grain's structure can be either a caryopsis or a utricle (Angold 1979). The pericarp or fruit coat surrounds the seed in a caryopsis and attaches closely to the seed coat, but in a utricle, the pericarp surrounds the seed like a sac and is linked to the seed at a single point. Caryopses are the primary cereal grains wheat, maize, barley, sorghum, rice, oats, and rye, whereas utricles are the millets finger, proso, and foxtail millets (Hoseney 1994; McDonough 2000).

9.3.1 Structure of the Finger Millet Grain

The utricular structure of the FM kernel (grain) was suggested by Angold (1979) and elucidated by McDonough et al. (1986). The FM kernel is roughly globular to oval (1–1.5 mm in diameter) in shape with average weight of 2.64 g of 1000 kernel (Angold 1979; McDonough et al. 1986). The FM kernel contains outer layers, the starchy endosperm and germ (Fig. 9.2).

9.3.2 Outer Layers

The outer layers of the FM kernel, according to McDonough et al. (1986), consist of a membranous cover that is loosely connected to the kernel at maturity and a testa that overlays an aleurone protein layer (Fig.9.3a). The authors determined that the cover is a flimsy membranous covering that is not attached to the episperm at any specific location and can be removed by rubbing or laundering, similar to what Angold (1979) and Hilu et al. suggested previously (1979). The cover appeared to be made up of several layers of tissue (McDonough et al. 1986). McDonough et al. (1986) also noted that the FM episperm layer's outward appearance was distinctive and distinct from that of other cereals. They discovered that FM episperm has five unique layers and range in color from red to purple. The initial layer was 1.5 m thick and autofluorescent blue, indicating that ferulic acid or a polymer was present. The second layer seemed striated and was made up of mound-like formations made up of portions of "interlocking" tissue. When the episperm was seen in cross-section, junctions were seen between the mound-like structures within the outer episperm layers, and the junctions were assumed to correlate to the interlocking sections seen from the surface. The second layer (5.5–17.5 m) was the thickest, had darker coloration than the lower layers, and most likely contained more phenolic resin components than the others. The third and fourth layers were approximately the same thickness (1.4–2.1 m) and color tone. The third layer (Fig. 9.3b) was characterized by unique wave forms throughout, but the fourth layer was mostly straight, with a few isolated wave patterns. The fifth layer was one meter thick and had a color that was noticeably distinct from the previous ones. The authors stated that the majority of the FM grain phenolics, as well as the tannins, were targeted

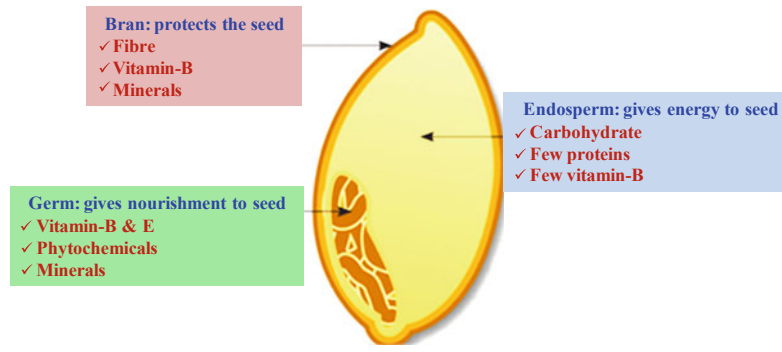

Fig. 9.2 Example of nutrient distribution and partitioning in developed seed (Adapted from: www.precisionnutrition.com/all-about-grains)

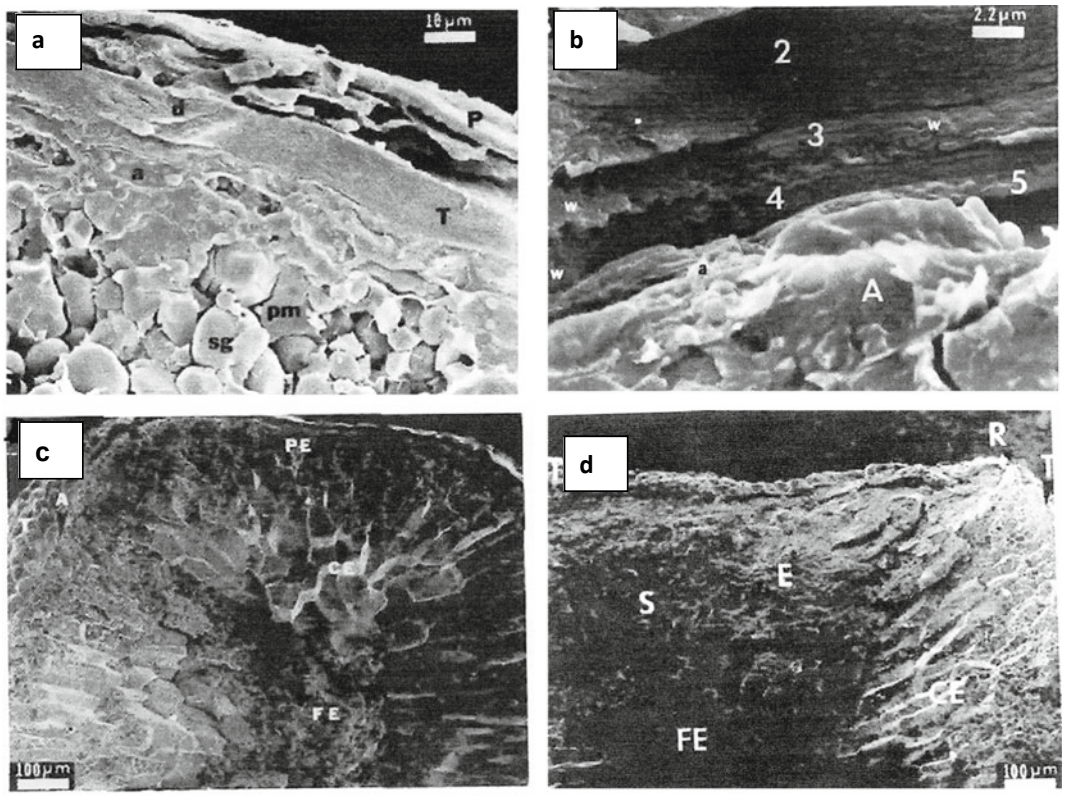

Fig. 9.3 Structure of anatomical parts of the finger millet grain (adapted from McDonough et al. 1986). **a** Pericarp, testa, aleurone layer and peripheral endosperm layers. P = pericarp; T = testa; a = aleurone; pm = protein matrix; sg = starch granule. **b** Four of the five testa layers, showing wave formations and contour striations. 1–5 = testa layers; w = wave formation; A = aleurone cell; a = aleurone cell wall. **c** Three discrete layers of the starchy endosperm. A = aleurone layer; PE = peripheral endosperm; CE = corneous endosperm; FE = floury endosperm. **d** Cross-section of the germ. R = ridge; T = testa; E = embryonic axis; S = scutellum; CE = corneous endosperm; FE = floury endosperm

within the episperm layer, although the location of the tannins was unknown.

9.3.3 Endosperm

The endosperm makes up the majority of the FM kernel's weight (McDonough et al. 1986). The aleurone layer of FM was discovered to be quite similar to that of maize, sorghum, and pearl millet. It was one cell layer thick and completely encircled the FM starchy endosperm. There are many aleurone bodies present, but no starch granules. The cell walls of aleurones autofluorescent, indicating the presence of phenolic chemicals. FM starchy endosperm contained separate floury and corneous layers, according to Angold (1979). Similar to what was discovered in sorghum, pearl millet, and maize, McDonough et al. (1986) identified three separate forms of starchy endosperm, the peripheral, corneous, and floury endosperm (Fig. 9.3c). The peripheral endosperm cells were the tiniest of the endosperm cells. The peripheral endosperm cell contents were firmly packed. A vast number of protein structures were contained in a protein matrix and linked to compound and simple starch granules (diameter: 8.0–16.5 m). Corneous endosperm made up the majority of the endosperm. It was mostly made up of compound

starch granules (3.0–19.0 m in diameter), with some simple starch granules thrown in for good measure. The starch granules were linked to patches of a protein matrix. Compound starch granules about 11–21 m in diameter make comprised the floury endosperm. There were only a few protein bodies and a protein matrix to be found. The starchy endosperm cell walls fluoresced brightly, showing the presence of phenolics.

9.3.4 Germ

Millet contains a comparatively small germ (270 × 980 m), according to McDonough (2000). McDonough et al. (1986) proposed that the FM germ was located in a deep depression surrounded by a distinctive ridge that wrapped around the entire germ (Fig. 9.3d). The hilum was placed adjacent to the germ, and the stylar was placed on the kernel's opposite facet. The scutellum stratum separated the scutellum from the tiny reproductive structure. The macromolecule bodies were found in the scutellum. It's worth noting that the FM grain may be difficult to mill due to its small size and the fact that its testa is securely attached to the endosperm (McDonough 2000).

9.4 Composition of the Finger Millet Grain

FM grain is supposed to be more nutritious than other cereal grains in terms of amino acids, minerals, and dietary fiber, according to the US National Research Council (1996). As previously stated, FM grain includes numerous phenolic chemicals, which may be beneficial to one's health due to their antioxidant characteristics (Dykes and Rooney 2006). Both genetics and the environment influence the chemical composition of FM grain (McDonough 2000). Despite the fact that Table 9.1 shows mean values, the proportions of grain chemical composition might vary greatly.

9.4.1 Carbohydrates and Dietary Fiber

Carbohydrates account for 70–76% of the total weight of the FM grain, according to Obilana and Manyasa (2002), with 7.9% cellulose, 61.8% starch, 0.8% reducing sugars, 4.9% pentosans, and 0.5% dextrins. In the endosperm, starch is usually found in the form of simple or complicated granules (McDonough et al. 1986). The major components of FM starch are amylose and amylopectin, which have similar MW to other cereal starches (Serna-Saldivar and Rooney 1995). Sucrose, raffinose, glucose, maltose, and fructose are among the sugars contained in FM grain (McDonough 2000). Glucose and sucrose account for 12.5 and 33% of the soluble sugars in FM grain, respectively.

FM grain has a higher total dietary fiber content (22.0%) than other cereal grains, which contain 12.6%, 4.6%, 13.4%, and 12.8% wheat, rice, maize, and sorghum, respectively. The fiber components of FM grain, like those of other cereal grains, are found in the cell walls (mostly endosperm and pericarp cell walls) (Serna-Saldivar and Rooney, 1995). Dietary fiber comprised 18.6% of the FM grain, according to Kamath and Belavady (1980), which included 4.6% cellulose, 6.1% non-cellulosic polysaccharides (1.5% water-soluble and 4.7% water-insoluble), and 7.9% lignin. FM grain has 1.4% soluble dietary fibre and 15.7% insoluble dietary fiber, according to Chethan and Malleshi (2007). Shobana and Malleshi (2007) found 22.0% total dietary fiber, 19.7% insoluble dietary fiber, and 2.5% soluble dietary fiber in their study. The non-cellulosic polysaccharide components of FM grain dietary

Table 9.1 Chemical composition of finger millet grain

Nutrients				Non-nutrients
Major nutrients (g/100 g[a])	Minerals (mg/100 g[c])	Amino acids (g/100 g protein[d])	Vitamins (mg/100 g[c])	Phenolic compounds[d]
Moisture: 12.0	Calcium: 358.0	*Essential amino acids*	Vitamin A (RE): 6.0	*Phenolic assay*[f]
Carbohydrate: 74.0	Chlorine: 84.0	Phe: 6.2	Thiamin: 0.2	Folin/Ciocalteu: 0.55–0.59
Protein: 7.3	Copper: 0.5	His: 2.6	Riboflavin: 0.1	Vanillin-HCl: 0.17–0.32
Fat: 1.3	Iodine (µg): 10.0	Ile: 5.1	Niacin: 1.0	Phenolic acids[g]
Total dietary fibre: 22.0[b]	Iron: 9.9	Leu: 13.5	Vitamin C: 1.0	Protocatechuic: 23.1
Ash: 2.6	Magnesium: 140.0	Lys: 3.7		Gentisic: 61.5
	Manganese: 1.9	Met: 2.6		p-OH Benzoic: 8.9
	Molybdenum (µg): 2.0	Thr: 5.1		Vanillic: 15.2
	Phosphorus: 250.0	Val: 7.9		Caffeic: 16.6
	Potassium: 314.0	*Non-essential amino acids*		Syringic: 7.7
	Sodium: 49.0	Asp: 7.9		Coumaric: 56.9
	Zinc: 1.5	Glu: 27.1		Ferulic: 387.0
		Ala: 8.0		Cinnamic: 35.1
		Arg: 5.2		
		Cys[e]: 1.6		
		Gly: 4.8		
		Pro: 6.7		
		Ser: 6.9		
		Tyr: 3.6		
		Trp[e]: 1.3		

[a] Obilana and Manyasa (2002)
[b] Shobana and Malleshi (2007)
[c] US National Research Council (1996)
[d] McDonough (2000)
[e] cysteine and tryptophan are not essential amino acids, but they can spare the requirement for methionine and phenylalanine, respectively
[f] mg/100 mg catechin equivalents, dry weight basis
[g] µg/mg

fiber appear to be predominantly non-starch polysaccharides arabinoxylans (pentosans), with glucose, xylose, galactose, and arabinose as important sugar contents, while mannose and rhamnose are minor constituents (Nirmala et al. 2000; Rao and Muralikrishna 2001).

9.4.2 Protein

Due to water availability, genotype, temperatures, soil fertility, and environmental factors present during grain development, the total protein content of FM grain varies from 4.9 to

11.3% (McDonough 2000). (Serna-Saldivar and Rooney 1995). When compared to brown FM grain variations, white FM grain varieties are said to contain higher protein (Virupaksha et al. 1975; Rao 1994). FM has a protein level of 7.3% (Table 9.1), which is similar to rice (7.9%) and lower or similar to other millets, sorghum, and wheat (11.0, 9.6, 9.0, 12.6 and 7.9%, respectively, pearl millet, teff, fonio, wheat, and sorghum) (Klopfenstein 2000; Obilana 2003). Prolamins are the most abundant protein component in FM grain, followed by glutelins (Serna-Saldivar and Rooney 1995). Prolamins are also the most abundant protein fraction in sorghum and other millets (Serna-Saldivar and Rooney 1995). (foxtail, pearl and proso millets). These fractions (glutelins and prolamins) are mostly found in the starchy endosperm's protein bodies and protein matrix, respectively (Serna-Saldivar and Rooney 1995). Lysine and other critical amino acids are abundant in albumin, glutelin, and globulin fractions (Serna-Saldivar and Rooney 1995). When compared to other millets, FM proteins are said to be more nutritionally balanced (Ravindran 1992). "Eleusinin," the primary protein fraction of FM grain, contains adequate quantities of cystine, tryptophan, methionine, and total aromatic acids, which are sometimes low in other cereals (US National Research Council 1996). FM is notably high in methionine, accounting for about 5% of total amino acid content (US National Research Council 1996). However, lysine is limited in FM grain, as it is in other cereals, but pearl and FM millets have the greatest lysine (McDonough 2000). Anti-nutritional factors such as phenolic chemicals (condensed tannins) and trypsin inhibitors, which may be found in FM grain, might reduce protein bioavailability (Serna-Saldivar and Rooney 1995; McDonough et al. 2000).

9.4.3 Lipids

Sorghum and millets, according to Serna-Saldivar and Rooney (1995), include a variety of lipids, including phospholipids, glycolipids, triglycerides, phytosterols, carotenoids, and tocopherols, which make up a minor fraction of the grains' proximate composition. In most cases, lipids are found in the scutellum. Polar, non-polar, and non-saponifiable lipids can be found in millets and sorghum, and they can be found as free, bound, or structured lipids. The non-polar lipids, which include triglycerides (fat/oil), are the most abundant. FM grain has a total lipid content of 5.2%, with the primary elements being oleic, palmitic, and linoleic acids (McDonough et al. 2000). FM grain has a lower fat content (1.3%) than sorghum and other millets, and is similar to 4.8, 2.0, 1.8, 1.1, and 2.8% pearl millet, tef, fonio, wheat, and sorghum, respectively (Obilana 2003). (Table 9.1). Because of its thin germ, FM grain has a low fat content (1.3%) (Serna-Saldivar and Rooney 1995). FM's low fat content may be important in that the grain has better storage qualities due to a lower tendency to go rancid.

9.4.4 Minerals

FM is a good source of minerals, especially calcium, which is 5–30 times higher than in other cereals (US National Research Council 1996). Iron, potassium, copper, magnesium, phosphorus, and sodium are also abundant (Obilana and Manyasa 2002) (Table 9.1). Minerals abound in the aleurone layer, pericarp, and germ (Serna-Saldivar and Rooney 1995). However, due to their interaction with anti-nutritional substances such as oxalic acid, phytic acid, and condensed tannins found in FM grain, the bioavailability of some of these minerals (e.g. divalent metal ions and phosphorus) may be reduced (Serna-Saldivar and Rooney 1995; McDonough 2000).

9.4.5 Vitamins

FM is high in water-soluble and lipid-soluble vitamins [thiamin, riboflavin, niacin, and maybe vitamin C, as well as tocopherols (vitamin E)]. (Serna-Saldivar and Rooney 1995; Obilana and Manyasa 2002; Serna-Saldivar and Rooney 1995; Obilana and Manyasa 2002) (Table 9.1).

The dried grain, on the other hand, is devoid of vitamin C. (Serna-Saldivar and Rooney 1995). Water-soluble B vitamins are concentrated in the aleurone layer and germ, while liposoluble vitamins are primarily found in the germ (Serna-Saldivar and Rooney 1995).

9.4.6 Phenolics, Flavonoids and Tanins

Phenolic acids, flavonoids, and tannins are among the health-promoting phenolic substances found in FM. The most common polyphenols are phenolic acids and tannins, with flavonoids making up a tiny percentage of the total. These compounds have recently attracted more attention due to their antioxidant and other nutraceutical characteristics. FM grains contain 0.3–3% polyphenols, with the majority (90%) localized in the seed coat, but a tiny fraction being present in the endosperm.

9.5 Nutrient's Partitioning

It observes specific differentiation of seed tissues into embryo, endosperm, and protein layer, and consequently, partitioning of the various nutritionally necessary bio-molecules, nutrient partitioning phenomena within the seed tissues may be a new area of interest to improve the standard of seeds (Nadeau et al. 1995). Previous research in the field of traditional genetics and breeding has made a significant contribution to the identification of factors affecting crop growth and grain filling. In addition to genetic and epigenetic variables, the environment has a significant impact on seed development. The primary biomolecules such as carbohydrates, proteins, lipids, and others are dispersed throughout the seed tissues; nonetheless, it is important to determine whether these components influence nutritional partitioning. Nonetheless, for a better knowledge of seed biology, genetic variables should primarily be investigated at the molecular level (Xie et al. 2015; Jing et al. 2016).

9.5.1 Nutrient Partitioning and Omics Approaches

The various omics-based approaches are the best method for understanding seed biology at the molecular to systems level, because they provide holistic views of complex biological phenomena in the seed system by interpreting the behavior of each constituent (gene, protein, and metabolites) and their interactions in seed. It can also identify a variety of innovative features that can be applied to seed biology studies (Fukushima and Kusano 2013; Kumar et al. 2015a, b). The following section of this chapter offers molecular insights into the complexities of nutrient partitioning and seed biology.

9.5.2 Molecular Status for Dissecting the Complexity of Seed Biology and Nutrients Partitioning

Seed development research has developed as a critical area of study in plant biological process biology (Lohe and Chaudhury 2002). Seed development is controlled by a number of genes and their interactions with their targets. B3 or HAP3 domains are found in seed development regulators that interact with the basic essential amino acid zipper (bZIP) and APETALA2 (AP2) transcription factors (TFs). Homeotic gene, NAM, ATAF, and CUC (NAC), Myeloblastosis (MYB), and plant hormone Response issue (ARF)-domains-domains are some of the TFs that play an important part in this strategy (Agarwal et al. 2011). Many genes play important roles, including LEAFY COTYLEDON2 (LEC2) (Stone et al. 2001), MYB (Dumas and Rogowsky 2008), GNOM (Grebe et al. 2000), SHOOT MERISTEMLESS (STM) (Long and Barton 2000), MONOPTEROS (Hardtke and Berleth 1998), and FACKEL. The ALTERED MERISTEM PROGRAMMING1 (AMP1) gene was discovered to play an important role in embryogenesis during pattern formation (Lohe and Chaudhury 2002), and the LEAFY COTYLEDON (LEC)

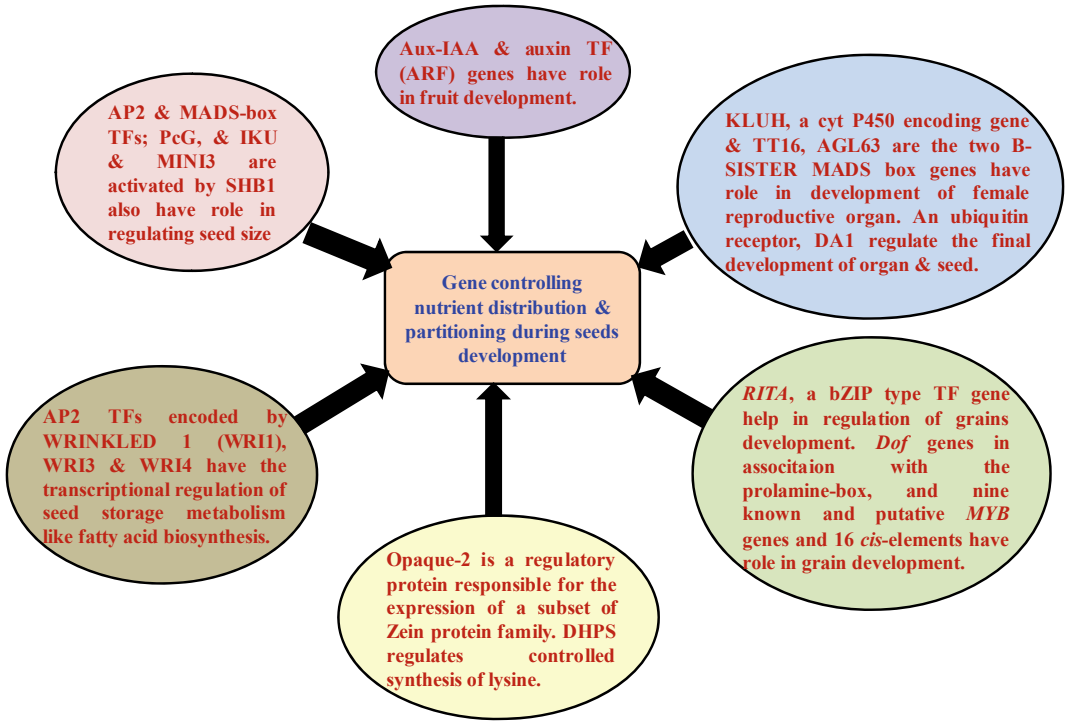

Fig. 9.4 Gene controlling nutrient distribution and partitioning during seeds development

genes LEC1, LEC1-LIKE, LEC2, and FUS3 are seed-specific TF genes that regulate seed developmental processes (Le et al. 2010) (Fig. 9.4).

Because seed developmental mechanisms differ across monocot and dicot plants, high-throughput transcriptome data analysis provides a first step toward understanding the molecular networks and pathways that act during seed development in specific compartments. In dicots and monocots, comparative systems-based co-expression network analyses will define evolutionarily conservative (FUS3/ABI3/LEC1) and divergent (LEC2) networks (Sreenivasulu and Wobus 2013).

Homeotic genes play an important part in the differentiation and development of the plant's various organs. In Arabidopsis, for example, AP2 is in charge of floral homeotic gene expression regulation. AP2 is expressed at the RNA level in all four types of floral organs, including petals, sepals, carpels, stamens, and developing ovules, in accordance with its genetic roles (Jofuku et al. 1994). B-sister genes have been demonstrated to be necessary for the proper differentiation of the ovule/seed in angiosperm species. Many genes, particularly those expressed in the seed, are still in the characterization or uncharacterized stage, and their roles remain uncertain. As a result, identification of seed-specific genes that could be exploited in biofortification programs to provide food and nutritional security for our civilization faces more obstacles. As a result, contemporary systems biology methodologies can help us gain a better understanding of seed biology.

9.5.2.1 A Hypothetical Model for Defining the Role of Various Calcium Transporter Genes Involved in Calcium Accumulation in Seeds

In the absence of a genomic resource in FM, rice and sorghum MPSS data were used to investigate the expression of different calcium transporter genes at various stages of seed development, and

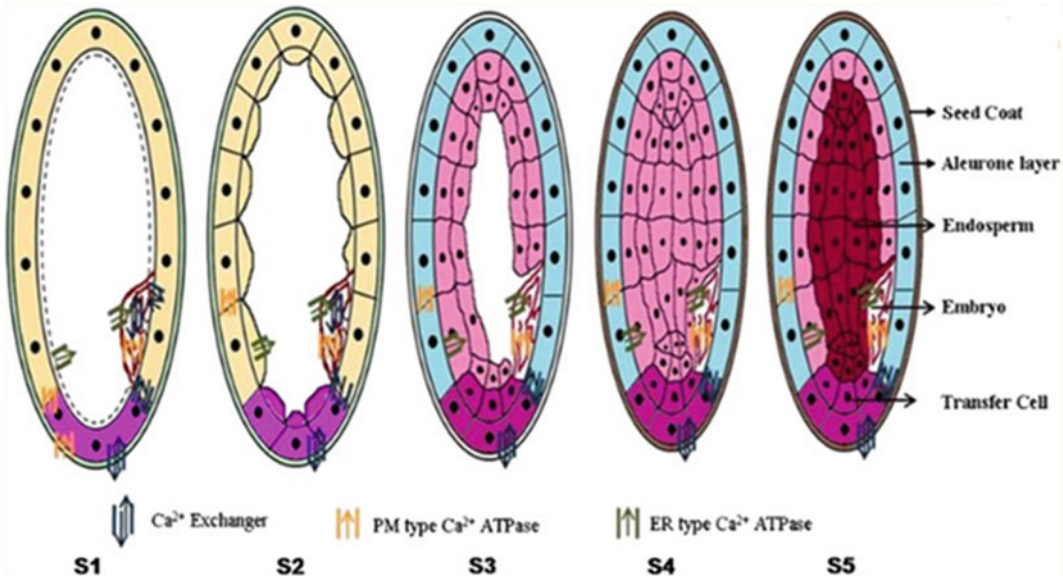

Fig. 9.5 A hypothetical model to show the possible presence of Ca^{2+} transporter genes that may be responsible for calcium accumulation in cereal grains during grain filling. (Adapted from Goel et al. 2012)

the results were used to identify the major transporters expressed at various stages of FM spike development (Goel et al. 2011). A possible model for calcium buildup in cereal grain seeds can be given based on expression analyses of calcium transporter genes and data from the literature (Fig. 9.5). In cereals, the 3n endosperm cell multiplies repeatedly after fertilization without establishing a plasma membrane, giving birth to the syncytium. A big vacuole's nucleus extends all the way around its edge. The plasma membrane is generated in the following cellularization stage, enveloping each nucleus. After that, the cells divide periclinally to create aleurone layer cells on the exterior and endosperm cells on the inside, completely covering the vacuole. Before undergoing apoptosis, the endosperm cells aggressively express seed-specific genes, such as TF genes, regulatory proteins, and genes involved in seed storage protein, glucose, and fatty acid accumulation. The aleurone cells create layers of cells to cover the endosperm and stay alive until germination. The majority of calcium transporter genes expressed in seed are expressed during the early stages of seed development and drop subsequently, indicating that the majority of calcium is mobilized through the transporters during the early stages of seed development. At the S1 stage of seed development, however, members of all three transporters (type IIA and type IIB Ca2 + ATPases and calcium exchangers) were present, and expression of all genes was reduced, with the exception of the two ER type ATPases, which had significant levels of expression until the S5 stage. We assumed that there must be channels in the membranes of transport cells (TC) at the basal region of the developing endosperm because they act as an entrance point for solutes from the mother plant to the developing endosperm. However, because no calcium channel genes have been expressed at this point, this activity could be performed by PM-type Ca2 + ATPases or exchangers. Three PM-type Ca2+ ATPases and four Ca2+ exchangers are expressed during the S1 stage, and calcium enters the TCs via some of these transporters found in the plasma membrane of TCs on the maternal tissue side. The incoming calcium is pumped into the developing coenocyte by ATPases and exchangers found in the PM of TCs on the other side (i.e., toward the syncytium). Calcium is pumped into the large vacuole by the action of ER-type ATPases located in the vacuolar membrane when the calcium concentration in the coenocytes

reaches a certain level, and because calcium cannot move back into the maternal plant, it is pumped into the large vacuole by the action of ER-type ATPases located in the vacuolar membrane. Calcium exchangers may potentially carry calcium from the syncytium to the developing embryo. Furthermore, other ER-type ATPases with high expression levels may pump calcium into the vacuoles of developing embryonic cells. There could also be Ca2+ ATPases with a similar function. One of the PM-type ATPases is expressed at high levels in the S1 and S2 phases, whereas the other two are expressed at lower levels. Because there is no aleurone layer at the S1 stage, higher-expressing ATPase cells are present in the TCs and are engaged in calcium transfer into the developing coenocytes. The other two ATPases are likely situated on the plasma membrane side of the aleurone layer, where they pump calcium into the aleurone layer from the beginning of the S3 stage until the conclusion of the S5 stage, or until the seed maturation stage (Goel et al. 2012). However, more experimental evidence is needed to determine whether the increase in calcium concentration detected in the seed coat is due to transporters present in the aleurone layer directing calcium to the seed coat or from the maternal tissue.

9.5.3 Potential Promises of Omics Approaches

The potential of "Omics" techniques open the door to new technologies that can aid in gaining an advantage by studying various components of biological systems that are essential for normal cell function. The application of integrative omics-based techniques is predicted to aid in the knowledge of seed biology and developmental processes (Fig. 9.6). These omics-based approaches include the use of genomics, transcriptomics, proteomics, metabolomics, glycomics, lipidomics, and other omics sciences to investigate biological molecules involved in the proper functioning of cells and their physiological mechanisms, as well as their responses to various environmental changes (Kumar et al. 2015a, b).

9.5.4 Phenomics: Characteristics Features of Seeds

Phenomics, which uses a modeling approach to obtain unbiased data from biological systems, will be critical in understanding the complexities of seed biology (Navarro et al. 2016). Seeds contain complicated traits that can be difficult to quantify (Gustin and Settles 2015). These are now required to understand the genetic basis of agriculturally relevant traits and to harness quantitative phenotypes. In resource-constrained contexts, screening for germplasm with high performance attributes would be easier. Plant phenomics has recently introduced and integrated new tools to better describe complex plant phenotypes. With the development of high-throughput phenotyping devices, it is now possible to obtain non-destructive phenotypic data from plants (Rahaman et al. 2015). Researchers have used computational algorithms to decipher the complexity of phenomics data such as plant senescence progression by analyzing distorted and blurred color images under high throughput conditions (Cai et al. 2016), as well as phenomic prediction of maize hybrids to obtain a viable alternative for genomic and metabolic prediction of hybrid performance (Cai et al. 2016; Edlich-Muth et al. 2016).

9.5.5 Genomics and Transcriptomics

The availability of whole genome sequence information for model plants such as *Arabidopsis* has enabled the development of tools to facilitate systems level integration of genes into functional units in massive interconnected biological networks and speed crop improvement (Venglat et al. 2014; Gupta et al. 2017). System biology techniques may now compare transcriptional networks across species that act throughout embryo and seed development, providing deeper insights into conserved and divergent gene networks (Bevan and Uauy 2013). It's also a good resource and reference for other seed systems, such as FM (Dean et al. 2011).

Crop plants have yet to fully leverage the global genetic and metabolic pathways involved

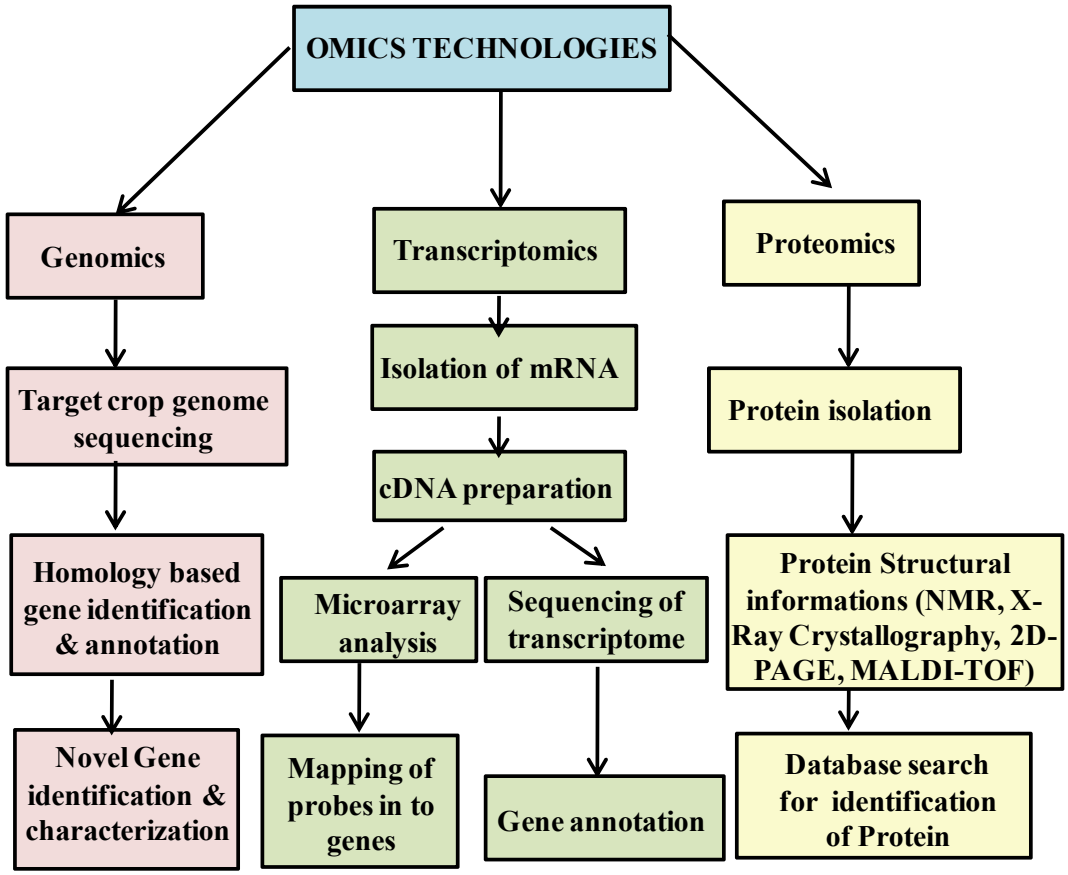

Fig. 9.6 Omics approaches for deciphering the secret of biochemical factories in seeds

in seed development and contribute to seed genetic improvement. The work is being progressed by gaining insights into molecular and biochemical pathways linked with gene expression, protein, and metabolite profiles during seed development in model and crop plants. Comprehensive datasets are being produced as a result of these integrated systems techniques and studies (Venglat et al. 2014). Seed size is governed by genetic variables that have been under continual selection during the evolution of various seed systems in flowering plants, as previously documented (Westoby et al. 2002). The expression of numerous known and suspected TF genes was analyzed during grain filling to investigate the involvement of TFs in the transcriptional regulation of grain filling. A set of TF genes with expression characteristics comparable to those of known grain filling genes was found using cluster analysis. RITA, a bZIP type TF gene, is one of the genes in this group that is strongly expressed in aleurone and endosperm tissues and may have a role in regulating gene expression in developing rice grains. Dof genes, whose gene products can activate the expression of seed storage protein genes by binding to the prolamine-box, and nine known and probable MYB genes are also included in this cluster (Kumar et al. 2009; Gaur et al. 2011; Gupta et al. 2011, 2017).

The size of the final seed is also influenced by the growth of the endosperm and seed coat. The transcription factors AP2 and MADS-box play

essential roles in Arabidopsis seed size regulation. The ap2 mutant's endosperm cellularization is delayed, resulting in larger embryo sacs and embryos with increased cell quantity and size. This larger seed characteristic was handed down through the maternal sporophyte and endosperm genome (Fang et al. 2012). Polycomb (PcG) protein-encoding genes are the second group of genes implicated in seed size regulation. This contains genes that make up the FERTILIZATION INDEPENDENT SEED (FIS) complex, which acts as a transcriptional repressor by methylating histones, as well as imprinting genes (DNA METHYLTRANSFERASE1 and DECREASE IN DNA METHYLATION2), which methylate the PcG genes (Hennig and Derkacheva 2009). HAIKU (IKU) and MINISEED3 (MINI3), which are activated by SHORT HYPOCOTYL UNDER BLUE1, are two genes that govern seed size (SHB1). In monocot crops, however, little is known about the molecular pathways that regulate endosperm cellularization (Figueiredo and Köhler 2014). The inner integument of developing ovules is heavily influenced by the expression of KLUH, a cytochrome P450 expressing gene. The two B-SISTER MADS-box genes TT16 and AGL63, which play roles in Arabidopsis female reproductive organ development, have been discovered to be negative regulators of seed coat differentiation. Larger seeds result from mutations in them (Prasad et al. 2010). Arabidopsis has a ubiquitin receptor called DA1 that controls the final seed and organ size, as well as the cell proliferation period. Seed size is reduced when DA1 is mutated, whereas seed size is increased when it is overexpressed. The function of WRINKLED 1 (WRI1), WRI3 and WRI4 in the control of fatty acid synthesis in numerous plant species, for example, has been shown by genetic study (Li et al. 2008).

9.5.6 Loss and Gain of Gene Function

The introduction of functional genomics technologies such as loss and gain of function in mutants has created a fantastic platform for studying seed biology. The Clustered Regularly Interspaced Short Palindromic Repeats-Cas9 (CRISPR-Cas9) technology has effectively changed the investigation of key gene activities (Rajendran et al. 2015). In seed biology, the method has more potential for identifying functional mutants of rate-limiting enzymes and critical regulatory targets of seed storage components, as well as revalidating models for building seed sinks for nutritional and health benefits (Sreenivasulu 2017).

9.5.7 Quantitative Trait Loci (QTLs) and Genome-Wide Association Studies (GWAS)

Quantitative trait loci analysis (QTLs) has been a precise and powerful way to determining loci and genes that manage essential and complex aspects connected with seed development in the post-genomic era (Macovei et al. 2017). While genome-wide association studies (GWAS) have lately gained popularity due to their ability to overcome numerous constraints of QTL analysis, allowing for a quantitative measurement of the connection between each genotyped marker and a phenotype of interest (Macovei et al. 2017). Several QTLs associated with seed dormancy have been studied in cereals (Wan et al. 2005; Gu et al. 2008; Hori et al. 2007; Sato et al. 2016; Imtiaz et al. 2008; Ogbonnaya et al. 2008) and need to be examined in FM.

9.5.8 Proteomics

The presence of high-abundance storage proteins limits the dynamic resolution of seed protein samples. Several methods for protein extraction have been developed over the last decade (Gupta and Misra 2016), and the use of proteomics approaches will aid in gaining a better understanding of seed biology. Key proteomics technologies such as MALDI-TOF, Edman sequencing, Q-TOF, LC–MS/MS, and others have emerged as the most important players in protein analysis (Deng et al. 2013). Proteomics is particularly useful for crops since it may reveal not just nutritional benefits, but also yield and how adverse conditions affect various aspects (Salekdeh and Komatsu 2007). Detailed proteomic analysis of rice leaf, root, and seed tissues using two independent technologies, 2DE followed by LC–MS/MS and MudPIT, resulted in the discovery of a large number of novel proteins (Ramalingam et al. 2015) involved in central metabolic pathways, transcription control, and mRNA and protein biosynthesis. Furthermore, because the protein composition and functional quality of cereal flour are significantly associated, the proteomics approach is ideal for discovering flour-making protein markers for suitable cultivars.

9.5.9 Metabolomics

Metabolomics has become a helpful diagnostic tool for phenotyping biological systems' biochemical phenotypes. Transcriptomics and metabolomics studies combined revealed similar variations in metabolite and transcript expression levels in diverse settings. DELAY OF GERMINATION (DOG) 1 is a seed-expressed gene that is necessary for dormancy induction. DOG1's function is not limited to dormancy, according to transcriptomics and metabolomics investigations, and it may possibly play many functions during seed development by interfering with the ABA signaling components in Arabidopsis thaliana (Dekkers et al. 2016). During tomato seed germination, however, considerable genetic diversity in metabolite abundance was identified, demonstrating that metabolic composition is linked to germination phenotypes and overall seed performance (Kazmi et al. 2017).

A considerable amount of data pertaining to mass spectra, compound names and structures, statistical/mathematical models, metabolic pathways, and metabolite profile data has been produced and maintained in the form of databases over the last few decades. Although data resources are distributed over the Internet, such databases complement each other and facilitate efficient growth in metabolomics (www).

Available metabolome datasets aid in summarizing the current state of related tool development, with an emphasis on the plant metabolome. Data sharing would pave the path for more accurate metabolomics interpretation and advancements in plant systems biology. Such a large amount of data can be used to extract key information about seed systems, which can then be used to engineer metabolic pathways responsible for nutrient production. It can also be used to develop strategies to increase or decrease the synthesis of high-energy compounds or the manufacturing of any other nutrients in the seed, depending on our needs (Fukushima and Kusano 2013; Kumar et al. 2015a, b).

9.5.9.1 Lipidomics

Lipidomics strives to quantitatively represent the many types of lipids in biological systems, as well as their molecular species. Lipidomics advancements allowed for a new degree of sensitivity and precision in quantitative lipid analysis. The ability of MS-based lipidomics to address the complexity of cell biology inquiries is critical (Brügger 2014; Horn and Chapman 2014). Recent research on Arabidopsis transgenic seeds has revealed where dihydroxy ascorbate (DHA) is collected and joined with other neutral and phospholipid fatty acids in growing as well as mature seeds (Zhou et al. 2014). Lipidomics techniques have the ability to examine the complexities of seed biology from a molecular to a systems level, allowing for the quantification of seed developmental processes that can be used to improve seed nutritional content through genetic engineering.

9.5.9.2 Glycomics-Thermodynamics Approach

Glycomics is the study of all the glycan structures found in a cell, tissue, or organism on a systemic level. It is one of the most promising developing technologies in the post-genomic era for characterization of biological systems. Studies using mass spectrometry-based glycomics and glycoproteomics techniques on N-glycan structures of lotus seed identified 19 N-glycan structures with high mannose (20%), paucimannosidic (40%) and complex forms (40%) and lotus convicilin storage protein 2 (LCP2), which has high mannose N-glycans and serves as a model system for deciphering the role of seed proteins and their glycosylation in food allergy (Dam et al. 2013). The seeds of plants with a high quantity of biomolecules such as carbohydrates, which include complicated information for conveying biopolymers, are gaining popularity among scientists (Hu et al. 2015). Glycosylation alterations have been observed throughout a number of important events, including embryogenesis and differentiation, and it may potentially play a role in seed formation. Understanding the role of glycan during seed development may therefore be useful in identifying important molecules that can be used to better understand the complicated biology of seeds and their developmental processes.

9.5.9.3 Vitamin Analysis

Vitamin analysis, like that of other chemicals present in seed tissues, can be done using a variety of detectors depending on their biochemical structures. Water-soluble vitamins can be determined simultaneously using an ion-pair reversed-phase HPLC separation method and an ESI–MS/MS detection system. It has also been demonstrated that it is effective in extracting complete vitamin content from meals. It has also been demonstrated that thiamine (B1), riboflavin (B2), nicotinic acid, nicotinamide, pyridoxine (PN), pyridoxamine (PM), piridaxal (PL), thiamine-monophosphate (TMP), riboflavine-5'-phosphate (FMN), and piridoxal-5'-phosphate (PLP) can all be separated using 0,05% (v/ (Engel 2009). These approaches can be used to detect the numerous vitamins contained in the seed, as well as their concentrations, which can help in the production of agri-food items with high nutritional value.

9.5.9.4 Minerals Analysis

The presence of several micro and macro nutrients in seed tissues makes them an important source of nutrition for humans. Mineral analysis is a widespread activity in the agricultural research field. Synchrotron X-ray microfluorescence was used to examine the in vivo mineral distribution profiles in rice grains, as well as alterations in those distribution patterns during advanced phases of germination (XRF). Mineral translocation from certain seed sites during germination was element specific as well. The mobilization of K and Ca from grains to growing roots and leaf primordia was found to be high, while the flow of Zn to these expanding tissues was found to be lower than that of K and Ca. At least during the first several days after germination, the mobilization of Mn or Fe was modest.

9.6 Systems Biology: A Holistic Approach to Seed Biology Data Integration and Analysis

In any organism, a system is made up of a set of components or elements such as genes, proteins, metabolites, and so on. These system components are designed to promote the flow of knowledge, either directly or indirectly, and to preserve the system's balance, which is essential for its survival and achievement of its goal. Systems biology is a holistic approach to studying its behavior through the integration, annotation, modeling, and analysis of high-throughput omics data provided by available omics platforms (Pathak et al. 2013; Kumar et al. 2015a, b).

Progress in "omics" technologies have produced a massive amount of molecular data about seed, and substantial efforts have been made to

decode biological systems as true systems in order to conduct affordable and cost-effective research in systems biology. Bioinformatics methods involving database handling, modeling, network analysis, and simulation result in significant improvement in systems level understanding of biological systems when high-throughput omics data is analyzed (Kumar et al. 2012; Gupta and Misra 2016; Pathak et al. 2017).

Systems biology is critical for dissecting the intricacy of each component and its particular function in seed developmental processes, as well as assisting in the increase of nutrient output through metabolic engineering, biofortification, and other biotechnological approaches that will lead to increasing contents of nutrients in the seed (Fig. 9.7).

The embryonic development of seeds is a large-scale metabolic conversion process. Photosynthates and amino acid precursors are imported and converted into oil, protein, and storage polysaccharides in this process. The developmental processes are genetically predetermined and influenced by external factors (Li et al. 2015). Current biological network models depend upon accumulation data, which makes it impossible to predict the entire picture of cellular metabolism (Gurwitz 2014). Previous omics-based studies using systems biology methods have looked into a group of unique genes and their molecular mechanisms by combining transcriptomics and metabolomics to decipher the global developmental and metabolic networks that find out the structural features and examined the biochemical composition of mature seeds of soybean (Li et al. 2015). Such research may aid in the identification of nutritional protein contained in seeds to combat hidden hunger, starvation, and preserve health, as well as the production of nutraceuticals products for societal gain.

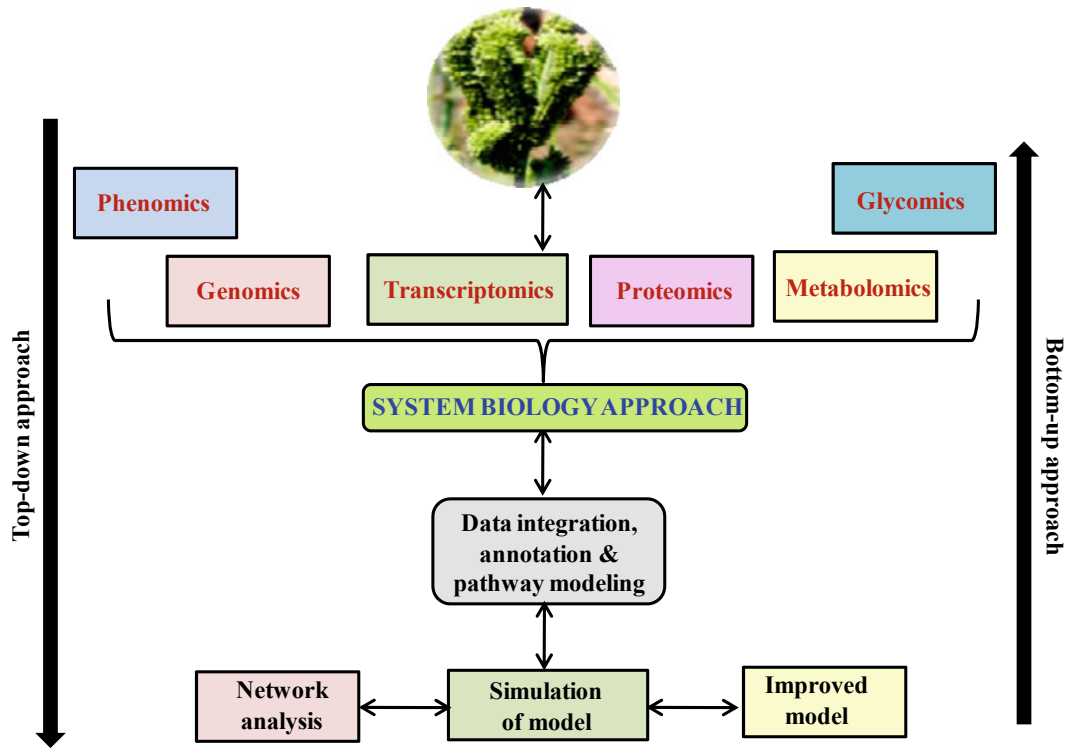

Fig. 9.7 Systems Biology methods are being used to decode the dynamics of seed and its developmental mechanism

9.6.1 Integrative Seed Systems Biology

It starts with sample collection followed by wet-lab experimentation using high-throughput omics platforms including genomics, transcriptomics, metabolomics, phenomics, glycomics, lipidomics, and data analysis, and continues with analytical methods, data integration and interpretation, and investigation of useful information. It aims to develop integrative models focused on data analysis and system organization at the molecular level, with a focus on detailed queries at specific scales. This strategy is also regarded as a "top-down" approach (Kumar et al. 2015a, b; Gupta and Misra 2016).

Recent advances in omics platforms have begun to provide information on a few primary genes/metabolites needed for seed development. mRNA abundance analyses and visualizations of during seed growth, maturation, and beyond are revealing global gene expression patterns that decide the final seed structure and composition in various crops (Watson and Henry 2005; Li et al. 2005; Furutani et al. 2006; Gallardo et al. 2007; Collakova et al. 2013; Palovaara et al. 2013). Accessible public domain information/data can be used for integration and analysis to explore the dynamics of seed biology at the molecular level in order to gain a full image of seed developmental processes.

9.6.2 Predictive Seed Systems Biology

Predictive systems biology or "Bottom up" approach focuses on the molecular mechanism and role of various components in biological/seed systems by building mathematical models and formulating hypotheses based on the detailed data provided by top-down approaches. Data retrieval from various database resources, literature mining, pathway modeling, network design, and perturbation analysis using systems biology techniques to predict the behavior of all the components present in systems are the key steps in the bottom up approach (Kumar et al. 2015a, b; Pathak et al. 2013; Gupta and Misra 2016).

The majority of currently available biological network models are focused on transcript accumulation data, which does not provide a full picture of cellular metabolism (Gurwitz 2014). As a result, combined omics dataset analysis is needed, and software is being developed to resolve the challenges of these "big data" analyses (Nadeau et al. 1995; Hur et al. 2013). Recent systems-based studies have clearly demonstrated the incorporation of transcriptomics, metabolomics, and metabolic flux analyses (MFA) for a deeper understanding of the metabolic and regulatory network that regulates soybean seed composition (Li et al. 2015). The MetNet systems biology platform was used to analyze data in order to establish hypotheses and predictive models related to the organization and control of metabolic networks (Wurtele et al. 2007; Sucaet et al. 2012). Additionally, 37, 593 Glycine max probes were annotated as model systems for studying soybean seed biology using sequence homology with *Arabidopsis* (Li et al. 2015).

9.6.3 Intermediate Approach in Seed Biology

The scientific community faces challenges in comprehending the complexities of biological processes. As omics-based high-throughput experiments assess the complexity of systems at multiple levels such as intercommunicating cell groups, interaction of multiple molecules in active pathways, and altered states to generate profiling data. This information will be used to bridge the difference between top-down and bottom-up approaches. Intermediate methods are used to integrate different biomolecules in an organized way from the cell to the system stage, allowing for the quantification of biological processes and the filling of unknown information for better understanding of biological systems (Butcher et al. 2004; Gupta and Misra 2016).

9.6.4 Tools and Databases for Seed Systems Biology

The development of databases resources and software packages for the study of biological systems is the most challenging field today because it requires a multidisciplinary team of researchers with strong backgrounds in fields such as biological sciences, physical sciences, chemical sciences, mathematical and statistical sciences, and computational sciences to create effective and user-friendly tools and databases. We identify some key computational systems biology databases and tools that should be used to decode the complexities of seeds at the molecular to systems level for characterization of important genes/proteins for the development of nutraceuticals and functional foods as well as other applications (Table 9.2).

Table 9.2 List of some important tools and databases of computational and systems biology

Softwares/databases	Application	Availability
R and bioconductor	It is a well-known software program for decoding the complexity of biological systems using next-generation sequencing data, functional genomics, network biology, and other applications	https://www.r-project.org/, https://www.bioconductor.org/
Cell designer	CellDesigner is a tool for designing, visualizing, and simulating biological systems' pathways	http://www.celldesigner.org/
Cytoscape	It is well-known systems biology software for high-throughput data integration and modeling, as well as visualization and analysis of biological networks	http://www.cytoscape.org/
Biological networks	It's a network systems biology tool for integrating and modeling multi-scale data and other applications	http://biologicalnetworks.net/Software/index1.php
Matlab	Matlab is a well-known program for biological system modeling and simulation	http://in.mathworks.com/products/matlab/?requestedDomain=www.mathworks.com
CLC genomic workbench	CLC Genomics Workbench is a piece of software that allows you to examine and visualize high-throughput omics data	http://www.clcbio.com/
PMR (Plant/Eukaryotic and Microbial Systems Resource)	It's a database of plant and eukaryotic microbe metabolomics data	http://metnetdb.org/PMR/
KEGG	KEGG is a well-known database resource for gaining a better grasp of the complicated functions and applications of biosystems	http://www.genome.jp/kegg/
GEO	GEO is a functional genomics data repository that houses gene expression data generated by high-throughput omics platforms. These data can be reused by the scientific community	http://www.ncbi.nlm.nih.gov/geo/
Array express	It's a functional genomics experimentation database that stores gene expression data from microarray and other high-throughput sequencing tools for researchers to reuse	https://www.ebi.ac.uk/arrayexpress/
Plant metabolic network	PMN is a database that contains information about plant metabolic pathways	http://www.plantcyc.org/

9.6.5 Application and Expected Outcomes

Genome sequencing of crop plants on a large scale, helped by high-throughput omics technologies, data integrations, modeling, and visualization of important genes and proteins, and by studying critical components present in seed with structural and functional details, simulation analysis methodologies have opened up new paths for bridging the gap in seed systems biology and identifying its undiscovered processes. When we use diverse genomic, transcriptomic, and other omics data about seeds for analysis through integrative and predictive approaches to develop seed as a model with complete information of each component present in the seed, and that information can be used to develop nutraceutical products, systems-based approaches will be very useful. Furthermore, it may contribute to future food and nutritional security (Kumar et al. 2015a, b).

9.7 Conclusions

To encourage translational research to engineer seed systems, it is critical to study the omics data of seeds, their integration, and annotation for identification of key components associated with nutrient loading during seed developmental processes, followed by their validation, using effective and precise molecular and systems biology techniques, which would provide a forum for researchers to investigate the secrets of biochemical seed factories in order to better understand their existence and produce sustainable seeds that can thrive in a variety of environments and geographical regions. Several genes and transcription factors affect the growth of the seed tissue system. The nutrient distribution event is governed by a complex network, which these omics-based tools are successfully exploring. System biology has tremendous potential in designing of research program for nutritional biology. From a very basic standpoint, we now have a well-established forum on which to continue seed biology research in order to improve nutritional quality of seed. The model plant system has sparked ideas for a wider prospectus and methods to make this more predictable. Thus, a comprehensive informational study is needed, which will open up a new horizon of molecular and developmental biology studies to aid nutrition biology research.

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Dr Anil Kumar He has conferred with several fellowships from National Academy of Biological Sciences, Confederation of Horticultural Associations of India, Indian Society of Agricultural Biochemists and also member of National Academy of Sciences, India

A Nutritional Crop Factory of Quality Seed Storage Proteins in Finger Millet for Combating Malnutrition

10

Apoorv Tiwari, Supriya Gupta,
Pramod W. Ramteke, and Anil Kumar

Abstract

Finger millet is a nutrition-rich crop to combat starvation, malnutrition, and water security for peoples. This enables to explore opportunities to increase finger millet production with enormous capacity for agriculture aimed at enhancing the health of malnourished people. Implementation in basic and translation studies by newer approaches has resulted in the overwhelming growth of scientific data. Strategic innovations in agriculture, i.e., enough availability of food has led to the growth and choice of high-yield varieties with the availability of quality proteins having enriched amount of essential amino acids, i.e., >40% of eight essential amino acids in the building blocks of seed storage proteins (SSPs) as per the recommendation of World Health Organization. SSPs offer nutritional proteins for people and animals as well as a source of nitrogen and sulfur for germinating seedlings. Therefore, SSPs are closely related to our life. Currently, OMICS and other high-throughput platforms have been used for the characterization of high-quality proteins in cereals and pulses. Considering the nutritional significance of finger millet, attempts were made first time in our lab to identify the seed storage proteins of finger millet using genomics-transcriptomics transition approaches and identified many variants of albumin, globulin, glutelin, and prolamin proteins with four proteins were having more than 40% essential amino acids. Gene sequence of two SSPs, albumin (*fima1) and* prolamin *(fimp2)* having 9% lysine and 15.8% methionine, respectively, were cloned and recombinants proteins have been expressed in *E. coli* system. This chapter provides an overview of SSPs, including their categorization, accumulation in seed cells, and breakdown of SSPs during seed germination. Multi-OMICS and in silico approaches are

A. Tiwari
Department of Computational Biology and Bioinformatics, Jacob Institute of Biotechnology and Bio-Engineering, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh 211007, India

A. Tiwari · S. Gupta · A. Kumar (✉)
Department of Molecular Biology & Genetic Engineering, G.B. Pant Univ. of Agriculture and Technology, U.S. Nagar, Pantnagar, Uttarakhand 263145, India
e-mail: anilkumar.mbge@gmail.com

A. Kumar
Rani Laxmi Bai Central Agricultural University, Uttar Pradesh, Jhansi, Uttar Pradesh, India

P. W. Ramteke
Department of Molecular Biology & Genetic Engineering, RTM Nagpur University, Nagpur, Maharashtra, India

Department of Life Sciences, Mandsaur University, Mandsaur, Madhya Pradesh, India

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helpful to identify the SSPs in finger millet along with the association studies to pin point the role of genes/alleles involved in grain protein accumulation besides prospects to produce bulk amount of recombinant proteins with nutritional superiority to use as nutraceuticals.

10.1 Introduction

Global cereal production is projected to expand by 375 Mt (Metric Tons), to reach 3054 Mt in 2029, mainly driven by higher yields (OECD-FAO Agricultural Outlook 2020–2029). Several short-term interventions have increased productivity but spoilt sustainability and eroded the resource base's ability itself, which leads to nutrient-deficient salty soil and a reduction in water supplies (Raza et al. 2019). Approximately one-third of the world's population lives in dried soil, which makes up 40% of the world's area. The World Bank report suggests that 815 million individuals globally face hunger as a challenge. In an agriculture-based country like India, the number of farmer suicides has risen to more than 50 a day on average, which depicts the sternness of the agriculture crisis even after a good production (Thomas and Taveriner 2017).

To satisfy world starvation (cereal demand) and increase farmers' incomes, sustainable crop substitutes are required and therefore the importance of millets in ensuring long-term food security cannot be overstated. The content of protein in millets is regarded to be equivalent or superior to the proteins of wheat (*Triticum aestivum*), corn (*Zea mays*), sorghum grain (*Sorghum bicolor*), and rice (*Oryza sativa*) (Kumar et al. 2018). Millets play a part in the design of contemporary foods such as multigrain and gluten-free cereals. Due to its richness in polyphenols and other bioactive compounds, millets are considered medicine for heart disease, diabetes, and higher stress and is also considering lower fat inclusion, releases sugars slowly (low glycaemic index) (Ugare et al. 2014). Finger millet is the sixth largest crop in provinces of Central, East Africa, and southern Indian regions, supplying vital nutrition (Vinoth and Ravindhran 2017). Finger millet is a grass belonging to Poaceae family and is frequently referred to as ragi in India. It has evolved as high nutritional value crop than other cereals and millets, primarily because of its elevated nutrients, vitamins, minerals, fiber, and quality proteins. Finger millet proteins have not been reported as allergenic as wheat. Finger millet can be produced and used as preventive medicine against osteoporosis and has calcium as high as 450 mg/100 g of grains (Puranik et al. 2017). Finger millet has numerous advantages like a large food in the livestock industry (Bhagwat 2019). This enables to explore opportunities to increase finger millet production in fields with huge capacity for agriculture aimed at enhancing the health of small-scale producers (Gupta 2017) (Fig. 10.1).

10.2 Food and Nutritional Security Require Adequate Protein

Proteins are substances that contain nitrogen and are composed of amino acids and serve as the muscle as well as other tissues' major structural component of the body. These are also used for the processing of hormones and hemoglobin. Proteins can also be used as fuel, but as an energy source, they are not the primary choice and required to be metabolized in the simpler form of amino acids for proteins to be used by the body (Hoffman and Falvo 2004). It has been identified that 20 amino acids are necessary for development and metabolism process. Among them 12 amino acids are considered non-essential, indicating the body will synthesize them and do not need to be ingested in the diet and the remaining 8 amino acids are not able to be synthesized in the body and are defined as necessarily implying to our diets (Lopez and Mohiuddin 2021). In order to fulfil the daily requirement for essential amino acids by the human body, a balanced intake of quality proteins are required in our diet. According to the recommendation of World Health Organization (WHO), the proteins having >40% essential amino acids in its structural composition are

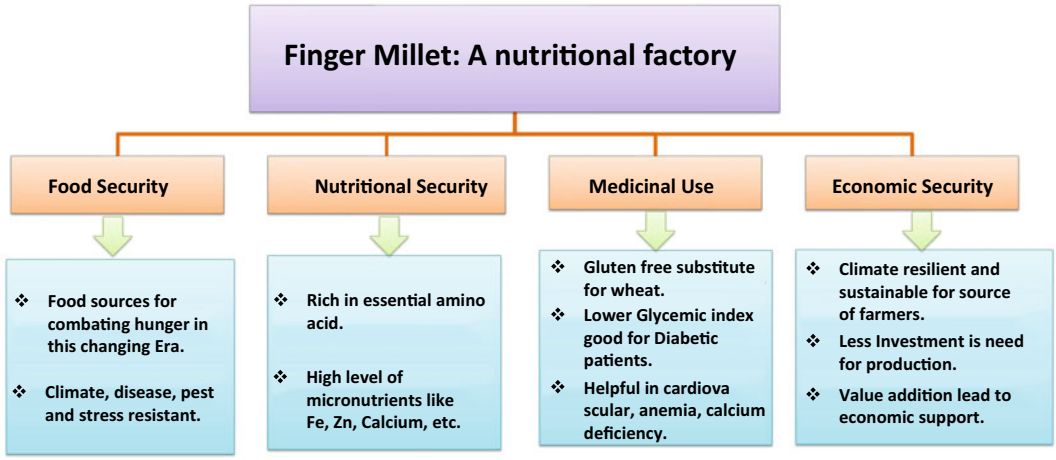

Fig. 10.1 Finger millet as a nutritional and economic security crop

referred to as quality proteins and nutritionally superior for not only combating protein energy malnutrition but also in turn augmenting the health security (Hoffman and Falvo 2004).

10.3 Protein for Life

Dietary methods for health promotion and independence sustenance in lives are required with a growing population partly by preserving muscle weight and endurance when individuals become old. New proof indicates that existing nutritional fiber intake suggestions may not be enough to accomplish this objective and that people may profit from enhancing their intake and incidence of high-quality protein consumption (Dwyer et al. 2015). However, there are doubts about the economic impacts of increased manufacturing of animal food, and substitute, more viable forms of food must be regarded. Protein is more required than other major macronutrients and has been uncertain if diets of plant protein impact the appetite of elderly people who are likely to become malnourished. In the last decade, a lot of attention was paid to plant nutrition (Lonnie et al. 2018). The protein requirements of aging populations (>40 years old), sustainable protein supplies, and dietary appetite-related consequences of plant protein were analyzed.

The ever-increasing demand for inherently protein-rich products forms a portion of an ecological discussion about encouraging more viable alternatives. In advanced nations, the elevated share of animal-based protein consumption increases safety and environmental concerns. The first of these is the enhanced danger of obesity, diabetes, cardiovascular disease death, and certain cancers in food-borne models characterized by elevated intakes of animal protein (Anand et al. 2015). However, it should be stressed out that the dietary model describes the whole diet and that all components in animal-based patterns (e.g., meat, fish, eggs, and dairy) have an equal and harmful effect on health cannot be stated (Battaglia et al. 2015; Birol et al. 2015; Campbell 2017).

The protein substance and the arrangement of amino acids differ between plant and cereal plant protein, methionine and cysteine are restricted, oats (lysine, tryptophan), nuts vegetables (cysteine, methionine, threonine), and ocean growth (histidine).

Investigating elective protein sources and progressing toward increasingly manageable, plant-based protein regimens has been an ongoing exploration need (Henchion et al. 2017). As indicated by the Food and Agriculture Organization (FAO) definition, practical weight control plans have “low natural effects which add to

nourishment and sustenance security and solid life for the present scenario." It has been very much reported that a plant-based diet can bring down the danger of cardiovascular disorder, sicknesses diabetes, hypertension, weight, metabolic disorder, and mortality, just as anticipate explicit kinds of malignant growth. Human food safety needs the processing of enough protein and dietary energy of high quality (Petrie et al. 2018).

10.4 Seed Storage Proteins of Finger Millet

Seed storage proteins are proteins that accumulate considerably in the growing seed and act as a reserve for nitrogen, carbon, and sulfur. During seed germination, these proteins are rapidly mobilized and provide the primary source of reduced nitrogen for developing seedlings. Storage proteins are not commonly involved in enzymatic functions. Despite the fact that storage proteins from different plants have different architectures, they all share some similar characteristics (Fujiwara et al. 2002). Storage proteins have a higher proportion in some tissues at a specific stage of development, which is one of their major features. Protein bodies are membrane-bound organelles that accumulate proteins and storage proteins are kept separate from the cell's metabolic compartments by being sequestered within protein bodies. Seed storage proteins are among the first proteins to be identified and are the primary source of plant proteins in the human diet. For example, in 1745 (Beccari 1745), wheat gluten was first extracted, and in 1859, Maschke had crystallized Brazil nut gluten. Nonetheless, literature on protein storage from seed goes back to the turn of the century, when Osborne (1924) classified them based on their solubility in water (albumins), distilled saline (globulins), fatty acid mixtures (prolamin), and diluted acids or alkaline solutions (glutyls). These were listed in classes and albumins, globulins, and prolamins are the key storage proteins for seeds (Shewry et al. 1995). In certain stages of development, plants produce storage substances such as starch, lipids, and proteins. The retention of proteins in the preservation of vegetative and reproductive tissues acts as a repository for the potential use of plants. SSPs are a group of proteins produced primarily during seed development and stored for the creation of embryos during germination in seeds that serves as nitrogen sources. The cereal grain's total protein content is 10–15% of its dry mass. SSPs normally occur inside the membrane of the vesicle (protein bodies, aleurone grains) in an aggregated state. Cereal seeds are synthesized as a nitrogen supply for germinating plants and used as a source of human and livestock nutrients. Because the SSP accounts for a considerable portion of the whole seed nutrition, the nutritional efficiency of the seed is linked to the value of the SSP and the structure. Soybean globulins are nutritionally and practically useful in the food industry (Tandang-Silvas et al. 2011). The protein content of seeds ranges from 10% (in cereals) to 40% (in nuts) (Shewry et al. 1995). The genes encoding the Triticeae's primary storage proteins have two origins: an ancient gene family found in the progenitor of monocotyledonous and dicotyledonous plants and a sequence of more recently evolved repetitions. Cereal seeds have a high concentration of storage proteins, which are degraded during germination to supply nutrients to the seedling (Gaur et al. 2018a). The cereals, which typically account for more than 50% of the total endosperm proteins, are analyzed in more detail. Prolamin storage proteins are usually maintained and organized into protein bodies within the ER (Endoplasmic reticulum) (called zeins in Zea mays and kafirins in sorghum) which are also abundant in seeds of crop plants with large amount of leucine and alanine (Otegui et al. 2006).

Because of the abundance and influence on seed consumption, seed storage proteins are the most studied plant proteins. These proteins differ greatly in structure and characteristics across and between species. Nonetheless, the great majority

are classified into only four categories based on their solubility and sedimentation factors. The most extensively distributed seed proteins are globulins, which constitute the majority of storage protein fractions in dicotyledonous plants as well as certain cereals (oats, Avena Sativa, and rice) (Liu et al. 2017). Because both forms of protein are stored in certain species, the comparable structures of 7S and 11S globulins may enable their packing together in the same protein bodies. In some species, however, either one or both kinds of globulin are stored with prolamins (cereals) or 2S albumins. Prolamins and 2S albumins are also members of a same protein superfamily, with the majority of members sharing a conserved pattern of cysteine residues in a tightly folded helical domain (Gell et al. 2017). However, there is limited overlap between 2S albumins and prolamins. The 2S albumins are abundant in dicotyledonous seeds but have yet to be identified in monocotyledonous plants, despite the fact that their existence in fern spores indicates an origin prior to that of monocotyledonous and dicotyledonous plants (Table 10.1).

10.4.1 Prolamins

Initially, the prolamin superfamily was defined on the basis of the preserved cysteine residue sequence in sulfur-rich seed storage prolamins, monocotylase-trypsin, and 2S storage albumins inhibitors. Some low-molecular-weight allergy proteins, including soybean hydrophobic protein, non-specified lipid transfer protein, and α-globulins, have also been described as belonging to this superfamily (Kawakatsu et al. 2010). A group of eight cysteine residues conserved cysteine skeletons, with a distinct Cys–Cys and Cys–X–Cys motif (X representing any other residue). In the case of alpha-amylase/trypsin inhibitors, there are two extra cysteine residues. Members of this superfamily share a common 3D structure, in addition to the prolamins for seed storage that characterize the insertion of an extensive repetitive domain. This contains a bundle of four disulfide bonds in the nsLTPs that are stabilized by a lipid-binding tunnel, which has degraded into 2S albumin structures. Many of these proteins can lead to their protection

Table 10.1 Properties of seed storage proteins

S. no	SSPs	Properties
1	2S Albumins	• Soluble in water • Molecular weight (Mw) typically ~10,000–15,000 • These proteins are post-translationally processed to produce large and small subunits having two intra-chain bonds inside the large subunit and two inter-chain disulfide bonds. They are not glycosylated, and certain constituents are high in methionine
2	7S Globulins	• These proteins are soluble in the dilute salt solutions • Typically trimeric proteins of Mw 15,0000–19,0000 • Subunits can be glycosylated and proteolytically digested • Cystine and methionine levels are low or non-existent
3	11S Globulins	• In dilute salt solutions, it is soluble. Mw 30,000–45,0000 hexameric proteins are typical. Mw 60,000 subunits are generally post-translationally processed to yield Mw 40,000 (acidic) and Mw 20,000 (basic) chains linked by one inter-chain disulfide bond • Cystine and methionine levels are low • Rarely glycosylated
4	Prolamins	• Structures vary greatly, with component Mw ranging from 10,000 to 10,000,000. Monomeric forms and high Mw polymers stabilized by inter-chain disulfide bonds are examples. Proline and glutamine are abundant, while lysine and, in certain circumstances, tryptophan, threonine, and methionine are deficient. There will be no glycosylation or proteolytic processing • When native and/or reduced, it is soluble in alcohol/water combinations • A source of cysteine and methionine

against proteolysis by maintaining their allergenic properties. As a consequence of the repetitive domain abundant in the amino acids of proline and glutamine, the cysteine skeleton and α-helical arrangement that are normally typical of the prolamin superfamily have degraded in seed storage prolamins (Balakireva and Zamyatnin 2016). The physical–chemical properties of these repetitive domains of the seed storage prolamins are dominated by a loose spiral structure made up of a complex ensemble of unfolded and secondary structures containing overlapping structures including β-turns and poly-L-proline II. Proteins are normally insoluble in diluted salt, either in their native state or after inter-chain disulfide bonds have been reduced (Kawakatsu and Takaiwa 2010).

10.4.2 2S Albumins

2S albumins are a leading class of seed storage proteins usually synthesized as single chains of 10–15 kda in the seed, which can be processed in a translational manner such that small and large subunits are commonly combined with disulfide bonds. The system of processing differs depending on species, with sunflower albumins being single-chain albumins and Brazil nut albumins being two-chain albumins. They can act as both industrial (due to dust inhalation) and dietary allergens (Moreno and Clemente 2008).

10.4.3 Lipid Transfer Proteins

The name of these proteins derives from the fact that they were found in plants because of their capacity to transport lipids in vitro, but their exact biological function in plants is unclear. They can play a role in plant defence because their expression is regulated by abiotic stress and they belong to pathogenesis-related protein group 14. They are present in plant outer epidermal tissues such as the peel of peach or apple fruits, and their lipid-binding properties have led to the theory that they are active in transporting the cutin and suberin monomers to plant outer tissues, where they polymerize to form outer waxy layers. They are the most widely distributed type of prolamin, found in a variety of plant organs such as seeds, fruit, and vegetative tissues, and have been labeled pan-allergens. As a result, in addition to being included in a variety of fruits and nuts, they have also been reported as allergens in the pollen of many plant species (Vergnolle et al. 1992).

10.4.4 Bifunctional Inhibitors

This class of allergens is limited to cereals, individual subunits that serve as inhibitors of, amylases from insects, trypsin or both, giving rise to the word bifunctional allergen. These proteins can serve as allergens in wheat flour allergies, such as baker's asthma, or in food sensitization through the gastrointestinal tract. They were first found in extracts made up of chloroform and water and are often referred to as CM proteins, but they are often soluble in water, dilute salt solutions, or alcohol and water mixtures (Radauer et al. 2008).

10.5 What Are the Quality Proteins from Plant Sources?

Adequate human nutrition is dependent on the consumption of a variety of nutrients included in the food. Proteins, which include essential amino acids, are necessary macronutrients. The nutritional quality of a protein supply might vary in terms of digestibility, amino acid composition, and bioavailability containing cysteine and methionine. Thus, with an ever-increasing global population, a contemporary problem is the consumption of low-cost, easily available proteins that fulfil environmental and social requirements. Several well-known plant protein sources may feed the human diet and aid in overcoming the population growth issue (Henchion et al. 2017). Plant proteins, depending on the source, may be lacking in some important amino acids. Cereals often have low Lys levels, whereas legumes have a sulfur amino acid deficit (Met and Cys).

However, pseudo-cereals (such as amaranth and quinoa) are high in Lys. Also, due to changes in climate and soil diversity, geographic altitude and latitude, precipitation levels, agricultural techniques, and varietal/cultivars, the same plant might vary in composition (e.g., macronutrients, such as protein and oil content, and amino acid profile) (Cremer et al. 2014).

Millets contain significant levels of essential amino acids, including Lys, beyond the amounts suggested by WHO/FAO/UNU for humans, making it a good alternative for increasing protein intake in diets. Finger millet is an important source of protein for India's rural population. As a result, the protein composition and quantity of this cereal are critical. Prolamin and glutelin are the main protein fractions based on solubility fractionation. White finger millet genotype has more prolamin but less glutelin than brown finger millet. The increase in protein content of finger millet varieties is mostly due to an increase in the grain's prolamin fraction, as has previously been documented in a few other types of cereal. The amino acid composition of finger millet protein reflects the usual tendency for prolamin rise with grain protein content increase (Sachdev et al. 2020). The negative association seen between lysine levels and protein content of finger millet can be explained to the prolamin fraction's significant lysine deficit when compared to the other protein fractions. Varietal variations in glutamic acid, proline, valine, leucine, and isoleucine levels are also consistent with variances in prolamin content. Differences in the amino acid content of different maize types and sorghum have been attributed mostly to rising percentages of prolamins as total protein increases. The essential amino acid content of a protein can be used to assess its nutritional quality (Huang et al. 2004). Finger millet protein is relatively low in lysine but has acceptable levels of other necessary and related amino acids. However, when finger millet is the only source of protein in the diet, the quantity of protein in the grain is insufficient. As a result, it is profitable to produce finger millet genotype with increased protein content (Zamaratskaia et al. 2020).

10.6 Synthesis, Deposition, and Regulation of Seed Storage Proteins

SSPs accumulate in the endosperm from the early accumulation storage stage to the late accumulation storage stage. SSPs are produced on the rough endoplasmic reticulum (ER) (Fig. 10.2), although the timing of protein production changes throughout seed development. Albumin and globulin protein fractions are synthesized in the early stages of seed development and are deposited in the abaxial cotyledon surface of embryo and aleuronic tissue, whereas prolamin and glutelin proteins are synthesized in the later stages of seed maturation and are deposited in the protein bodies and protein matrix (Fontanini and Jones 2002).

During seed growth, the regulatory network of transcription factors, hormones, miRNA, and other genes organizes the accumulation of SSPs. The endoplasmic reticulum (ER) produces seed storage proteins, which are then transferred to protein storage vacuoles (PSV), where they are predominantly kept. The mature SSPs are delivered in two ways: Golgi-dependent and Golgi-independent. Seed growth and development are inextricably linked to the buildup of seed storage reserves. The seed growth method begins with twofold fertilization. The embryo and endosperm are protected by maternal integuments and a possible seed coat. Organic nutrients like sucrose, amino acids, and potassium are imported from the maternal tissue to the seeds during the early stages of development via vascular tissue within the funiculus, which terminates in the chalazal portion of the seed coat. The released nutrients are largely stored in the endosperm, which provides sustenance to the growing embryo. Transcriptional regulation of SSP accumulation occurs during the seed development phase. The transcription factors (TFs) LEC1, LEC2, FUS3, and ABI3 are known to play a role in seed development, SSP aggregation, and regulating the expression of several other metabolic pathway regulators in Arabidopsis (Gacek et al. 2018) (Fig. 10.3).

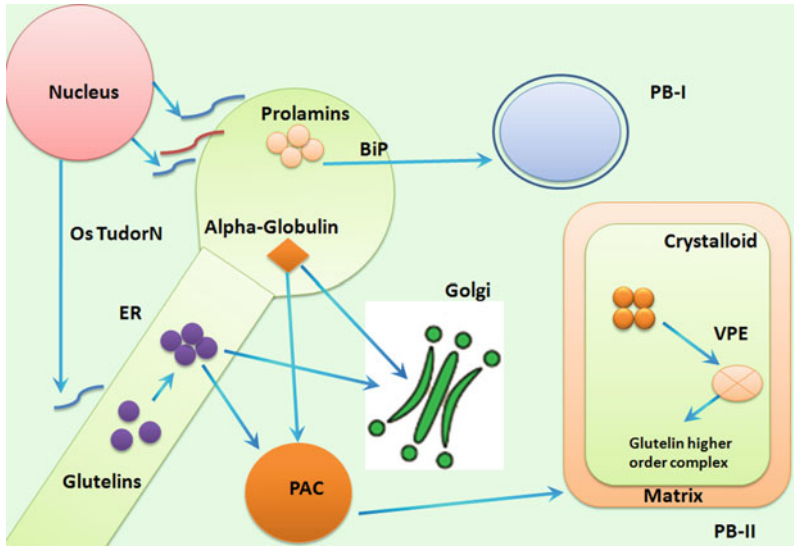

Fig. 10.2 Synthesis, trafficking, and deposition of different SSPs

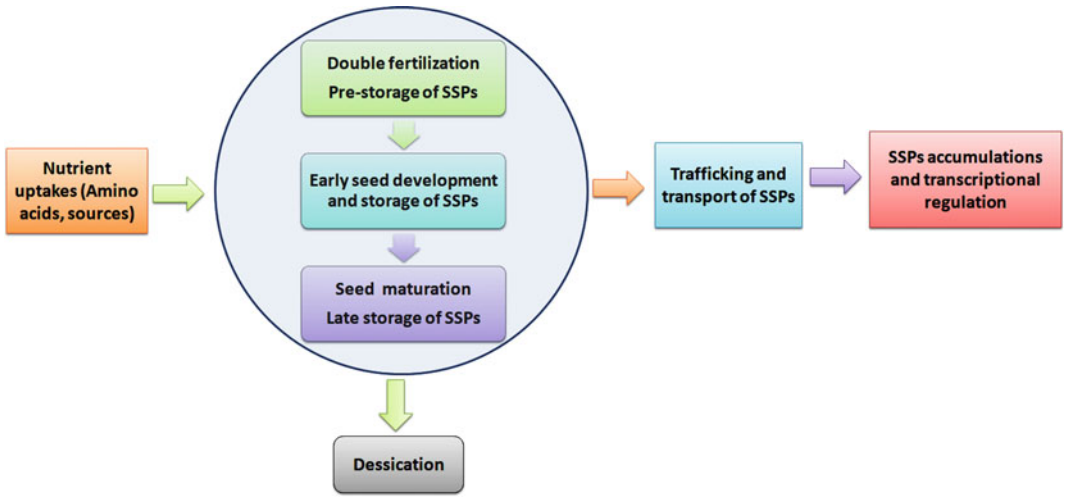

Fig. 10.3 Transcriptional regulation, accumulation, and translocation of seed storage proteins (SSPs)

10.7 Use of Omics Approaches for Studying Seed Storage Proteins

Advent of *OMICS* approaches has revolutionized the field of nutritional biology and use of such genomics-transcriptomics transition, proteomics, metabolomics, genotyping by sequencing, and other high-throughput technologies are being used in deciphering complex process of seed development, biology, and nutritional traits for understanding the mechanisms of accumulation of nutrients and SSPs during the different stages of seed development (Kumar et al. 2015).

10.7.1 Genomics for Identification of SSP Genes and Cloning of Quality Protein Genes

Seeds are the sources of a wide range of foods, magnesium, and amino acids, including phosphates and leucine**.** Different genomic resources/properties for finger millet have been made available in NCBI. Plant breeders will benefit from the genome sequencing initiative in terms of allele selection, genetic mapping, and recognition of candidate genes for agronomically significant traits. The availability of finger millet genomic resources will expand novel breeding opportunities to solve future problems in finger millet improvement. (Hittalmani et al. 2017). PBF (prolamin box-binding factor) transcription factors regulate the seed storage proteins of cereals (Gupta et al. 2011). However, advancements in laboratory techniques like as high-throughput sequencing and mass spectrometry have resulted in an increase in the output of protein information. This has demanded precise annotation, categorization, characterization, and decoding of these sequences biological function (Radhika and Rao 2015). Recently, the genome of finger millet is sequenced and efforts have been done to find and annotate the genes coding for SSPs. The experimental findings from the whole genome sequencing and assembly procedure of a finger millet cultivar yielded 1196 Mb, which accounts for approximately 82 percent of the overall predicted genome size. The existences of 85,243 genes were discovered by genomic technologies, and one-half of the genome is repetitive in nature. When compared to other Poaceae plants, the finger millet genome had higher co-linearity with foxtail millet and rice. Functional annotation and transcription factor mining showed that the finger millet genome contains a significant number of drought tolerance genes. In a recent study, we have identified 18 SSPs using genomic and transcriptomic approaches. Using NGS applications, a set of 18 SSP genes were identified in finger millet transcriptome and their physical location in the genome was defined. A comprehensive analysis was done by annotation and characterization of identified SSPs, while their expression pattern was analyzed by calculating fragments per kilobase of transcript per million mapped reads (FPKM). Molecular functions of all 18 SSPs were predicted using the gene ontology approach by mapping the blast, InterPro results. It was found that the genes were involved in different molecular functions like nutrient reservoir activity, molecular function regulation, enzyme regulatory-inhibitory activity, lipid, ion binding, and immunoglobulin binding (Hittalmani et al. 2017 and unpublished data).

10.7.2 Transcriptomics for Studying the Expression of SSPs in Developing Spikes

The finger millet has a volume of transcriptome series acquired under various stress conditions and for the consistency of the crop. There have hardly been attempts to combine specific genotypes, such as dry and saline effects, into the transcriptome (Rahman et al. 2014). In our lab, we also attempted for the first time to describe the de novo assembly of transcriptome data from two-finger millet genotypes that differed in seed protein and calcium content. The transcriptome data collected may be utilized to uncover genes expressed throughout spike formation as well as to build useful molecular markers. The annotated genes are characterized using transcriptomics and proteomics techniques based on the transcriptome data (Kumar et al. 2014). The data of developing spikes transcriptome is quite useful in the identification and annotation of seed storage protein (SSP) genes. A total of 10 SSP genes were identified rich in essential amino acids. The recent advancement of the molecular biology approach became useful to find out novel and multifunctional genes from the transcriptome data and further cloning. The two out of four quality proteins identified using developing spikes transcriptome later their presence in genome of finger millet and having >40% essential amino acids were cloned in our labs and expressed in *E.coli* expression system (filed

patent no. 201711006104). The four quality proteins identified in developing seeds of finger millet have been depicted in Fig. 10.4. One such gene has 9% lysine and another having 15.8% methionine in its amino acid composition and can be harnessed for nutritional quality improvement of other staple cereals lacking lysine and methionine as one of the essential amino acids (patent no. 201811033469) (Fig. 10.4).

Attempts were made to clone as per transcriptome data, the full open reading frame sequence of the lysine-rich albumin *fimA; fi:* finger, *m:* millet, *A-1:* albumin *1* and methionine-rich *fimP2; fi:* finger, *m:* millet, *P-2:* prolamin, high methionine-variant 2, containing 9% and 15.7% methionine, respectively. After cloning, the expression construct was made for heterologous expression. The recombinant proteins *FIMA1* and *FIMP2* expressed in the bacterial system can be further purified for nutraceutical purposes. The unique properties of the protein were disclosed by using computer-based in silico tools and validated subsequently through experimental approaches. Such gene can be employed for the development of nutraceuticals through genetic engineering and heterologous expression in a bacterial system (Le et al. 2016). The cloning and expression strategies have been given in Fig. 10.5.

Two patents have been filed for both the *FIMA1* and *FIMP2* storage proteins. The amino acid analysis of cloned genes revealed 45 and 46.5% EAAs including lysine (9%) and methionine (15.8%) in the cloned SSPs. The recombinant protein was expressed in bacteria and it was isolated and purified. Albumin-1 and Prolamin-2 are further characterized for its physicochemical properties. The importance of the protein having high lysine and methionine content is the target entity to use as a nutraceutical for improving human nutrition. Further in silico approaches were utilized to identify its unique properties. The tissue-wide expression analysis was performed using quantitative PCR analysis for determining the specificity of temporal and spatial expression (Fig. 10.5). Functional properties of the protein taken in the diet of

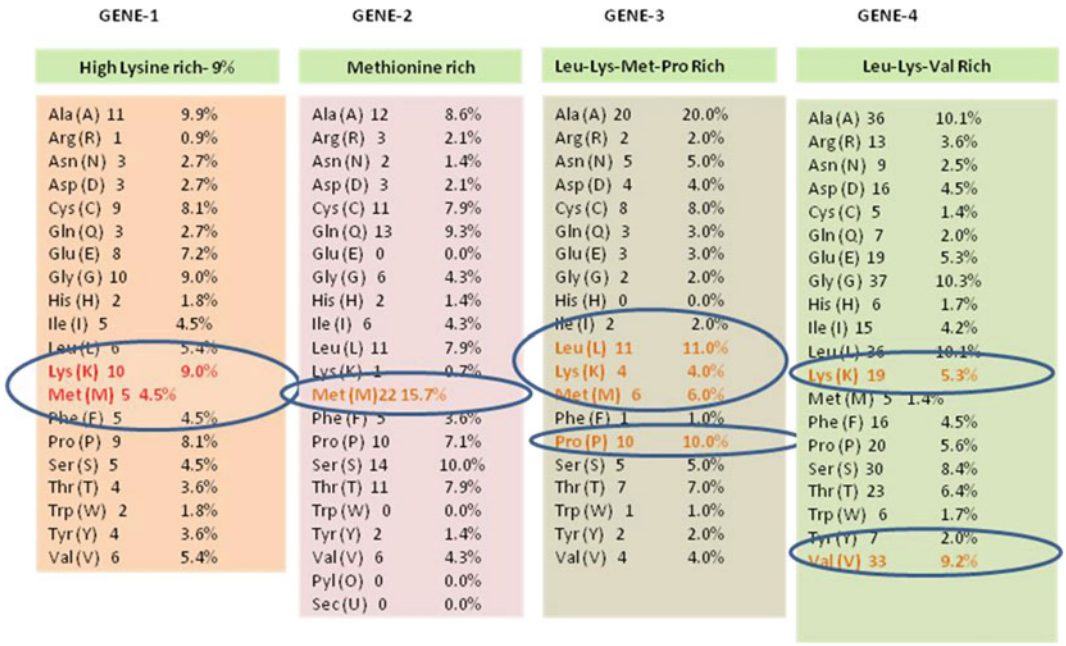

Fig. 10.4 Identification of candidate gene(s) having nutritional potential (high amount of essential amino acids in finger millet proteins)

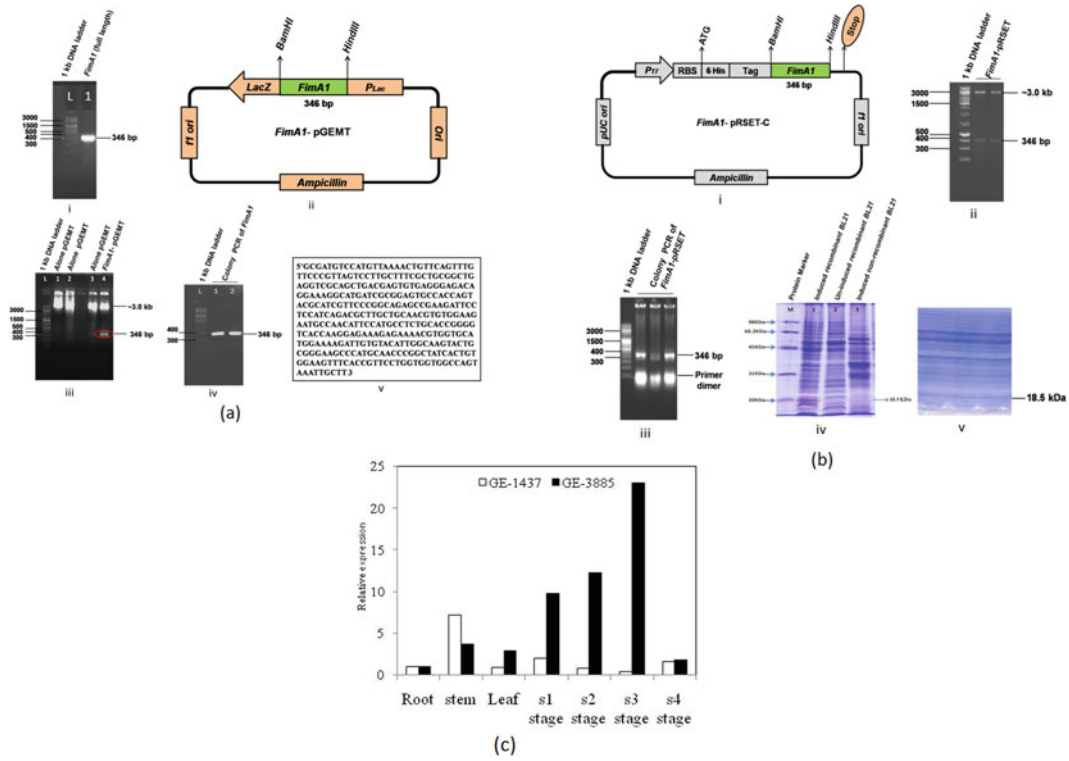

Fig. 10.5 Cloning of seed storage proteins enriched in essential amino acids in an expression vector; (**a**) schematic representation of the *fimA1*-pGEMT clone harboring the full-length *fimA1*; (**b**) schematic representation of the *fimA1*-pRSET-C clone harboring the full length *fimA1*, III and IV represent *BamHI* and *Hind III* restriction sites, respectively; (**c**) expression of FIMA1 protein in different stages of developing spikes of finger millet

the human system can be further elaborated by advanced research methods, of nutritional science and molecular biology.

In our lab, we identified and described the mRNA expressing *OPAQUE2* (O_2) similar TF from finger millet (FM) (*Eleusine coracana*) (*EcO2*). Full-length *EcO2* mRNA was derived by utilizing conserved primers developed by aligning O_2 mRNAs from other cereals, as well as 3′ and 5′ RACE (rapid amplification of cDNA ends). A 1248-nt ORF in the full-length *EcO2* mRNA codes for the 416 amino acid O_2 protein. Domain research showed the existence of the BLZ and bZIP-C domains, which are common in O_2 proteins (Gaur et al. 2018b). *EcO2* protein shared high sequence similarity with barley BLZ1 protein, according to phylogenetic study of *EcO2* protein with other bZIP proteins found using finger millet transcriptome data and O_2 proteins from other cereals. *EcO2* transcripts were used in the root, stem, leaves, and seed growth stages. In addition, the expression profiles of *EcO2* and a prolamin gene were examined during the seed development stages of two FM genotypes (GE-3885 and GE-1437), which differed in grain protein content (13.8 and 6.2 percent, respectively) grown under increasing nitrogen inputs to investigate nitrogen responsiveness and the role of *EcO2* in regulating seed storage protein gene expression. *EcO2* gene was more abundant throughout the S2 stage of seed development, and its expression increased as nitrogen intake increased. At all nitrogen inputs, the Ec-prolamin gene was significantly mediated in the high-protein genotype (GE-3885). These findings point to the existence of nitrogen responsiveness regulatory elements, which may play a role in protein accumulation in FM

genotypes by regulating *EcO2* expression in response to plant nitrogen status (Gupta et al. 2018). In addition to this DOF transcription family, well-characterized plant-specific transcription factors having diverse roles, has also been identified in finger millet. The expression profile of these *EcDof* transcription factors has shown their role during different stages of plant development (Gupta et al. 2018).

10.7.3 Proteomics for Sequential Extraction and High-Throughput Approaches for Analyzing SSPs

In our lab, we compared the seed proteomes of finger millet and rice to learn more about the nutritional and stress-related proteins that accumulate during the growth of finger millet seeds. Using Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS), seed proteins from finger millet and rice were analyzed, and total 453 and 437 proteins were detected, respectively. Comparative analyses found that finger millet and rice both have 25 and 9 proteins that are special to them. Seventeen and five of these unusual proteins were seed storage proteins, respectively (SSP). These SSPs in finger millet were the gliadins, zeins, and avenins. The remaining special proteins in both crops were implicated in abiotic and biotic stress resistances. Of the 428 common proteins, 175 had a higher relative abundance in finger millet (unpublished data).

A BLAST-based homology search was used to identify the full-length gene of *Eleusine coracana* alpha prolamin (Ec-prolamin). Phylogenetic study of Ec-α-prolamin and associated prolamin genes from various cereals and millets reveals Ec-α-prolamin clustering in a separate cluster. Secondary structure prediction shows that Ec-α-prolamin has 59.4% alpha helix structure, which is a structural hallmark. Aside from that, the protein contains a balanced proportion of all essential amino acids. According to qPCR expression study, the accumulation of Ec-α-prolamin transcripts in developing finger millet seeds rises before seed maturity. Western blotting with a monospecific anti-prolamin antibody confirmed the presence of a 22 kDa band in the S3 and S4 phases of growing spikes. The heterologous production of isolated full-length Ec-prolamin might be exploited to develop nutritionally enhanced functional food items as well as value-added industrial goods (Fig. 10.6).

Further expression of calcium-binding proteins was also analyzed in developing spikes to investigate their functional role in the accumulation of calcium in developing seeds (Kumar et al. 2014; Singh et al. 2016).

To further explain the remarkable high grain calcium buildup in finger millet grains, a calmodulin (*CaM*) gene that is strongly expressed during growing spikes of the high grain calcium genotype was reported. Using 5'-3' RACE, the full-length *CaM* open reading frame (ORF) was extracted, and the predicted protein sequence indicated the presence of four unique EF motifs. Phylogenetic research revealed that the finger millet *CaM* (*Eleusine coracana* calmodulin [*EcCaM*]) is related to the rice *CaM 1-1*. Southern hybridization indicated the presence of at least four copies of the *CaM* gene in different locations of the "AABB" finger millet genome. According to immune detection utilizing monospecific polyclonal anti-*EcCaM* antibodies, *EcCaM* is localized in the embryo and aleurone layer and accumulates in greater levels in the high grain calcium genotype compared to the low grain calcium genotype. *In silico* analysis also found that *EcCaM* interacts with aquaporin, implying that calcium is most likely supplied to growing spikes by mass flow of water. These findings imply that enhanced *CaM* expression can stimulate the downstream calcium transport mechanism in the aleurone layer, resulting in larger calcium buildup in grains of high grain calcium genotype (Puranik et al. 2017) (Fig. 10.7).

A clearly visible blue color band of 48 kDa stained by Stains-all was eluted and identified as calreticulin using nano-liquid chromatography-tandem mass spectrometry (nano-LC-MS) (CRT). Based on the top hits of peptide mass

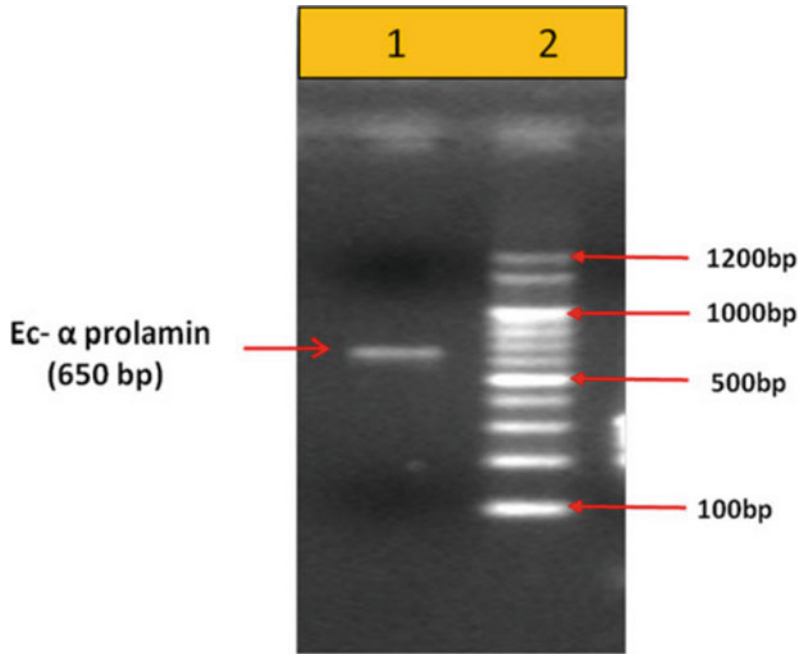

Fig. 10.6 PCR-based amplification of the Ec-prolamin gene from the finger millet GE-3885 genotype. Lane 1 represents a 100-bp DNA ladder, whereas Lane 2 represents a 650-bp amplified gene

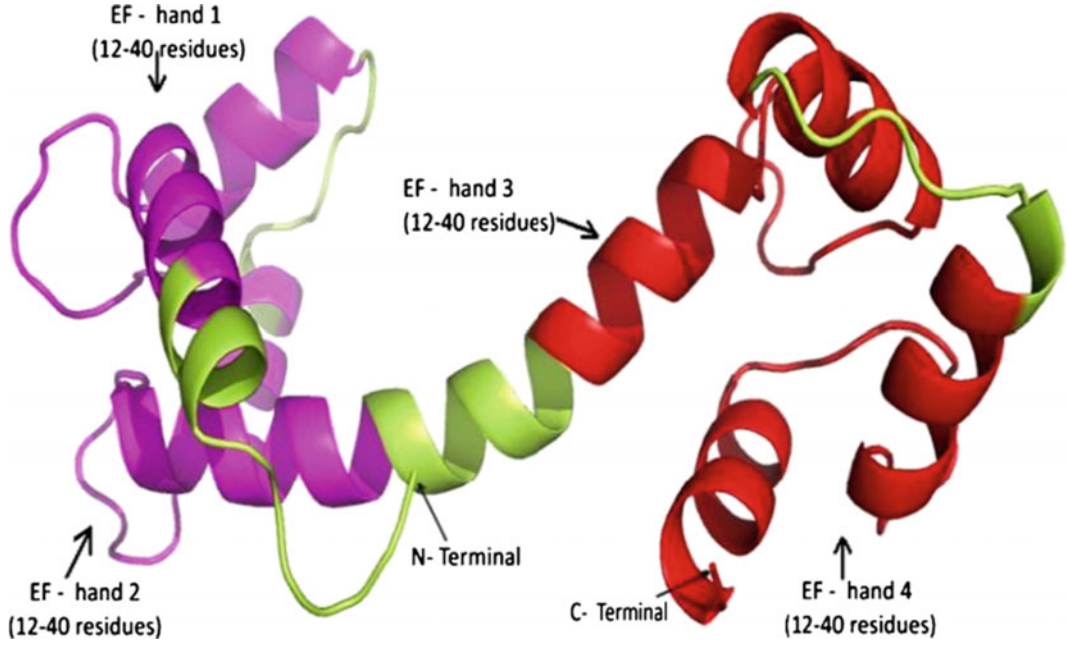

Fig. 10.7 Using Modeller 9.10, a ribbon model of the protein encoded by the isolated EcCaM gene (finger millet Calmodulin protein) was created

fingerprinting data, conserved primers were constructed to isolate the CRT gene from finger millet utilizing calreticulin sequences from other cereals. The deduced nucleotide sequence analysis of a 600 bp amplicon revealed up to 91% similarities to *CRT* gene(s) of rice and other plant species and was named *EcCRT1*. *EcCRT1* transcripts profiling revealed varying levels of relative expression at various stages of spike growth. *EcCRT1* transcripts and protein were identified to be more abundant in later stages of growing spikes, which might be ascribed to enhanced translational synthesis of *EcCRT1* protein during finger millet seed development. Higher synthesis of this *CaBP* at later phases of grain filling may be responsible for calcium sequestration in the endoplasmic reticulum of finger millet grains (Singh et al. 2016) (Fig. 10.8).

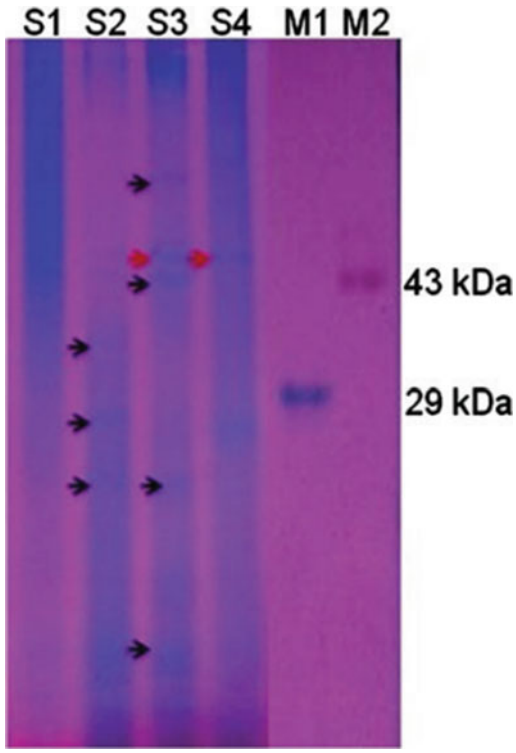

Fig. 10.8 Protein profiling of CaBPs in finger millet genotype GPHCPB-45 spikes at various phases of development using Stains-all staining. (M1, M2: native PAGE molecular weight protein marker; S1, S2, S3, and S4: spike development phases.)

10.7.4 Molecular Marker-Assisted Breeding

Continuous research efforts in the fields of molecular breeding, plant biotechnology, and genomics have resulted in massive amounts of data being generated using high-throughput sequencing technologies in recent years (Fig. 10.9).

Furthermore, genome-wide genetic variation study has aided in understanding the molecular and genetic foundation of nutritional quality characteristics in finger millet (Sharma et al. 2018). Because of their uniform distribution, ubiquitous nature, biallelic character, and high heritability, SNPs (single nucleotide polymorphisms) are thought to be the best option among all molecular markers. Furthermore, alterations in gene function may result from mutations at a single nucleotide level within genes, which may be responsible for phenotypic variances. As a result, identifying SNPs associated to essential nutritional characteristics such as SPC (seed protein content) and related traits such as DM (days to maturity) and GY (grain yield) (Tiwari et al. 2020).

Protein content phenotypic diversity was found high in different finger millet genotypes. However, no attempts have been undertaken too far to study both the genetic architecture and functional significance of genetic loci controlling SPC in finger millet. To unravel the complex genetic architecture of SPC and related characteristics, genomics-assisted breeding was used to identify and introgress candidate genes/QTLs (quantitative trait locus) regulating this essential quality attribute in various crop plants. However, little emphasis has been paid to the discovery of SPC-regulating genes/QTLs that may be exploited in marker-assisted genetic improvement of finger millet (Tiwari et al. 2020).

A study by Tiwari et al. (2020) was designed to identify genes/QTLs regulating SPC and associated characteristics in finger millet by utilizing SNPs identified by GBS (genotyping by sequencing) of a naturally varied population of finger millet. One of the main methods used to identify and develop specific qualities is

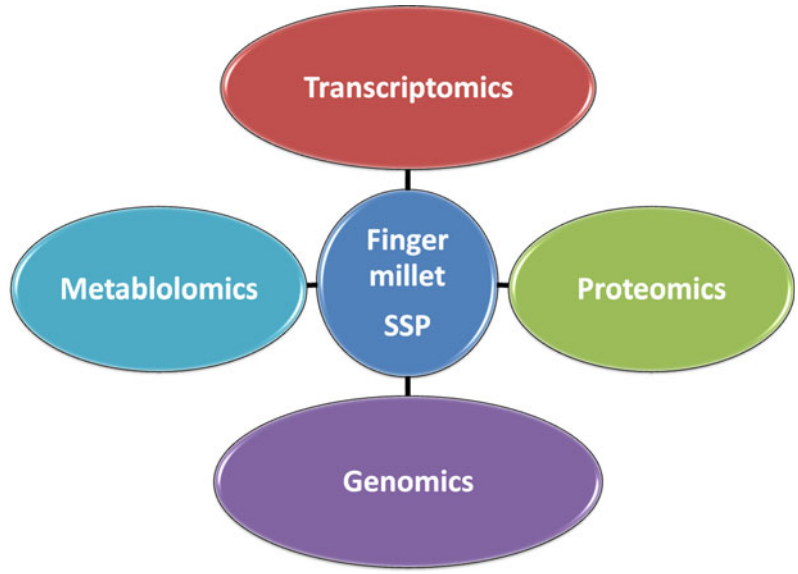

Fig. 10.9 Different NGS approaches to annotate the SSPs of finger millet

molecular markers. An extensive range of molecular marker systems has been identified by DNA markers, which are typically used as part of the plant breeding program (Jiang et al. 2015). The use of molecular markers has revolutionized the speed and reliability of plant genetic analysis which has allowed the molecular replication of crops in effect. The development of marker mechanisms and their respective methods have advanced immensely over the last three decades. The center of the molecular genetics stage was quickly increased in recent years by markers based on SNPs due to their abundant genomes and their comfort to high-performance detectives and platforms. The sequence of knowledge in public libraries dominates SNP discovery methods. A few studies for analyzing genetic ability and QTL in finger millets with molecular markers are available. The analysis of the strong hereditary heterogeneity is important for improving the yield, as it exposes the sensitivities of the hereditary relations and offers inspections of reproductive populations**.** Hereditary and efficient variety study recognizes inherited genotype ties around the world and assists in identifying appropriate genotypes for reproductive systems (Babu et al. 2017). For finger millet produced in Asia and Africa under various climatic conditions, analyses for genetic diversities require genome variability between genotypes and subsequently increase the population.

10.7.5 Identification of QTLs for SSP

A study of the complex genetic architecture of SPC (seed protein content) and related traits such as DM and GY of 113 diverse finger millet genotypes was carried out in our lab at two geographically different locations in India (Uttarakhand), i.e., Almora (E1) and Pantnagar, using an integrated genomic-based breeding strategy and significant variations between SPC, DM and GY genotypes were found in both places (Tiwari et al. 2020).

For the MTA (marker-trait association) study, a set of genome-wide SNPs has been identified by genotyping. NCBI blasts of common SNP markers identified 5, 3, and 5 most effective genomic areas for SPC, DM, and GY, respectively. The SNP encoding the aspartyl protease gene exhibited the greatest SPC interaction and was chosen as the most promising candidate for protein content variation in finger millet. As ATP was revealed to be the influential gene, the ATP synthase gene linked by GY and DM catalyzes the addition of a phosphate to ADP, collecting energy from the proton gradient. Five SPC-

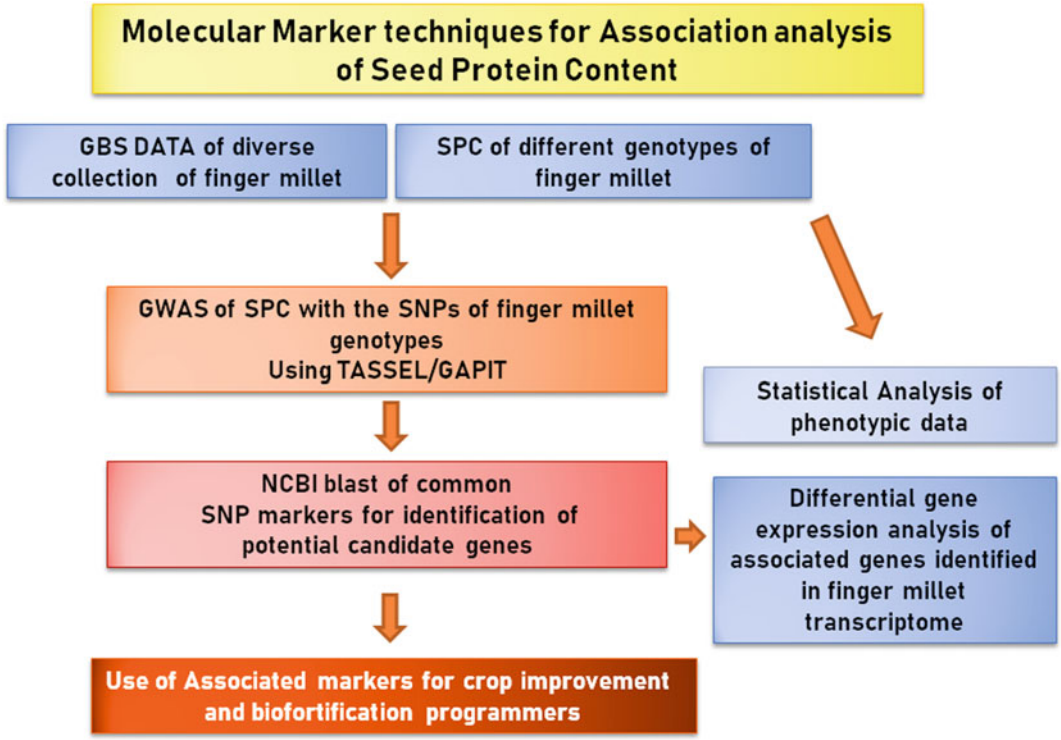

Fig. 10.10 A sequential flow of association studies for SSP trait in finger millet

related genes had higher expression levels in the high protein-containing genotype than in the low protein-containing genotype (Fig. 10.10) (Tiwari et al. 2020).

10.8 Bioactive Peptides

Dietary-derived peptides have the potential to be functional food components that may be included into health-promoting diets aimed at the prevention and management of a variety of chronic illnesses. The discovery of bioactive peptides (BPs) in intact proteins that have a favorable impact on bodily processes will have an impact on health. BPs derived from millet seeds such as sorghum bicolor, foxtail, buckwheat, finger millet, pearl millet, chia, and quinoa, as well as their biological activities such as antimicrobial peptides, anticancerous peptides, antioxidant peptides, antidiabetic peptides, and antihypertensive peptides, have been reported (Orona-Tamayo et al. 2019).

The BPs are inert inside the parent protein sequence but become active once released. Peptides can be released in a variety of ways, including breakdown by digestive enzymes, proteolytic bacteria, and enzymes generated from microbes or plants (Udenigwe et al. 2012). The usefulness and efficacy of BPs, such as their small intestine absorption and bioavailability in target tissues, are largely determined by their size, intrinsic amino acid composition, sequence, and other characteristics such as charge, hydrophobicity, and rate of hydrolysis. As a result, their generation/production is a critical phase that requires more research and attention (Chai et al. 2020).

In another interesting study using extraction of finger millet functional ingredients (FFI) using

different protein extraction buffers and isolated ingredients and their enzymatic hydrolyzed fraction were used to access its influence on calcium uptake studies using Fura 2 fluorescent dyes under in vitro intestinal mimicking environment of $CaCO_2$ cell culture system. The results were found that FFI in its hydrolyzed fraction is promoting higher calcium uptake compared to whole FFI indicating that finger millet seeds not only contain higher calcium contents but also have calcium-binding proteins which facilitate higher uptake of calcium through human gut (unpublished data).

The traditional in vivo techniques for generating, identifying, and validating BPs are time-consuming and labor intensive. Because of its focused approach, low time consumption, quick pace of results collection, cost-effectiveness, and bioinformatics/in silico analysis might be a strong tool for BPs discovery. Bioinformatic techniques enable the identification of possible BPs among dietary proteins. Elucidating protein sequences is an important stage in the development of BPs, and molecular docking is used to assess the therapeutic potential of novel BPs produced from food and to monitor protein–ligand interactions (Panyayai et al. 2019). Many peptide databases are now available for BPs like FeptideDB which is a computational aid for assessing peptide bioactivities (Panyayai et al. 2019). Identification of derived BPs could be a potential source for targeting many diseases.

10.9 Concluding Remark and Future Prospects

Seeds are source of wide range of foods, magnesium phosphates, and amino acids, including leucine and lysine. Finger millet is an annual herb widely cultivated which provides a large amount of protein as well as high calcium. Identification of seed protein contents provides an improved biofortification path and boosts the finger millet nutritional value for cultivation. These are the beginnings that need to be explained by modern approaches to the biology of computational systems, including genomics, transcriptomics, and proteomics to improve the finger millet crop's nutritional quality. Thus, *OMICS*, high-performance genotyping, and sequencing technologies are expected to contribute not only toward improving breeding practices but also improving the quality and quantity of such crops. Gene sequence of two SSPs, albumin (*fima1), and* prolamin *(fimp2)* having 9% lysine and 15.8% methionine, respectively, was cloned and recombinant proteins have been expressed in *E. coli* system which is a remarkable output of these *OMICS* technologies. The functionally annotated SNPs have been used to identify genes that regulate important agricultural traits in finger millets. This information would be an asset for potential finger millet breeding research. The SNP genotyping allows breeders to choose parents and to introgress rare germplasm alleles. The SNPs identified and associated with these genes could be used for the cloning of full gene sequences, precise mapping, and ultimate reproductive support programs for the marker to incorporate alleles in local genotypes. The genomic regions found may be targeted in finger millet for marker exploration. Using the GWAS (genome-wide association studies), new essential genetic information for these agronomic characteristics was discovered. In the potential biofortification of finger millets, the established loci and candidate genes will serve as promising targets. The recent findings would become a milestone for selection of genotypes containing high proportions of these seed storage proteins or analysis of their amounts by genetic engineering which could lead to improved nutritional quality of finger millet. Further, identification of active bioactive peptides in finger millet using bioinformatics tools could be a potential source for targeting many diseases.

Competing Interest The authors declare that there are no competing interests.

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Finger Millet Genome Analysis and Nutrient Transport

11

T. Maharajan, T. P. Ajeesh Krishna, S. Ignacimuthu, and S. Antony Ceasar

Abstract

Ever-increasing population and malnutrition in the developing countries increase the demand for the poor people's food and nutritional security. Millets can survive in the current erratic climatic conditions as they require minimum water, short cultivation period, and are tolerant to drought and diseases. Millets possess several rich nutrient components and potential health benefits. Among the millets, *Eleusine coracana* (L.) Gaertn (finger millet (FM)) is valued as a crop of short growth duration, nutritious and rich source of minerals and vitamins. Low availability of nutrients such as nitrogen (N), phosphorus (P), calcium (Ca), and zinc (Zn) in soils affects the growth of shoot and root and yield. Therefore, an alternative strategy is required for improving nutrient use-efficiency in FM under low nutrient conditions. Identification of membrane-bound transporters helps for the efficient uptake, translocation, and remobilization of nutrients in FM. Only a draft genome of finger millet is available till date, which hampers the complete identification of nutrient transporters in FM. Only a few nutrient transporters have been identified in various tissues of FM so far. In this chapter, we discuss the details of the draft genome sequences of FM. We also present the details on the N, P, Ca, and Zn family transporters identified so far in FM. Proper exploitation of FM genome will pave the way for functional genomic studies and helps to develop new and improved FM varieties in future. This will limit the use of non-renewable synthetic fertilizer and improves the nutrient use-efficiency (NUE) of FM and other crops in low-input agriculture soils in the developing countries including Asia and Africa.

11.1 Introduction

Finger millet (*Eleusine coracana* (L.) Gaertn.; FM) ($2n = 4$, $x = 36$) belonging to the family Poaceae and the genus *Eleusine* is an annual herbaceous cereal crop widely grown in African and Asian countries. It is an important food crop in dry areas of Eastern Africa, India, Sri Lanka, China, and other Asian countries (Fakrudin et al. 2004). From the cultivation point of view, it is the sixth largest crop mainly among the rural populations of Africa and India and fourth important crop among millets globally (Devi

T. Maharajan · T. P. Ajeesh Krishna · S. A. Ceasar (✉)
Department of Biosciences, Rajagiri College of Social Sciences, Kerala, Kochi 683104, India
e-mail: antony_sm2003@yahoo.co.in

S. Ignacimuthu
Xavier Research Foundation, St. Xavier's College, Palayamkottai 627002, India

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et al. 2014; Ceasar et al. 2018). Among the millets, FM has the highest percentage of essential amino acids like tryptophan, threonine, arginine, and histidine (Devi et al. 2014). Also, it has high carbohydrate content, protein, crude fiber, and has several potential polyphenols related to health benefits (Chethan and Malleshi 2007). It also possesses the highest amount of amino acids such as valine, threonine, and lysine among the millets (Ravindran 1991; Sripriya et al. 1997). Its calcium (Ca) content (344 mg/100 g) is tenfold higher than wheat (*Triticum aestivum*), maize (*Zea mays*), and rice (*Oryza sativa*) and three times higher than milk (Kumar et al. 2016). It also contains higher contents of other minerals such as phosphorus (P), iron (Fe), and manganese (Mn) compared to other cereals (Ceasar and Ignacimuthu 2011).

Nutrient deficiency [(nitrogen (N), P, and zinc (Zn)] affects the growth and yield of finger millet (Yamunarani et al. 2016; Ramakrishnan et al. 2017; Maharajan et al. 2019). For example, N deficiency decreased the shoot tiller number, number and length of crown root and lateral root but with no consistent changes in root hair traits (Goron and Raizada 2015). Low P stress (10 μM) modified the root architecture, viz., decreased primary root length and increased lateral root and root hair development (Ramakrishnan et al. 2017; Maharajan et al. 2019). Similarly, dry weight of shoot and root and yield were severely affected by low P supply (Maharajan et al. 2019). Zn is one of the key micro-nutrients, and Zn deficiency resulted in stunted growth, delayed seed maturity, appearance of chlorosis, shortened internodes, and petioles with malformed leaves (Yamunarani et al. 2016). In future, nutrient loss will be one of the major abiotic stresses affecting the finger millet growth and yield. Therefore, awareness is needed from biotechnologists, agronomists, and plant breeders to develop newer FM varieties to acclimatize in low nutrient soils. In this chapter, we discuss the functional genomics and molecular marker studies reported on nutrient transport in finger millet.

11.2 Finger Millet Genome

Generally, millets received less attention for genome sequencing and modern genetic studies when compared to other cereals due to the cultivation and consumption in less developed countries in Asia and Africa. Apart from foxtail millet (*Setaria italica*), other millets have less annotated or no genome sequences so far (Ceasar et al. 2018). So, FM has been categorized as an orphan crop for modern genetic studies (Goron and Raizada 2015). Presence of complex genome with large size, tetraploidy, and cultivation in less developed countries delayed the genome sequencing in FM. Its genome size is estimated to be 1,593 Mb. The first draft genome sequence of FM was released in 2017 (Hittalmani et al. 2017). Unfortunately, unlike foxtail millet, complete annotation is still not yet available for FM. As a result, high-resolution genetic studies (both forward and reverse) are still lacking for this crop unlike other major cereals like rice. Studies related to the identification and characterization of nutrient transporters are still not attempted in FM.

Whole-genome draft sequence of FM genotype ML-365 was released in 2017 (Hittalmani et al. 2017). The genome assembly consisted of 5,25,759 scaffolds (>200 bp) with N50 length of 23.73 Kb, and the average scaffold length of 2,275 bp (Hittalmani et al. 2017). The same work also reported the assembly of transcriptome for well-watered (53,300 unigenes) and low moisture stressed (1,00,046 unigenes) plants of genotype ML-365. Nearly 64% of unigenes assembled were functionally annotated. Overall, 2,866 drought-responsive genes were associated with major TF families across 19 Pfam domains. About 1,766 genes were identified as resistance genes for various diseases and 330 genes were found to be involved in Ca transport and accumulation (Hittalmani et al. 2017). The genome sequence of FM was found to have greater co-linearity with those of foxtail millet and rice in Poaceae species. The genome sizes of *E. coracana* subspecies coracana and *E. coracana*

subspecies Africana found to be relatively similar (Hittalmani et al. 2017).

Hatakeyama et al. (2018) reported the whole-genome assembly of FM genotype PR-202 (IC:479,099) using a novel polyploidy genome assembly workflow. This study identified the genome size of FM to be 1.5 Gb and the assembled genome to be 1,189 Mb which is estimated to cover 78.2% genome. The whole genome consisted of 2,387 scaffolds with the N50 value of 905.318 Kb having maximum sequence length of 5 Mb. The FASTA file format of final scaffolds and annotation is publicly available at National Center for Biotechnology Information (NCBI) (biosample number: SAMD00076255). This study identified 62,348 genes among which nearly 91% genes are functionally annotated and 96.5% are found to be single-copy genes (Hatakeyama et al. 2018).

Although complete annotation is not yet available for FM genome and protein sequences are yet to be identified, the draft sequences of ML-365 and PR-202 could be used for the identification of genes and proteins involved in nutrient transport. Tools like comparative genomics and open reading frame (ORF) finder could be exploited for the BlastN-based identification of key genes and proteins involved in nutrient transport, using draft genome sequences.

11.3 Nutrient Transporter

Membrane-bound transport proteins play a key role in the acquisition and redistribution of nutrients in plants. It has been predicted that specialized plant membrane transporters can be used to enhance yields of staple crops, increase nutrient content, and increase resistance to key stresses, including salinity, pathogens, and aluminum toxicity which may help to improve the yield (Schroeder et al. 2013). Each nutrient enters into the plant by a specialized membrane-bound nutrient transporters. Most of these proteins and genes are well characterized in model plants like Arabidopsis (*Arabidopsis thaliana*) and rice. Macronutrients have specific transporter for the acquisition of each nutrient and micro-nutrients like copper (Cu), Fe, and Zn have common nutrient transporters. These transporters are well studied in many other cereals like rice (Table 11.1). For example, in rice, nitrate (NO_3^-) is transported by NO_3^- transporter (NRT) family transporters (Ouyang et al. 2010; Léran et al. 2014; Islam 2019), phosphate is acquired from the soil solutions primarily by phosphate transporter 1 (PHT1) family transporters (Paszkowski et al. 2002; Wang et al. 2014), potassium (K) transporters play a vital role in uptake and transport of K (Gomez-Porras et al. 2012), sulfate (SO_4^{2-}) is taken up by plants via SO_4^{2-} transporters (SULTR) (Buchner et al. 2004; Kumar et al. 2011), acquisition of ammonium (NH_4^+) in plants is undertaken by the NH_4^+ transporters (AMTs) (Hao et al. 2020), and zinc-regulated, iron-regulated transporter-like proteins (ZIP) family transporters are mainly involved in uptake, transport, and distribution of Zn Fe Mn, cadmium (Cd2), cobalt (Co), copper (Cu), and nickel (Ni) (Ramesh et al. 2003; Chen et al. 2008; Krishna et al. 2020). However, these nutrient transporters are not yet characterized in FM. Only gene expression of some of these nutrient transporters was studied in FM so far and protein characterization of these transporters is still not undertaken.

11.3.1 Nitrate Transporter

The NO^{3-} is an essential source of N for most of the cultivated crops (Forde 2000). NO_3^- is absorbed from the soil by NRT family of transporters. Plant NRTs were first identified and functionally characterized more than 20 years ago (Fan et al. 2017). They are encoded by four gene families, NRT1/peptide transporter family (NPF) (Léran et al. 2014), NRT2 (Krapp et al. 2014), chloride channel (CLC) (Bi et al. 2007), and slow anion channel-associated 2 (SLAC2)/SLAC homologues (SLAH) (Negi et al. 2008). Among the four families, NRT1 and NRT2 are involved in NO_3^-uptake, transport. and remobilization from root to shoot under NO_3^- limitation condition in rice (Chen et al. 2016; Fan et al. 2017). The NO_3^-transporter was first identified

Table 11.1 Details on genome size, year of genome sequencing, studies on various nutrient transporters reported in rice, Arabidopsis, foxtail millet, and finger millet are listed with references

Name of the crop	Arabidopsis (*Arabidopsis thaliana*)	Rice (*Oryza sativa*)	Foxtail millet (*Setaria italica*)	Finger millet (*Eleusine coracana*)
Size of genome	125 Mb	430 Mb	490 Mb	1593 Mb
Year of draft genome sequence release	**2000** (Kaul et al. 2000)	**2005** (International Rice Genome Sequencing, 2005	**2012** (Zhang et al. 2012; Bennetzen et al. 2012)	**2017** (Hittalmani et al. 2017) **2018** (Hatakeyama et al. 2018)
Year of complete annotation	**2003** (Rhee et al. 2003)	**2013** (Kawahara et al. 2013)	**2012** (Bennetzen et al. 2012)	Not yet available
Nitrate transporter studies	**AtNRT1** (Tsay et al. 1993a, b; Mounier et al. 2014; Mao et al. 2014) **AtNRT2** (Chopin et al. 2007; Orsel et al. 2002; Kotur and Glass 2015) **AtNAR2** (Okamoto et al. 2006; Orsel et al. 2006) **AtCLC** (De Angeli et al. 2006; Monachello et al. 2009)	**OsNRT1** (Hu et al. 2015; Lin et al. 2000) **OsNRT2** (Feng et al. 2011; Liu et al. 2014) **OsNAR2** (Fan et al. 2017)	**SiNRT1** and **SiNRT2** (Ahmad et al. 2018; Nadeem et al. 2018) and **SiNAR2** (Nadeem et al. 2018)	**EcNRT1** and **EcNRT2** (Gupta et al. 2013)
Phosphate transporter studies	**AtPHT1** (Mitsukawa et al. 1997; Misson et al. 2004; Ayadi et al. 2015) **AtPHT3** (Jia et al. 2015; Hamel et al. 2004) **AtPHT4** (Guo et al. 2008; Cubero et al. 2009) **AtPHT5/VPT1** (Liu et al. 2016) **AtPHO1** (Secco et al. 2012)	**OsPHT1** (Paszkowski et al. 2002; Jia et al. 2011; Secco et al. 2013) **OsPHT2** (Shi et al. 2013) **OsPHO1** (Secco et al. 2010)	**SiPHT1** (Ceasar et al. 2014)	**EcPHT1** (Pudake et al. 2017; Maharajan et al. 2019)
Ca uptake, transport, and accumulation studies	**Ca exchanger** (Singhet al. 2015a, b)	**Ca exchanger** (Yadav et al. 2015; Singh et al. 2015a, b; Kamiya et al. 2006) **Ca transporter** (Goel et al. 2011; Hao et al. 2020)	Nil	**Ca exchanger** (Mirza et al. 2014) **Ca sensor** (Singh et al. 2014) **Ca transporter** (Singh et al. 2015a, b; Chinchole et al. 2017)
Sulfate transporter studies	**AtSultr1** (Yoshimoto et al. 2003; Takahashi et al. 2000)	**OsSultr1** (Godwin et al. 2003; Kumar et al. 2011) **OsSultr2** (Buchner et al. 2004)	Nil	Nil

(continued)

Table 11.1 (continued)

Name of the crop	Arabidopsis (*Arabidopsis thaliana*)	Rice (*Oryza sativa*)	Foxtail millet (*Setaria italica*)	Finger millet (*Eleusine coracana*)
	AtSultr2 (Takahashi et al. 2000; Maruyama-Nakashita et al. 2015) **AtSultr3** (Kataoka et al. 2004a; Cao et al. 2013) **AtSultr4** (Kataoka et al. 2004b) **AtSultr5** (Buchner et al. 2004)	**OsSultr3** (Zhao et al. 2016; Sacchi and Nocito 2019) **OsSultr4** (Kumar et al. 2011) **OsSultr5** (Buchner et al. 2004)		
Ammonium transporter studies	**AtAMT1** (Sonoda et al. 2003; Ninnemann et al. 1994; Loqué et al. 2006) **AtAMT2** (Sohlenkamp et al. 2002; Neuhäuser et al. 2009)	**OsAMT1** (Su-Mei et al. 2012) **OsAMT2** (Suenaga et al. 2003) **OsAMT3** (Li et al. 2009)	**SiAMT1** (Nadeem et al. 2018; Ahmad et al. 2018)	Nil
Magnesium transporters	**AtMGT1-10** (Chen et al. 2009; Li et al. 2001; Schock et al. 2000)	**OsMGT1-9** (Saito et al. 2013; Li et al. 2016)	Nil	Nil
Zinc transporter studies	**AtZIP1 to AtZIP12** (Grotz et al. 1998; Wintz et al. 2003; van de Mortel et al. 2006; Assunção et al. 2010)	**OsZIP1 to OsZIP16** (Chen et al. 2008; Ishimaru et al. 2005; Yang et al. 2009; Ramesh et al. 2004; Lee et al. 2010a,b)	**SiZIP1to SiZIP7** (Alagarasan et al. 2017)	**ZIP1** (Chandra et al. 2020)
Iron transporter studies	**AtIRT1** and **AtIRT2** (van de Mortel et al. 2006; Shanmugam et al. 2011; Henriques et al. 2002) **AtIRT3** (Lin et al. 2009)	**OsIRT1** (Ishimaru et al. 2005, 2006; Bughio et al. 2002) **OsIRT2** (Ishimaru et al. 2006; Bughio et al. 2002)	Nil	Nil
Copper transporter	**AtCOPT1 to AtCOPT6** (Sancenón et al. 2003; Vatansever et al. 2017)	**OsCOPT1 to OsCOPT6** (Yuan et al. 2011)	Nil	Nil
Genome-wide association studies for nutrient transport	Nil	**Nitrate** (Tang et al. 2019) **Sodium and molybdenum** (Yang et al. 2018) **Sulfur** (Pariasca-Tanaka et al. 2020)	Nil	Nil

Abbreviations used Ammonium transporter (AMT), copper transporter (COPT), chloride channel (CLC), iron-regulated transporters (IRT), megabase (Mb), magnesium transporter (MGT), nitrate assimilation-related protein (NAR), nitrate transporter (NRT), phosphate 1 (PHO1), sulfate transporter (Sultr), zinc-regulated, iron-regulated transporter-like proteins (ZIP), vacuolar phosphate transporter 1 (VPT1)

and characterized in the model plant of Arabidopsis (Tsay et al. 1993a, b). Transcription factor prolamin-binding factor DNA binding with one finger only (*PBF Dof*) is involved in gene regulation of seed storage proteins during seed development (Vicente-Carbajosa et al. 1997; Diaz et al. 2002; Dong et al. 2007). Notably, the *PBF Dof* transcription factor is a key regulator in the accumulation of grain protein and yield through regulation of key enzymes of C/N metabolism involved in source to sink relationship during grain filling (Gupta et al. 2012). Also, the *PBF Dof* transcription factor regulates the expression of NO_3^- transporters (Gupta et al. 2013). The transcription factor of DOF involved in regulates the NO_3^- transport.

Expression pattern of *PBF Dof* was analyzed in various tissues like root, stem, and flag leaf at the vegetative stage and developing spikes of three-FM genotypes [PRM-1 (brown), PRM-701 (golden), and PRM-801 (white)] with differing in seed protein content and color (Gupta et al. 2011). Interestingly, the expression of *PBF Dof* was higher in the developing spikes compared to root, stem, and flag leaf in all three genotypes. Likewise, six genes (*E. coracana high-affinity NRT2 (EcHNRT2)*, *E. coracana low-affinity NRT1 (EcLNRT1)*, *E. coracana* NO_3^- *reductase (EcNADH-NR)*, *E. coracana glutamine synthetase (EcGS)*, *E. coracana glutamine oxoglutarate aminotransferase (EcFd-GOGAT)*, and *EcDof1* involved in NO_3^- uptake and assimilation were studied in two contrasting FM genotypes (GE-1437 (low-protein content) and GE-3885 (high-protein content)) (Gupta et al. 2013). Among them, *EcLNRT1, EcNADH-NR, EcGS, EcFd-GOGAT,* and *EcDof1* were expressed in the leaves of GE-3885 under N deficiency (Fig. 11.1). Compared with the GE-1437, expression of *EcHNRT2* was also strongly induced in both roots and shoots of GE-3885 genotype under low N conditions (Fig. 11.1) (Gupta et al. 2013). This study indicates that high-protein content genotype is a quick sensor of N compared with the low-protein content genotype (Gupta et al. 2013). The same group also analyzed the expression pattern of *EcDof1* and *EcDof2* in the root and shoot tissues of the

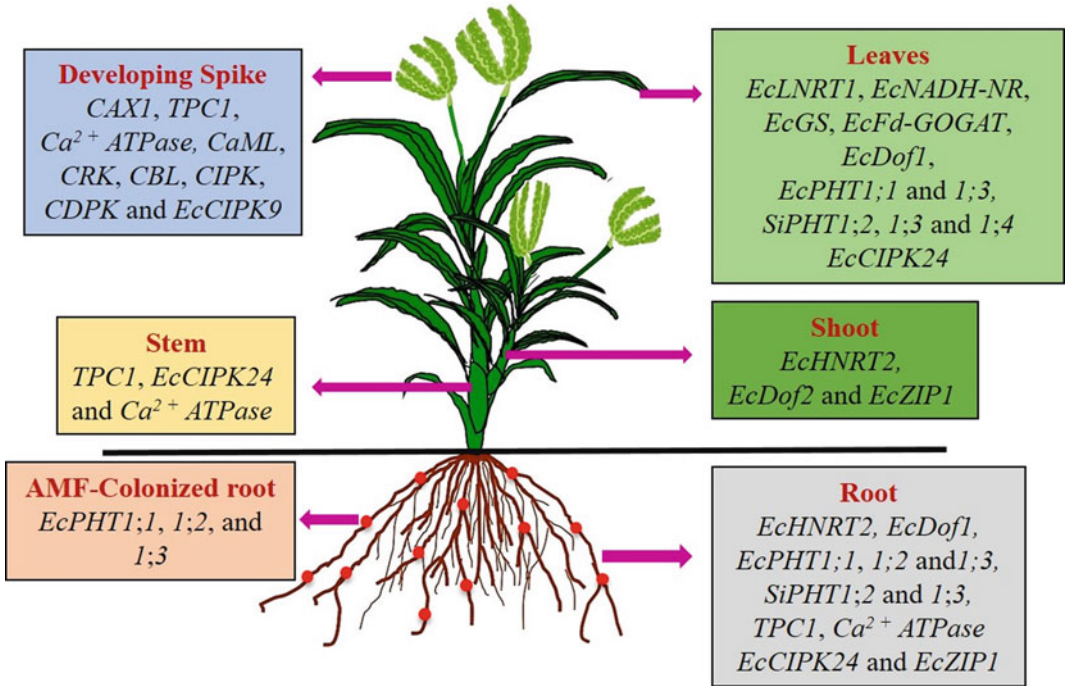

Fig. 11.1 Nitrate (N), phosphate (P), calcium (Ca), and zinc (Zn) family transporters analyzed in direct and AMF-mediated (indirect) pathways in finger millet. Expression patterns of N, P, Ca, and Zn transporters in shoots, leaves, stem, developing spikes, roots, and AMF colonized roots are listed in rectangular boxes. The AMF colonized roots are indicated in red circular structures in the root zone. Abbreviations used: Arbuscular mycorrhizal fungus (AMF); *calmodulin (CaM)*; *CaM-independent protein kinases (CDPK)*; *CaM-dependent protein kinase (CaMK)*; *CAX*,$Ca2^+/H^+$ *antiporter*; *Calciuneurin B-like protein (CBL); EcHNRT2*; *E. coracana high-affinity nitrate transporter*; *E. coracana low-affinity nitrate transporter (EcLNRT1)*; *E. coracana* NO_3^-*reductase (EcNADH-NR,)*;. *coracana glutamine synthetase (EcGS,E)*; *E. coracana glutamine oxoglutarate aminotransferase (EcFd-GOGAT)*; *DNA binding with one finger only (EcDof)*; *Phosphate transporter1 (PHT1)*; *two pore channel1 (TPC1)*; and *zinc-regulated, iron-regulated transporter-like proteins (ZIP)*

same two genotypes under low and high N conditions (Gupta et al. 2014). The *EcDof2* expression level was higher in shoots of GE-1437 under low N compared to GE-3885 (Fig. 11.1). *EcDof1* expression level was higher in roots of GE-3885 under high N conditions compared to the GE-1437. However, the *EcDof1*/*EcDof2* ratio was higher in the roots of GE-3885 than in GE-3885 (Gupta et al. 2014). Only these are the works available related to NO_3^-transporter in FM so far. Identification and functional characterization of key transporters like NRT family is not yet reported in FM. Since FM is a crop with rich nutrient profile, identification and functional characterization of key genes involved in NO_3^- transport will help to identify the primary role and cross-talks of NO_3^- with other nutrients. Notably, many low- and high-affinity NO_3^-transporters were identified and functionally characterized in Arabidopsis (Tsay et al. 1993a, b; Muños et al. 2004) and rice (Hu et al. 2015; Islam 2019). For example, overexpression of NRT1; 1 (dual-affinity transporter) and NRT1; 2 (low-affinity transporter) in Arabidopsis improved the uptake of $NO_3^-$from the soil under high N condition (Wang et al. 2012; O'Brien et al. 2016). Similarly, overexpression of *OsNRT1;6* in rice increased NO_3^-influx rate, acquisition of $NO_3^-$by root and transfer of NO_3^- from root to shoot under high NO_3^-condition (Xia et al. 2015). NRT2 family is the second NO_3^- transporter family in plants. Totally, seven members in Arabidopsis (Chopin et al. 2007; Orsel et al. 2002) and five in rice (Lin et al. 2000; Feng et al. 2011) were identified and characterized for NRT2s. NRT2 family plays a vital role in acquisition of NO_3^- from the soil under low N condition. For example, *OsNRT2; 1, 2; 2* and *2; 3* were expressed mostly in root tissues of rice. Among these three transporters, OsNRT2; 1 and 2; 2 are involved in the acquisition of NO_3^- from the soil and OsNRT2; 3 is involved in the transport of NO_3^- from root to shoot (Feng et al. 2011). *OsNRT2; 4* was expressed mainly in shoots and involved translocation of NO_3^- from root to shoot and remobilization of NO_3^- from older leaves to younger tissues under low NO_3^- condition in rice (Cai et al. 2008; Fan et al. 2017). Like Arabidopsis and rice extensive study on NO_3^- transporters in FM need to be undertaken to understand and improving N acquisition efficiency (NAE) and N use-efficiency (NUE).

11.3.2 Phosphate Transporters

The *PHT* members are grouped into five families, viz., PHT1, PHT2, PHT3, PHT4, and PHT5 (Roch et al. 2019). Among the five members, only four *PHT1* family genes (*EcPHT1;1 to EcPHT1;4*) were described in three genotypes (ragi korchara, khairna, and VHC 3611) of FM (Pudake et al. 2017). In this study, rice's *PHT1* family gene sequences were used to identify and analyze the expression of the *PHT1* family genes in FM. The *EcPHT1;1* to *EcPHT1;4* genes were expressed in FM under the arbuscular mycorrhizal fungus (AMF) symbiosis and low P stress conditions (Pudake et al. 2017). When the root of FM is colonized with AMF, *EcPHT1;1, EcPHT1;2*, and *EcPHT1;3* were highly expressed in root of ragi korchara and leaves of khairna variety at the seedling stage (Fig. 11.1). However, the expression of *EcPHT1;1, EcPHT1;2*, and *EcPHT1;3* genes were similar in the genotype VHC361 when the plants are grown with and without AMF colonization. In all three genotypes, *EcPHT1;2* gene was not expressed in leaves. Among the four genes, *EcPHT1;4* did not express in leaves and roots of non-mycorrhizal seedlings of all three genotypes, but the expression was higher in roots where AMF colonization percentage was high. Under low Pi stress, the *EcPHT1;1* gene was highly expressed in 6-day-old leaves and roots compared with the control (Fig. 11.1). *EcPHT1;2* also highly expressed in roots under low Pi stress, but this gene was not expressed in leaves during the seedling stage. *EcPHT1;3* gene expressed in both leaves and roots was found to be more responsive under low Pi stress conditions (Fig. 11.1) (Pudake et al. 2017). Also, *EcPHT1;4* gene was not expressed in leaves and roots of FM genotypes under low Pi. These genes can serve to be an important resource for the better understanding of P use-efficiency (PUE). Now, the whole-genome

assembly of FM is available. This may help to identify more PHT1 family members in FM and for high-throughput studies to dissect the phosphate transport mechanism in this crop. Recently, we have analyzed the expression pattern of 12 *PHT1* family genes (*SiPHT1*;*1* to*1*;*12*) in FM under low and high Pi conditions using foxtail millet gene-specific primers (Maharajan et al. 2019). In our study, *SiPHT1*;*2*, *1*;*3,* and *1*;*4* genes were expressed in leaves of FM under low Pi conditions (Fig. 11.1). Furthermore, *SiPHT1*;*2* and *1*;*3* were expressed in roots of FM under low Pi conditions (Fig. 11.1). Interestingly, the expression level of *SiPHT1*;*2* and *1*;*3* were > onefold higher in both leaf and root tissues under low Pi compared to high Pi conditions (Maharajan et al. 2019). More than 10 *PHT1* family genes have been identified in various crop plants (Nussaume et al. 2011; Baker et al. 2015; Roch et al. 2019). The regulation and homeostasis of Pi are well studied during the last decade in model plants like rice and Arabidopsis (Ceasar 2020).

Many detailed studies are available on Pi transporters in model cereal crop rice (Table 11.1). In view to understand the Pi transport mechanism holistically in rice, Paszkowski et al. (2002) were the first one to identify 13 *PHT1* genes (*OsPHT1*; *1–1*; *13*) in Nippon bare genotype of rice under low and high Pi conditions. Among the 13 *OsPHT1* genes, *OsPHT1*;*1* was a key member which was expressed abundantly and constitutively in different cells of roots and shoots. The high-affinity *OsPHT1;1* was constitutively expressed in the shoots irrespective of Pi concentration and expression in the root was slightly elevated under low Pi condition (Seo et al. 2008). Further the histochemical analysis of β-glucuronidase (GUS) expression in transgenic plants indicated the expression of OsPHT1;1 in leaves, stems, and roots indicating their involvement in the acquisition and mobilization of Pi at the basal level regardless of Pi concentrations (Seo et al. 2008). Interestingly, the overexpression of *OsPHT1;1* gene in rice enhanced Pi acquisition. Under Pi sufficient condition, *OsPHT1;2* was expressed in roots and interestingly, Pi resupply resulted its expression in roots (Secco et al. 2013). The overexpression study of *OsPHT1;2* with the GUS reporter gene revealed that the OsPHT1;2 was localized exclusively in the stele cells and its transgenic studies in yeast Pi uptake mutant and *Xenopus oocytes* showed its function of OsPHT1;2 in the translocation of the stored Pi in the plant apart from Pi uptake (Seo et al. 2008; Ai et al. 2009). OsPHT1;4 played a vital role in the increased Pi uptake, translocation of Pi from root to shoot, and enhanced mobilization of Pi from other tissues to panicles (Ye et al. 2015). The expression study of high-affinity OsPHT1;6 revealed that it was upregulated predominantly in roots under low Pi condition and study with the GUS reporter gene showed that it was localized in both epidermal and cortical cells of younger primary and lateral roots (Seo et al. 2008; Ai et al. 2009; Secco et al. 2013). Similarly, transgenic studies in yeast Pi uptake mutant and *X. oocytes* revealed that OsPHT1;6 played a broader role in both Pi uptake and translocation of the stored Pi in the plant (Ai et al. 2009). The high-affinity OsPHT1;8 was upregulated predominantly in roots, especially in phloem of both root and leaf blade under low Pi condition and was also expressed in various tissue organs from roots to seeds regardless of Pi concentration (Jia et al. 2011; Li et al. 2015). Its overexpression resulted in higher Pi concentration in both roots and shoots pointing to its role in Pi uptake and Pi homeostasis in rice that are essential for the growth and development (Jia et al. 2011). Its characterization by RNA-interference (RNAi) proved its role in P redistribution from senescent or older leaves to metabolically active sink organs such as young leaves, growing roots, and developing seeds regulating the Pi homeostasis (Li et al. 2015). Knockdown of OsPHT1;8 showed its role in P accumulation in young leaves and endosperm and P transport from vegetative organ to entire seed that includes endosperm, embryo, and phytic acid which ensured normally the requirement of Pi for seed germination (Li et al. 2015). The high-affinity *OsPHT1;9* and *1;10* were expressed in root epidermis, root hairs, lateral roots and mesophyll and vasculature in leaves regardless of Pi

conditions and were redundantly functioned in Pi uptake which was confirmed by transgenic experiments with yeast and *X. oocytes* (Secco et al. 2013; Wang et al. 2014). The preferential expression of *OsPHT1;11* in stamen just before flowering helped in rice development (Liu et al. 2011), *OsPHT1;12* in roots (Seo et al. 2008), and *OsPHT1;13* in flag leaf after the heading stage led to loading of Pi in reproductive organs (Liu et al. 2011), which revealed primary role of these transporters in Pi uptake and translocation under low Pi conditions.

Among the small millets, the complete and annotated whole-genome sequence was first made available for foxtail millet which serves as a perfect model to study PHT1 in other millets (Ceasar et al. 2014, 2017; Ceasar 2019). The *PHT1* family transporter genes were identified and expression characterized in foxtail millet (Ceasar et al. 2014). Expression of 12 *SiPHT1* genes was analyzed in leaves, roots, and shoots of 15- and 30-day-old seedlings of foxtail millet grown under low and high Pi conditions and AMF and non-AMF colonized plants. Among the 12 genes, *SiPHT1*;*1*, *1*;*2*, *1*;*3*, *1*;*4, 1*;*11,* and *1*;*12* were expressed in shoots, *SiPHT1*;*2* and *1*;*3* in leaves, and *SiPHT1*;*2* and *1*;*4* in roots of 15-day-old seedlings of foxtail millet grown under both high and low Pi conditions. Furthermore, six SiPHT1 family members (SiPHT1;1, 1;2, 1;3 1;4 1;7, and 1;8) were characterized by complementation in *Saccharomyces cerevisiae* PHO84 mutants (ScPHO84). Complementation study with ScPHO84 mutants revealed that all these transporters could mediate Pi transport (Ceasar et al. 2017). Down-regulation of *SiPHT1*;1 and *1;3* through RNAi strategy significantly reduced the contents of total P and Pi in shoots and roots and increased the number of lateral roots. Similarly, down-regulation of *SiPHT1;2* strongly reduced the total P and Pi contents in root and shoot tissues (Ceasar et al. 2017). The above studies on PHT1 transporters would aid understanding the complex Pi transport system in small millets in order to improve Pi uptake, mobilization, and its efficient utilization in the Pi-deficient soils in Asia and Africa ensuring global food security. Minimum studies in FM call for further research on *PHT1* family genes and their characterization, post-translational regulation of Pi transport, plant–fungal signaling in the AMF-regulated Pi uptake, and remobilization mechanisms to improve PAE and PUE in the low-input agriculture soils. Draft genome sequences of FM (Hatakeyama et al. 2017; Hittalmani et al. 2017) will pave the way for the identification and functional characterization of all other *PHT1* family genes in FM in future.

11.3.3 Ca Transporters

Ca is a vital macronutrient for plant growth and development as well as for humans and animals. Ca is a third most important nutrient available in soil and is required for normal growth of plants. Generally, plants absorb Ca by roots from the soil solution, which reaches the shoots through the xylem stream. In FM, maximum Ca is present in aleurone layer followed by seed coat and embryo (Nath et al. 2013). Ca level is also associated with higher expression of Ca-signaling transporter genes (Carter et al. 2004). Although there is no active transpiration stream within cells of the mature embryo, nutrient transfer between maternal and filial tissues is restricted to the apoplast (Patrick and Offler 2001); therefore, changes in apoplastic Ca levels of the maternal plant could be reflected in the mature embryo or seed coat, which may be governed by Ca^{2+} transporter genes. The Ca transporters are involved in the uptake and transport of Ca in the cells, while Ca sensors are involved in the regulation of Ca transporters. Some Ca-signaling and transporter genes were identified in vegetative and reproductive stages of FM. For instance, two contrasting FM genotypes [GP-45 (high Ca accumulating) and GP-1 (low Ca accumulating)] were used to study the expression levels of various Ca transporter family genes such as $Ca2^{+}/H^{+}$ *antiporter* (*CAX1*), *two pore channel1* (*TPC1*), *calmodulin (CaM)-stimulated type IIB* $Ca2^{+}$ *ATPase* (*CaM IIB* $Ca2^{+}$ *ATPase*), and two *CaM-dependent protein kinase* (*CaMK1* and *CaMK2*) genes in FM (Mirza et al. 2014). Among these, *CAX1* was found to be

expressed in the late stages of spike development. The *TPC1* and *Ca^{2+} ATPase* were expressed in the root, stem, and developing spike of FM. This study revealed that CAX1 could be responsible for accumulating high concentrations of Ca in seeds; TPC1 and Ca^{2+} ATPase might be involved in the uptake and translocation of Ca. Same group also analyzed the expression pattern of 82 Ca sensor family genes including *Calmodulin* (CaM) and *Calciuneurin B-like protein* (*CBL*), *Ca^{2+}-dependent* and *CaM-independent protein kinases* (*CDPKs*), *SOS3/CBL interacting protein kinases* (*SIPKs/CIPKs*), *CaM-dependent protein kinases* (*CaMKs*), *Ca^{2+}/CaM-dependent protein kinases* (*CCaMKs*), *CDPK-related protein kinases* (*CRKs*), *phosphoenolpyruvate* (*PEP*), and *carboxylase kinase-related kinases* (*PEPRKs*) in the developing spikes of GP-1 and GP-45 genotypes of FM (Singh et al. 2014). The expression analysis revealed that 24 genes (7 encoded for *CaML*, 2-*CRK*, 5-*CBL*, 7-*CIPK,* and 4-*CDPK*) and 11 genes (5 encoded for *CaML*, 2-*CRK*, 3-*CIPK*, and 1-*CDPK*) were highly expressed in the developing spikes of GP-45 and GP-1 genotypes, respectively. Interestingly, *EcCIPK9* was highly expressed in the developing spike of GP-45 when compared to the GP-1 genotype (Fig. 11.1) (Singh et al. 2014). The same group also analyzed the expression pattern of 19 *Ca^{2+}* transporter family genes (11 *Ca^{2+} ATPases*, 7 Ca^{2+}/cation exchangers, and 1 Ca^{2+} channel) in the developing spikes of these two FM genotypes (Singh et al. 2015a, b). Chinchole et al. (2017) found that *EcCIPK24* gene was highly expressed in root, stem, and leaf tissues of the GP-45 genotype compared to the GP-1 genotype (Fig. 11.1). This study suggests that *EcCIPK24* can play an important role in high-seed Ca accumulation (Chinchole et al. 2017). Much of this research was done before the availability of whole-genome sequence of FM. Such genes have not been analyzed further through extensive studies. The previous studies on analyzing the expression levels of Ca uptake, translocation, and accumulation of genes in FM were performed without functional characterization and the identified candidate genes are merely a prediction. The complete sequences of proteins involved in Ca accumulation are still not available. Furthermore, overexpression of Ca-signaling transporter genes may help to increase the levels of Ca in the seeds. Hence, characterization of such genes is vital to understand and improve the Ca accumulation which will help for carrying over this trait onto millet and non-millet cereals.

The clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) genome editing tool which is projected to play a vital role in functional genome studies and crop improvement. CRISPR/Cas9 will be useful in the editing of genes associated with Ca accumulation in FM in future. CRISPR/Cas9 system has emerged as an alternative tool to RNAi for the precise knockout of the genes of higher plants. The overexpression or knockout study will help to understand the exact role of Ca accumulation gene in seed/grain. The high Ca content FM genotype will help to reduce Ca deficiency in worldwide particularly.

11.3.4 Zn Transporter

Zn transporters are involved in Zn uptake in numerous plants including *A. thaliana* (Plaza et al. 2007), rice (Ishimaru et al. 2005; Bashir et al. 2012), barley (*Hordeum vulgare*; (Pedas et al. 2008), soybean (Moreau et al. 2002), and tomato (Eckhardt et al. 2001). The improvement of seed Zn content is possible by modulating the metal transporters like ZIP family transporters that facilitate the uptake, translocation, and storage of Zn in plants. Recently, *EcZIP1* gene was identified from the whole-genome sequence of FM (Chandra et al. 2020). The expression level of *EcZIP* was analyzed in root, shoot, root–shoot zone, and flag leaf of six FM genotypes (VHC3582, IE3618, IE6240, VL330, GE724, and VHC3893) (Chandra et al. 2020). Among these genotypes, expression level of the *EcZIP1* gene was higher in root, shoot, root–shoot zone, and flag leaf of GE724 compared to the other genotypes. Till date, only one gene was identified and expression analyzed from the whole-genome

sequence of FM. However, such studies were widely performed in rice and many ZIP genes were functionally characterized. Totally, 16 ZIP transporters were identified and some of the transporters are functionally characterized in rice. Three *OsZIP* (*OsZIP1*, *OsZIP3,* and *OsZIP9*) transporters were found to be responsible for Zn uptake from the soil (Ramesh et al. 2003; Meng et al. 2018). Similarly, *OsZIP4*, *OsZIP5,* and *OsZIP8* transporters are involved in the translocation of Zn from root to shoot as well as OsZIP4 and OsZIP8 responsible for grain filling (Ishimaru et al. 2005; Lee et al. 2010a, b, c; Meng et al. 2018). *OsZIP9* gene was highly expressed in roots under Zn-deficiency conditions (Huang et al. 2020). The *OsZIP9* was localized at the root exodermis and endodermis and it functions as an influx transporter of Zn and contributes to Zn uptake under Zn-limited conditions in rice. Knockout of *OsZIP9* decreased the plant growth and Zn concentration in both root and shoot under Zn-deficient condition (Huang et al. 2020). *OsZIPs* could be used for crop improvement in rice for the enrichment of Zn in grains through the biofortification process. Studies on rice ZIP transporters call for more such research in FM to identify and characterize the functionally important Zn transporters for the efficient transport and biofortification of Zn. Researchers need to focus more on the identification and characterization of ZIP transporters in FM for optimized nutrient transport and biofortification in FM.

11.4 Genome-Wide Association Studies (GWAS) for Nutrient Traits in Finger Millet

The genomic information is essential for genome-wide association studies (GWASs) in crops. The next-generation sequencing (NGS) tool is useful for high-throughput and accurate genome (re)sequencing and to detect the genetic basis of phenotypic variations in crops. Therefore, it is helpful for understanding the genome organization of crops. The GWASs are useful to identify the genetic variants across the genomes of individuals and identify their genotype–phenotype associations (Tam et al. 2019). It is helpful to identify the genotypic variations of quantitative and qualitative traits among the phenotypes and helps for improving the crop genome with desirable traits through genome-assisted breeding (GAB) programs.

FM is a future crop for strengthening both food and nutritional aspects due to its climate resilience features. Many abiotic stresses especially nutrient deficiency affect FM production (Ceasar et al. 2018). The whole-genome sequence of FM genotype ML-365 (Hittalmani et al. 2017) and PR-202 (Hatakeyama et al. 2017) provide the basic foundation of GWASs in FM. The FM germplasm consisting of more than 22,799 accessions is available worldwide (Goron and Raizada 2015). Many researchers identified nutrient stress-tolerant genotypes in the mini-core collection of FM germplasm based on their phenotypic responses (Krishnamurthy et al. 2014, 2016; Ramakrishnan et al. 2017). Due to the advent of NGS selection of breeding materials has changed from phenotype-based to genotype-based selection. Genome-based characterization and GWASs are helpful for selection of best breeding materials in crop improvement. Till date, only very little effort was taken in GWASs in FM. Lack of whole-genome sequence in FM hampered the efficiency and opportunity for GAB so far. GWASs in FM are essential for crop improvement in future as it a crop with rich nutrient profile.

Only limited reports are available on GWAS in FM to dissect the nutrient-related traits. Genotyping-by-sequencing (GBS) technology was used to evaluate the agro-morphological traits of 113 FM genotypes with the identification of the single nucleotide polymorphism (SNP) markers linked to grain yield and its component traits (Sharma et al. 2018). Totally, 109 novel SNPs found to be associated with important agro-morphological traits in FM. Five SNP marker sequences showed homology to candidate genes of rice and foxtail millet. The candidate genes are responsible for flowering, maturity, and grain yield traits (Sharma et al. 2018). Recently, Swati et al. (2020) evaluated the

six nutritional traits (Fe, Zn, Ca, Mg, K, and Na) using 190 FM genotypes by GBS technology and identified the marker-trait associations (MTAs). Totally, 169,365 SNPs were generated from GBS and out of these 418 SNPs are linked with nutritional traits. In this study, 18 SNP markers showed homology with candidate genes having putative functions in binding, remobilization/transport of metal ions (Swati et al. 2020). These studies provide the genotypic variation of the phenotypes for the selection of best breeding materials to improve grain yield, nutritional content, and nutrient use-efficiency in FM. Only mini-core FM accessions were used to characterize the genotypic variation so far. Therefore, germplasm characterization through GBS technology provides the GWASs and genome variation of FM. The GWASs are abundant in other crops when compared to FM. GWASs for many agronomical traits have been reported in rice (Chen et al. 2014; Yang et al. 2014), maize (Hao et al. 2011; Xue et al. 2013), and barely (Cockram et al. 2010; Pasam et al. 2012). The importance and implications of GWAS in FM are represented in Fig. 11.2. Researchers need to focus more on GWASs in FM for the development of improved variety for nutrient stress tolerance.

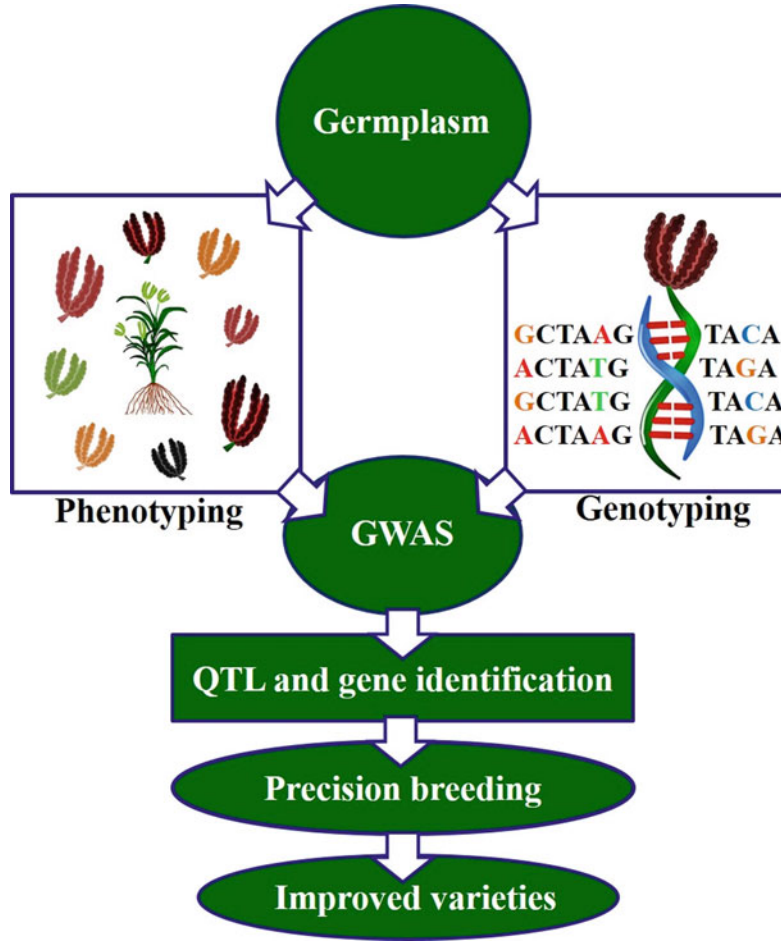

Fig. 11.2 Role of genome-wide association studies (GAWS) in finger millet improvement through precision breeding. This flowchart describes the GAWS useful for finger millet germplasm characterization based on genotype–phenotype methods for the identification of QTL and genes for developing improved varieties

11.5 Conclusion and Future Prospects

FM is a crop with rich nutrient profile compared to other major cereals. Its whole-genome sequence, annotation, and functional genomic studies are delayed due to its complex genome and cultivation in less developed countries. Plasma membrane-bound nutrient transporters play important role in the acquisition of nutrients from soil solutions, transport from root to shoot, redistribution to other parts, and storage in seeds. Various transporters are involved in uptake and transport of each nutrient in crop plants. Such transport proteins are well characterized in model cereal like rice. Despite possessing high content of essential nutrients like Ca, studies on nutrient transporters of FM are still lagging far behind especially due to lack of annotated genome sequence of FM. Identification of nutritionally important traits by GWAS also not exploited much in FM. The available draft genome sequences and complete annotation of genome in the near future may aid for the accelerated functional genomic studies to characterize the nutrient transporters and improve the FM. Characterization of nutrient transporters of FM not only helps to improve the FM but may also improve the nutritional value of other millets and non-millet cereals in future.

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Finger Millet as Input Use Efficient and Organic by Default Crop

12

Supriya Gupta, Sanjay Mohan Gupta, Kavita Gururani, Subodh Sinha, Rajeev Gupta, and Anil Kumar

Abstract

Nitrogen (N) is the mainly used nutrient source in agriculture that not only resulted in a significant increase in yield but also in considerable negative impacts on the environment. This impeded the demand for identification of nitrogen use efficient crops that enables uptake, utilize and remobilize the available N more efficiently (increased uptake efficiency) or low N-responsive genotypes having high nitrogen use efficiency (NUE) and grain yields (increased utilization efficiency). One way to tackle these problems is through the intervention of modern genetic engineering tools used for the essential nutrient enrichment of staple crops with improved NUE combined with superior agronomic traits. The genetic and genomic potential of suitable nitrogen use efficient crops like *Eleusine coracana* L. Gaertn (finger millet; FM), which is considered as input use efficient and organic by default crop can be used as source donor for this purpose. FM is well known for its exceptionally high calcium, rich amounts of protein, and important mineral (Fe, Zn, K, Mn, Zn, etc.) as compared to other major cereals. Apart from excellent nutraceutical value, it survives on almost no N-input and has a good NUE, which makes it an ideal model for developing strategies to make nitrogen use efficient crops. With the advent of modern functional genomics, proteomics, and association-based approaches; several regulatory gene networks controlling the NUE have been identified in FM. In plants, NUE is a complex trait and this chapter focused on the N-metabolism, their regulation in presence of nitrate as N-source, carbon: nitrogen (C:N) interactions and key genes/transcription factors (TFs) that influence the regulation of N-metabolism and ultimately grain yield.

12.1 Introduction

Nitrogen (N) is an important macronutrient that is crucial for the growth and development of plants and thus is a crucial determinant of the

S. Gupta · K. Gururani
Department of Molecular Biology and Genetic Engineering, G. B. P. U. A. & T., Pantnagar 263145, India

S. M. Gupta
Defence Institute of Bio-Energy Research (DIBER), DRDO, Min of Defence, Haldwani 263139, India

A. Kumar (✉)
Director Education, Rani Lakshmi Bai Central Agricultural, University, Jhansi 284001, India
e-mail: anilkumar.mbge@gmail.com

S. Sinha · R. Gupta
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Patancheru, Hyderabad, Telangana 502324, India

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crop productivity in all ecosystems. Plants cannot utilize gaseous air N_2 (except through symbiotic N-fixing microorganisms) and require dissolved forms of reactive N such as nitrate, urea, ammonium, and from organic or inorganic fertilizers (Vitousek et al. 2002). The capability of utilizing available soil N varies from plant to plant and plants being sessile have to respond and adjust biologically to the availability of soil N that differs in form and concentration. This opens opportunity for improvements in crop nitrogen use efficiency (NUE) at the genetic level for sustainable agriculture and food security (McAllister et al. 2012). Poor NUE results from either limited ability of the plant or excessive/inefficient use of fertilizers that not only threatens crop productivity but also has major ecological penalty including N-pollution of surface and ground, affecting fisheries, causing eutrophication, disturbing biodiversity and ecosystem, etc. (Sinha et al. 2020).

One way to tackle these problems is through the intervention of modern genetic engineering tools used for the essential nutrient enrichment of staple crops by using genetic and genomic potential of suitable nitrogen use efficient crops like *Eleusine coracana* L. Gaertn (finger millet; FM). FM is an annual herbaceous cereal crop, which is well known for its exceptionally high calcium, rich amounts of protein, and important mineral (Fe, Zn, K, Mn, Zn, etc.) as compared to other major cereals and also required less agricultural inputs since organic by default (Kumar et al. 2015a; Akbar et al. 2018). Apart from excellent nutraceutical value that makes it an exceptional model for exploring vast genetic and genomic potential of this crop that helps us in developing strategies for making nutrient enriched crops (Gupta et al. 2017). Also, the growing world population increases the demand for food that results in the overuse of inorganic N-fertilizers that are hazardous to the environment. Thus, to minimize the ecological pollution and for satisfying the high global demand for organic produce, it requires the low N-responsive crop genotypes with higher NUE and grain yields (Andrews and Lea 2013). Since other cereal genomes have a limited gene pool for high NUE, research on thoughtful the mechanism of the high NUE of FM is essentially required.

Presently, the NUE for crop plants is of great anxiety but due to lack of information about precise regulatory mechanisms to explain NUE in crop plants hampers the goal of productivity. Plant NUE can be defined in various ways such as N-uptake efficiency (NUpE), N-utilization efficiency (NUtE), agronomic efficiency of fertilizer N (AE), apparent N-recovery rate (ANR), N-physiological use efficiency (NpUE), N-remobilization efficiency (NRE), and N-transport efficiency (NTE). However, for most practical purposes, plant physiologists consider definitions of NUE that rely on N-uptake from external sources but internal remobilization is known to be significant in crops (Xu et al. 2012). Thus, the identification of potential candidate genes/proteins in the regulation of NUE will provide biomarker(s) for determining the genotypic potential for optimization of N-input in agriculture (Kumar et al. 2009a; Gururani et al. 2020). FM can survive on almost no N-input with high NUE and also accumulates high amounts of proteins in its grains. Thus, it proves that the FM crop has devised unique regulatory controls to attain high NUE under low N-conditions.

This chapter highlights the various plant biological processes that could determine NUE, as well as the promising strategies for improving NUE at the plant that will be supportive to strengthen the nutritional security and food.

12.2 Finger Millet: A Crop Organic by Default

FM (Fam. Poaceae) is a native crop of Central Africa that mainly grown as a cereal grain in the semi-arid tropics and subtropics regions of East Africa and Southern India under rainfed conditions. This crop is tolerant to a wide range of environmental temperature and can survive significant levels of biotic and abiotic stresses (Gupta et al. 2017). It is grown mainly by marginal farmers and serves as a food security crop due to its high-nutritional value and excellent

storage qualities (stored without any pesticides), which makes it an ideal grain for food crisis prone areas. It is habitually grown on marginal soils in rainfed conditions and irregular drought spells have a negative effect on yield. It is considered to be nutritionally rich and is one of the cheapest sources of dietary energy in the form of carbohydrates and proteins (Kumar et al. 2018). It is highly rich in calcium and due to the high proportion of fiber; it has hypoglycemic effects that make the products of this crops suitable for consumption by heart and diabetic patients (Gupta et al. 2011). The cultivation of small millets including FM is organic by default and capitalizes on the low usage level of synthetic fertilizers and pesticides-organic farming. FM can respond very well under low N-conditions therefore, it can be considered that this crop has NUE. Thus studying the molecular basis and finding the genes involved for high NUE and higher yield even at low N-input may help a lot in reducing the N-fertilizer application and therefore increased production economically (Kumar et al. 2015b).

12.3 Nitrogen Use Efficiency (NUE) in Crop Plants

NUE can be defined as the plant ability to utilize the available N-resources into its productivity. It includes various processes such as N-uptake and assimilation, remobilization within the cell, and balance between storage and current use at the cellular and at plant level. In agriculture terms, it is the optimal utilization of nitrogenous matter (fertilizers) for plant protein content, growth, and yield. As we all know atmospheric N gas is not utilized by higher plants, except by some symbiotic legumes. NUE can be estimated as grain yield/available N-content (soil + N-fertilizer). NUE is governed by various genes and many external and internal factors including soil N-availability, etc. Broadly, there are two main components of NUE; firstly (1) efficiency of absorption (uptake); and secondly (2) utilization efficiency of N-absorbed into produce grain protein (Moll et al. 1982). The nitrogen uptake efficiency (NUpE) can be defined as plant N-content/available N-content (soil + N-fertilizer), while nitrogen utilization efficiency (NUtE) is defined as grain yield/plant N-content. Both components are controlled by a combination of environmental (denitrification, volatilization and/or leaching, etc.) and genetic factors (latent ability of genotypes/cultivars, soil microflora, etc.) across genotypes (Francis et al. 1993). Not only these factors, but also N-metabolism is another major contributing factor, which affects NUE.

In order to improve agricultural productivity, many technologies and practices have been tried in past for improving NUE. Some of the important opportunities are (i) increasing yield potential through crop management and genetic improvement; (ii) optimum utilization of availablemN through balanced nutrition; (iii) multiple N-applications to match N-requirements of plant in the growing season; (iv) using slow- and controlled-release fertilizers in field; (v) use of fertilizer additives to reduce N-losses; (vi) site-specific N-management prescriptive, corrective; (vii) use of decision support systems such as computer-based models or simple field assessment tools and interpretation aids, etc.; (viii) genetic improvement in N-recovery or NUtE of some underutilized crops; and (ix) Use of green organic manuring in crops (Giller et al. 2004; Gupta et al. 2012b).

In addition to these the inherent efficiency of the plant to utilize available N-inputs for crop productivity must be tackled biologically. Since overuse of inorganic N-fertilizers is environmentally hazardous causing poisoning of rivers and coastal waters (Sinha et al. 2020). Thus, the designing of crops with higher N-efficiency is essentially required and also poses a big challenge for molecular breeders. Marker assisted selection (MAS) of genotypes related to low and high N-inputs will allow us to enhance agricultural productivity. It will also in turn led to the discovery of novel N-responsive genes and their *cis* and *trans* acting gene elements that may be used as biomarkers for determining of genotypic potential of NUE (Kumar et al. 2009a).

12.4 Nitrogen Uptake, Assimilation, and Remobilization

12.4.1 Nitrogen Sources and Uptake

Plants have evolved many mechanisms to acquire N at low concentrations and also to use different forms of N. Plants can assimilate N in inorganic (nitrate and ammonia) and organic (organic N-compounds, urea, etc.) forms. Nitrate (NO_3^-) is the main source of N for most of the plants (Vitousek et al. 2002). In plants, only a proportion of the absorbed NO_3^- is assimilated in the root, while the rest being transported upwards through the xylem for assimilation in the shoot for their internal remobilization and translocation between different plant organs (Xu et al. 2012). However, the excess NO_3^- accumulated in the vacuole and lost to the soil solution by efflux across the PM (Miller and Smith 1996). Thus, an essential element in the process of NO_3^- integration in plant tissues is the trafficking of the NO_3^- ion across the PM membranes.

12.4.2 Nitrate Uptake and Assimilation

Nitrate (NO_3^-) uptake by plant root cells is the main step of N-metabolism and has been widely studied at the molecular and physiological levels (Orsel et al. 2002). The NO_3^- acquired is partly utilized in the roots but largely transported to the shoots, which have the photo-synthetically accumulated reducing power to convert it into nitrite with the help of nitrate reductase enzyme present in the cell cytoplasm. After that nitrite is translocated into the plastids, where it is converted into ammonium (NH_4^+) by nitrite reductase enzyme. Finally, the NH_4^+ ions are integrated into amino acids by glutamate synthetase (GOGAT) plastidic and glutamine synthase (GS2) cycle, with the help of 2-oxoglutarate from photosynthetic C-fixation, followed by further transformations through amino-transferases (Masclaux-Daubresse et al. 2006) (Fig. 12.1). It is called primary N-assimilation or metabolism, which depends heavily on carbon–nitrogen (C–N) balance. The NO_3^- uptake system in plant must be flexible and vigorous since plant has to transport sufficient NO_3^- to meet out the total N-demand. Therefore, in order to work efficiently the plants have evolved a transport system, which is active, multiphasic, and regulated. The needed energy that drives NO_3^- uptake comes from the H^+ gradient maintained across the plasma membrane by the H^+-ATPase. Plants have different types of transporters families (high-affinity transport system or HATS and low-affinity transporters or LATS) in their roots to take up NO_3^-/NH_4^+/urea from the soil, as well as in the shoots for their internal remobilization and translocation between various tissues and organs (Xu et al. 2012). Plant root architecture is very important bearing on NUE, due to the major role of roots in N-sensing and acquisition in plants (Chakraborty and Raghuram 2011). Also, NH_4^+ assimilation requires less energy as compared to NO_3^- and NH_4^+ is the preferential form of N-uptake when plants are under N-deficiency (Gazzarrini et al. 1999). But, excessive NH_4^+ uptake into plants cells may be lethal (Kronzucker et al. 2001). In plants NH_4^+ uptake is facilitated by the presence of transporter proteins. Both HATS and LATS are responsible for NH_4^+ uptake and are present in plant roots, which are constitutive and do not seem to be significantly induced by NH_4^+ (Glass et al. 2002). So far many NH_4^+ transporters have been characterized in several crops (Gaur et al. 2011).

12.4.3 Nitrogen Remobilization

The plant leaf serves as a sink for N during the vegetative growth of the plants and also serves as a source for remobilization of N during seed development, which undergo senescence during seed setting (Okumoto and Pilot 2011). The cytosolic glutamine synthase (GS1) plays important role in this secondary N-metabolism/-remobilization (Goodall and Tobin 2013). The leaf proteins and in particular photosynthetic proteins of plastids are extensively degraded

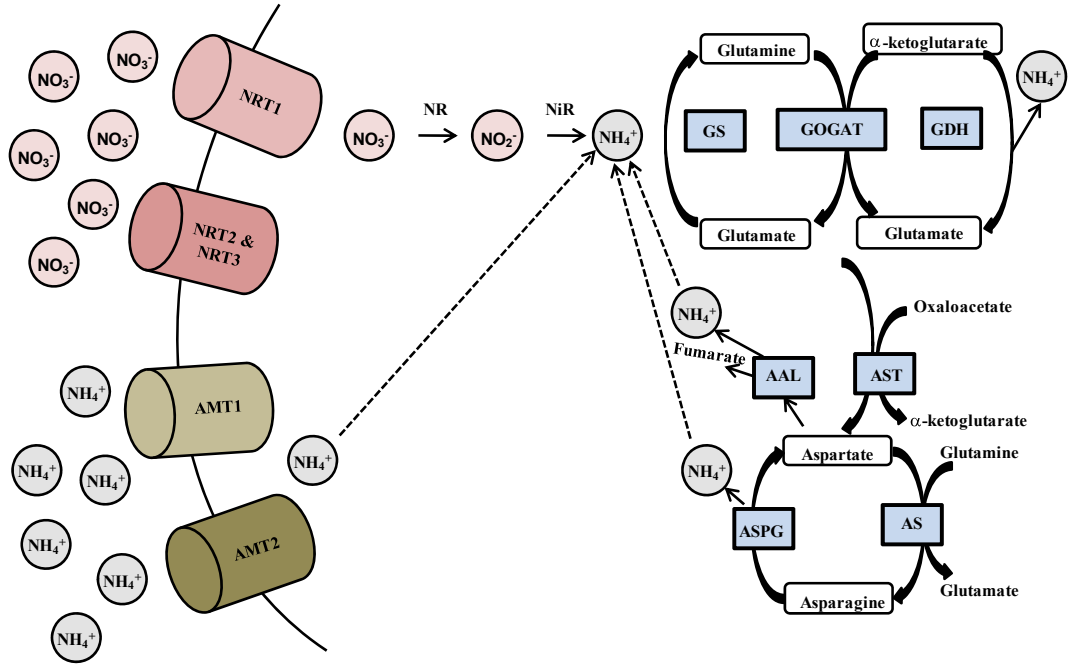

Fig. 12.1 Schematic representation of biosynthetic pathway of N-uptake and -utilization to generate amino acids in cereals (AMT1: High-affinity ammonium transporter; AMT2: Low-affinity ammonium transporter; NTR1/PTR: Low-affinity nitrate transporter; NTR2 and 3: High-affinity nitrate transporter; NR: Nitrate reductase; NiR: Nitrite reductase; GS: Glutamine synthetase; GDH: Glutamate dehydrogenase; GOGAT: Glutamine Oxoglutarate aminotransferase; AST: Aspartate aminotransferase; AS: Asparagine synthetase; ASPG: Asparaginase; AAL: Aspartate ammonia-lyase)

during senescence, providing an enormous source of N that plants can use to supplement the nutrition of growing organs like new leaves and seeds. The N-remobilization has been studied in many crops by "apparent remobilization" method, which is the determination of the amount of total N present in the different plant organs at different times of development and through ^{15}N long-term labeling (Diaz et al. 2008). The N-uptake and assimilation during the grain filling period is generally in sufficient for the high demand of the seeds. The remobilization steps, occurring successively in the different plant organs, are needed to route N to the seeds. The contribution of leaf N-remobilization to wheat, rice, or maize grain N-content is cultivar dependent, which varies from 50 to 90% (Masclaux-Daubresse et al. 2010). Also, N-remobilization is environment dependent and favored under limiting nitrate supplies (Diaz et al. 2008). Leaf senescence seems then to have a special role in N-availability and its mobilization during grain filling (Uauy et al. 2006). However, delaying in leaf senescence may result in prolongation of photosynthesis, which increases grain yield and carbon filling into seeds. Breeding plants have then to cope with the dilemma that delayed senescence may lead to higher yields but also decreases in N-remobilization efficiency (NRE) and finally decrease in grain protein content. On the other hand, increasing N-remobilization has the advantage of reusing N from the other vegetative parts and of lowering N-loss in the dry remains.

12.5 Cross-Talk of Nitrogen and Carbon Metabolism

The C and N-metabolism are associated with each other, leading to interdependence. Depending on physio-chemical properties of soil, NO_3^- and NH_4^+ are inorganic N-sources taken up by higher plants through their root system which are further assimilated inside the root and shoot system of plant involving the reduction of NO_3^- to NO_2^- and then to NH_4^+, followed by NH_4^+ assimilation into amino acids. Reduction of NO_3^- and NO_2^- occurs in roots as well as shoots depending on species and external conditions, but is spatially distributed between the cytoplasm for NO_3^- reduction and plastids/chloroplasts for NO_2^- reduction. Reduction of NO_3^- into NO_2^- is catalyzed by nitrate reductase (NR) enzyme. Two classes of genes, i.e., *Nia* genes encoding the NR apoenzyme and *Cnx* genes encoding the MoCo have been identified so far. The NR enzyme from corn root and barley has been found to be associated with plasma membrane (PM-NR). Reduction of NO_3^- is followed by NO_2^- translocation to the chloroplast and then reduction to NH_4^+ by the second enzyme of the pathway, i.e., the nitrite reductase (NiR). The *Nii* genes encoding the NiR enzyme varying in number from one to two copies have been cloned from various species (Meyer and Stitt 2001). Assimilation of NH_4^+, either derived from NO_3^- reduction or from photorespiration or amino acid recycling, is mainly takes place by the GS/GOGAT pathway in the plastid/chloroplast (Lea and Forde 1994). The glutamine synthetase (GS) catalyzes the conversion of NH_4^+ and glutamate molecule into glutamine, which further reacts with 2-oxoglutarate to synthesize two molecules of glutamate by enzymatic catalysis of glutamine 2-oxoglutarate amino transferase [or glutamate synthase; GOGAT] (Unno et al. 2006). The two classes of nuclear genes code for GS, firstly GLN2; present as a single nuclear gene in all the species studied so far that codes for the chloroplastic GS2, and secondly GLN1; a gene family codes for cytosolic GS1 isoforms (e.g., five in maize), which are temporally, spatially, and developmentally regulated and present in different organs such as roots, leaves, or stems. GLN2 is thought to catalyze the primary assimilation of NH_4^+ resulted from NO_3^- reduction in both C3 and C4 plants and also in re-assimilation of it synthesized during photorespiration in C3 plants. Interestingly it has been observed that NH_4^+ flux through the photorespiration pathway in the leaves of C3 plants is almost 5-tenfold higher than that of produced from NO_3^- reduction (Keys et al. 1978). However, the GLN1 gene family regulates NH_4^+ recycling during developmental changes like leaf senescence and in glutamine synthesis for its transport into the phloem sap (Bernard and Habash 2009). The two different forms of glutamate synthase present in plants, viz., NADH-GOGAT and Fd-GOGAT that require NADH and ferredoxin, respectively, as electron donors (Vanoni et al. 2005) and widely localized in leaf chloroplasts and in plastids of non-photosynthetic tissues, respectively. Another cytosolic enzyme asparagine synthetase (AS) catalyzes the ATP-dependent transfer of the amido group from glutamine to aspartate molecule to make glutamate and asparagine and also can use ammonia as a substrate (Masclaux-Daubresse et al. 2006) (Fig. 12.2). The diurnally controlled ASN gene family of *Arabidopsis* (ASN1, ASN2, and ASN3) contributes in N-storage and its transport for allocation of N-resources. The overexpression of *ASN1* gene in transgenic Arabidopsis causes enhanced N-status in seed protein content (Lam et al. 2003). It has been confirmed that ZmASN4 seems to have direct associations with CLC-a, PEPC, TPS, SUS, and regulatory genes GLK5, GLK8 Ser/Thr phosphatase, and Zn finger when using *ZmASN4* as seed gene (Jiang et al. 2018). Carbamoyl phosphate, a precursor of citrulline and arginine is produced by carbamoyl phosphate synthase (CPSase) within plastids using bicarbonate, ATP, and NH_4^+ or the amide group of glutamine (Potel et al. 2009). Though GDH shows its predominant activity in deamination of glutamate in plant cells, which is shown to be indispensable for plant survival in dark conditions (Miyashita and Good 2008), in response to high levels of NH_4^+ under stress it has

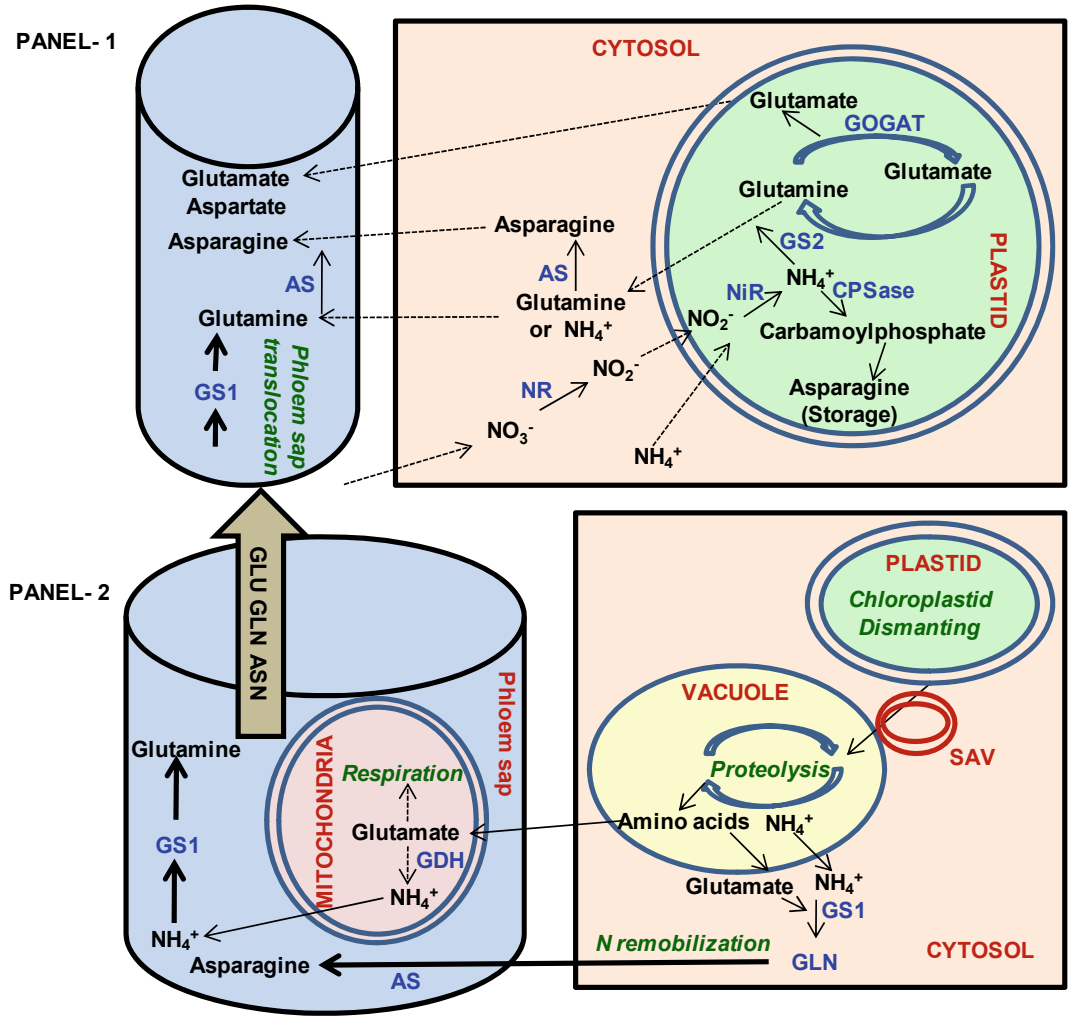

Fig. 12.2 Role of key enzymes participating in nitrogen metabolism in young (Panel 1) and Senescing leaves (Panel 2). Panel (1) Nitrate reductase (NR), Asparagines synthetase (AS) and Glutamine synthetase 1 (GS1) are present in cytosol while nitrite reductase (NiR), Glutamine synthetase 2 Isozyme (GS2), Glutamate synthase (GOGAT) and Carbamoyl phosphate synthetase (CPSase) are within the plastids/chloroplast. Panel (2) Senescence-associated events include chloroplast degradation and translocation of plastids proteins to the central vacuole via senescence-associated vacuole (SAV) trafficking. Amino acid recycling is carried in mitochondria and cytosol of mesophyll cells. GS1, AS, and Glutamate dehydrogenase (GDH) are the major enzymes involved in biosynthesis of glutamine, asparagines, and glutamate in the phloem

been observed that mitochondrial NADH-glutamate dehydrogenase could also otherwise incorporate NH_4^+ into glutamate (Skopelitis et al. 2006). NR, NiR, and GOGAT need either NADH or ferredoxin (Fdx) as co-factor for its enzymatic catalysis whereas glutamine synthetase and asparagine synthetase need ATP. C-skeletons and keto-acids are essential to form organic N in the form of amino acids, therefore, availability of C-skeletons controls N-assimilation through NH_4^+ condensation and the supply of Fdx, ATP, and NADH as products of respiration, photosynthesis and photorespiration pathways.

Genome-wide expression studies under different C/N condition have proven that C-sources like different sugars regulate the expression of

transporters involved in nitrate, ammonium, and urea also enzymes involved in N-assimilation (Malamy and Ryan 2001). As far as nitrate is concerned it not only acts as N-source but also acts as signal molecule and determines various processes of growth and development of plants including the expression of C-metabolism genes (Wang et al. 2003). C and N alone have been found to repress or induce specific genes but together they are unable to show the same effect (Gutierrez et al. 2007). Thus, the C/N balance is maintained by networking of genes associated with metabolism involved in signal transduction, hormone pathways, and protein synthesis.

12.6 Role of NUE in Relation to Yield and Grain Protein Content

The nitrogen harvest index (NHI) is defined as the ratio of seed-N to total shoot-N. It is also considered to be one of the good measures to know how efficiently plant utilizes acquired N for production of seed protein. N-remobilization from vegetative parts of the plant accounts in between 60 and 92% of grain N. As observed, the percent of N-remobilized to the grain is less under high N-application but this also depends on the intensity of post-anthesis N-uptake and environmental conditions. Bogard et al. (2011) showed that increasing uptake of N after anthesis was a major factor in increasing grain protein content (GPC). They demonstrated that synchronization of N-demand and supply in plants, and the relationship of N-supply with other environmental factors will influence GPC. The early senescence governed by NAM (no apical meristem) TF has been found to be linked to the improved N-content in wheat grains related to the high GPC locus (Gpc-B1). Generally a negative correlation between grain yield and GPC has been observed, which may be a barrier for improvement in protein accumulation. Because, seed protein cannot adequately form without available N; thus, a supply of N is a prerequisite for high protein yield. It has also been observed that changes to the N-availability to the plant at critical developmental stages can also increase the NUE (Kumar et al. 2009a). The best strategy forward is to deal with factors responsible for regulation of N-metabolism alongside N-reallocation to developing seeds.

12.7 Molecular Mechanisms Involved in NUE and Their Regulation

Plants constantly sense changes in N-availability both in external nutrient/soil media as well as inside various organs (below and above-ground part) and respond appropriately by modulating morphology of the belowground part, i.e., roots (Sinha et al. 2018) and gene expression in both parts. Plants employ multiple routes for the systemic and long-distance signaling and communication of N-status and availability. These depend on nitrate itself (nitrate-specific signaling), phytohormones, small peptides, etc. Recent studies proposed that nitrate-specific signaling functions mainly in the context of the synthesis of nucleic acids and amino acids, which includes the control of expression of many of genes.

PII has been shown to play a central regulatory role in N-sensing and signaling in bacteria and archaebacteria through interacting with several key proteins involved in N-metabolism at protein level (Huergo et al. 2013). For instance, PII proteins binds with the key effector metabolites 2-OG (2-oxoglutarate), ATP, and ADP. Crystal structure revealed that flexible T-loops of PII facilitate interaction with the target protein. Conformation change and covalent modification are major post-translational modifications of PII protein through, which it interacts with different target proteins in response to changes in cellular energy status and, C and N-sources in cyanobacteria. A PII-like protein/homologue, GLB1, has been studied in Arabidopsis and castor bean, which causes accumulation of anthocyanins and decreased ability to sense or metabolizes glutamine when overexpressed (Hsieh et al. 1998). Studies indicate the strong role of PII in regulation of arginine biosynthesis and possibly in sensing of

internal N-levels (Ferrario-Mery et al. 2008) and approximately tenfold increase in PII transcripts has been shown in the early to late stages of seed development, suggesting a link between protein storage and PII (Uhrig et al. 2009). GLB1-PII knock-out mutants proved their important roles in N-uptake and -assimilation. The control of carbon transport by PII in *Synechocystis* sp. PCC 6803 was studied in a PII mutant. PII homolog in FM genome has been identified, which indicates the possibility of it to be a regulatory molecule in cereals too. The current understandings revealed that the roles of nitrate-hormone cross-talk in plant development (Ristova et al. 2016).

12.7.1 Regulation of Nitrogen Uptake, Assimilation, and Remobilization by Nitrate and Carbon Availabilities

Multiple mechanisms along with nitrate availability constitute NO_3^- uptake and signaling which is controlled by feedback repression by N-status and stimulation by photosynthesis regulation (Forde 2000). Plant integrates the different processes including N-uptake by the root system and N-assimilation to match the nutrient demand of it and the expression and/or activity of transport system and enzymes that is modulated by external stimuli/stresses/nutritional status of the plant through various regulatory mechanisms. One of the mechanisms is operated at the transcriptional level, which includes the induction by substrate and repression by endogenous N-assimilates and results in an up-regulation with low N and a down-regulation with high N. Accordingly, several NRTs and AMTs along with *Nia* and *Nii* genes were found to be transcriptionally repressed by N-metabolites like glutamine (Meyer and Stitt 2001). Depending on the external N-concentration and their distribution, expression of different NH_4^+ and NO_3^- transporters is induced/repressed. In higher plants, four families of NO_3^- transporters were identified (Krapp et al. 2014). Nitrate transporter 1 (NRT1/PTRs), nitrate transporter 2 (NRT2), chloride channel (CLC) a/b, and slow anion channel-associated 1 homolog 3 (SLAH3), which are located right from root epidermal cells to newly developing seeds. Nitrate taken by roots was transported via various types of NO_3^- transporter 1 (NRT1/NPF) and NO_3^- transporter 2 (NRT2). Source-to-sink phloem remobilization of NO_3^- have been reported that involve different transporters [NRT1.7, NRT1.9, NRT2.4, and NRT2.5] (Gupta et al. 2012b, 2013); NRT1.4/NPF6.2 in its storage in petiole (Fan et al. 2009); CLC a/b in accumulation in vacuoles (De Angeli et al. 2006); NRT1.6 and NRT2.7 during NO_3^- accumulation in seeds (Chopin et al. 2007); NRT1.11 (shoot specific); CHL1/NPF6.3 and SLAH3 in stomatal functioning; NPF2.3 in NO_3^- translocation to shoots under salt stress; NRT1.12 in xylem-to-phloem transfer for nitrate redistribution (Hsu and Tsay 2013); and NPF5.5 in embryo N-accumulation (Taochy et al. 2015). Most of the LATS and HATS are inducible except NRT2.5 (HATS) and NRT1.2 (LATS), which are constitutive transporters (Kotur and Glass 2015). Proteins of AMT/Rh/Mep family mediate NH_4^+ transport across membranes (Santos et al. 2017). NRTs and AMTs differ in their biochemical properties, tissue localization, and regulation at transcriptional level. NARs (Nitrate Assimilation Related/NRT3) are another type of membrane protein, which interact and are required for the activity of some high-affinity nitrate transporters.

Transcriptome studies showed that NO_3^- regulates N-uptake and its assimilation through a regulator of gene expression, which further coordinating for C- and N-metabolism (Kumar et al. 2014). Photosynthesis stimulates the N-uptake and -assimilation (Kanwal et al. 2014) which suggest its direct correlation with C-status. Therefore, identification of *cis* elements responsible for N and C signaling interactions will provide new opportunity for manipulating the pathway for enhancing NUE (Palenchar et al. 2004). Also, NO_3^- regulation has been proven to be independent of sugar regulation pathways like hexokinase signaling. Thus, over-expression of

AtNRT2.1 and *AtNRT1.1* was prejudiced by the concentration of glucose 6-phosphate (Wirth et al. 2007). On other hand, diurnal regulation of the *Nia* transcripts is also governed by phytochrome and sugars (Lillo 2008). HY5 and its homologue HYH, (bZIP family TFs), were reported to play an important role in phytochrome dependent light-activated expression of NR and NRT2.1. In Arabidopsis, evidences showed that SnRK1s (Snf1-related protein kinases) are central key regulator that integrates transcriptional networks during stress that are inactivated by sugars (Baena-Gonzalez and Sheen 2008). In addition to phosphorylation, chloroplastic N-assimilation enzymes such as NIR, GS2, and Fd-GOGAT were also redox regulated through the thioredoxin system (Krouk et al. 2010).

12.7.2 Role of Nitrate and Hormones in Nitrogen Signaling

Nitrate (NO_3^-) one of the essential nutrients plays a significant role as a crucial signal molecule during plant growth, development, and stress responses is center of communication among plant intrinsic programs and the environment. In response to NO_3^- availability plants have evolved elaborate adaptive sensing including membrane and cytosol sensing, signaling and regulatory network, TFs and their interactions, and NO_3^--responsive regulatory elements (Ho et al. 2009) with the interaction of environment, for survival, fitness, and reproduction. A series of NO_3^- transporters are involved in the uptake of NO_3^- ions and their distribution throughout the plant parts (Crawford and Glass 1998). Past studies revealed that NO_3^- signaling determined the N-status using numerous pathway and processes, particularly hormone signaling pathways (Krouk 2016). NO_3^- availability and NUE of crops determined the lateral root growth and development. NO_3^- induces genes coding for assimilatory enzymes, NR and NiR with the involvement of NLP7 TF, while glutamine represses them. An N-dependent regulation of nitrate reductase and glutamine synthetase is modulated by 14-3-3 TF. CCA1 TF, which is involved in the regulation of circadian clock coordinates the response of nitrate assimilatory genes. NO_3^- as a signal regulates the transcription of wide range of nitrate-responsive genes that require *cis* regulatory sequences or nitrate response elements**.** Such regulatory sequence, A [G/C]TCA core sequence motif, preceded by a 7-bp AT rich region, has been reported in *Arabidopsis thaliana* and birch on the basis of promoter deletion analyses in NO_3^- and nitrite reductase (Raghuram et al. 2006).

Integration of extrinsic signal and intrinsic responses during different stages of plant growth and development is critically regulated by endogenous phytohormones. NO_3^- and hormonal signaling regulates morphological, physiological features at molecular levels and affects important agronomic traits, especially nutrient-dependent adaptive root system architecture (Bellegarde et al. 2017). From studies it has been revealed that NO_3^- signaling partly regulates the biosynthesis, de-conjugation, transport, and signaling of hormones. It was reported in Arabidopsis that direct effect of N-availability on regulation of TAA1 (along with its homologs such as TAR1 and TAR2) at the first step of IAA biosynthesis. However, PIN-FORMED PROTEIN 7 (PIN7) and TAR2 are found to be crucial among NLP7-activated genes along with NO_3^- assimilation genes such as *NiR, NIA1*, *FNR2,* and *NRT2.1* by studying genome-wide transcriptional profiling (O'Malley et al. 2016). Repressed auxin accumulation was observed during LR primordia emergence and numbers in *tar2* mutants under limited N-conditions (Vidal et al. 2015).

N-status regulates cytokinin metabolism and its translocation that changes root water permeability and root pressure in response to NO_3^- uptake (Hoarau et al. 1996) and also may regulate the accumulation of cytokinin (CK), as observed in roots of barley and Arabidopsis (Takei et al. 2002). Previous studies revealed that His-Asp phosphor relay systems (two-component regulatory systems) are involved in CK perception and signaling (Haberer and Kieber 2002). Transcript profiling of genes of CK-metabolism and signaling showed up-regulation of *CYP735A2* and *IPT3* transcripts with high

NO_3^- involved in the biosynthesis of *trans*-zeatin-type (*t*Z-type) CK in roots. NO_3^- seems to be a major factor for controlling *CYP735A2* and *IPT3* expression and also root system architecture under various abiotic stresses and shaping NO_3^--dependent spatiotemporal CK distribution in plants and further to induce CYTOKININ RESPONSE FACTORS (CRFs) (Rashotte et al. 2006; Ramireddy et al. 2018).

In Arabidopsis, a low-affinity NO_3^- transporter (NRT1.2/NPF4.6), i.e., ABA-importing transporter (AIT) 1, also has been found to be involved in post-germination growth and cellular ABA transport during seed germination (Kanno et al. 2012). It might also be helpful in regulation of stomatal aperture in inflorescence involving transfer of ABA from vascular tissues to guard cells. NO_3^- signaling and ABA degradation showed their direct molecular link in seed germination (Fan et al. 2016) and catabolism of ABA and *CYP707A2* expression was found to be regulated by NLP8 (conserved nitrate regulators), which is indispensable for NO_3^--induced seed germination (Footitt et al. 2013). High NO_3^- also stimulates the release of bioactive ABA from inactive storage form e.g., ABA-glucose ester (ABA-GE) (Ondzighi-Assoume et al. 2016). NO_3^- along with GA regulates nutrient availability and floral induction (Castro-Marín et al. 2011) and GA also regulates the expression of *NPF3.1* in endodermis by suppressing its transcription, which is promoted by ABA (Tal et al. 2016). NO_3^-, GA, and ABA play an important role to determine seed dormancy and germination times (Alboresi et al. 2005).

12.8 Mastery of Transcription Factors Involved in C: N Metabolism

Many factors are responsible for regulation of gene expressions; among them transcription factors (TFs) and RNA binding proteins are notable. The importance of TF as a tool for the dissection and manipulation of multigenic traits has fuelled the development of new TF-based technologies and approaches that will benefit both gene discovery and crop improvement.

The Dof (DNA binding with one finger) transcription factors (TFs), which is a master regulator in the accumulation of seed protein and yield characteristic through regulation of many rate limiting enzymes of C/N-metabolism pathways (Gupta et al. 2011, 2012a; Gaur et al. 2018). Dof proteins bind on, particularly the AAAG core motif (Plesch et al. 2001) present in many promoter regions, which have been involved in the regulation of various genes like light-regulated and stress/phytohormone-responsive genes, etc. (Ward et al. 2005; Yanagisawa 2000) (Fig. 12.3). Recent past studies proved that several members of the *Dof TF* gene family act like key regulators by simultaneously regulating the various genes involved in nitrogen (NR, GOGAT, GS, etc.) and carbon (PK, PEPC, CyPPDK) metabolism (Yanagisawa 2000; Kumar et al. 2009b; Gupta et al. 2013, 2014a, b, 2018).

Also, nitrate-responsive DNA regulatory elements and transcriptional regulators have been identified (Wang et al. 2012). One of the best studied TFs is NIN-LIKE PROTEIN 7 (NLP7) that directly binds to the upstream promoter region of N-responsive genes to regulate their expression (Konishi and Yanagisawa 2013). In C4 plants, NLP is a recently identified family, having consensus motif conserved RWP-RK domain, with the function in N-regulation of nodule organogenesis and development as discussed in lotus and legume plants (Schauser et al. 2005).

Another gene of interests in this series is phytochrome-activated MYB-related TF, e.g., circadian clock associated 1 (*CCA1*). As a TF, CCA1 potentially affects plant metabolism directly or indirectly via being a part of circadian clock oscillator. *CCA1* binds to the promoter of light-harvesting chl *a/b* protein (CAB1) and thereby regulates the expression of genes in the carbon-based metabolism including starch storage and its conversion into sugars and downstream pathways. In addition to regulate the C-availability, *CCA1* also influences the expression of N-metabolism genes, viz., *GLN1;3* and *GDH1*. Other TF that has shown its role in NUE is HAP3, heme activator proteins (HAP) are

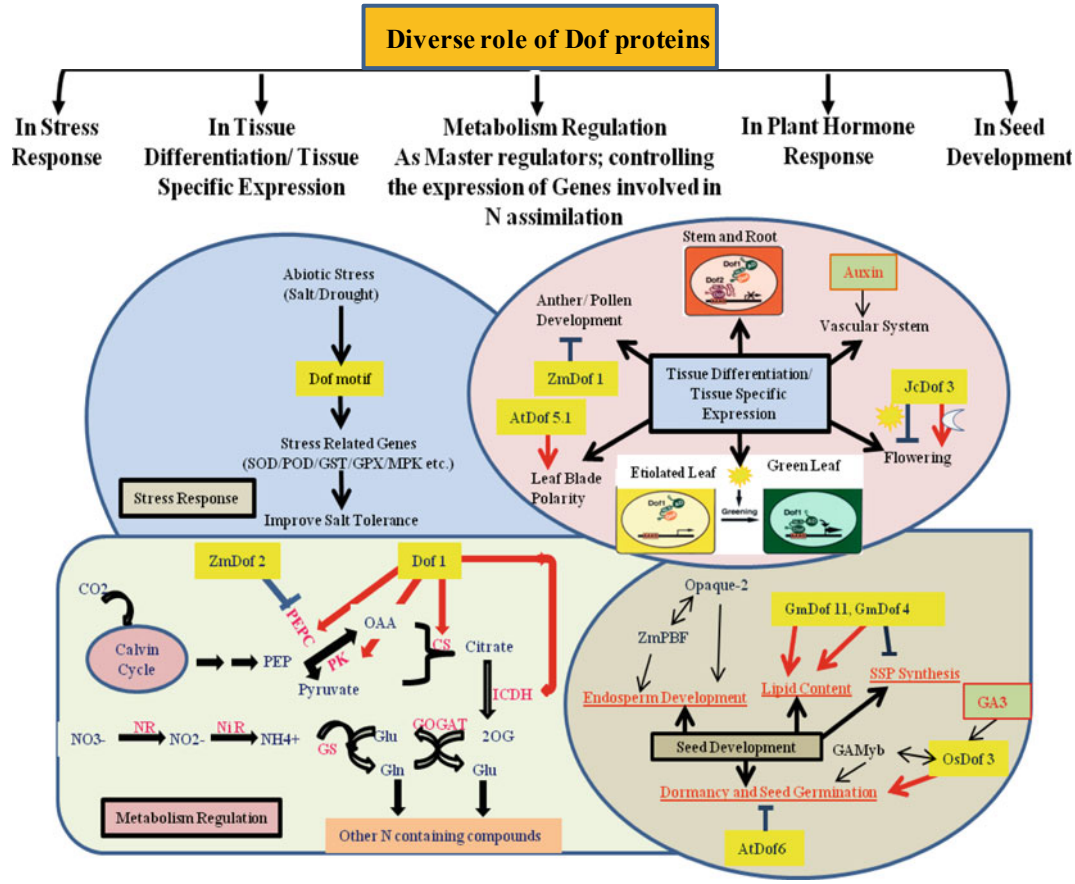

Fig. 12.3 The DoF proteins: master regulator of genes involved in carbon and nitrogen metabolism. A proposed model representing the divergent roles of *Dof* genes

involved in regulating flowering time in plants and implicated in NUE in yeast. In yeast, the Hap2-3-5-Gln3 complex transcriptionally activates the expression of both GDH1 and ASN under N-limiting conditions (Hernández et al. 2011), indicating possibilities of interaction among plant HAP proteins/complexes with N-assimilation enzymes.

12.9 Strategies for Enhancing NUE in Plants

In most of the plant which are grown aerobically, roots predominantly absorb NO_3^- as N-source from the soil, which aside from being assimilated to a minor amount into amino acids in the root, is transported to the above-ground part, i.e., leaf, for reduction and further assimilation. Uptake and transport of NO_3^- and its further reduction into NH_4^+ is highly energy requiring process suggesting that NUE efficient crops could save lots of energy along with improvement in biomass and yield. Irrespective of the approaches available, the challenges in improving NUE include reduction in N-losses, improvement in N-uptake, translocation and assimilation, augmentation the fine tuning of N-supply and demand, and finally yield enhancement. Improving NUE in cereal crops is one of the major initiatives taken by both private and public sector researchers as the outcome will not only save the economical loss of farming community but it would also save environment degradation by

minimizing chemical fertilizers use. The current section deals with some of the approaches and their impact on crop NUE.

12.9.1 Integrated Omics Approaches to Improve NUE

Systems biology approach is an emerging research area that takes advantage of various "omics" approaches, viz., transcriptome, proteome, metabolome, ionome fluxome, etc. to further analyze various datasets in an integrated manner using various bioinformatics tools (Kumar et al. 2015b, 2016). System biology enables us to understand the N-regulatory networks connecting genomic information to agronomic traits such as biomass production or yield. Studies report the integration of N-regulated gene-expression analysis, proteomics, and metabolite data analysis. Among several key molecular factors identified, regulatory genes such as AtCIPK8 (Hu et al. 2009) or TFs such as NLP7 (Marchive et al. 2013) have been identified using whole-genome/transcriptome approaches, which were shown to play important role in NO_3^- sensing and signaling in Arabidopsis. Therefore, identification of the regulatory and structural elements controlling the molecular and physiological changes associated with NUE would be highly beneficial.

Gupta et al. (2018) has identified and characterized 48 putative Dof TF using genome transcriptome data of FM. Among these, more than 50% *EcDof* genes showed differential expression during seed development process. Also, data obtained from gene profiling experiments along with proteomic studies using 2D-PAGE and mass spectrometry have been carried out to assess the role of N-concentration on protein levels (Jorrin-Novo et al. 2009). Mass spectrometry-based proteome and phosphoproteome could serve to identify the impact of N-deficiency stress on the proteome of maize and wheat (Bahrman et al. 2004; Facette et al. 2013). In some other studies, the protein profile of plants grown under low- or high N-supply like the roots and shoots of maize, barley, rice, and Arabidopsis were also demonstrated (Kim et al. 2009; Møller et al. 2011; Amiour et al. 2012; Wang et al. 2012). Although the regulation of plant N-starvation responses at the post-translational level is limited but interestingly, phosphorylated nitrate reductase (NR) is targeted for inactivation by 14-3-3 proteins, while unphosphorylated NR remains active. The phosphorylation and dephosphorylation have been demonstrated to change the kinetics of a low-affinity nitrate transporters (NRT1.1) in Arabidopsis.

The current knowledge of metabolome response to N-nutrition is limited. Overexpression of the maize TF Dof1 in rice, caused elevation of malate and 2-OG levels whereas down-regulation of isocitrate levels without causing any change in citrate and phosphoenolpyruvate levels (Yanagisawa 2002). N-starvation in maize leaves, resulted in decrease amino acids and organic acids content, while carbohydrate content, antioxidant activity, and secondary metabolite level increases. Metabolite profiling of transgenic plants with enhanced NUE has also been reported. Rice plants over expressing *AtDof1* and barley alanine aminotransferase (*alaAT*) showed enhanced level of amino acids such as asparagine and glutamine. Data further suggests that cellular metabolite pool played an important role in N-metabolism. Also, the expression of transgenes affects these pools, thereby conveying improved NUE. Therefore, it is plausibly considered that amalgamation of "omics" data could enhance the understanding of complex regulatory networks underlying important phenotypic traits such as NUE and yield (Kaufmann et al. 2011).

12.9.2 Transgenic Efforts to Manipulate NUE

Recently developed, transgenic approaches not only provide platform to investigate the contender genes critical for NUE by over expressing them, but also validate the role of such genes by mutational studies (Table 12.1). The following sections describe various genetic engineering strategies for enhancing NUE (Fig. 12.4).

Table 12.1 A list of candidate genes having potential roles in improving NUE in crops

Gene	Gene family	Source	Role in improving NUE	References
Transcriptional factors				
DOF1/ *DOF2*	DNA-binding one zinc finger	FM	Increased growth, photosynthetic and NH_4^+-assimilation, enhance grain protein, N and light responsiveness, C–N-metabolism	Kumar et al. (2009b, 2014), Gupta et al. (2011, 2012a, 2014a, 2018; b)
SAT1	bHLH TF	Soyabean	Nodulization to improve NH_4^+ transport and N fixation	Chiasson et al. (2014)
NFY	Nuclear TF Y	Arabidopsis	Increase nitrate transport	Zhao et al. (2012)
APO	F-box protein	Rice	Grain yield improved per plant	Terao et al. (2010)
NAC	NAM/ATAF/CUC	Wheat	Improve Zn, Fe, and grain protein content	Uauy et al. (2006)
Transporter genes				
NRT	Nitrate transporter	FM	N-uptake and assimilation	Gupta et al. (2013)
AMT	NH_4^+ transporter	FM	Increased NH_4^+ uptake	Gaur et al. (2011)
STP13	Hexose transporter	Arabidopsis	Growth, biomass, and N use increased by application of exogenous sugar	Schofield et al. (2009)
LHT	Lysine histidine transporter	Arabidopsis	Improved plant growth under low N condition	Hirner et al. (2006)
N-Assimilation genes				
NR and *NiR*	NO_3^- reductase and Fd-nitrite reductase	FM	Relationship of NUE with enzyme activity of N-uptake and assimilation	Gupta et al. (2012b)
GS		FM	NUE increased under high N condition	Yanagisawa (2000), Gupta et al. (2013)
GOGAT		FM	N-uptake and assimilation	Gupta et al. (2013)
Signaling genes				
ENOD	Early nodulin like protein	Rice	Increased total amino acids content and dry biomass; seed yield	Bi et al. (2009)
SMG1	Mitogen-activate kinase kinase	Rice	Improve panicle density and grain size	Duan et al. (2014)
DEP1	G-protein g subunit	Rice	N-uptake and assimilation; grain yield increased	Sun et al. (2014)
SnRK	SNF1-related kinase	FM	Higher NUpE	Kanwal et al. (2014)

12.9.2.1 Exploitation of Genes/Transporters

Very encouraging results have been reported in rice wherein nitrate transporter genes have been used to improve overall NUE. In rice high Os*Nrt2.3* expression enhances the capacity of pH buffering of the plant as well as uptake of N, iron, and phosphorus. Whereas overexpression of Os*NRT2.3b* results improvement in NUE and grain yield (Fan et al. 2016) demonstrated the increased total N-accumulation at anthesis and maturity, and grain N-content in transgenic rice as compared to wild type when Os*NRT2.1* is expressed under the control of Os*NAR2.1* promoter, whose relative expression pattern was found to be more in root. Likewise, transgenic

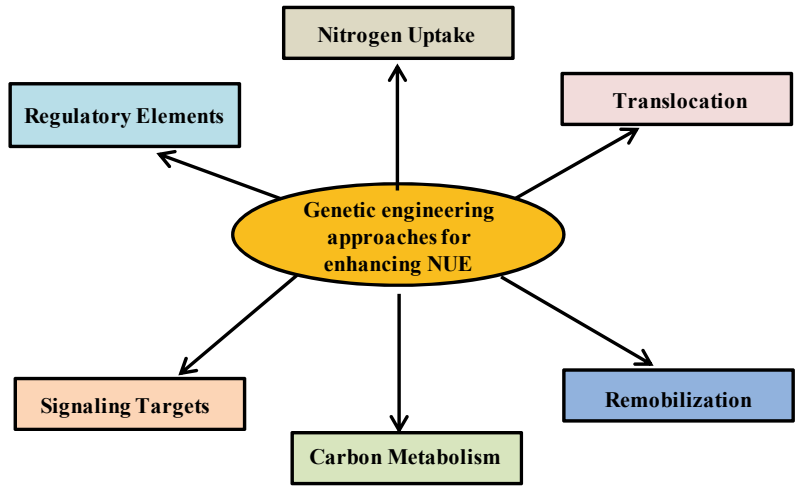

Fig. 12.4 Genetic engineering approaches for enhancing nitrogen use efficiency (NUE)

lines expressing pOs*NAR2.1*:Os*NRT2.1* or pUbi:Os*NRT2.1* constructs showed the increased total biomass including yields of ~38% and ~21% compared with wild type (WT) plants, respectively (Chen et al. 2016). The agricultural NUE (ANUE) of the pOs*NAR2.1*:Os*NRT2.1* lines was increased to ~128% as compared to WT plants. The dry matter transfer into grain increased by 46% relative to the WT. Similarly in transgenic tobacco, overexpressing *NpNRT2.1* gene was almost similar to their WT levels, however, an increase in NO_3^- influx suggests that modification of nitrate uptake (Up) machinery is not essentially lead to concomitants improvement in nitrate utilization (Ut) or NUE, though different plants respond differently in presence of overexpressed transporters (Fraisier et al. 2000).

NR being considered as rate limiting steps in nitrate assimilation. Efforts have been made to enhance the NUE by manipulating genes like NR and NiR, which gives varied result. Tobacco plants expressing NR constitutively, demonstrated delayed drought induced loss in rapid recovery of N-assimilation and NR activity. Deregulation of NR gene in transgenic tobacco and potato showed a reduction in nitrate levels (Djannane et al. 2002). Furthermore, NO_3 availability regulates the flux through the pathway of N-assimilation, the transformants of NR were observed to be better prepared in terms of available NR protein that efficiently restored N-assimilation. Overexpressing *NiR* genes studied in tobacco and Arabidopsis plants showed increase level of NiR transcript but decreased enzyme activity, which was accredited to post-translational modifications (Hoshida et al. 2000). Therefore, improvements of NUE by overexpression of NR/NiR remain a mystery since different crops respond differently.

Another transgenic approach involves improvement of NUE via manipulation of plastidic GS2 and Fd-GOGAT genes that resulted in low success. Transgenic tobacco plants with twofold overexpressed GS2 showed enhanced photorespiration and tolerance to high-intensity light. Overexpressed GS2 in rice (Hoshida et al. 2000) and tobacco improved re-assimilation of ammonia in tobacco (Migge et al. 2000). Research on barley mutants having reduced Fd-GOGAT gene expression revealed a decrease in leaf protein content, Rubisco enzyme activity, nitrogenous metabolites, and also low NO_3^- content (Hausler et al. 1994).

Ectopic expression of pea GS1 in tobacco provided an alternative route, for the re-assimilation of photo-respiratory NH_4^+, thereby improving N-assimilation efficiency and plant growth (Man et al. 2005). In rice and alfalfa, studies have been conducted to modulate the expression of NADH-GOGAT, using transgenic knock-in and knock-out approaches (Schoenbeck et al. 2000), which further implicated the role of

GS1 in the export of N via phloem in senescing leaves (Yamaya et al. 2002). However, in spikelets and developing leaf, NADH-GOGAT is involved in utilization of glutamine transported from senescing organs. Secondary NH_4^+ assimilation genes appear to be good candidates for improving NUE, but extensive research is required across crops under different conditions.

12.9.2.2 Manipulation of Signaling Targets

In 2004, Yanagisawa group developed transgenic Arabidopsis lines overexpressing maize *Dof1*, involved in expression of several C-metabolizing genes linked with organic acid metabolism. These *Dof1* transformants showed increase in N-content up to ~30% with improved growth and enhanced levels of amino acids, under low N-conditions. Higher levels of enzyme activities and transcripts for PEP carboxylase and pyruvate kinase were also reported, without compromising any reduction in NR, GS, and GOGAT activities. Up-regulation of C- and N-metabolism genes under influence of Dof1 overexpression opens possibilities of improving NUE through *Dof* TFs. The attempts to manipulate signaling/regulatory proteins have also been made like manipulation of MADS box proteins that govern nitrate induced changes in root architecture (Zhang and Forde 1998).

12.9.2.3 Over-Expression of *Dof1* Transcription Factor: Strategy to Enhance NUE in Cereals

One of the most important implications of functions of *Dof TFs* was shown by Yanagisawa et al. (2004), which set a new era of metabolic engineering and regarded Dof transcription factors as "master regulators". Yanagisawa et al. (2004) developed Arabidopsis transgenic lines carrying overexpressed *Dof1* gene of maize. Transgenic Arabidopsis plants over-expressing maize *Dof1* induced transcript accumulation of genes encoding enzymes of C-skeleton production and results suggested cooperative modification of C- and N-metabolisms on the basis of their intimate link. Elemental analysis of N revealed 30% increase in N-content in *Dof1* overexpressed transgenic plants, indicating an increase in net N-assimilation. Results high lightened the utility of *Dof TFs* in engineering the C- and N-metabolism of plants to improve their growth under low N-conditions. A similar experiment was carried out where the maize *Dof1* was transformed into rice. As expected, *ZmDof1* overexpression in rice induces gene expressions like *PEPC* genes, modulates C- and N-metabolites, enhances growth, and increases N-assimilation under low N-conditions (Kurai et al. 2011).

12.9.3 miRNAs to Improve NUE

Considerable investigations have been carried out for the identification and characterization of N-starvation responsive miRNAs in maize, Arabidopsis, rice, and common bean plants. miR156 up-regulated in rice, maize, and Arabidopsis, but miR160 and miR447 in rice and Arabidopsis. miR156/157 has been observed to control the expression of SBP-like (SPL) transcription factor or squamosa promoter binding-protein (SBP) family that regulates vegetative phase change, fertility, flowering, and leaf formation. Similarly in Arabidopsis, miR164 controls development of lateral root growth by controlling the expression of NAM/ATAF/CUC (NAC) transcription factor 1, whereas miR167 targets ARF6 and ARF8. miR169, miR397, miR399, and miR408 were reported down-regulated in common bean, maize, and Arabidopsis. Interestingly, miR164 is down-regulated in beans, while up-regulated in maize. These, studies on miRNA discovered that how plant genes respond to low N-stress and many approaches to manipulate genes for improving NUE. As revealed on maize studies, the occurrence of miRNA-mediated control of gene expression could be one of the important components for NUE that needs further extensive research (Zhao et al. 2012).

12.9.4 N-Fertilizer Application Management

The meticulous understanding of the genetic and metabolic control of N-attainment and recycling during plant growth and development is required not only to improve crop productivity and quality, but also to reduce excessive use of N-fertilizers. Earlier management of fertilizer practices in wheat demonstrated that application of a same amount of N-fertilizer in more than two splits doses under field conditions enhanced the availability of N at later growth stages (Abdin and Abrol 1993). Kumar et al. (2009b) proved that the influence of N-inputs on the expression of *Dof TF* in wheat and its co-relationship with NH_4^+ assimilating and photosynthetic efficiency. Studies on mustard genotypes having contrasting NUE showed genotypes with higher utilization efficiency will not only transport N but also utilize N-efficiently. Such cultivars are required since they can be grown under deficient or limited N-supply for sustainable agriculture systems (Ahmad et al. 2014). In maize, studies carried on different recombinant inbred lines or genotype based on NUE components, chromosomal regions, and putative contender genes have confirmed key factors, which might be responsible directly or indirectly for controlling yield and its components, under different concentration of N-fertilizers (Hirel et al. 2007). Therefore, searching for N-efficient genotypes, which grow well at low N-level with high yield potential can further provide better management of the applied N-fertilizer.

12.9.5 QTL Approach to NUE

NUE is a complex quantitative trait, which depends on many external and internal factors such as soil N-availability, photosynthetic carbon fixation to provide C-skeleton for amino acid biosynthesis, respiration to provide energy, and many more. Also, it is controlled by a large number of loci acting alone or together, depending on conditions such as nutritional, environmental as well as developmental. Identification of genotypic and phenotypic variability will be helpful to understand the genetic basis of NUE related to yield and other traits for marker assisted breeding.

The first QTL studies were conducted by Obara et al. (2001) for analyzing NUE in rice. Identified QTL's associated with the NUE co-segregated with NADH-GOGAT and GS1 in rice. Results showed seven loci, co-segregated with GS1 activity and six loci, co-segregated with NADH-GOGAT. After that, recombinant inbred maize lines grown under high and low N-conditions were used for QTL studies pertaining to N-uptake, remobilization, grain protein content, and yield, Results of QTL analysis reported that many desired NUE traits co-segregated with GS genes, particularly Gln4 on chromosome 5 of maize (Gallais and Hirel 2004). Also, QTL mapping was carried by Quraishi et al. (2011) in wheat showed genomic regions consisting of genes for GS and GOGAT linked to NUE. Genomic regions segregating for NUE have been found to be linked to genes controlling dwarfing (Rht-B1 and Rht-12), photoperiod sensitivity (Ppd-A1 and Ppd-B1), a UDP-glucose phosphorylase (UDP-GP), and also vernalization (Vrn-A1 and Vrn-D1). Identified genomic regions depict synteny between sorghum, maize, and rice indicating evolutionarily conserved regions for NUE exist in cereals genome.

Marker-based technology for identifying and mapping quantitative trait loci (QTL) could be emerged as a powerful approach that leads to the development of better understanding of genetic phenomena (Kumar et al. 2009a). Quantitative genetic studies linked with use of molecular markers can be a smart way for identifying genes involved in the genetic differences of NUE trait. Molecular markers have undoubtedly accelerated progress of improvement of crop species which can better adapt under various biotic (disease and insect) resistance and abiotic (low N fertilization, drought, and frost) stresses. QTLs mapping for identification of NUE have been done in Arabidopsis, rice, maize, barley and their association with plant N-status has been discussed (Hirel et al. 2007). Thus, it is assumed that quantitative studies of genetic unpredictability for NUE using

molecular markers combining agronomic and physiological studies will be used in coming days to identify newer genes or loci involved in the regulation of these complex metabolic pathways. Also, it will be helpful in determining their interconnection with C-assimilation and recycling pathways to select genotypes, which is assimilate or remobilize N more efficiently.

12.9.6 Conclusions and Future Prospects

Expression profiling of transcription factors, regulatory enzymes, and other elements that could be key regulators N-metabolism could be one of the futuristic means of improving NUE in cereals and other plants, as they tend to regulate multiple downstream factors. Genome-wide identification of *Dof TFs* and their gene-expression analyses revealed that *Dof TFs* acts as master regulator, not only in the expression of photosynthetic and N-metabolism genes which could improve N-assimilation efficiency of crop plants but also have potential to enhance the biomass, which could in turn increase yield. Using omics-based approaches researchers were able to identify novel candidate gene/elements that could introgress to commercial cultivars for development of elite varieties useful to farmers. Further, QTL analysis can be a promising tool to identify candidate genomic regions corresponding to NUE in response to grain yield. Biomarkers associated with high grain yield and high NUE should be identified so that breeders were able to use them as selection criteria for identification of N-efficient and high yield potential crops for breeding.

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Molecular Basis of Biotic and Abiotic Stress Tolerance in Finger Millet

13

Radha Shivhare, Anil Kumar, and Charu Lata

Abstract

Finger millet (FM; *Eleusine coracana* (L.) Gaertn.) is one of the major millet crops generally tolerant to adverse climate-induced constraints. However, substantially available genetic resources hold potential for FM improvement to various environmental stresses. The continuous efforts for the development of techniques that quantify abiotic and biotic stress would pave the path for our understanding of finger millet molecular responses. Advances in next-generation sequencing and biochemical methods have only recently allowed us to begin to comprehend the complexity of the molecular processes that underpin these responses in finger millet. Earlier studies also address the potential genomic tools of finger millet, namely functional molecular markers, DNA sequences, and genetic maps, but the knowledge is scarce to help develop the effective and enhanced climate-smart finger millet varieties. Compared to other major cereal crops, such as rice, wheat, maize, barley, and sorghum, the genomic knowledge available for this crop is very low resulting in very few studies related to genetic and genomic aspects, partially due to the late availability of its whole-genome sequence (WGS). Here, we review recent progress in our understanding with an emphasis on functional, transcriptomic, and genomic studies to comprehend the molecular bases of drought and temperature stresses in finger millet. Advances in genetic and genomic studies together with the WGS of finger millet will help to strengthen the food security in developing countries like Asia and Africa.

13.1 Introduction

Finger millet (*Eleusine coracana* (L.) Gaertn.) is a member of the Poaceae family. It is a C_4 allotetraploid ($2n = 4x = 36$, AABB), self-pollinated crop with a genome size of 1,593 Mb (Goron and Raizada 2015). It is a low-input orphan crop that evolved over 5000 years, mainly in Africa and Asia as subsistence farming (Fuller and Hildebrand 2013; Bandyopadhyay et al. 2017). It is an

R. Shivhare
CSIR-National Botanical Research Institute, Rana Pratap Marg, Lucknow 226001, India

A. Kumar
Rani Lakshmi Bai Central Agricultural University, Gwalior Road, Jhansi 284003, India

C. Lata (✉)
CSIR-National Institute of Science Communication and Policy Research, 14 SatsangVihar Marg, New Delhi 110067, India
e-mail: charulata@niscair.res.in

R. Shivhare · C. Lata
Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, India

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annual herbaceous cereal crop that intends to play a crucial role in climate-smart agriculture (CSA) and improve grain production, especially in the developing world, in the face of global climate change (Bandyopadhyay et al. 2017). With exceptional nutritional values and storage characteristics, it functions as an excellent food security crop (Ramashia et al. 2018). This millet crop is known for its high calcium (Ca) content [∼0.34% in whole plants] as compared to most other cereals where Ca ranges between 0.01 and 0.06% (Kumar et al. 2016; Gupta et al. 2017). Though food protection can be provided by crops like rice and wheat, millets, especially finger millet, have nourishing properties greater than those of rice and wheat and thus have been suggested to help improve nutritional stability in developing countries like Asia and Africa (Lata 2015; Shivhare and Lata 2016, 2017; Puranik et al. 2017).

Global temperature change is taking place so rapidly, and it is obvious that global agriculture has increasingly experienced vulnerability in recent decades due to unpredictable climate shapes, particularly higher temperatures and water stress (IPCC 2014, 2018). Studies have indicated that an increase in temperature of 2 °C or more without mitigation would have an adverse effect on the development of cereal crops in tropical areas (IPCC 2014). Similar to other crops, most of the FM biological processes are temperature-sensitive (Jena and Kalli 2018). Overall, climate change has already gravely affected the appropriate land distribution for several crops, contributing to a considerable decline in agricultural systems productivity for marginal and resource-poor farmers (Adhikari et al. 2015). In addition, substantial evidences indicate a decline in the extent of land suitable for the production of cereal crops, including FM worldwide due to worsening abiotic stresses, especially drought, salinity, and warmth (FAO 2015; Sudan et al. 2015). Adaptation would be required to tackle the intensifying climate challenges using new varieties of crops resistant to temperature and climate extremes, drought, salinity, floods, etc. (Shivhare and Lata 2016, 2019). Consequently through CSA, the essential varied genetic and genomic resources of FM will become the centre for reinforcing the resistance of the crop to adversative conditions due to global climate change (Bandyopadhyay et al. 2017). Therefore, the present review offers the latest information on how genetic, genomic resources as well as molecular basis of stress tolerance in FM could contribute to the transition to CSA in order to ensure augmented and sustainable crop production. With the rising demand for millets, it is becoming increasingly important to equip them with multi-stress tolerance genetic tools through advances in molecular stress response using comparative and functional genomics. To that end, we hope to present an outline of general plant defence systems and the causal regulatory network(s) in this study, with an aim to highlight their applied potential through genetic engineering and molecular breeding. This review article specifies the molecular and functional genomic approaches used in previous studies, including identification of characteristic quantitative trait loci (QTLs) and genes, and transcriptomic studies to improve finger millet genetic composition for higher yield production under adverse environmental conditions.

13.2 Nutritional Value and Medicinal Uses

Among main course cereal crops, the United States National Academies have recognized finger millet as one of the most nutritious "super cereal" (National Research Council 1996). Finger millet is substantially ample in minerals from the nutritional point of view with higher micronutrient density in comparison to wheat and rice, the world's main cereal grains (Vadivoo et al. 1998). Finger millet is the lushest source of calcium with approximately 10 times higher Ca content as compared to brown rice, wheat, maize, etc. It is also having a higher amount of fibre and iron, making this crop more nutritious than other frequently used cereal crops. Finger millet is an ample source of essential amino acids, such as lysine and methionine which are necessary for

good health and development and are usually absent in many other plant-based foods (McDonough et al. 2000). Moreover, it also comprises significant quantities of α-linolenic acid and *cis*-linoleic acid, the two important polyunsaturated fatty acids which help in the proper functioning of the central nervous system (Birchet al. 2007; Jacobson et al. 2008). Both water-soluble and liposoluble vitamins such as thiamin, riboflavin, niacin, and tocopherol are also reported in finger millet (Obilana and Manyasa 2002).

Since small-holder farmers with limited agronomic resources primarily in developing countries, often grow and eat finger millet, the crop is also frequently called a "crop for the poor" or a "famine grain" (National Research Council 1996). Despite its excellent nutritional benefits, the nutraceutical applications of finger millet as a potential functional food have made only modest progress.

13.3 Tolerance to Abiotic Stresses

Production and productivity of all crops are significantly affected by abiotic stresses, and finger millet is no exception. Millets, however, are typically considered to survive in unfavourable environmental conditions influenced by a wide range of abiotic stresses such as drought, high temperature, salinity, waterlogging, and poor soil fertility relative to most other cereals (Shivhare and Lata 2017). The frequency of vulnerability varies from species to species to different abiotic stresses. Stress adaptation is a dynamic process that is controlled at different points in plant cells, including physiological, cellular, and molecular levels (Dida and Devos 2006). The area of research concern to agricultural scientists has long been the delineation of the mechanisms of stress tolerance and adaptation in crop plants. Finger millet is known as one of the most valuable crops because of its capacity to thrive with fewer resources and high nutritional composition (Gull et al. 2014). It has been ignored for many years despite these unique features. Fortunately, the richness of genetic diversity of this crop species has been preserved. Agricultural output losses, food insecurity, and malnutrition are important concerns all over the world as a result of climatic change's negative effects. World Summit on Food Security suggested that food production will need to increase by at least 70% by 2050 to support the world's population growth (Gupta et al. 2017). In this situation, because of their exceptional ability to grow under low humidity, high temperatures, and poor soils, scientific research has turned its attention towards finger millet.

While finger millet is usually thought to be abiotic stress-tolerant, it is necessary to establish unique genetic resources from this crop that could be valuable in plant breeding programmes. It could be significant to identify patterns of genetic variability in finger millet germplasm, and collections with respect to tolerance towards biotic and abiotic stresses could also be crucial. GenBanks of African, Asian, European countries, and the United States have a large repository of finger millet accessions. The largest finger millet germplasm collection is in India, followed by Ethiopia (Dwivedi et al. 2012). Enormous morphological, genetic, and genomic diversity could exist among finger millet germplasm and their core collections (Kumar et al. 2015). Using molecular markers, calcium dynamics, tryptophan aggregation, association mapping, and disease resistance have been characterized. However, the characterization of finger millet abiotic stress tolerance using molecular markers, genes, short RNAs, gene editing, and other methods is still limited, allowing researchers to investigate its diverse germplasm including wild and cultivated accessions (Table 13.1) (Yadav et al. 2014; Babu et al. 2014a, b, c, d, e, f).

13.4 Resistance to Biotic Stresses

For decades, finger millet has been known to be disease-resistant, and in the last 15 years, various blast-resistant finger millet lines have been developed to determine the immunity sources (Ramappa et al. 2002). Further, the molecular mechanism of resistance to pests and diseases is also determined by the physical and chemical

Table 13.1 Genotypes available for abiotic stress tolerance and biotic stress resistance in finger millet

Abiotic stress		References
Drought	ML-365, ML 181, GPU 28, IE 4073, IE 4797	Dwivedi et al. (2007), Reddy et al. (2018)
Salinity	GPU 48, Trichy 1	Dwivedi et al. (2007), Vijayalakshmi et al. (2014)
Biotic stress		References
Blast resistance trait (*Magnaporthe grisea*)	Gautami, GPU-76, GPU 28, OEB-532, GE# 281, 568, 1409, 1546, 3024, 3058, 3060 and MR 6; KNE 409, IE 287, GPU-67, IE 976,IE-4795; IC 43335; MR 33, KNE 814, KNE 1149, KMR 9 and KMR 3; Gulu E, Seremi 1, Seremi 2, Pese 1, SX8, SEC915; KNE# 620, 629, 688, 814, 1034, and 1149; VL 149, VL 146, NW Himalayan, VHC3997, VHC3996 and VHC3930,DM-7, PR-202, VR-948,BR-2, TNAU-1063, RAU-8, TNAU-1066, PPR-2885, and IE-4709, KNE 688, KNE409, Gulu E, SX8, SEC 915, KNE 1098, and 1098	Lenne et al. (2007), Dwivedi et al. (2007), Mgonja et al. (2007), Babu et al. (2014a, b, c, d, e, f), Ramakrishnan et al. (2016), Ganesha et al. (2018), Saha et al. (2017)

composition of the grains. The grain's physical structure serves as the first defensive line against contamination and infestation. A significant restriction that has been found to decrease mould infestation is small size and grain hardness. Grain mould resistance is further influenced by cell wall composition, seed phenols (e.g. ferulic acid), pigmented testa, and glume colour composition (Kavitha and Chandrashekar 1992). Seetharam and Ravikumar (1993) found that brown coloured grains (resistant cultivars) had considerably more total phenolic content than white grains (susceptible cultivars). Similarly, the importance of polyphenols, namely, p-coumaric acid and flavonoids, has been reviewed by Chethan and Malleshi (2007) in finger millet. These studies showed a clear relationship between blast disease and phenols content. Plants often react against fungal infection via generating a wide range of harmful substances such as phytoalexins that could be one of the potential tools to improve the grains' defence capabilities (Snyder et al. 1991). Prolamins are storing proteins found in finger millet grains that are organized into protein macromolecules that act as a functional and nutritional shield against insect and fungal protease digestion (Gupta et al. 2011). In the last few years, many bioactive compounds generated against pathogen response have also been characterized in millets (Gatehouse and Gatehouse 1998). A plentiful supply of pathogenesis-related (PR) proteins reported in the finger millet grains, of which some may be present in the protein bodies also, is required for pest control. In addition, cereals and millets crops also contain many inhibitors of protein enzymes that act on essential digestive hydrolases of the insect's gut. For instance, a number of phytophagous insects are regulated by α-amylases and proteinases (Gatehouse and Gatehouse 1998). The finger millet α-amylase/trypsin inhibitor (RBI) is the sole inhibitor of storage insects with dual functions (Stroblet al. 1995). Further, fractions of ammonium sulphate obtained from finger millet grain isolate examined against α-amylases of quite a few storage and other insect pests revealed α-amylase inhibition against *Callosobruchus chinensis* (the pulse beetle) to a range of 8.0–69.9% (Payan 2004; Sivakumar et al. 2006).

13.5 Delineating Molecular Basis of Abiotic and Biotic Stress Tolerance in Finger Millet

Stress management is an extremely dynamic and inter-dependent process that is critical for a plant's survival under adverse conditions, as evidenced by "omics" approaches and high-throughput sequencing technologies. Further, it

has also been established that the stress resistance mechanism is mediated by a complex of regulatory and signalling molecules that may operate in concert and/or in opposition. Millets include a wealth of critical genes and regulatory proteins that, together with their highly adaptable features, can be used to improve crop varieties that are resistant to stress. Finger millet is the most climate tolerant among small millets, capable of growing in a varied range of harsh environmental conditions. As a result, they are known as "farmer-friendly" crops because they generate higher returns than other crops that are affected by climate change. Furthermore, from the breeder's viewpoint, these are the sources of traits that may improve the durability of other widely grown cereal crops. From the agricultural biotechnology standpoint, its "hardy" nature has piqued the interest of researchers all over the world, who are exploring and utilizing it in order to improve tolerance towards stress in several economically vital crop plants (Kumar et al. 2015). The language of finger millet's DNA must be deciphered to fully comprehend the molecular basis of the plant's abiotic stress tolerance capacity. This chapter discusses contemporary genomics and proteomics strategies for using the genetic makeup of FM in related crops to improve resilience and productivity under less-than-ideal growing conditions.

13.6 Genetic and Genomic Resources

13.6.1 Functional Molecular Markers, Genetic Linkage Maps, and Trait-Genetics

Finger millet has limited genomic resources compared to other major cereals, which hinders the crop's ability to evolve and become more efficient (Saha et al. 2016). In Table 13.2, the properties of numerous finger millet genomic tools available at the National Center for Biotechnology Information (NCBI) are shown. A very limited number of expressed sequence tags (ESTs) is reported in finger millet in comparison to major cereals (Ceasar et al. 2018). There are only 1934 ESTs reported in the finger millet, which is over 100 times smaller than for rice and maize, and up to 50 times less than for barley. Although most other cereal crops have multiple genome assemblies, however, finger millet only has one (ASM218045v1). A limited number of proteins have been isolated and reported in finger millet as equated to maize, rice, and wheat. Until now no single nucleotide polymorphism-based (SNP) markers have been reported in the finger millet genome. Further, in the upcoming years, the recently released finger millet WGS will contribute to the development

Table 13.2 Summary of DNA-based markers available in finger millet-related abiotic and biotic stress tolerance

Polymorphic information content						
Marker types	Number of genotypes	Number of markers	Mean	Range	Associated trait	References
Genic SSRs	190	58	0.385	0.186–0.677	For the identification of genotypes resistant to blast	Babu et al. (2014a, b, c, d, e, f)
SSRs	105	20	0.53	0.09–0.88	For the identification and selection of drought-tolerant genotypes	Dramadri (2015)
SRAP	67	12	0.243	0.116–0.459	For the identification of blast-resistant genotypes	Saha et al. (2017)
SSR RAPD ISSR	2	21 12 2	All polymorphic		For the identification of diverse parental lines and drought-tolerant genotypes	Krishna et al. (2020)

of advanced and high-throughput technologies accelerating research in this important crop across all fields. A small number of transcriptome-based studies have been conducted in finger millet against some stresses and for grain calcium concentration. There have been a few attempts to sequence the transcriptome of particular genotypes exposed to different stressors like drought, salinity, and blast disease. Yet, in most of these investigations, detailed characterization of essential genes still has not been done, and the results have merely been submitted as raw reads.

A major limiting factor for the development and productivity of finger millets is blast disease triggered by *Magnaporthe grisea*. For molecular breeders, the discovery of QTLs/genes associated with essential physiological characteristics such as blast resistance will be valuable for introgressing certain genes into region-specific popularly cultivated germplasm (Panwar et al. 2011).

Molecular markers allied with resistance against FM blast disease were reported, which might be used in marker-assisted selection to create blast-resistant genotypes (Table 13.2). Furthermore, comparable levels of synteny were discovered between the FM and rice NBS-LRR areas, with nearly identical locations when plotted on a chromosome map of rice. Out of 15 EST-based SSR primers, eight were found to be polymorphic amid the selected resistant and susceptible genotypes of finger millet, and the sequencing results showed their identity with the distinctive kinase-2 and kinase-3a of rice and R-genes motifs of finger millet (Babu et al. 2014a, b, c, d, e, f). Through the application of a comparative genomics approach for blast-resistant genetic analysis, in total 58 genetic SSR markers were obtained from several rice blast EST sequences. Babu et al. (2014d) reported that these 58 SSR markers might be used to divide 190 diverse finger millet accessions into four categories on the basis of their resistance response to blast disease. While trying to categorize different genotypes of finger millet into various clusters on the basis of their sensitivity to blast disease, a good correlation was observed amongst PCA analysis, population structure, and phylogenetic tree (Babu et al. 2014b, c). On the basis of association studies between SSR marker data and that of the data for neck blast, leaf blast, and finger blast, five important QTLs for neck and finger blast diseases were discovered. The FMBLEST32 genetic SSR primer developed from the *Pi5* rice blast gene gives broad-spectrum resistance to *M. grisea* and RM262, and is found to be closely linked to the QTLs for finger blast. Similarly, MLM was used to map the relationship revealing seven QTLs including one QTL for neck blast, and three QTLs each for finger blast and leaf blast. Similarly, both methods revealed a linkage of FMBLEST32 and RM262 with all finger millet blast diseases. By defining 10.5% of the phenotypic variance, the SSR marker UGEP53 was correlated with finger blast (Babu et al. 2014b). Genes for finger and neck blast resistance were discovered on the second and sixth finger millet chromosomes indicating them to be the main clusters of genes responsible for blast resistance (Babu et al. 2014b). Further, the discovery of molecular markers might also be employed for precise mapping, finger millet marker-assisted breeding (MAB) and full-length blast gene cloning.

13.6.2 Genome-Wide and Transcriptomic Approaches

Finger millet's genetic ability is an invaluable opportunity for learning about the mechanism of stress tolerance (Kumar et al. 2015). While finger millet is deemed to be tolerant to various abiotic stresses, there is very little knowledge on how genomics can be used in this crop (Gupta et al. 2017). Furthermore, to evaluate the specific traits present in finger millet and utilize them for crop improvement, a few transcriptome and genome-wide association studies have also been performed.

Recent transcriptomics and genomic studies under drought stress showed the induction of *Farnesyl pyrophosphate, Ec-apx1, EcDehydrin7, ATFP6, EcNAC1, Metallothionein, Protein phosphatase 2A, RISBZ4, Farnesyl phosphate*,

and *NAC 67*. In the finger millet cultivar ML-365, RNA sequencing, assembly, and qRTPCR identified 2866 drought-sensitive genes, with ZFHD, ABF, NAC, MYB, MYC, WRKY, AREB, NF-Y, and GRF transcription factors (TFs) being the most important (Hittalmani et al. 2017). Transcriptome analysis of leaf tissue of Trichy 1 under salt stress revealed the enhanced expression of several categories of functional genes related to cell signalling, transcription factors, transporters, osmotic homeostasis, and biosynthesis of compatible solutes was observed in the salt-tolerant genotype, whereas diminished expression of genes related to flavonoids biosynthesis was also observed (Rahman et al. 2014). The invention of the "genome zipper", which compares totally sequenced and annotated genomes with multiple data sources emanating among less well-studied animals, and thus aids in generating a near effective gene order in a genome that is partially sequenced (Mayer et al. 2011). Additionally, functional and structural genomics together can be utilized to completely characterize a genome. Genome-wide expression profiling can be used to identify candidate functional genes and different TFs linked with desirable agronomic traits for both the abiotic and biotic stress tolerance.

13.6.3 Identification and Characterization of Abiotic and Biotic Stress Responsive Genes

In comparison to other major cereal grains, attempts to improve finger millet genetics have been lagging. In order to improve nutritional quality and tolerance to abiotic and biotic stresses, improved genetic transformation of millets has been considered necessary (Ceasar and Ignacimuthu 2009) (Table 13.3). A background study on finger millet transformation using a biolytic method was done to compare the efficacy of the b-glucuronidase (FtuidA) expression regulated by b-glucuronidase/Flaveria trinervia b-glucuronidase

Table 13.3 Functional characterization of important candidate genes associated with stress response in finger millet

Gene	Gene function	Source	Plant/organism tested	Type of tolerance	References
EcNAC1	NAC transcription factor	Finger millet	Tobacco	Drought, salt and osmotic stress	Ramegowda et al. (2012)
Ec-apx1	Ascorbate peroxidase	Finger millet	Bacteria	Drought stress	Bhatt et al. (2013)
EcDehydrin7	Dehydrin protein	Finger millet		Drought and heat stress	Singh et al. (2014)
NAC 67, bZIP, WRKY29, AP2, MYB and *NAM family*	Transcription factors	Finger millet	Rice	Drought, and salt stress	Rahman et al. (2014)
EcGBF3	G-box binding factor 3	Finger millet	Arabidopsis		Ramegowda et al. (2017)
EcbZIP17	bZIP transcription factor tethered with endoplasmic reticulum (ER) membrane	Finger millet	Tobacco	Salt stress	Ramakrishna et al. (2018)
EcTAF6	TATA-binding protein (TBP)-associated factors (TAFs)	Finger millet	Rice	Drought, and salt stress	Parvathi et al. (2019)

(FtuidA)/ribulose-1,5-biophosphate carboxylase small subunit (RbcS) gene promoter of five gene promoters (Gupta et al. 2001). A few studies have suggested that transformation settings be optimized for optimal transformation and regeneration, with the majority of these studies relying on the *Agrobacterium*-mediated transformation. Even few studies have also been published on finger millet transformation utilizing functionally active transgenes. Below the specific reports are discussed.

Using the *SbVPPase* (sorghum vacuolar HC-pyrophosphatase) gene through *Agrobacterium*-mediated transformation, a salt-tolerant finger millet transgenic was developed and evaluated for the effectiveness of growth under salt stress. Another transgenic finger millet line was created by overexpressing the *AtAVP1* (*Arabidopsis thaliana* vacuolar HC-pyrophosphatase) and *PgNHX1* (*Pennisetum glaucum* NaC/HC antiporter) for salinity stress resistance using *Agrobacterium*-mediated transformation. In contrast to wild plants, the transgenic lines of finger millet displayed improved salinity tolerance (Jayasudha et al. 2014). The overexpression lines of finger millet transformed with serine-rich protein (*PcSrp*) gene of *Porteresia coarctata* were exhibited normal development, flower, and seed setting under 250 mM NaCl stress (Mahalakshmi et al. 2006). Further, *Agrobacterium*-mediated transformation was also used to overexpress a bacterial mannitol-1-phosphate dehydrogenase (*mtlD*) gene to produce transgenic finger millet (Hema et al. 2014). *mtlD* gene overexpressing finger millet transgenic plants outdid control/wild-type plants in terms of drought and salinity tolerance, as well as osmotic stress resistance and chlorophyll preservation. Only a few research studies on transgenic overexpression in finger millet giving resistance to blast and other abiotic stressors are readily available. More extraneous genes must be screened by overexpression/transgenic technology/gene editing in order to generate finger millet lines resistant to multiple stressors as the majority of the studies have concentrated on the introduction and phenotyping of foreign genes under particular stress only.

Using an antifungal protein (PIN) gene from prawns, a transgenic finger millet resistant to leaf blast disease has been developed (Latha et al. 2005). Under the influence of the CaMV35S promoter, the chemically synthesized *PIN* gene was cloned into plasmid pPin35S. Similarly, in order to improve leaf blast resistance, the rice Chitinase11 gene (*Chi11*) was inserted into the finger millet genotype GPU45 via *Agrobacterium*-mediated transformation (Ignacimuthu and Ceasar 2012). The transgenic lines of finger millet immune to leaf blast disease helped to establish the two initial studies which established that genetically engineered lines overexpressing foreign genes displayed enhanced resistance to leaf blast. However, no transgenic finger millet has been found to be resistant to neck and finger blasts. In order to generate transgenic finger millet lines resistant to wide-ranging fungal infections, researchers need to screen a large number of other possible antifungal genes and utilize the same in gene pyramiding.

13.6.4 Small RNAs Mediated Stress Tolerance Response

Small RNAs are non-coding RNAs that regulate the transcriptional and post-transcriptional expression of gene function. Several sRNAs have been reported to date including small interfering RNAs (siRNAs), microRNAs (miRNAs), small nucleolar RNAs (snoRNAs), small nuclear RNAs (snRNAs), etc. (Lata and Shivhare 2017). Two main groups of small RNAs involved in gene regulation are endogenous siRNAs and miRNAs. miRNAs control the expression of specific transcripts and/or genes by linking to reverse target sequence and cleaving the target RNA, while siRNAs do the same and also through direct DNA methylation (Khraiwesh et al. 2012). To date, multiple investigations have been performed to better explain the function of miRNAs in several abiotic and biotic stresses, such as drought (Zhou et al. 2010), salinity (Liu et al. 2008; Sunkar et al. 2006), UV-B radiation (Zhou et al. 2007), and bacterial infection (Navarro et al. 2006) in various model plants.

However, only a restricted number of studies on the characterization of stress-responsive miRNAs in other millet crops have recently been published. Qi et al. (2013) conducted a genome-wide transcriptomic study in foxtail millet (*S. italica*) under drought stress wherein whole seedlings of *S. italica* Yugu1 of both the control and drought-stressed treatments were used to build and sequence two RNA and sRNA libraries. sRNAs with different lengths of nucleotide (nt) sequence were discovered, out of which 24-nt sRNAs were the most common, followed by 21-, 22-, and 23-nt sRNAs. The abundance of 24-nt siRNAs was also low across the genic areas, showing that they serve a detrimental role in regulating gene expression in response to drought stress, according to the study of Qi et al. (2013). Sequencing of two small RNA libraries from the *S. italica* Yugu1 shoot tissues resulted in the discovery of several miRNAs including 43 conserved and 172 novel, and two miRNA precursor candidates (Yi et al. 2013). The complete chromosomal position, duration, pre and mature miRNA sequences, secondary structure, and targeted gene data of known sit-miRNA were being made accessible through the Foxtail millet miRNA Database, an open-access web resource (Khan et al. 2014; http://59.163.192.91/FmMiRNADb/index.html). The above studies shed light on the function of the *S. italica* miRNAome in abiotic stress response. Small RNAs must also be identified and characterized in finger millet in order to determine their functions in stress regulatory pathways.

13.6.5 Proteomics Studies

Proteomics is an alternative functional genomics method that is quite promising and significant in targeting specific stress-responsive proteins for improved stress resistance of important agricultural crops. Stress activates a variety of ion transporters, signalling cascades, and regulatory proteins, and identifying the proteins involved in these processes will help researchers better understand the special properties of millet crops that can be leveraged to boost production and productivity. Several researchers have emphasized the importance of organ-specific proteome analysis in identifying proteins that commonly get deposited in diverse plant organelles and intracellular compartments in response to various abiotic stimuli, thus playing a key role in plant stress adaptation and responses (Hossain and Komatsu 2013).

Mass spectrometry (MS) has enhanced accuracy and throughput when used in conjunction with advanced proteomic technology(s). Jacoby et al. (2013) devised a system for ranking the relative importance of stress-responsive proteins. Advanced MS platforms have ushered in a newfangled era in proteomic analysis which turn out to be an important method for studying protein interactions and post-translational modifications (PTMs) to understand various processes related to cellular functions (Cox and Mann 2007). In a number of laboratories, LC-based proteomic analyses are becoming more popular. However, due to the scarcity of genomic data, crop proteomics has been slowed significantly. Proteomics techniques, however, have not revealed much about the stress tolerance potential of finger millet yet. A lack of finger millet genomic data is the most likely reason for this, however, scientific developments continue to raise new expectations for achieving the aim of sustainable agriculture.

13.7 Conclusion and Future Perspectives

Finger millet is a hardy and nutrient-rich crop mainly grown and devoured by the resource-poor farmers of developing Asian and African countries. Although finger millet was once thought to be a climate-resilient crop, however, current research has revealed that it is also prone to drought, salinity, and low nutrition stress, besides blast disease infection. Very few studies on the characterization of functionally significant finger millet genes have been conducted. A move to climate-risk-resilient climate-smart agriculture (CSA) production methods is required to guarantee improved and sustainable finger millet

production. The prudent use of climate-smart breeding to utilize FM genetic and genomic tools could considerably contribute to a switch to CSA. As a result, scientific efforts should concentrate on trait-specific screening of FM's vast genetic capital. Because of growing environmental pressures, this could help distinguish germplasm with better alleles suitable for breeding requirements. In particular, it is expected that redesigning climate-smart breeding methods based on the recent repository of high-quality FM genomic data would revolutionize the discovery of SNPs as a large scale high-throughput molecular breeding tools. The novel FM genomic resources are expected to augment crop improvement, including the examination of key characteristics responsible for nutrient enhancement and stress tolerance using GWAS, SNP-based genetic diversity studies, reverse genetic studies using mutant analysis, genome editing techniques such as CRISPR/Cas9, augmented functional genomics studies with reporters including GFP for localization, and high-throughput proteomics will help to classify the proteins linked to vital agronomic purposes. The adoption of such CSA climate-resilient FM cultivars would increase the productivity of long-term crops while maintaining essential ecosystem services.

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Genetic Transformation for Crop Improvement and Biofortification

14

Sanjay Mohan Gupta, Supriya Gupta, and Anil Kumar

Abstract

Food and nutritional security (also termed as hidden hunger) for fast-growing worldwide populations is a major worry of public health issue in a majority of developing countries. The solution to combat this issue is through the intervention of modern genetic engineering tools used for the essential nutrient enrichment of staple crops along with their yield. *Eleusine coracana* (L.) Gaertn (finger millet; FM) is an annual herbaceous cereal crop, which is well known for its exceptionally high calcium, rich amounts of protein, and important minerals (Fe, Zn, K, Mn, Zn, etc.) as compared to other major cereals. In addition to its high nutraceutical value, it is also resistant to various abiotic and biotic stresses, which makes it an excellent model for studying the vast genomic and genetic potential of this crop. It will help us in developing new strategies for making climate-flexible crops. Since, this crop is almost self-pollinating that makes difficultly in crosses between different strains used for crop improvement in breeding programmes. Therefore, genetic transformation is the method of choice for crop improvement that is also expected to help to understand unique molecular mechanisms in FM that may be useful for the development of functional designer (nutrient fortification) food of other cereals. This chapter highlights the various approaches used for FM crop improvement and biofortification that will certainly improve food and nutritional security. Progress made so far on genetic manipulation of FM for crop improvement and biofortification, and biosafety regulatory prospects for the development of transgenic crops is also discussed.

14.1 Introduction

Food and malnutrition (nutritional security) are presently the two foremost problems being faced worldwide. As per the UN, 2007 report, the global population is expected to reach ten billion by 2050. Also, with the rapid degradation of cultivable land, decreasing resources for agricultural water availability, and abiotic stresses (salinity, drought, etc.), there is an urgent requirement to revolutionize the present farming practises. World Health Organization (WHO) has identified

S. M. Gupta (✉)
Defence Institute of Bio-Energy Research (DIBER), DRDO, Min of Defence, Haldwani 263139, India
e-mail: smg555@rediffmail.com

S. Gupta
Department of Molecular Biology and Genetic Engineering, G. B. P. U. A. & T., Pantnagar 263145, India

A. Kumar
Director Education, Rani Lakshmi Bai Central Agricultural University, Jhanshi 284001, India

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malnutrition problems due to deficiency of several micronutrients and proteins. Mineral deficiencies in human populations are one of the greatest health concerns for the current worldwide population that is causing ~462 million adults to be underweight, and around 45% of deaths among children below 5 years of age are associated with malnutrition. Therefore, the urgent demand for the production of functional food (nutrient enriched) is crucial for the prevention and/or treatment of diseases (Vinoth and Ravindhran 2017; Lockyer et al. 2018).

To meet this challenge, millets are one of the good options, which are generally grown under marginal lands and require comparatively less agricultural inputs. They offer quality food proteins, which are not only nutritionally rich but also possess nutraceutical properties. Among the millets, *Eleusine coracana* (L.) Gaertn (finger millet; FM) occupies a prime place due to its exceptional nutritional and medicinal properties (Gupta et al. 2017; Sharma et al. 2017). It contains high-quality proteins (5–12%), vitamins, fiber, minerals, low fat, high low glycemic index of malted grains, and exceptionally higher calcium (Ca^{2+}) content having about 0.34% in whole grain as compared with 0.01–0.06% in other cereals (Kumar et al. 2016; Sharma et al. 2017). This is good for controlling the blood glucose levels of diabetics (Gupta et al. 2017). Apart from these, FM is highly adaptive to local climate, and also can efficiently withstand various biotic and/or abiotic stresses. Due to these qualities, it is an excellent model for exploring genomics and a reasonable bio-resource for gene mining for many quality traits. The selected potential candidate genes responsible for high nutritive values and stress tolerance could be utilized for biofortification (designer crops) of other cereals by using genomics-assisted breeding and transgenic approaches for addressing nutrient security issues of the growing global population (Kumar et al. 2018a, b).

Little genomic information is available for this crop as compared to other cereals such as maize, rice, and barley. This leads to limited genetic and genomic efforts having been undertaken for the improvement of this crop. Therefore, genetic manipulation studies on FM for enhancing nutrient content (biofortification) are essentially required. Also, FM is an almost self-pollinating crop that makes it difficult in crosses between different strains used for crop improvement in breeding programmes. Therefore, genetic transformation is the method of choice for crop improvement that is also expected to help to understand unique molecular mechanisms in FM that will be used for the nutrient fortification of other cereals. Presently, transcriptome data of developing spikes of FM genotypes is available (Singh et al. 2014; Kumar et al. 2015a) that can be utilized for biofortification programmes to understand the holistic unique molecular mechanism of protein accumulation during the grain filling in FM genotypes. Recently, "Omics"-based approaches, nutri-genomics and nutri-genetics, etc. have opened new strategies for solving the problem of malnutrition by increasing the knowledge of nutrient-gene interaction aiding nutritional research (Kumar et al. 2015b). Once using omics-based approaches, the key ingredients were recognized in millets; the information can be utilized in future to design healthy foods that add value in addition to traditional diets (vanOmmen and Stierum 2002).

This chapter highlights the progress and prospects of FM resource utilization for genetic transformation for crop improvement and biofortification. It includes the details on various approaches such as genomics-assisted breeding, genetic improvement through transgenics, and mapping of quantitative trait loci (QTLs) for traits used for FM crop improvement programmes, which will be definitely helpful to address issues related to food and nutritional security problems of developing world.

14.2 Genetic Transformation: Methods and Potential

Genetic transformation or transgenic technology refers to a set of techniques allowing artificial insertion (excluding insertion of genes through

pollination) of a desirable gene(s) across taxonomic boundaries into a host organism (Gupta et al. 2013b). Plant transgenic technology includes various techniques that are required for stable integration of the foreign DNA (transgene) into the host plant (genetically modified, GM; or genetically engineered, GE) genome and its subsequent expression (Kumar et al. 2014a). This technology provides a possibility of not only bringing in a desirable trait from other plants, but also has the liberty of adding traits from other kingdoms' species. The dependency on GM crops is now rapidly increasing, since this technique permits need-based accelerated evolution of crops for betterment. This technology offers several opportunities to introduce genes conferring resistance to biotic and abiotic stress tolerance, nutritional biofortification of crop, introduce male sterility, manipulate biochemical pathway of flower pigmentation, delay fruit ripening, production of plant metabolites, increase NUE efficiency, crop yield, molecular farming (production of therapeutic agents, vaccines, biopolymers, antigens, monoclonal antibody, etc.) and many more. So far, the efforts of genetic transformation in FM are not adequate and still, there is lots of scope for crop improvement and biofortification. Some of the important traits for genetic transformation in FM are discussed in Fig. 14.1.

The desired traits can be introduced into a plant by many methods (Gupta et al. 2016). We can broadly classify all techniques into two main categories, firstly, **(i) Vector-mediated gene transfer** that involves plant gene vectors (Ti plasmid-based vector, plant viral-based vector) for the transfer of genes to plants from other organisms, and secondly, **(ii) Direct DNA transfer or vectorless gene transfer** that involves chemical or physical methods of gene transfer to plant. Physical gene transfer methods (also known as DNA-mediated gene transfer; DGMT) use the direct transfer of DNA to plant through several methods such as electroporation, microinjection, gene gun (microprojectile bombardment), silicon carbide whiskers, ultrasonication, UV-based laser microbeam irradiation, and liposome-mediated transformation. However, chemical gene transfer methods use direct gene transfer of DNA to plants with the help of various chemical agents such as calcium phosphate, polyethylene glycol (PEG), and polycations.

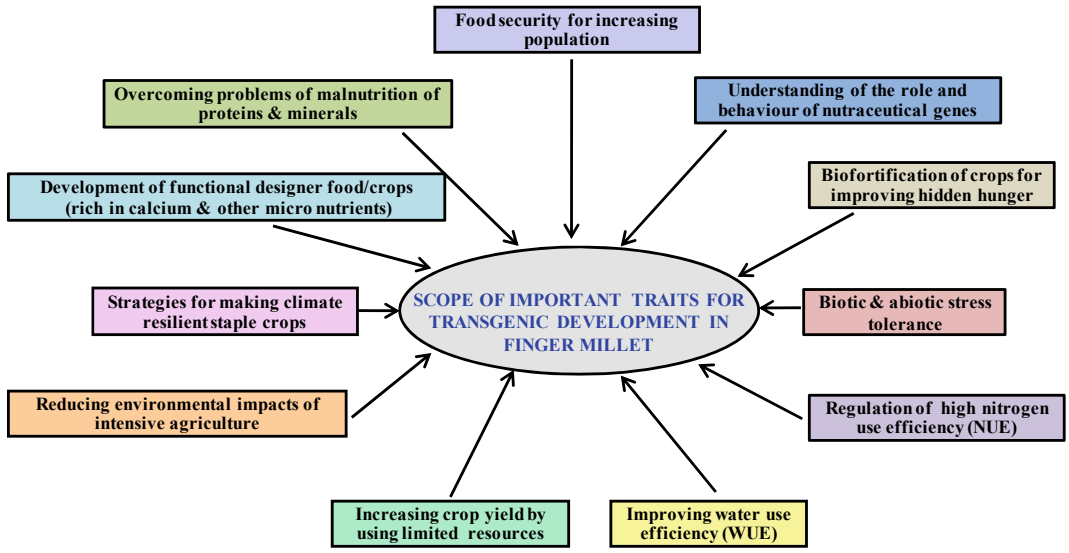

Fig. 14.1 Important traits for genetic transformation and biofortification in finger millet

14.3 Efforts on Genetic Transformation for Finger Millet Improvement

14.3.1 In Vitro *Regeneration Studies in Millets*

Standardization of reliable and reproducible in vitro regeneration method is an essential requirement for crop improvement through genetic engineering/transformation. It requires optimization of various factors such as selection of explant type, composition of the growth medium, plant growth regulators (PGRs) dose, plant environmental growth conditions, and many more. In FM, in vitro regeneration studies are fewer as compared to other cereals. Since millets are main crops of developing countries, which are having limited resources, therefore, investment toward improving these crops by genetic transformation techniques is limited. However, the first in vitro regeneration studies in millets were performed in proso-, pearl- finger-, and kodo-millets (Rangan 1976). Later on, many groups have developed regeneration protocols for Agrobacterium-mediated transformation and propagation using different FM explants like somatic tissue (Rangan 1976), root (Mohanty et al. 1985), leaf base segments (Mohanty et al. 1985; Gupta et al. 2001), mesocotyl (Eapen and George 1990), immature embryos (Yemets et al. 2013), embryogenic seed (Kothari et al. 2004; Jagga-Chugh et al. 2012), and shoot tips (Ceasar and Ignacimuthu 2008, 2011) were used. In the year 2015, attempt was made to regenerate FM by direct organogenesis using shoot apical meristem (Satish et al. 2015). They have also developed an efficient in vitro regeneration protocol for indirect organogenesis in four Indian FM genotypes by using PGR and spermidine chemicals (Satish et al. 2016b). The use of seaweed liquid extracts is helpful in enhancing regeneration rate that was also reported in FM (Satish et al. 2016a). Later on, a direct plant regeneration protocol was also developed in three FM genotypes (Babu et al. 2018). Recently, Jamra et al. (2021) showed endogenous phytonutrient, phytochemical, and phytohormone levels modulate *in vitro* callus induction and plant regeneration in FM genotypes.

In general, it was concluded from these studies that the shoot apex explant was the best explant for efficient in vitro regeneration due to its easy accessibility, availability, and rapid regeneration of multiple shoots as compared to other explants (Ceasar and Ignacimuthu 2008). However, there are fewer reports available so far in FM for in vitro regeneration through protoplast, anther culture, and protoplasmic fusion that can help in developing the haploid lines. The development of protoplasmic fusion may also help to improve the hybrid variety of FM because the recent genome editing methods such as whole-genome sequencing (WGS) and clustered regularly interspaced short palindromic repeats (CRISPR) use protoplast-based mediated transformation methods. Thus, standardization of an efficient protoplast-based in vitro regeneration system in FM is essentially required to achieve these tasks at a higher level.

14.3.2 Genetic Transformation in Finger Millet

As compared to other cereals, only a little handful of genetic successful transformation events were reported in FM to date, which are restricted to improving biotic and abiotic stress tolerance in FM (Table 14.1). However, the first genetic transformation efforts in FM were made by Gupta et al. (2001) that used the gene-gun method for comparing the transformation efficiency of five gene promoters (CaMV35S)/rice actin gene; ActI/maize ubiquitin; (UqI)/ribulose-1,5-biohosphate carboxylase; RbcS/Flaveria trinervia; b-glucuronidase/FtuidA) on the expression of the GUS reporter gene. After that, a few studies reported on the optimization of efficient transformation conditions by using agrobacterium-mediated transformation methods (Ceasar and Ignacimuthu 2011; Sharma et al.

2011; Jagga-Chugh et al. 2012; Satish et al. 2017). Later on, various efforts were made for developing biotic stress tolerance in FM such as resistance to leaf blast disease by using chemically synthesized antifungal protein (PIN) gene of prawn transferred to plant through the biolistic method (Latha et al. 2005). Similarly, the rice Chitinase11 gene (*Chi11*) was transferred to the GPU45 genotype of FM by agrobacterium-mediated transformation for leaf blast resistance trait (Ignacimuthu and Ceasar 2012). In both studies, the developed transgenic plants over-expressing foreign genes showed resistance to leaf blast disease as compared to control plants. However, genetic transformations studies by including other potential antifungal genes and also attempts at gene pyramiding are required for the development of a wide spectrum of fungal disease-resistant transgenic plants in FM.

Apart from biotic stress tolerance, FM has been also considered as a drought-hardy crop, therefore, efforts have been made to characterize the candidate genes responsible for abiotic stresses (drought, salinity, dehydration, cold, UV, etc.) tolerance. In this attempt, the serine-rich protein (*PcSrp*) gene from Porteresia coarctata's was over-expressed in FM under salt stress (Mahalakshmi et al. 2006). The developed transgenic FM exhibited normal growth, under saline (250 mM NaCl) stress (Mahalakshmi et al. 2006). In another study, the NAC gene (*EcNAC1*) was up-regulated in response to salinity stress and was suggested to be involved in salt and drought stresses tolerance (Ramegowda et al. 2012). Later on, many more drought-responsive genes have also been characterized by using drought-responsive transcriptome in FM (Ramegowda et al. 2017). Another potential gene, *EcGBF3*, was over-expressed in *A. thaliana*, which showed improved tolerance to saline, drought, and osmotic stresses (Ramegowda et al. 2017). Likewise, Rahman et al. (2016) also reported that over-expression of *EcNAC67* TF in rice with improved tolerance to drought and salinity. Furthermore, seven more drought-responsive genes, namely farnesylated protein *ATFP6*, farnesyl pyrophosphate synthase, metallothionein, RISBZ4, and protein phosphatase 2A, were over-expressed in GPU-28 genotype of FM under drought stress (Parvathi et al. 2013). Another drought-responsive, TBP Associated Factor6 (*EcTAF6*) gene was also identified and its expression in response to various abiotic (NaCl, PEG, and oxidative) stresses was investigated in FM genotype GPU-28 that showed significant tolerance as compared to control plants (Parvathi and Nataraja 2017). Similarly, Bhatt et al. (2013) reported over-expression of the *Ec-apx1* gene improved drought tolerance. An *AC2H2* type of zinc finger transcription factor (TFs) gene isolated from foxtail millet also improved salinity, dehydration, and cold stress tolerance in FM (Muthamilarasan et al. 2014). Similarly, Bhatt et al. (2013) reported over-expression of the *Ec-apx1* gene improved drought tolerance (Table 14.1).

An *AC2H2* type of zinc finger transcription factor (TFs) gene isolated from foxtail millet also improved dehydration, salinity, and cold stress tolerance in FM (Muthamilarasan et al. 2014). In addition to these, a salt-tolerant FM was developed by using sorghum vacuolar HC-pyrophosphatase (*SbVPPase*) gene through agrobacterium-mediated transformation (Anjaneyulu et al. 2014). Likewise, transgenic FM expressing a bacterial mannitol-1-phosphate dehydrogenase (*mtlD*) gene also showed better growth under salinity and drought stress as compared to control plants (Hema et al. 2014). Bayer et al. (2014) showed over-expression of *HvTUB1*, *TUAm1* genes improved resistance to herbicides (dinitroaniline family) tolerance in FM. Jayasudha et al. (2014) also produced a transgenic FM by introducing *Arabidopsis thaliana* vacuolar HC-pyrophosphatase (*AVP1*) and NaC/HC antiporter of *Pennisetum glaucum* (*PgNHX1*) for salinity stress tolerance. Likewise, Singh et al. (2015b) characterized the drought-responsive *EcDehydrin7* gene and showed its over-expression studies in transgenic tobacco that improved tolerance to drought stress in FM. Later on, two abiotic stress-responsive TFs belonging to the Basic helix-loop-helix (bHLH) family (*EcbHLH57*) and the bZIP family (*EcbZIP60*) were identified in GPU-28 genotype of FM under salt, drought, and oxidative stresses (Babitha et al.

Table 14.1 List of recent efforts on genetic transformation for crop improvement in finger millet

S. No	Gene name	Source of the gene	Transformation technique	Transgenic trait	References
1	CaMV35S/ActI/UqI/RbcS/Ft uidA	Finger millet	Biolistic	Promoter efficiency in GUS reporter expression	Gupta et al. (2001)
2	Antifungal protein (PIN)	Prawn	Biolistic	Transgenics resistant to leaf blast disease	Latha et al. (2005)
3	*PcSrp*	*Porteresia coarctata*	Biolistic	Salt tolerance	Mahalakshmi et al. (2006)
4	CaMV35S/uidA/nptII	Bacteria	Agrobacterium-mediated	Establishment of transformation efficiency	Sharma et al. (2011)
5	CaMV35S/uidA/hptII	Finger millet	Agrobacterium-mediated	Establishment of transformation using shoot apex	Ceasar and Ignacimuthu (2011)
6	Chitinase (*Chi*11)	Rice	Agrobacterium-mediated	Leaf blast disease resistance	Ignacimuthu and Ceasar (2012)
7	CaMV35S/hptII	Bacteria	Biolistic	Optimization of biolistic mediated transformation protocol	Jagga-Chugh et al. (2012)
8	*EcNAC1*	Finger millet	Agrobacterium-mediated	Enhanced abiotic stress tolerance	Ramegowda et al. (2012)
9	Metallothionein	Finger millet	Agrobacterium-mediated	Induced in drought stress	Parvathi et al. (2013)
10	Farnesylated protein ATFP6	Finger millet	Agrobacterium-mediated	Induced in drought stress	Parvathi et al. (2013)
11	Farnesyl pyrophosphate synthase	Finger millet	Agrobacterium-mediated	Induced in drought stress	Parvathi et al. (2013)
12	Protein phosphatase 2A	Finger millet	Agrobacterium-mediated	Induced in drought stress	Parvathi et al. (2013)
13	RISBZ4	Finger millet	Agrobacterium-mediated	Induced in drought stress	Parvathi et al. (2013)
14	*Ec-apx1*	Finger millet	pET23b vector-mediated	Expression increased under drought	Bhatt et al. (2013)
15	C2H2 type of zinc finger transcription factors (TFs)	foxtail millet	Agrobacterium-mediated	Salinity, dehydration, and cold stress	Muthamilarasan et al. (2014)
16	SbVPPase	*Sorghum bicolor*	Agrobacterium-mediated	Salt tolerance	Anjaneyulu et al. (2014)
17	*mtlD*	Bacteria	Agrobacterium-mediated	Tolerance to drought and salinity	Hema et al. (2014)
18	*HvTUB1*, *TUAm1*	Finger millet	Biolistic and Agrobacterium-mediated	Resistance to herbicides of the dinitroaniline family	Bayer et al. (2014)
19	*PgNHX1*, *AVP1*	Finger millet	Agrobacterium-mediated	Salinity tolerance	Jayasudha et al. (2014)
20	*EcDehydrin7*	Finger millet	Electroporation	Abiotic stress tolerance	Singh et al. (2015a, b)

(continued)

Table 14.1 (continued)

S. No	Gene name	Source of the gene	Transformation technique	Transgenic trait	References
21	*EcbHLH57*	Finger millet	Agrobacterium-mediated	Tolerance to salt, oxidative and drought stress	Babitha et al. (2015)
22	Monodehydroascorbate reductase	Finger millet	Agrobacterium-mediated	Abiotic stress (drought, salt, and UV) tolerance	Sudan et al. (2015)
23	*NAC 67*	Finger millet	Agrobacterium-mediated	Salt and drought stress tolerance	Rahman et al. (2016)
24	*EcJAZ*	Finger millet	Agrobacterium-mediated	Tolerance to biotic and abiotic stress	Sen et al. (2016)
25	*EcCIPK31-like*	Finger millet	Agrobacterium-mediated	Drought tolerance	Nagarjuna et al. (2016)
26	CaMV35S/hptII	Finger millet	Agrobacterium-mediated	Optimization of transformation using direct plant regeneration	Satish et al. (2017)
27	*EcGBF3*	Finger millet	Agrobacterium-mediated	Osmotic, salt, and drought stresses tolerance	Ramegowda et al. (2017)
28	*EcTAF6*	Finger millet	Agrobacterium-mediated	NaCl, PEG, and oxidative stresses	Parvathi and Nataraja (2017)
29	PHT1 phosphate transporter family *SiPHT1*	Foxtail millet	Agrobacterium-mediated	Phosphate uptake and signaling	Baker and Ignacimuthu (2017)
30	*EcbZIP17*	Finger millet	Agrobacterium-mediated	Tolerance to saline and heat stresses	Ramakrishna et al. (2018)

2015). The over-expression studies using monodehydroascorbate reductase and *EcJAZ* genes of FM also showed significant tolerance to various abiotic (drought, salt, and UV) stresses (Sudan et al. 2015; Sen et al. 2016). Similarly, Nagarjuna et al. (2016) characterized CBL interacting protein kinase31 (*EcCIPK31*-like) gene that is responsible for drought tolerance in FM. In addition, *SiPHT1* phosphate transporter gene over-expression studied showed improved phosphate uptake and signaling in FM (Baker and Ignacimuthu 2017; Ceasar 2019). Also, a novel endoplasmic reticulum-specific bZIP TF gene of FM (*EcbZIP17*) was also over-expressed in tobacco, which showed tolerance to heat and saline stresses as compared to control plants (Ramakrishna et al. 2018) (Table 14.1).

In conclusion, most of these genetic transformation studies conducted so far in FM are focused on the transfer of candidate genes for improving resistance to biotic and abiotic stresses. However, more precise resolution studies such as subcellular localization of foreign genes and fusion of promoters of FM with reporter genes (GUS, GFP, etc.) are yet to be carried out in FM. The WGS technique will be helpful to conduct such studies, especially those focusing on the isolation of native promoters for functional analysis by fusing them with reporter genes. It will be helpful for the functional validation of key genes and their promoters involved in abiotic and biotic stress tolerance. In addition to these, more attempts are needed in future to address the nutritional quality improvement of FM.

14.4 Other Genetic Approaches for Finger Millet Improvement

In addition to genetic transformation or transgenic approach, there are other genetic tools that are also employed widely for improving FM which are briefly discussed below (Fig. 14.2).

Molecular marker-assisted breeding has now become an essential tool to select genetically distinct germplasm for hybridization programmes for FM improvement. Although several markers have been developed in FM in the recent past, such studies are restricted to limited genotypes used. Among these, a randomly amplified polymorphic DNA (RAPD) marker has been used to study the genetic diversity of FM (Karad et al. 2013; Ramakrishnan et al. 2016). Later on, other markers such as RFLP and simple sequence repeat (SSR), expressed sequence tags (ESTs), and Cytochrome P450-gene-based markers have also shown genetic relationships among different geographical genotypes with respect to traits like yield, calcium and protein content, etc. (Panwar et al. 2010a, b; Kumar et al. 2012; Babu et al. 2014; Obidiegwu et al. 2014; Yadav et al. 2014). Additionally, in the shortage of genome sequence in FM as compared to other cereals (viz., rice, maize, and sorghum), comparative genomics allows us to project map-based orthologous candidate quantitative trait loci (QTLs) of interest that can be used for nutritional improvement of FM (Srinivasachary et al. 2007). This approach has been used to develop EST-SSRs from the opaque2 modifiers for grain protein and from the calcium transporters and calmodulin for calcium contents to analyze genetic diversity for these traits (Babu et al. 2014). After that, putative QTL for calcium content in FM grains using association mapping has been also studied by several workers

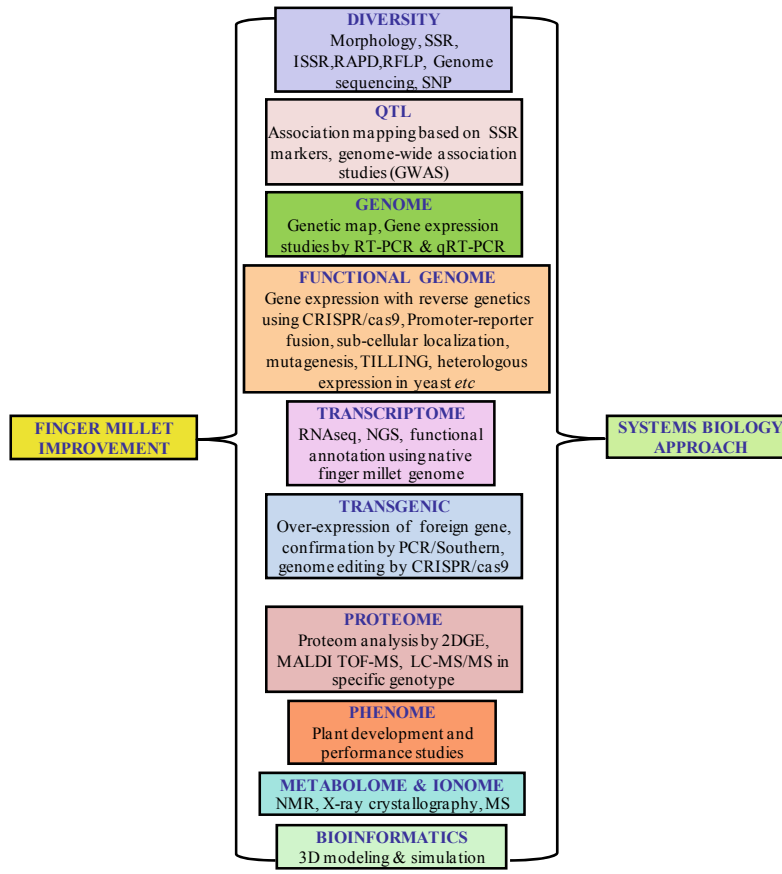

Fig. 14.2 Different genetic approaches for finger millet improvement

(Nirgude et al. 2014; Kumar et al. 2015c). The first genetic linkage map of FM covered 1,100 cM of the genome across 27 linkage groups (LGs) by using 212 RFLP markers (Gale and Devos 1998). Later on, the more detailed map was also generated by using several types of molecular markers like RFLP, AFLP, EST, and SSRs by several workers (Dida et al. 2007; Ren et al. 2013; Bharathi 2011; Tang et al. 2016). Optimistically, the WGS will aid in the development of SNP-based diversity analysis in FM accessions, which will further assist to boost future breeding programmes in FM for biofortification in terms of mapping and tagging important QTLs and creation of new genetic maps (Ceasar et al. 2018).

Transcriptomics or gene expression profiling techniques can also be used to identify candidate genes involved in many biological processes. In the past, microarray and serial analyses of gene expression (SAGE) were the only approaches available for gene expression studies but now with the introduction of Next Generation Sequencing (NGS) and RNA-sequencing, has emerged as new tool for high throughput sequencing of cDNA (Soneson and Delorenzi 2013; Wolf 2013). These advanced tools can be used for studying nearly every gene-regulating nutritionally important traits in many crops including millets (Kumar et al. 2015a; Akbar et al. 2018). Also, using meta-analysis of available gene expression data, a hypothetical model was made for elucidating the distribution and transport of calcium in FM (Goel et al. 2012). In another study in FM, large-scale transcriptome analysis has enabled the identification of 82 unique calcium sensor genes from developing inflorescence from genotypes differing in grain calcium content (Singh et al. 2014; Kumar et al. 2015a). Also, gene expression of calmodulin and Cax1 transporter genes identified them to be highly expressed in the high grain calcium genotype of FM (Kumar et al. 2014b; Mirza et al. 2014).

The proteomics technique can be used to study the structure, function, and expression of a complete set of proteins (Kussmann et al. 2006) and is considered as a coupler between the transcriptome of an organism and the ultimate responsive metabolome. The most broadly used technique for proteomics is 2-D gel electrophoresis which is being used to separate the complex mixture of proteins. Also, mass spectrometry (MS) can serve as a more specialized protein identification tool (Fuchs et al. 2005; Kussmann et al. 2006; Wang et al. 2006). Although there are fewer reports so far of protein profiling for characterizing nutritive proteins, some studies identify proteins involved in calcium accumulation in FM. However, elevated immuno-detection of calmodulin and calreticulin proteins in the embryo and aleurone layer of high grain calcium FM genotype was correlated to stimulation of higher calcium accumulation during grain filling in developing seeds (Kumar et al. 2014b; Singh et al. 2016). Thus, the identification of more FM proteins of nutraceutical importance through this approach is required in the coming future.

Metabolomics and ionomics are other "omics"-based technologies that allow the analysis of low molecular weight biomolecules and elemental composition synthesized or consumed by a biological system, respectively. It can also provide a direct functional statement of a plant's nutritive value by determination of its ionic contents, metabolic biochemistry, and phytochemicals (Sumner et al. 2003). So far in FM, many polyphenols have been identified by various methods such as high-performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR), and electrospray ionization mass spectrometry (ESI-MS). Some of these include benzoic acid (viz., gallic, *p*-hydroxybenzoic, ferulic acids, protocatechuic, and vanillic), cinnamic acid (viz., syringic, *p*-coumaric acids, and *trans*-cinnamic), and quercetin (Chandrasekara and Shahidi 2010; Banerjee et al. 2012). Variation in nutritional content due to transport of minerals and ions and differential imbibitions depend not only on the organism's physiological state but are also interrelated to its genetic makeup. Therefore, more efforts in profiling metabolomes in FM are required to expand this knowledge to other metabolites of nutritional importance.

Bioinformatics and systems biology approach is a multidisciplinary approach that

aims to integrate biological sciences with computer studies and characterize the wealth of transcriptome, proteome, and metabalome data and simulate them to forecast an informational pathway (vanOmmen and Stierum 2002; Kumar et al. 2015b). In the past, a mechanistic model in Arabidopsis was developed to identify key quantitative attributes for plant architecture and development (Mündermann et al. 2005). Later on, this approach was also used in studying C4 and C3 photosynthesis networks in maize and rice, respectively, thereby providing a framework for improved carbon fixation in other crops (Wang et al. 2014). This attempt helps in the formulation of a systems biology hypothesis which, in future, can be extrapolated to explore more nutraceutical properties of FM (Kumar et al. 2018b).

14.5 Biofortification: Tackling Micronutrient Deficiencies

The development of crops that by harvest have accumulated higher amounts of a particular micronutrient than standard crops is known as biofortification (Lockyer et al. 2018). Biofortification is the development of nutrient-rich crops without disturbing any agronomic performance and important traits (Saltzman et al. 2013). Biofortification can be done by following two strategies, firstly, (1) by improving the accumulation of nutrients in milled grains; and secondly, (2) by reducing the antinutrients to increase the bioavailability of minerals. Various approaches have been adopted for biofortification in crops that are discussed below.

14.5.1 Various Approaches for Nutrient Biofortification in Finger Millet

Biofortification can be achieved in three ways (Hirschi 2009; Vinoth and Ravindhran 2017). **(1) Agronomic biofortification**—use of additional micronutrient-rich fertilizers that are temporarily taken up by the crop; **(2) Conventional breeding biofortification**—selecting plants that naturally contain higher amounts of a micronutrient of interest for cross-breeding methods to produce crops with desirable enriched nutrient traits; and **(3) Transgenic biofortification**—inserting genes needed for the accumulation of a micronutrient that would not naturally exist in that particular crop (Fig. 14.3).

14.5.1.1 Agronomic Biofortification

This method is simple and inexpensive that uses the application of fertilizers containing essential mineral micronutrients. However, it is governed by several factors like the soil composition, application method, mineral mobility in the plant, and its accumulation site. Therefore, this strategy has been successful in only limited crops and is suitable for particular geographical locations. Also, the micronutrient fertilizers must be applied regularly which makes this method costly and potentially harmful to the environment. Thus, such strategies are applicable to specific crops and cannot be universally applied as a strategy to improve nutritional enrichment (Fig. 14.3).

14.5.1.2 Conventional Breeding Biofortification

This approach relies on improving the level and bioavailability of essential minerals in crops by using their natural genetic variation (Govindaraj et al. 2019). It includes the discovery of genetic variation affecting heritable mineral traits, checking their stability under different seasons, and the feasibility of breeding for increasing mineral content without affecting other quality traits like yield, etc. This approach utilizes some advanced molecular biology tools like marker-assisted selection (MAS) and quantitative trait locus (QTL) maps to accelerate the identification of better nutrient-rich varieties. It also makes use of the greater genetic variability, which can be induced by chemical treatments or irradiation, known as "mutation breeding", for improving mineral content and in some cases higher yield in crops (Fig. 14.3).

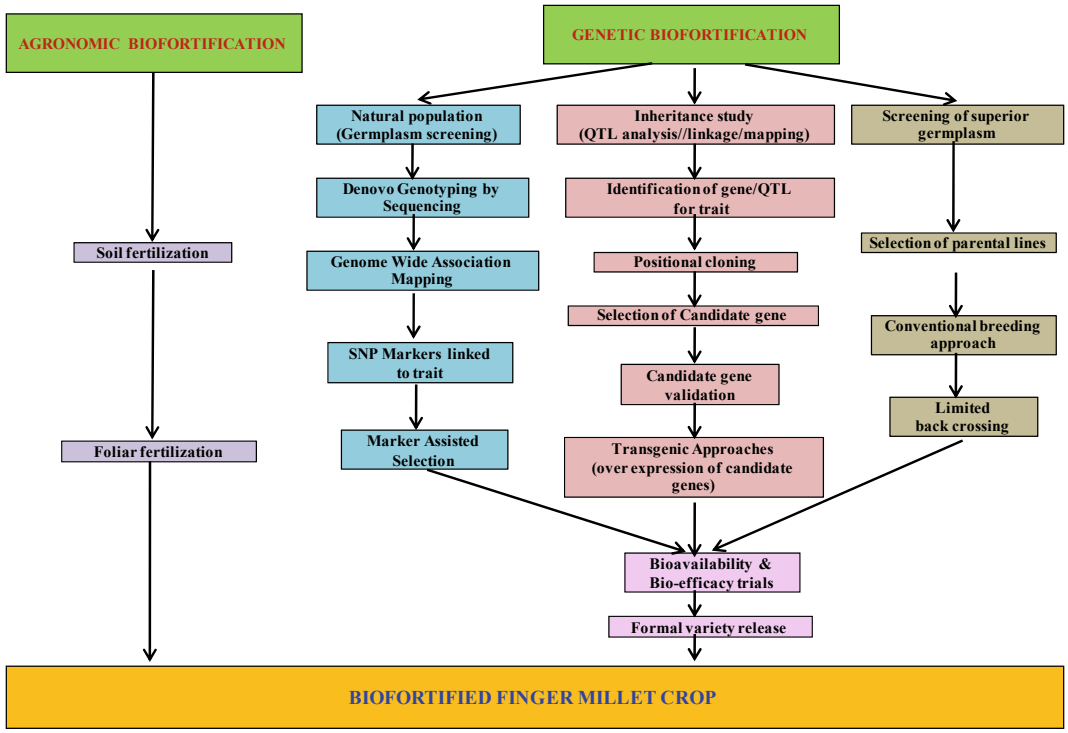

Fig. 14.3 Various approaches for nutrient biofortification in finger millet

14.5.1.3 Transgenic Biofortification

Genetic engineering is the latest approach to mineral deficiency and uses advanced biotechnological techniques to introduce genes directly into the crops for nutrient biofortification (Kumar et al. 2018a). Unlike conventional breeding, which is limited to genes that can be sourced from sexually compatible plants, it has no taxonomic constraints and even artificial genes can also be used for improving economic traits. The transgenic trait is added without normal biological reproduction, but once successfully incorporated into the plant it becomes inheritable through normal reproduction. This chapter highlights only this approach in detail including various efforts on micronutrient biofortification in FM in a subsequent section (Fig. 14.3).

14.5.2 Recent Transgenic Efforts on Micronutrient Biofortification in Finger Millet

Micronutrient biofortification through genetic modification of crops is a recent approach adopted by many workers for addressing the challenges and issues related to malnutrition (hidden hunger). Some of the important transgenic efforts that lead to the biofortification of

Table 14.2 The various events that lead to biofortification of micronutrients in crops

S. No.	Name and source of gene	Target plant	Biofortification of micronutrients	References
1	*Arabidopsis thaliana* (AtCAX1)	Tobacco	Enhance Ca^{2+} content	Hirschi (1999)
2	Yeast vacuolar Ca^{2+}/H^{+} antiporter, (VCX1)	Tobacco and Arabidopsis	Enhance Na^{+} and other ions sensitivity	Hirschi (2001)
3	*Triticum aestivum* (LCT1)	Tobacco	Protection from Cd^{2+} toxicity	Antosiewicz and Hennig (2004)
4	*Arabidopsis thaliana* (AtsCAX1)	Tomato	Enhances Fe^{3+}, Cu^{2+}, Mg^{2+}, Zn^{2+}, and Mn^{2+}	Park et al. (2005)
5	*Arabidopsis thaliana* (AtsCAX2b)	Bottle gourd	Enhance K^{+} and Na^{+} ion content	Han et al. (2009)
6	*Arabidopsis thaliana* (AtCAX1)	Rice	Architectural changes in starch granule synthesis	Yi et al. (2012)
7	Dhanashakti hybrid, ICRISAT	Pearl millet	Increase in Fe^{3+} and Zn content	Cercamondi et al. (2013)
8	Ca^{2+} signaling, sensor, uptake, translocation, and transporter genes	Rice; finger millet	Uptake and transport of calcium	Goel et al. (2012); Mirza et al. (2014); Singh et al. (2015a); Sharma et al. (2017); Akbar et al. (2018)
9	Zn++ transporters genes	Millets	Enhance zinc content in grain	Vinoth and Ravindhran (2017)
10	Iron and zinc transporter genes	Finger millet	Enhance bioavailability of iron and zinc	Chandra et al. (2020)

micronutrients in crops including millets are discussed in Table 14.2.

14.5.2.1 Genes Involved in Calcium (Ca^{2+}) Biofortification

Major cereals contain low levels of Ca^{2+} and several attempts in the recent past were made for the development of biofortified crops with enhanced amounts of Ca^{2+} that resulted in varied results (Hirschi 2009). Therefore, there is a required dissecting of the complex molecular mechanism that plants employ to acquire and store Ca^{2+} in edible tissues. Some of the successful transgenic reports for calcium biofortification in crops have been listed in Table 14.2. In tobacco plants, the *AtCAX1* gene was overexpressed that showed enhanced accumulation of Ca^{2+} (Hirschi 1999). Likewise, yeast vacuolar Ca^{2+}/H^{+} antiporter, (*VCX1*) gene was expressed in Arabidopsis and tobacco, the transgenic plants expressing VCX1 showed increased (up to 50%) tonoplast-enriched Ca^{2+}/H^{+} antiport activity as well as significantly higher accumulation of $Ca2^{+}$ content (Hirschi 2001). After this, several transgenic events were made by expression of various CAX proteins such as Arabidopsis *CAX1 (AtsCAX1)* in rice (Yi et al. 2012), *AtCAX4* in tomato (Park et al. 2005), *AtsCAX2b* in bottle guard (Han et al. 2009), and about 300% increase in calcium (Ca^{2+}) were reported. High Ca^{2+} accumulation in FM has been mainly attributed to the Ca^{2+} sensor genes that have been proposed as candidates for targeted Ca^{2+} enhancement in FM varieties (Singh et al. 2016; Sood et al. 2016). A hypothetical molecular model for Ca^{2+} transport from soil to seed was also proposed, which

is based upon genes that are differentially expressed in contrasting FM cultivars (Mirza et al. 2014; Kumar et al. 2014b, 2015a; Singh et al. 2014, 2015a; Sharma et al. 2017). Also, a few past studies reported the accumulation of Ca^{2+}-binding protein (Calreticulin) and CaM protein during grain filling stages of FM (Kumar et al. 2014b; Singh et al. 2016). In addition, it has been also shown that by activating EcCAX1b protein, *EcCIPK24* can also play an imperative role in high seed Ca accumulation (Chinchole et al. 2017). However, more efforts on high-resolution studies like CRISPR/Cas9 are required that may be helpful to develop mutants with defects in key genes of Ca transport and grain filling since this technique demands WGS to avoid any off-target effects (Hittalmani et al. 2017). It will definitely help in dissecting the complex mechanisms involved in Ca^{2+} transport in FM.

14.5.2.2 Genes Involved in Nitrogen (N) Metabolism

Fewer studies have reported on the key genes involved in N transport, which can be manipulated for N-biofortification in FM. Among these, prolamin-binding factor DNA binding with one finger only (*PBF-Dof*) TF gene involved in the regulation of seed protein storage was analyzed in different tissues at the vegetative stage and developing spikes of three FM genotypes (PRM-1, PRM-701, and PRM-801) (Gupta et al. 2011). Interestingly, the grain protein content of these genotypes was directly related to higher expression of *PBF-Dof* at the early stages of growth (Gupta et al. 2011). Similarly, the characterization of six key genes involved in N-uptake and absorption were analyzed in two genotypes (GE-1437, low-protein and GE-3885, high-protein) of FM, namely nitrate reductase (*EcNADH-NR*), low-affinity nitrate transporter (*EcLNRT1*), high-affinity nitrate transporter (*EcHNRT2*), glutamine oxoglutarate amino transferase (*EcFd-GOGAT*) glutamine synthetase (*EcGS*), and DNA *EcDof1* (Gupta et al. 2013a; Gaur et al. 2018). This study proved that GE-3885 might be a quick sensor of N as compared to the low-protein genotype (Gupta et al. 2013a). After that, the same group also studied the expression pattern of *EcDof1* and *EcDof2* genes in the same FM genotypes and suggested that both Dof genes are having opposite roles in the regulation of genes related to C– and –N metabolism (Gupta et al. 2014).

14.5.2.3 Genes Involved in Carbon (C) Metabolism

In FM, the expression analysis was performed for some C-metabolism genes, namely Rubisco (*RBCS*), phosphoenol pyruvate carboxylase (*PEPC*), chlorophyll a/b binding protein (*Cab*), malic enzyme (*ME*), phosphoenol pyruvate carboxykinase (*PEPC-k*), pyruvate dikinase (*PPDK*), pyruvate kinase (*PK*), sucrose phosphate synthase (*SPS*), and 14-3-3 and sensor protein kinase 1 (*SnRK1*). Also studied of co-expression of these genes with Dof1 were done in two genotypes of FM under light–dark conditions (Kanwal et al. 2014). This study confirms that the expression of Dof genes was oscillated in both genotypes with control by an endogenous clock. However, some genes like *Cab*, *RBCS*, and *PPDK* showed no oscillations that might be due to induction by light. Also, the expression of *Dof1* along with other genes involved in the C-metabolism gene was found more in the higher grain protein FM genotype (GE-3885) that proves that Dof1 regulates the expression of light-inducible genes and controls the grain protein content in FM (Kanwal et al. 2014; Gupta et al. 2018). However, more studies on WGS are required to identify and characterize more genes involved in C metabolism in FM.

14.5.2.4 Genes Involved in Phosphate (P) Transport

The expression analysis of four phosphate transporter 1 (*EcPT1* to *EcPT4*) genes involved in P-transport was analyzed in three genotypes (Khairna, Ragi Korchara, and VHC3611) of FM (Pudake et al. 2017). The expression of these genes was checked under various regimes of inorganic phosphate (Pi). It was found that *EcPT1* expression was fivefold higher in leaves and roots under depleted Pi as compared to wild plants, while the *EcPT3* gene was induced under

phosphate stress in both roots and leaves. On the other hand, *EcPT4* genes were found to be induced by arbuscular mycorrhizal fungus (AMF) in roots (Pudake et al. 2017). So far, only four *EcPT1* genes have been studied in FM, however, it is accepted that each plant contains more than 10 such genes (Baker and Ignacimuthu 2017). Even in, foxtail millet it was found that such 12 PT genes have been characterized for expression pattern, P transport assay, and in planta function by down-regulation through RNAi technology (Ceasar et al. 2017). In the coming future, WGS studies will be more helpful for the genome-wide identification and functional characterization of all PT genes in FM.

14.5.2.5 Genes Involved in Zinc (Zn) Accumulation

Zinc (Zn) deficiency results in diarrhea, impairment of physical growth, and suppressed immune function (Gibson et al. 2008). Genetic enhancement of grain Zn content is possible by modulating the metal transporters, which facilitate Zn uptake, translocation, and storage. Members of Zn-regulated transporters regulate the transporter-like protein (ZIP) family that contributes to Zn homeostasis by either uptake or remobilization in intracellular compartments (Guerinot 2000). The ZIP transporters enhance Zn uptake in many higher plants including Arabidopsis (Plaza et al. 2007). In the past, initial success in transgenic development for grain Zn accumulation was reported in rice (Ramesh et al. 2004). After that, high zinc accumulating FM transgenic plants were also produced by overexpression of *OsZIP1* gene that showed higher Zn content as compared to control plants (Ramegowda et al. 2013). Interestingly, higher Mn accumulation was also recorded in the gains of these FM transgenic plants as compared to wild plants (Ramegowda et al. 2013). The *in planta* transgenic events also showed higher Zn accumulation in transgenic seeds by up-regulation of ZIP transporters. The expression of heterologous Zn transporters needs further detailed investigations to enhance grain Zn content in FM.

14.5.2.6 Iron Fortification

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has developed the popular open pollinated variety (OPV) ICTP 8203 that was improved to create the first biofortified ICTP 8203-Fe variety in pearl millet (Cercamondi et al. 2013). It was also found that the iron absorption from ICTP 8203-Fe pearl millet when consumed as porridge was higher (7%) than initially assumed for setting breeding target levels (5%). Similarly, another study also validated these bioavailability results in women of childbearing age in Benin (Saltzman et al. 2013).

In conclusion, although significant efforts were made in FM biofortification, the major gaps in knowledge with respect to biofortification are still lacking. Therefore, more effective studies and efficacy trials are needed to confirm and understand the importance of cross-nutrient synergies. Also, additional delivery and marketing research is required for improving the effectiveness of delivery and marketing strategies in ensuring maximum adoption and consumption of biofortified crops in FM in the coming future (Chandra et al. 2021).

14.6 Biosafety Regulatory Decision Points for Development and Release of a Transgenic Crop in Finger Millet

Adoption of genetically modified (GM) crops in agriculture is rapidly increasing now these days (Falck-Zepeda et al. 2012). Presently, approximately 52 countries have granted approvals for field trials of GM crops; among them, Japan ranks first followed by the USA, Canada, Australia, Mexico, New Zealand, the Philippines, European Union, and China. However, in other developing countries including India, many of the GM crops are still at biosafety regulatory approval and evaluation stages (Falck-Zepeda et al. 2012). Biosafety deals with the safe use of GMOs in laboratory conditions, in confinement field trials and in general, unconfined

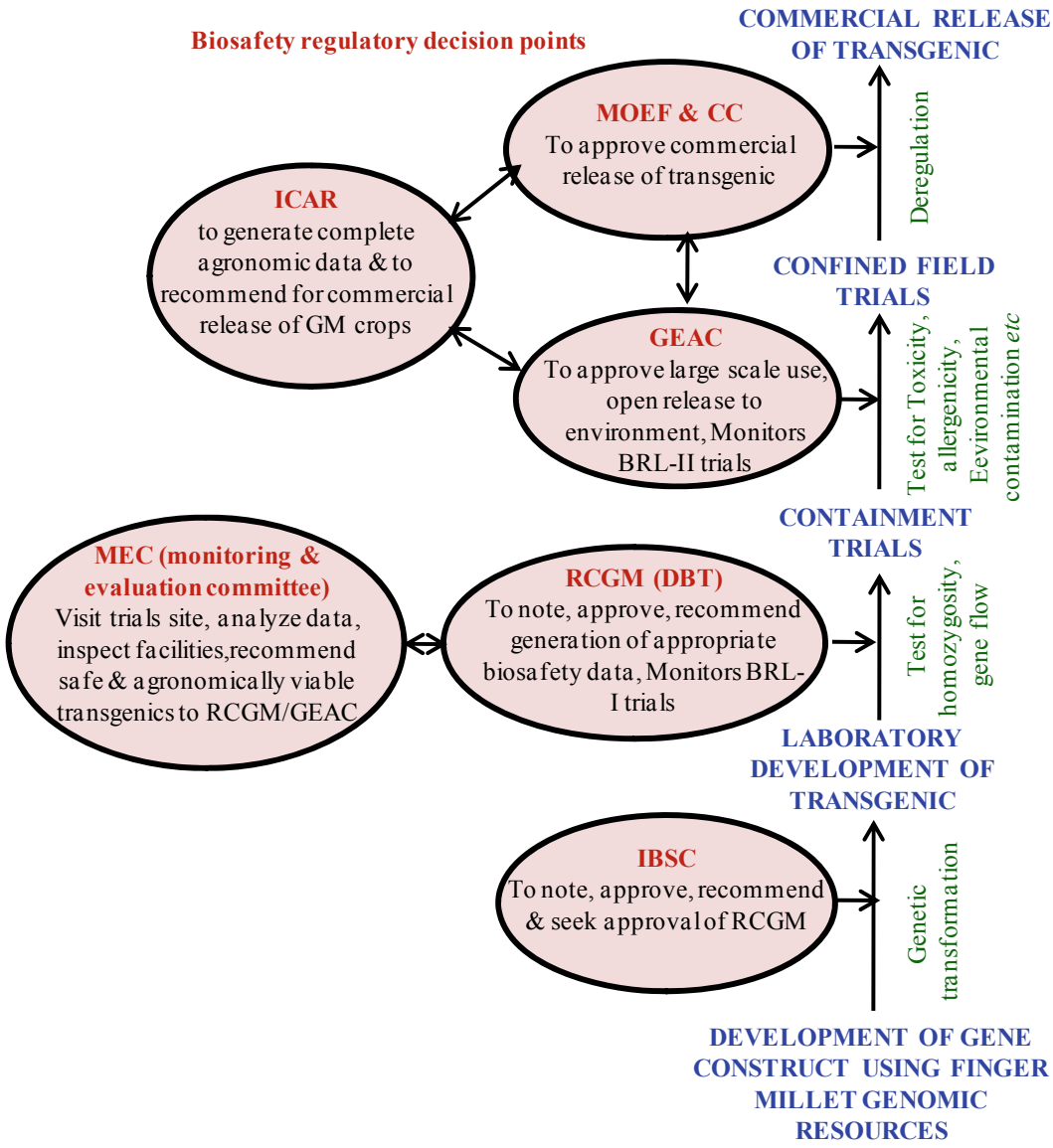

Fig. 14.4 Biosafety regulatory decision points for different stages of development and release of a transgenic crop in finger millet

introductions into the environment (Komen 2012). The several biosafety regulatory decision points needed for the development and release of a transgenic crop in FM in India are depicted in Fig. 14.4. Any developed GM crops have to undergo vigorous biosafety evaluations during containment and confined field trials, and at all stages, food, feed, and environmental safety issues are monitored strictly. To address these safety issues, several international agreements and treaties have been formulated so far, which regulate advancements in GM events in India. Some of the important policies such as Convention on Biological Diversity (CBD), intellectual property rights (IPR), and International Union for the Protection of New Varieties of Plants (UPOV) are covered under the Cartagena Protocol.

14.6.1 The Cartagena Protocol

As such, there are no universal international laws that exist, which govern genetically modified organisms (GMOs). Different countries have different rules and regulations for the release of any transgenic events. However, several developed countries are following all regulations governing GMOs with the formulation of Cartagena Protocol of Biosafety. In the year 2000, the Cartagena protocol on biosafety to the Convention on Biological Diversity (Biosafety Protocol) was signed by 131 government/parties in Canada to protect biodiversity from potential environmental effects of the trans-boundary movement by genetically modified living organisms (LMO). Later on, various organizations such as WHO, FAO, and WTO provided further regulation of the products of GM biotech through the intervention of various worldwide agencies. For developing countries, Cartagena Protocol on Biosafety (CPB) is the first point for the formulation of their internal biosafety regulations that decide biosafety assessments is an important part of the sustainable development of mankind. However, some countries designed policies against unidentified risks, while other countries designed the policies to get maximum benefits from the GM technology (Wafula et al. 2012). Therefore, it may vary from country to country as per the country's needs basis.

In India, the activities related to GMOs are regulated under the "Rules for the manufactures, use/import/export and storage of hazardous organisms/GM organism or cells notified under the Environment Protection Act 1986, commonly called Rules 1989". These rules and regulations are implemented jointly by the Ministry of Environment and Forest (MOEF) and Department of Biotechnology (DBT) with the involvement State government's agency. In India, presently the following seven competent authorities are involved handling various aspects of GMOs (Fig. 14.4).

i. Institutional Biosafety Committee (IBSC).
ii. Review Committee on Genetic Manipulation (RCGM).
iii. Monitoring and Evaluation Committee (MEC).
iv. Genetic Engineering Approval Committee (GEAC).
v. Recombinant DNA Advisory Committee (RDAC).
vi. State Border Coordination Committee (SBCC).
vii. District Level Committees (DLCs).

Among these, RDAC is advisory in the role; IBSC, RCGM, and GEAC are of regulatory functions; and SBCC, DLC, and MEC are for monitoring purposes. In addition to this, DBT has issued several guidelines for applicants, who are seeking approval for the environmental release of GM crops in India as per Rules 1989. These guidelines address various key elements of the safety assessment of foods and/or livestock feed, which may be developed from GM crops. The detailed biochemical evaluation reports are submitted to concern regulatory bodies, i.e. RCGM and GEAC, from time to time or when required. In addition to these, DBT has also prepared five protocols each based on international best practices including guidance and peer-reviewed publications from the FAO, WHO, Codex Alimentarius Commission (CAC), ILSI, and Organisation for Economic Co-operation and Development (OECD).

14.7 Conclusion and Future Perspectives

FM is considered as a nutraceutical crop that seems to be an ideal solution for addressing hidden hunger or malnutrition problems. Besides its nutritional benefits, its ability to abide by various abiotic and biotic stresses makes it a good model for exploring the vast genomic and genetic potential for crop improvement and biofortification of other crops. It also provides a reasonable bio-resource for gene mining for many complex traits of economic importance. The identified potential candidate genes/proteins responsible for high yield, stress tolerance, and

high mineral accumulation can be isolated from FM and utilized for improving other cereals, which definitely pave way for the development of designer crops for a sustainable future.

Until now, limited progress has been made in millets mainly due to lack of appropriate genomic information in these crops. However, high throughput sequencing platforms and integration of various advanced omics strategies have proven to be able to overcome the complexity of large and complex FM genomes. It will also help to understand the regulation of genes/proteins/metabolites responsible for the high nutrient accumulation and stress tolerance at transcriptional, post-transcriptional, and epigenetic levels. Thus, integration of knowledge on "Omics" technologies (phenome, genomics, transcriptomics, proteomics, metabolomics, ionomics, etc.) could promote millets as model systems for advancements in biofortification of other crops for nutritional security of the growing world population. In the coming future, the chances of the development of a super cereal may also be possible by incorporating many agronomical traits into a single FM genotype. Thus, utilization of current advances in Omics technologies (system biology) will definitely improve the present scenario of FM research.

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Novel Prospective on Suppression of Ageing by the Consumption of Finger Millet

15

Anil Kumar, Madhu Rani, Rashmi Kumari, Pallavi Shah, Shalini Mani, and Salej Sood

Abstract

Ageing process is a crucial phenomenon of life that deals with the proceedings of deadly and unmodified changes by impairment of biomolecules (Proteins, Lipids, and DNA), cells, tissues, and organs. It is an inescapable event but it could be delayed via both genetic and dietary interventions. The proper use of natural compounds along with regulation of diet can be beneficial in overcoming premature death and age-related diseases. Natural compounds have been known to suppress the stress responsive pathways and induce the longevity pathways (DNA damage repair pathway). Phytochemicals represent high anti-ageing potential and other health-promoting properties. Among all plants/cereals, minor cereals like millets are a rich source of calcium, iron, methionine, high fibre, polyphenols, and secondary metabolites which exhibit various health-beneficial properties including anti-ageing potential. In this book chapter, *prima facie* we will address the role of phytochemicals that are found in millets. The main emphasis will be on finger millet and we will summarize the critical reports that are relevant to the significant health benefits along with anti-ageing properties of phytochemicals from finger millet. Further, we will also discuss the underlying mechanisms of deferment of ageing and age-related diseases by phytochemicals of millets via affecting the processes like genetic repair, protein glycation, and stress responsive pathways. Furthermore, we will shed light on the well-established phytochemicals for their significant use in anti-ageing drugs. In conclusion, we suggest and promote awareness about the development of novel formulations/combinations based on millets to utilize their anti-ageing potential for human welfare.

A. Kumar (✉)
Rani Lakshmi Bai Central Agricultural University, Uttar Pradesh, Jhansi, Uttar Pradesh, India
e-mail: anilkumar.mbge@gmail.com

M. Rani · R. Kumari
Genome Biology Laboratory, Department of Biosciences, Jamia Millia Islamia, New Delhi 110025, India

P. Shah
Indian Veterinary Research Institute, Uttar Pradesh, Izatnagar, Bareilly, Uttar Pradesh, India

S. Mani
Centre for Emerging Diseases, Department of Biotechnology, Jaypee Institute of Information Technology, Uttar Pradesh, Noida, Uttar Pradesh, India
e-mail: shalini.mani@jiit.ac.in

S. Sood
Crop Improvement Division, ICAR-Central Potato Research Institute, Shimla, Himachal Pradesh, India
e-mail: salej.sood@icar.gov.in

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15.1 Introduction

The past few decades have seen an enormous growth in research work elucidating those certain major components in diet influence ageing and associated age-related diseases. These dietary components include a myriad of constituents like saturated, polyunsaturated, and trans-fatty acids healthy for the heart, fibre to improve glycemic index in diabetes, calcium and vitamin D for osteoporosis and other bone malformations, and many more. With ever-increasing interest in the role of nutrition for maintaining good health, phytochemicals especially polyphenols and flavanols and dietary fibre have caught attention due to their numerous health benefits. Millets are a rich source of such healthy antioxidant polyphenols and dietary fibres (Shlisky et al. 2017; Kumar et al. 2021a).

Non-communicable chronic diseases (NCDs) such as obstructive pulmonary disease, cardiovascular disorders, type II diabetes, dementia, some cancers, and osteoporosis are often associated with old age fibres (Taylor 2017). Being a complex multicomponent process, many theories have been proposed to explain ageing. These theories include the free radical theory of ageing also known as the mitochondrial theory of ageing, the telomere shortening theory, and the protein translational modification theory (Santos and Lindner 2017). Antioxidants and protein modifying compounds present in diet often influence different pathways and components as explained in these theories and ultimately either delay or help overcome ageing. Earlier reports have also explained the effect of dietary factors on epigenetic modifications such as direct influence on gene expression by affecting histone modification, DNA methylation, activation of nuclear receptors, and by membrane receptor signalling cascades (Ribarič et al. 2012). With a better understanding of how dietary components influence molecular mechanisms involved in ageing such as sirtuins pathway, insulin/insulin-like growth factor (II S) signalling cascade and mammalian target of rapamycin (mTOR)pathway (Altintas et al. 2016; Chen et al. 2020; Yu et al. 2021) have opened newer prospects in the field of nutraceuticals to overcome ageing.

Millets being an abundant source of healthy phytochemicals, minerals, and dietary fibres offer various health benefits ranging from antioxidant, anti-cancerous, antimicrobial, anti-diabetic, and anti-ageing properties. Amidst different varieties of millets, finger millet has a significantly high nutritional value (Taylor 2017). Previous work has also reported the preliminary anti-ageing effect of finger millet grains (Dykes and Rooney 2006; Hegde et al. 2002; Kakkar and Bais 2014; Khan et al. 2015; Pei et al. 2016; Shobana et al. 2010; Zhang et al. 2011). In this present book chapter, we address the nutraceutical properties and composition of natural compounds from finger millets. We describe the effect of their phytochemicals on ageing process and age-related disorders along with their molecular insights. We also shed light on famous phytochemicals for their use as anti-ageing drugs. In conclusion, we suggest the development of novel formulations/combinations based on millets to utilize their anti-ageing potential for human welfare.

15.2 General Information About Millets

Millets belong to the family *Poaceae* and are often considered as "coarse cereals" similar to sorghum because of their tough grain texture, rendering their processing as well as cooking inconvenient when compared to general cereals like rice and wheat (Hassan et al. 2021). They are mostly grown as small seed grain-producing cereals in Asian and African countries for the past 10,000 years (Bhat et al. 2018). Millets are resilient plants often distinguished by their remarkable abilities such as resistance to pests and diseases, stress tolerance, and high adaptability (Kumar et al. 2018).

A huge portion of the total world's millet produce comes from India, Africa, and China. The Asian and African millets include finger millet (*Eleusine coracana*), foxtail millet (*Setaria*

italica), pearl millet (*Pennisetum glaucum*), barnyard millet (*Echinochloa spp.*), kodo millet (*Paspalum scrobiculatum*), proso millet (*Panicum miliaceum*), and little millet (*Panicum sumatrense*) (Rao et al. 2017). Whereas, Tef (*Eragrostic tef*) and Fonio (*Digitaria exilis*) are indigenous to Africa (Bhat et al. 2018). Regions where millets are grown are well evident from their common names like foxtail millet is known as Italian millet, proso millet is called as French millet, and barnyard millet is often known as Japanese barnyard millet. Millets are mostly used for food purposes in developing countries but are mainly used as feed ingredients in developed countries.

40% of the world's total millet production is pearl millet with large grains (ICRISAT 2007; Mariac et al. 2007). Aside from pearl millet, the rest of the millets are known as small or minor millets (FAO 2012). Finger millet is mainly grown in Southern Asia mainly in India and Nepal and in Eastern and Southern African countries like Uganda, Kenya, the Democratic Republic of the Congo, Zimbabwe, Zambia, Sudan, Tanzania, Nigeria, and Mozambique.

15.2.1 Nutrient Composition

Nutritional composition of food influences the overall health and well-being of humans. When consumed as food, millets provide a rich source of healthy phytochemicals and micronutrients with innumerous health benefits (Singh et al. 2012; Hassan et al. 2021). They possess a high nutritional value in comparison to several main cereals like wheat, rice, sorghum, and maize. Millets are nutritionally significant as they contain a high amount of roughage (18%), a high calcium content (0.38%), and rich content of phenolic compounds (0.3–3%) (Rao et al. 2017). Millets contain proteins abundant in essential amino acids such as tryptophan, threonine, and sulphur-containing amino acids (Shah et al. 2021a, b). They possess a high fat content especially unsaturated fatty acids in comparison to other cereals (Nithiyanantham et al. 2019). The average nutrient composition of different millets is shown in Table 15.1.

Different millet varieties possess 65–75% of carbohydrates in their grains, 7–12% of protein, 2 to 5% of lipid content, and 15–20% of dietary fibre. Amidst the different millet varieties, pearl millet has the highest lipid and protein content. Finger millet on the other hand is a rich source of distinctive amounts of sulfur-containing amino acids, calcium, and pyridoxine.

All millets are a rich source of Vitamin B complex. Their rich nutritional value has made millets an indispensable part of the healthy food regimen (Liu et al. 2012). Similar to cereals, millets are also poor in lysine content, and a millet-based diet, thus, had to be supplemented with lysine enriched vegetables and animal proteins. The rich dietary fibre content (>90%) of millet grains is made up of non-starch polysaccharides found in both the seed bran as well as endosperm.

Millet-based traditional food items are a major part of the food consumed in Central America, Africa, and India. The majority of prepared food items are flour-based and include items like porridge, pancakes, snacks, and other deep-fried products. Further, millet grains are used to prepare sweet or sour local liquor, some non-alcoholic beverages, and decorticated grains, prepared similar to rice by boiling (Bhat et al. 2018; Taylor 2017). Different types of millets are consumed in different seasons of the year based on their availability and nutritional content by local people around the world.. Millets are a rich source of both soluble and insoluble dietary fibres contributed by both seed coat as well as endosperm cell walls. Dietary fibres offer numerous physiological benefits especially the health of the gastrointestinal tract and hence millets can be used in the preparation of functional and healthy foods. Further, bioactive compounds found in millets are also recognized for their health-beneficial effects, such as antioxidant, anti-diabetic, and anti-ageing properties (Banerjee et al. 2012; Kumar et al. 2016, 2002; Shahidi and Chandrasekara 2013; Shobana et al. 2007; Sripriya et al. 1997).

Table 15.1 Nutrient composition of various millets (g/100 g db and mg/100 g db). All values represent the mean of reliable published data

Millets	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Carbohydrate (g)	Fat (g)	Protein (g)	Fibre (g)	Ash (g)	Energy (kcal)	Ca (mg)	Fe (mg)	Zn (mg)
Kodo millet	0.2	0.1	2.0	74	4.05	9.7	5.2	3.3	353	35	1.7	2.2
Finger millet	0.4	0.2	1.1	67	1.5	8.9	15.2	2.6	336	350	3.9	3.1
Fonio millet	0.2	0.2	1.2	62	3.3	8.4	18.2	3.4	379	20	2.1	1.5
Foxtail millet	0.6	0.1	3.2	62	4.0	11.2	9.4	3.3	351	31	2.8	2.9
Little millet	0.3	0.1	3.2	76	4.5	15	2.5	5.4	329	17	9.3	5.3
Pearl millet	0.4	0.2	2.8	69.5	5.1	11.8	13.8	2.2	363	42	11.0	3.3
Barnyard millet	0.3	0.1	4.2	74	5.2	11.0	13.6	4.5	300	22	18.6	3
Proso millet	0.4	0.3	4.5	70	7.25	12.6	13.1	2.7	316	15	2.2	2.4

Source Adapted from Kumar et al. (2021b), Taylor (2017), Serna Saldivar (2018), Kumar et al. (2018), Selvi et al. (2015), Shankaramurthy et al. (2019), Renganathan et al. (2020)

15.2.2 Millet Phytochemicals

Millet phytochemicals are mostly secondary metabolites such as polyphenols which chiefly include phenolic acids, flavonoids, and tannins reported in different parts of the millet grain. These polyphenols are responsible for the potent antioxidant activity of millet grains (Rao and Muralikrishna 2002; Sreeramulu et al. 2009). Other smaller portion of phytochemicals consists of organosulphides, indoles/glucosinolates/sulphur compounds, terpenes, betalains, protein inhibitors, other organic acids, and phytates. The presence of flavonoids and tannins assigns different colours to their seed coats and some possess coloured endosperm. Millet starch differs from cereal starch in crystallinity and the organization of amylose and amylopectin. Millet starch gelatinizes at a higher temperature than that of rice and wheat. This property makes the digestion of millet-based food items slow as compared to other cereals.

15.2.2.1 Millet Polyphenols

Polyphenols are secondary metabolites often characterized by the presence of multiple phenol rings (Manuja et al. 2013. A variety of polyphenols have been obtained from several plants and a vast majority of them are derived from either phenylalanine or shikimic acid. Classification of polyphenols is based on the number of phenol rings and on the basis of the arrangement of these rings. Polyphenols basically consist of phenolic acids (benzoic acid and cinnamic acid derivatives), flavonoids, lignans, and stilbenes (Figs. 15.1 and 15.2). Polyphenols present in millets are among the most marketed dietary supplements. Previous works have established an abundance of polyphenols in the finger millet seed coat in comparison to other major cereals like rice, barley, wheat, and maize (Viswanath et al. 2009).

Millet Phenolic Compounds

Phenolic compounds in millet grains can be found in both free soluble as well as insoluble bound forms that are either derived from hydroxycinnamic acid or hydroxybenzoic acid. Glycosides are the major form of phenolics found in finger millet. The majority of bound insoluble phenolics are cinnamic acid derivatives attached to the polysaccharides in the cell wall by esterification. On the other hand, soluble forms include either free non-conjugated forms or phenolic compounds esterified or etherified to soluble carbohydrate molecules (Shah et al. 2021a, b). In finger millet, protocatechuic acid is the major free phenolic acid (45.0 mg/100 g) and ferulic acid is the major bound phenolic acid (19 mg/100 g) (Rao and Muralikrishna 2002). The finger millet seed coat is most abundant in benzoic acid-based phenolic compounds such as vanillic acid, syringic acid, gallic acid, protocatechuic acid, and *p*-hydroxybenzoic acid (Chandrasekara and Shahidi 2010; Hegde et al. 2002; Rao and Muralikrishna 2003). The rest consists of either cinnamic acid derivatives such as *trans*-cinnamic acid, *p*-coumaric acid, caffeic acid, and sinapic acid followed by condensed tannins or flavonoids like quercetin (Chethan and Malleshi 2007; Shah et al. 2021a, b).

Shobana et al. (2009) performed a detailed direct infusion electrospray ionization mass spectrometry (ESI MS) of the extract from finger millet seed coat and described the presence of kaempferol, phloroglucinol, luteolin, naringenin, apigenin, catechins, epigallocatechins, malic acid, diadzein, and catechin gallates. Of all the millets studied, finger millet has the highest catechin content followed by foxtail, little, pearl, and proso millets (Shah et al. 2021a, b). The darker seed coat colour of finger millet varieties from Northern Malawi has been associated with the abundance of polyphenols and has been correlated with their higher antioxidant potential in comparison to lighter coloured varieties. Thus, darker counterparts are more valuable for use as functional foods with naturally higher amounts of antioxidants (Xiang et al. 2019a).

Millet Flavonoids

Flavonoids are important classes of polyphenols that help regulate plant growth, reproduction, and function. Earlier work has reported the role of flavonoids in protecting plants against both abiotic and biotic stresses by imparting pest

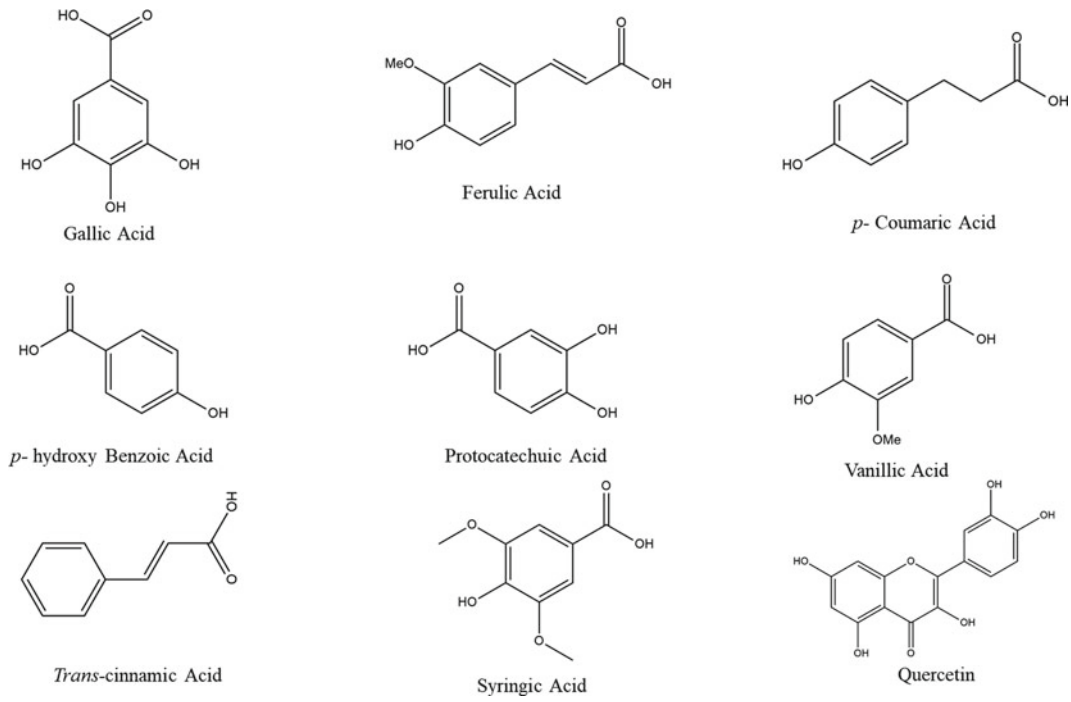
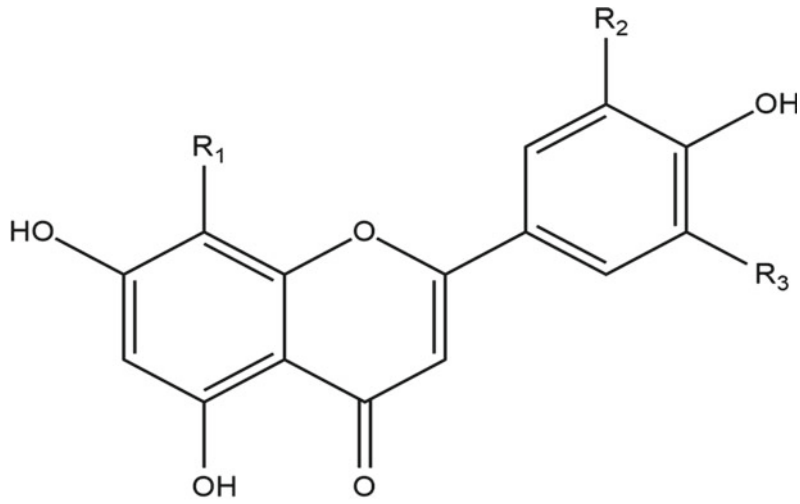


Fig. 15.1 Major phenolic compounds found in millets

Fig. 15.2 Basic structure of major flavonoids found in millets



Vitexin : R_1 =Glc, R_2 =H, R_3 = H
Orientin : R_1 =Glc, R_2=OH, R_3= H
Apigenin: R_1 =H, R_2 = H, R_3= H
Luteolin : R_1=H, R_2=OH, R_3= H
Tricin : R_1=H, R_2 =OCH_3, R_3 = OCH_3

resistance, pigmentation to grains, and UV protection and by affecting physiological processes like germination and auxin regulation (Panche et al. 2016). Flavonoids can be found either in bound or free forms as monomeric or polymeric forms. Unlike most cereals where bound flavonoids are in majority, the millet flavonoids are mostly free forms with the exception of teff (Shumoy and Raes 2016). Being less abundant, monomeric flavonoids have been less extensively studied in comparison to polymeric flavonoids such as condensed tannins. Of all the millet varieties, finger millet is the only known variety to be rich in condensed tannins (Dykes and Rooney 2006).

Millet Micronutrients

Micronutrients viz., minerals and vitamins, play a crucial role in maintaining human health. Micronutrients deficiency and their imbalance have been correlated with various age-related diseases in humans like osteoporosis, obesity, diabetes, etc. Across the globe, billions of people suffer from one or other age-related chronic micronutrient deficiencies (MNDs) and many million are malnourished (Beal et al. 2017; Kumssa et al. 2015). Millets possess the highest mineral content, i.e., 1.7–4.3 g/100 g in comparison to cereals like rice and wheat. Minerals play crucial roles in the body such as in the formation of strong bones and teeth, maintaining structure and rigidity, blood clotting, muscular contractions, oxygen transport, the transmission of nerve signals, regulation of heartbeat, and maintaining fluid balance (Kulkarni et al. 2018). Developing countries often face the problem of calcium and iron deficiency. This has led to issues like osteoporosis and anaemia in India. Finger millet has a very high calcium content (340 mg/100 g) almost eight times higher than major cereals wheat and rice and is almost and hence can be used to overcome age-related calcium deficiency diseases such as osteoporosis (Puranik et al. 2017; Sharma et al. 2017). Similarly, a rich iron content as reported in pearl millet and barnyard millet can help in protection from anaemia. The highest contents of thiamin in foxtail millert is a good source of zinc (4.1 mg/100 g) and iron (2.7 mg/100 g) and can help combat zinc-associated diseases and anaemia. Zinc and iron both boost the immune system as well (Kulkarni et al. 2018; Kumar et al. 2018). Millets are also abundant in β-carotene and Vitamin B complex like riboflavin, niacin, and folic acid. Barnyard millet has the highest content of riboflavin (4.20 mg/100 g) whereas foxtail millet has the richest source of thiamine (0.60 mg/100 g) (Table 15.1). Accordingly, the consumption of millets in the diet ensures wholesome nourishment to overcome age-related deficiencies and diseases (Chandel et al. 2017).

Aside from being laden with healthy phytochemicals and micronutrients, millets are often reported to contain anti-nutrients like phytates, certain polyphenols, and tannins. These anti-nutrients reduce the mineral bioavailability by chelating multivalent cations such as Ca, Zn, and Fe. The rich content of protease and amylase inhibitors further adds to this drawback and causes reduced digestibility of millet grains. Methods are being devised to increase digestibility and enhance nutritional content and bioavailability of nutrients along with the reduction in anti-nutrients and increase in the accumulation of nutrients in milled grains (Vinoth and Ravindhran 2017). Modern-day gene-editing tools and genetic engineering can also be utilized for the improvement of millet varieties for better nutrient accumulation in grains and reduction in the synthesis of anti-nutrients.

15.3 Health-Promoting Properties of Millets

Millets can be considered a better option over cereal grains due to the presence of nutraceutically important phytochemicals and micronutrients which impart them enormous health benefits. In comparison to other cereals like wheat and rice, millets consist of a large amount of fibre and a low amount of simple sugars which are required to maintain a low glycemic index. It

has been revealed that millets have anti-diabetic activity as they aid in the maintenance of low blood sugar levels over other cereals like wheat and rice (Kumari and Sumathi 2002). Millets also show the presence of magnesium in large amounts which is an important co-factor for enzymes that mainly participate in the uptake of glucose and insulin secretion. Magnesium is well known to suppress the influence of migraines and reduce the risk of heart attacks. Hypocholesterolemic activity of millets has been found due to the presence of niacin in them. The decrease in C-reactive protein and unhealthy lipids has been observed by regular consumption of millets which protects against cardiovascular diseases. The rich phosphorous content in millets ensures proper cell structure. Apart from functioning in the bone mineral matrix, phosphorous is also a key commodity for adenosine triphosphate (ATP) which is the energy currency of the cell. Besides the aforementioned health-beneficial properties, millets also exhibit antioxidant and anti-ageing activity. This is in agreement with antioxidant and anti-ageing activities of different millets as shown by Chandrashekhra and Shahidi (2010). Regulation of oxidative stress is a crucial step in controlling health issues like diabetes, cancer, neurodegenerative disorders, cardiovascular problems, and age-associated diseases. Administration of antioxidants in the diet can play a major role in overcoming oxidative stress. Millets offer a valuable source of rich natural antioxidants and thus hold great promise to overcome age-associated diseases.

15.4 Anti-ageing Effects Mediated by Millet Bioactive Compounds/Phytochemicals

Plant-based phenolics and flavonoids are potent antioxidants with numerous health benefits. Plant antioxidants play a crucial role in the attenuation of damage induced by lipid peroxidation, a process that plays a key role in cancer and ageing. Oxidation-induced damage of lipids and fatty acids is prevented by the stable radical intermediates provided by antioxidants (Lobo et al. 2010). The polyphenols present in the millet seed coat act as reducing agents by quenching free radicals, chelating metal ions, and quenching singlet oxygen (Banerjee et al. 2012). Polyphenols achieve their antioxidant nature because of their ability to donate hydrogen atoms from hydroxyl groups on phenol rings to electron-deficient free radicals. Oxidants and antioxidants can be balanced by polyphenols, thus, imparting them their strong antioxidant nature. Henceforth, these compounds show different health benefits and can primarily act as anti-ageing compounds as well. Numerous health benefits that arise from the antioxidant nature of polyphenols have made them "lifespan essentials" (Chandrasekara and Shahidi 2010). Throughout Asian and African countries, millets are consumed as conventional food with recipes based on whole edible millet grain. Numerous reports and work done suggest that phytochemicals and polyphenols from millets can be safely consumed without any adverse health effects. Polyphenol content and composition vastly depend on the millet variety and these have major health attributes and nutraceutical properties (Chandrasekara and Shahidi 2011). Several other reports have proven the hypoglycemic, anti-ulcerative, and hypocholesterolemic properties of millet polyphenols (Kumari and Sumathi 2002; Hegde et al. 2002).

Varying amounts and types of polyphenols in different varieties of millets act as potent antioxidants which are important for exhibiting anti-ageing properties. Millet grains have been reported to contain dimers and trimers of ferulates which are strong antioxidants (Chandrasekara and Shahidi 2011). Anti-glycation properties, i.e., effectiveness against diabetes mellitus which is the main age-related disorder have been reported for phenolics from Italian millet, barnyard millet, finger Italian millet, and South Korean millet. Finger Italian millet showed the highest content of phenolics and flavonoids when compared with Italian millet and Barnyard millet and hence can play a role of nutraceutical rich in antioxidants (Ofosu et al. 2020). Coloured varieties of finger millet found in the Northern Malawi region have been reported to show significantly higher antioxidant

activity when compared to the white variety due to the abundance of phenolic compounds, tannins, and flavonoids in them (Xiang et al. 2019b). In another study, the defatted foxtail millet (DFMB) was able to scavenge the free radicals like superoxide anions (Amadou et al. 2011).

Two flavones namely, luteolin and tricin, were reported in Japanese barnyard millet (Watanabe 1999). Luteolin and its glycosides possess antioxidant, anti-inflammatory, anticancer, and anti-arrhythmic activities properties (Lin et al. 2008). Similarly, tricin has been reported for its anti-tumour properties and antimetastatic properties (Yue et al. 2020). Finger millet showed the highest total flavonoid content in defatted meals followed by kodo and foxtail millets. Ferulic acid exhibits very strong antioxidant, free radical scavenging, and anti-inflammatory activity found in the edible flours of small millets (Shahidi et al. 1992; Castelluccio et al. 1995).

Several chronic diseases such as diabetes, cardiovascular disease, cancer, and cataract often related with ageing arise as a result of free radical production in excess and lipid peroxidation. Rowan et al. (2018) have reported that non-enzymatic glycosylation, whereby a chemical reaction occurs between the aldehyde group of reducing sugar and the amino group of proteins, plays a major role in the complications of diabetes and ageing. Free radicals often induce non-enzymatic glycosylation and cross-linking of collagen whereas free radical scavengers such as polyphenols inhibit these reactions (Fu et al. 1992). Thus, any compound which can neutralize these free radicals can in turn exhibit the anti-ageing effect. In a recent study, anti-diabetic properties of different millets varieties such as finger millet, proso millet, white finger millet, kodo millet, and foxtail millet cultivated in Srilanka were evaluated (Senevirathne et al. 2021). The inhibition of both early and middle glycation and reversal of anti-glycated products by these millets further with anti-α-amylase activity played a crucial role in maintaining blood glucose levels in diabetic patients.

The effects of the antioxidant properties of millets on oxidative stress and glycemic status in alloxan-induced rats were also studied (Hegde and Chandra 2005). The finger millet-fed rats showed a significant decrease in blood glucose and cholesterol level and a significant reduction in tail tendon collagen glycation. Alloxan-induced diabetic groups when fed on a finger millet diet showed increased levels of enzymatic and non-enzymatic antioxidants with reduced lipid peroxides. This was correlated with the abundant amounts of polyphenols present in finger millets. In another report, the effects of methanolic extracts of finger millet and kodo millet on glycation and cross-linking of collagen were studied (Hegde et al. 2002). It can, thus, be deduced that finger millet possesses potent therapeutic efficacy for use as dietary supplements for the prevention of glycation-induced complications, as in diabetes or ageing.

Normal aerobic respiration and substrate oxidation often produce free radicals as a byproduct on a regular basis in our body. The excessive accumulation of these radicals is, however, associated with damage to vital biomolecules such as carbohydrates, lipids, proteins, and DNA, ultimately leading to various age-related diseases (Darley-Usmar and Halliwell 1996). Polyphenols show rich antioxidant properties either by scavenging of free radicals, chelation of metal ions, quenching of singlet oxygen, or by inhibiting the reactive oxygen species (ROS) production.

The antioxidant properties have been also reported from phenolic extracts of several varieties of millet such as (foxtail, kodo, finger (Ravi), finger, proso, pearl, and little) (Chandrasekara and Shahidi 2011). Additional properties of these millets were also found, like the quenching of singlet oxygen inhibition of lipid peroxidation and suppression of DNA scission.

Several studies have also focused on the pharmacological importance of finger millet. The exogenous supply of finger millet to diabetic rats helps in fast wound healing, antioxidant character of skin, and generation of nerve growth factor (Rajasekaran et al. 2004). In another study, antioxidant property of whole finger millet by significantly scavenging the DPPH, H_2O_2, and NO has been revealed (Ajiboye et al. 2017). The catechin, Ferulic acid, vanillic acid, and

resveratrol (phenolic acids) from millet varieties showed preventive influence on human erythrocyte peroxidation and protein (Palaniswamy and Govindaswamy 2017).

During ageing, ROS-mediated oxidative stress regulates numerous diseased conditions. Therefore, ageing can also be demarcated as low energy and high oxidative stress state. The proper use of millet affects the ROS level and helps curb age-related pathological conditions. As per literature, a few studies have been explored about the molecular mechanism for anti-ageing effect of millets. Therefore, the probable mechanism of anti-ageing by millets can be well explained via various theories of ageing and also available preliminary supported data in the context of anti-ageing effects of millets. We can hope for the significant candidate/bioactive peptides/natural products from diverse species of plants that can exhibit anti-ageing potential. As per available data, the faith is created in consuming millets for providing the antioxidant properties and anti-ageing properties to combat age-related diseases by influencing the signalling pathways like stress responsive pathway, genetic repair, and protein glycation. Next, we have described many ageing-related theories where millets have shown both antioxidant property as well as ageing potential by affecting these pathways.

15.5 Molecular Prospects of Ageing

15.5.1 Free Radical Theory of Ageing

This theory is based on the property of ageing and anti-ageing which specified that upsurge in the accumulation of endogenous oxygen radicals might cause impairment of macromolecular constituents of the cell rendering cells as well as also inhibit the various organs functions that finally promote the demise via senescence (Shields et al. 2021). In 1972, the mitochondrial theory of ageing proposed that during mitochondrial respiration generation of several reactive oxygen species results in the destruction of various macromolecules such as mitochondrial DNA which is due to the mutations that occur in mitochondrial DNA (Kregel and Zhang 2007). These studies have been demonstrated in various experiments by the usage of animal models and tissue. A shred of evidence recognized that millet grains contain numerous natural antioxidants which are useful in increasing the life expectancy of an organism by fighting with an increase in the number of free radicals (Majid et al. 2020).

15.5.2 Telomere Shortening Theory

Premature ageing is associated with high oxidative stress that results in the accumulation of oxidative DNA damage in telomeres. According to telomere shortening theory, it has been concluded that loss of telomeres leads to ageing (Razgonova et al. 2020). Malfunctioning of telomeres leads to the initiation of cellular senescence which is due to the exodus of the cell cycle after several cell cycles (Libertini et al. 2021). It has been revealed that the length of telomeres becomes shorter after each consequent cellular proliferation that disrupts the ends of the chromosome which is known as double-stranded breaks. Additionally, it has also been analysed that DNA damage is also involved in the revoke of ageing. Moreover, in aged organisms, a variety of mutations also augment the chance of tumorigenesis and age-related disorders. Consistent with various studies it has been reported that lots of reasons increase the damage of DNA that is liable for ageing and allied diseases (Fig. 15.3). Henceforth, it has been suggested that bioactive compounds found in the millets work as effective antioxidants that may decrease the oxidative stress level, thus, reducing the telomere shortening rate.

15.5.3 Post-translational Modifications (PTMS)

Abnormal functions of proteins can be observed in ageing due to the formation of disorganized protein and unusual sort of post-translational

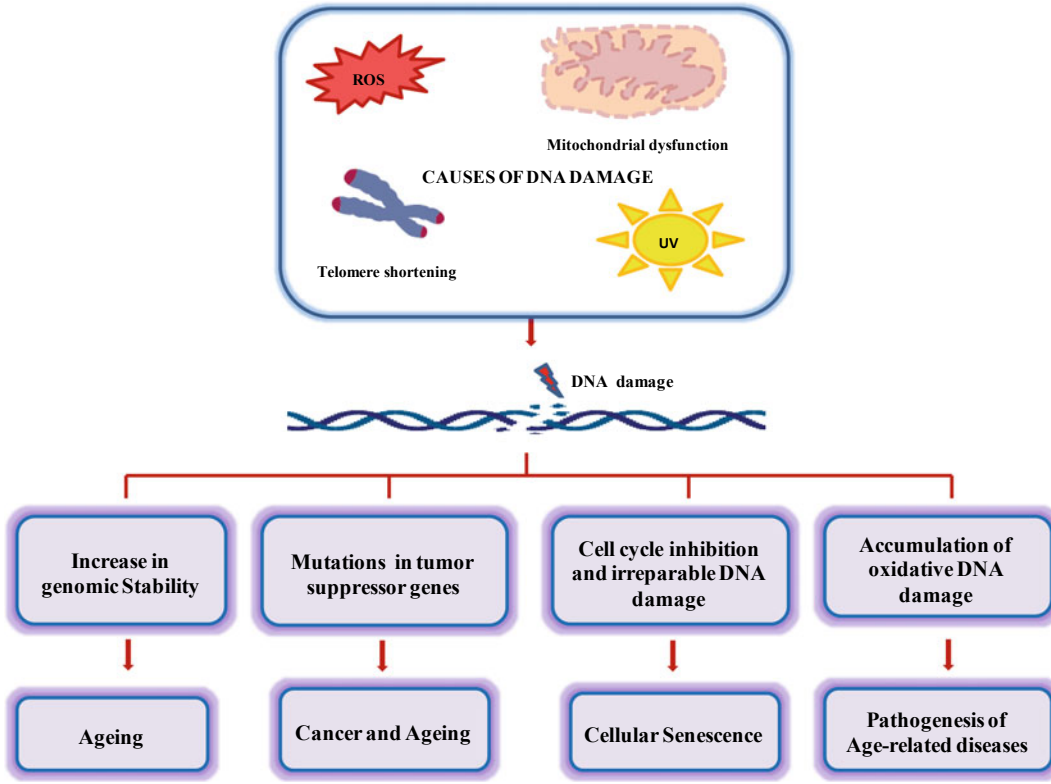


Fig. 15.3 Various types of DNA damage lead to premature ageing and age-related diseases

modifications (PTMs) including phosphorylation, glycosylation, acetylation, sumoylation, ubiquitination, and neddylation that finally result in the accretion of inactive, denatured, misfolded, and mutated proteins in cells of aged organisms. The post-translational modifications of proteins are primarily accountable for the stability, activity, and also interactions between protein–protein that affect both metabolism and cellular function (Santos and Lindner 2017). It has been observed that different compounds influence the PTMs of different proteins and help in maintaining cellular function during the ageing process (Lan et al. 2016; Peleg et al. 2016). For many years, a large number of potent drugs and natural compounds including millets and millet-based products have been studied for their anti-ageing potential. It was reported that millet extracts inhibited the glycation of collagen protein and rescue its protein function (Hegde et al. 2002).

15.6 A Novel Prospective for Designing Anti-ageing Drugs by Gathering Information from Millets and Natural Products from Other Plants

It has been found that fruits, vegetables, and grains are rich sources of numerous biologically active compounds that possess anti-ageing properties. These compounds are liable for controlling many genes, and several mechanism pathways are involved in the process of ageing that may eventually interrupt the ageing process. Yet, the complete mechanism of action of the biologically active compound of millets has not been observed but primary data show that the extract of millet influences the lifespan of an organism as well as the disease that is accompanied with ageing. Therefore, it is important to

have elaborated examinations of millet bioactive compounds and mechanism of action as anti-ageing compounds. Many studies suggested that premature ageing and ageing disorder are supposed to be linked with errors in the crucial cellular pathways like the insulin/insulin-like growth factor signalling (IIS) the mammalian target of rapamycin mTOR and sirtuins molecular networks. Though, various essential pathways involved in the process of ageing required many studies in context with the impact of biologically active compounds of millet extracts that can be appropriate for in-vitro model systems. Millets are the natural richest source of antioxidants that possess nutraceutical properties and numerous active food constituents that provide improvement in health and decrease disease risk. Still, further validation is needed to conduct concerning human subjects and animal models so that it might be useful in the welfare of their activity and health (Fig. 15.4).

The recently developed drugs have the capacity to bind with the target enzyme of cellular progression linked with ageing. MLN4924, Nutulin-3, and HDAC are the few natural potent drugs that have been identified that their effect helps reduce ageing and its associated disorder; however, no drug is sustained in the market that can be endorsed for use to halt ageing and its symptoms. The ability of drugs can be identified by using absorption, distribution, metabolism, and toxicity (ADMT) predicting tools to escape the risk of failure of the drug in clinical trials. It has been revealed that various natural and synthetic compounds, for example, Caffeine, Resveratrol, Rapamycin, Quercetin, Acetyl L-Carnitine (ALC), Indole propionamide (IPAM), and Epicatechin have played an impending part

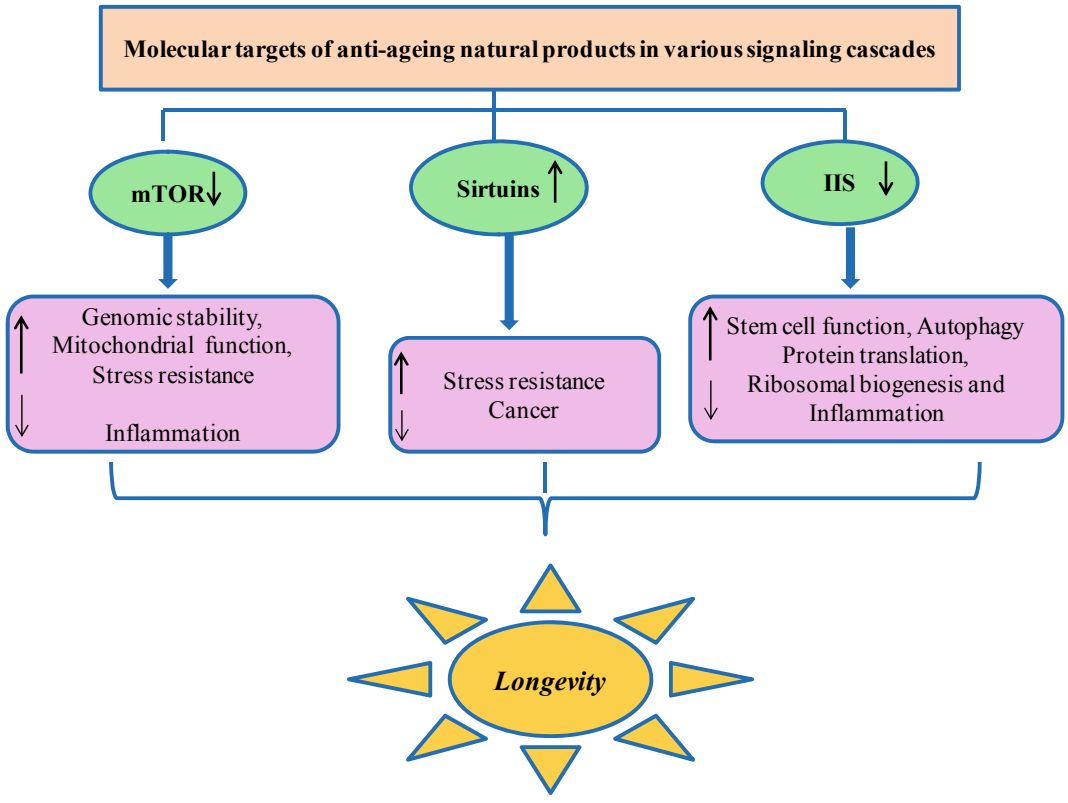


Fig. 15.4 Delay of Ageing process and inhibition of age-related diseases via anti-ageing natural products from millets by regulation of various cell responsive pathways through influencing their targeted molecules

in the regulation of ageing development via triggering targets by several drugs. These bioactive compounds have numerous types of mechanisms of action that vary from target to target by which they initiate the anti-ageing reaction. Lots of ageing research have been conducted on a variety of animal models such as a rat, mice, *Drosophila melanogaster, Caenorhabditis elegans,* and worms. So, this is important to studies on humans to know the effect of the compound as anti-ageing by detecting the mechanism of action and their interaction with the target. Still, further studies are required to validate the target and mechanism of action relevant to millets on anti-ageing property. In addition, to design and develop more specific high potential drugs against any target, a bioactive compound having anti-ageing properties possibly be used as a lead molecule. In this regard, computational tools can be useful in deciphering the chemical modification which is a requisite to optimize the activity of the lead molecule. Hence, via lead optimization potent candidate drugs can be endorsed for clinical as well as in-vitro trials.

15.7 Conclusion

Implementation of a balanced diet with nutritional supplements in daily routine aids in the facilitation of enhanced longevity and good health. So far numerous phytochemicals have been revealed for delaying ageing and age-related diseases. Among all cereals, millets are a crucial source of proteins, carbohydrates, minerals, and low fat content which authenticates their use in the regular diet for a healthy lifespan. The anti-ageing factor of millets is well recognized due to the presence of total phenolics, flavonoids, and other natural compounds in large amounts in them. Therefore, nutraceutical and functional foods from millets might be developed for daily consumption which will aid in the slow down of the ageing process and combat age-related diseases. The exploitation of millets for the identification, isolation, and characterization of bioactive molecules is critical for their future use in delaying the ageing process. Further, future work involves the authentication of their molecular mechanism by the wet lab and in-silico analysis. More studies on the influence of characterized bioactive compounds can be determined in in-vitro systems (cell- or tissue-specific manner) followed by animal models to rule out any possibility of safety concerns. Finally, the therapeutic potential of bioactive compounds will be evaluated for their future use in personalized medicine.

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Holistic Value Chain Approach in Finger Millet

16

B. Dayakar Rao, E. Kiranmai, D. Srenuja, and Vilas A Tonapi

Abstract

Finger millet is widely grown as a cereal in the arid areas of Africa and Asia. It is commonly known as *ragi* in India. It has good health benefits and contains rich in nutrients like calcium, dietary fibre, protein and slow-releasing carbohydrates compared to rice and wheat, which are essential and aimed at good health. However, their consumption as direct food has come down past few decades due to changing farming systems, low productivity, lack of processing technologies, lack of awareness of its health benefits, time-consuming, laborious process and lack of policies. There is a need to create a mode for bringing back these ancient grains to our plate. In this view Indian Institute of Millets Research has established a value chain approach with innovative interventions to create demand for finger millet. The interventions were started on-farm to create awareness among farmers and were supported by FPOs. Processing intervention has been developed on finger millet to develop diversified value-added ready-to-eat and ready-to-cook products by retrofitting the existing machinery, intervention on marketing for branding and labelling finger millet foods as health products. Entrepreneurship development and capacity-building programmes such as training rural/urban entrepreneurs and women groups on processing technologies, product preparation, marketing, popularization etc., and identification of entrepreneurs are linked up with other stakeholders. Policymakers are to be sensitized to the health and nutritional benefits of processed millet foods on target populations. The farmers, consumers and stakeholders will be benefited from this holistic value chain model.

16.1 Introduction

The value chain includes a full range of activities required to bring the product or services through intermediate phases from production to consumption. The value chain activities cover raw material supplies, processing, packaging, marketing, distribution and support to the end-user in the market where the markets could be local, regional, or global (Kapinsky and Morris 2001). The value chain approach will help understand the dynamics of value creation at different value chain stages. Value chain analysis is a controlling tool for understanding the key determinants of competitiveness (Kapinsky 2000). Establishing a holistic value chain model for underutilized

B. D. Rao (✉) · E. Kiranmai · D. Srenuja · V. A. Tonapi
ICAR-Indian Institute of Millets Research, Hyderabad, India
e-mail: dayakar@millets.res.in

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nutri-cereal crops like finger millets is very much needed in the pandemic state.

Finger millet (*Eleusine coracana* L.) is a chief millet crop grown in India and has higher productivity among all the millets. Finger millet (FM) is produced in different parts of the country, and Karnataka state tops in production followed by Tamil Nadu, Uttar Pradesh, Maharashtra, Andhra Pradesh and Orissa. It is grown in semi-arid and sub-tropics of the world under rain-fed areas. It is more densely packed with dietary fibre, fat, minerals and folic acid than common crops like rice, wheat and corn. FM is consumed as the food which delivers a nourishing diet, especially for people doing hard graft. Straw makes valuable fodder for milking animals. Grain can also be malted and pounded into flour. This can be used as a basic ingredient in cakes or porridge and wholesome infant foodstuff. FM is considered an especially wholesome food and the best suitable diet for diabetic people.

FM value chains (at rural and urban levels) encompass various stages, beginning with input supply and continuing through product handling, processing, packaging, distribution and marketing. As products move successively through various stages, transactions happen between various factors in terms of material, money, technical information, etc. and the value is progressively added.

In the olden days, food production is being replaced by practices more akin to manufacturing processes, with greater coordination across farmers, food processors, retailers and other stakeholders in the value chain. Moreover, value addition and efficient marketing determine the success of most production-oriented development programmes. Therefore, particularly in the farming sector where farmers are dependent on many external agencies and unable to plan the marketing of their product, the profit margins are under severe pressure, often resulting in failures. Small farmers' problems are the lower scale of operation, outdated technologies, lack of financial support, poor technical information, guidance and communication e-linkages with the market, and exploitation by the middlemen. Small farmers are heavily exploited by a series of intermediate traders procuring agricultural inputs and marketing their fresh produce.

The agricultural inputs required by small farmers being small in quantity, they tend to procure from local traders, which is about 20–30% higher than the price paid by large farmers, who procure larger quantities. They further suffer due to the inferior quality of the inputs and delay in procurement. The high cost of borrowing may further increase the cost of these inputs. The lack of appropriate technology is another major consequence for small farmers. Firstly, it is a time-consuming process for small farmers to adopt innovative technologies and great notions to multiply crop production with a high yield. Secondly, small farmers being resource-poor and semi-literate are very hesitant to implement novel knowledge. There are various examples of new varieties which promise a very high harvest but fail to meet the anticipation either due to uncertain weather conditions or newly evolving pests and infections. Often, small farmers cannot procure critical inputs well in time, resulting in a significant drop in the yield. The objective of any food value chain is to deliver maximum value to the end-user at the least possible total cost and create a competitive advantage. Hence, a value chain model is necessary for the agricultural system of nutri-dense foods like FM too. This book chapter will provide the whole idea about the value chain of FM establishment (Fig. 16.1).

Fig. 16.1 FM panicles

16.2 Why Value Chain Model Required in FM?

As discussed above, generally FM grains are used as food and feed in several countries. Currently, FM grains are used in food industries to manufacture multiple FM-based value-added products. But over the years, the area under FM has decreased in almost all the millet-growing countries. Similarly, in India, over the past three decades, the area under millets has fallen due to the higher demand and profitability of competing for crops (Seetharama et al. 2007). Although FM is nutritionally rich, its consumption is declining significantly (Dayakar et al. 2010). To augment the production and consumption of FM-based products, the creation of a value chain model is highly needed.

Mackay et al. (1997) defined value chain analysis as studying the full range of activities required to bring the product or services from production to final consumers. But according to Sturgeon (2001), value chains have three dimensions: organizational, spatial and the type of actors involved. From the organizational aspect, value chains are complex and dynamic or simple, depending on their sustained supply of various critical inputs, i.e., human resource requirements, capital equipment and services. The second dimension is spatial, which involves some global value chains and is sometimes referred to as "global commodity chains" (Daviron and Gibbon 2002) because they operate internationally. The third dimension is the production factors in the value chain; these involve the firms that participate in the chain. The factors can either be producers (in the agricultural production value chain), suppliers, retailers/wholesalers or lead firms.

The value chain model essentially involves bringing all the stakeholder's involvement together on a mutual platform, with significant value creation at the end of each stage. The farmers are our primary stakeholders and should be guaranteed higher economic returns and benefits in the long run. There is a solution for demand creation for millet cultivation through value addition and commercialization of FM-based processed products. Hence, it has fair amounts of nutritive values and enthralling health-promoting benefits that could involve the private sector, public research institutes, policymakers, entrepreneurs and consumers to revive its demand. It became imperative to reorient the research and development efforts on these crops to revive demand through value addition with diversified processing technologies, nutritional evaluation, awareness and backward integration eliminating the intermediaries. Production to consumption system (PCS) value chain model needs to be developed for specific products and markets to augment such efforts.

16.3 Development of Holistic Value Chain Model for FM

To revive the demand for FM in India, the Indian Council of Agricultural Research (ICAR)–Indian Institute of Millets Research (IIMR), Hyderabad, led a consortium and has undertaken several interventions to bridge the identified gaps in different aspects of on-farm production, processing diversification, nutritional certification, promotion and marketing of FM in the Indian market. This challenge has empowered a seamless value chain with all the stakeholders linked together, especially the poor dryland farmers linked with the markets. In this regard, the ICAR–IIMR as the lead institute has built multiple linkages with prestigious bodies in both government and private sectors. These are National Institute of Nutrition (NIN) and State Agricultural Universities (SAUs). Similarly, Defence Food Research Laboratory (DFRL), Central Food Technological Research Institute (CFTRI), Central Institute of Agricultural Engineering (CIAE), Central Institute of Post-Harvest Engineering and Technology (CIPHET), Indian Institutes of Technology, Kharagpur (IIT), National Institute of Food Technology Entrepreneurship and Management (NIFTEM),

and Indian Institute of Food Processing Technology (IIFPT), Imperial Tobacco Company of India Limited (ITC), Britannia Industries Pvt Ltd., etc.

16.4 Individual Components of the FM Value Chain Explained Below

16.4.1 On-Farm FM Production

The Sarada Valley Development Samithi (SVDS), a non-governmental organization (NGO), has taken initiation by mobilizing farmers and bringing change in their mindset towards diversified crops like millets instead of monocrops. Mobilization of farmers includes various communication aids like pamphlets, documentary movies, posters, regular village-level meetings, and proper vision development of promoter farmer-members. ICAR–IIMR, Hyderabad supplied good quality FM seeds to the Visakha Millets farmer-producer organization members in the last season. They witnessed the bumper yields due to the awareness of good management practices in FM cultivation. ICAR–IIMR has also provided technical guidance to establish a primary processing unit for millets at the farm gate, with the help of which the farmers could get a better price for their produce. The farmers were allowed to attend field days and exposure visits to ICAR–IIMR, to learn more about the production, processing and value-added FM technologies. The members of the farmer-producer organizations have also come up with the branding of their products.

ICAR–IIMR associated with the eco-club to create attentiveness in farmers about the significance of forming a farmer-producer company through pamphlets, leaflets, etc. ICAR–IIMR nurtured a farmer-producer organisation to conduct various demonstration and capacity-building programmes for the farmers to understand the package of practices in FM. The farmers-producing organizations have taken technical guidance from ICAR–IIMR to establish a primary processing unit for millets so that the farmers could receive a good amount for their produce. The Mahbubnagar farmer-producer organization has taken steps to brand its products made from millets. This is one of the appreciable results under ICAR–IIMR effort towards the creation of value chain.

16.4.2 Processing Interventions in FM

Processing involves the conversion of raw FM grains into any palatable forms such as ready-to-cook (RTC) or ready-to-eat (RTE) products. Processing is an essential component to create demand for millets by offering a wide range of healthy, tasty and convenient options to consumers. Traditionally, FM has been consumed in flour-based foods such as *roti*, *mudde* and *ambali*. Normally, for making FM *roti*, its flour is mixed with hot water to partially gelatinize the starch (to improve its binding nature), kneaded into dough, flattened and baked on a hot pan by indirect contact of heat. The product develops a characteristic aroma and swells during baking, forming two distinct layers similar to wheat *chapati*. Mostly, it is consumed soon after its baking; otherwise, it turns leathery and chewy after a few hours after preparation.

On the other hand, for the preparation of *mudde*, in small quantities, flour is mixed with water and then boiled. Next, a predetermined amount of flour is added and left in the form of a heap; heating could be continued so that the flour experiences partial steaming for a few minutes, and then it is mixed well with boiling slurry to a smooth consistency. Finally, it is shaped into balls. The thin *porridge* or *ambali* of the millet is normally a mild fermented product. For its preparation, the FM flour is mixed with water and a small quantity of buttermilk and the mix is left overnight for cooking the next day. It improves the bioavailability of the minerals and imparts a little sour taste.

There is a lot of inconvenience in preparing these types of traditional foods and requires skill and considerable time. Today, the proportion of working men and women is increasing while the

disposable family income is increasing. Consumers are looking for a wide range of alternatives for their health, taste, quickness and convenience. There is an urge to develop RTE and RTC FM products for the present generation population to avoid drudgeries.

The research efforts are stimulated towards the development of RTE and RTC products through different processing technologies for better utilization of FM. Processing technologies for FM include milling, flaking, puffing, popping, baking, extrusion etc. Some processing technologies will also enhance the nutrients and their bioavailability.

16.4.2.1 Milling

It is the most important step to separate germ, bran and endosperm. The endosperm will be processed into different particle sizes (likewise fine, medium and coarse), which is the basic ingredient for other product processing technologies. It is done by pulverizing technique in a stone mill, iron disc or emery-coated disc mills, or hammer mills (Fig. 16.2).

16.4.2.2 Decortication

This technology is done recently for FM to increase its value. It is also known as debranning/husking. This method has been used to remove bran/husk of cereal grains, but the decortication process was not required for FM due to its seed coat attached intact to soft endosperm. The parboiling process is used to decorticate an FM that hardens the grain endosperm and resists the impact that occurs mechanically. The decorticated FM can be cooked and consumed like normal rice.

Fig. 16.2 FM flour

16.4.2.3 Popping

Popping is one of the former methods to make pops from FM. In this process, 3–5% of water is added to the FM and tempered for 2–4 h to attain the desired moisture content, and then pop it by agitating in the sand at 230 °C. This process develops a desirable aroma because of the Maillard reaction between sugars and amino acids present in it. It also gives milky white with maroon strikes appearance, which attracts the consumers. Popped FM is a pre-cooked ready-to-eat product and can be pulverized and mixed with protein-rich sources to prepare nutritious supplementary food for the children. But popping contaminates the product with sand particles used as heat transfer media and affects its eating quality. Air popping is a suitable mechanical device that has been successfully explored to overcome this drawback, but this method lacks aroma characteristics compared to that using sand. Popped FM can be prepared at the household, community or industrial level.

16.4.2.4 Puffing

Gun-puffed grains are formed by subjecting the grain to a sudden pressure, under which the interior portion of the grain seeks to equilibrate with the surrounding lower-pressure atmosphere and forces the grains to expand quickly or "puff". The production of gun-puffed grain involves pretreatment, puffing, screening, drying/roasting and cooling. Puffing can be performed with manual single-shot guns, automatic single-shot, automatic multiple-shot guns or continuous guns. In manual single-shot guns, the pre-treated grains are loaded into the gun's opening and the lid is closed. When the gun begins to rotate, gas burners heat the sides of the gun chamber causing the grain's moisture to convert into steam. When the lid opens, the immediate change in pressure causes the grains to swell. Single-shot automatic pistols work on the same principle,

Fig. 16.3 FM puffs

except that steam is injected directly into the chamber of the gun. Multi-shot pistols have multiple barrels mounted on a wheel that rotates slowly so that each barrel passes the load and firing positions at the correct time. The loading, vaporizing and firing process for any barrel is identical to that of the single-shot pistol. After puffing the grain, it is sieved and dried before packaging. The end product is very porous and absorbs moisture quickly and easily, so it must be packaged in materials with good moisture barrier qualities (Fig. 16.3).

16.4.2.5 Extrusion

It is a modern method of food processing applied to solve the problems associated with the processing of small cereal-based products in terms of physical state, quality, functionality and shelf-life extension. Extrusion is a process of gelatinization and cooking of the product completely until it is fully cooked, which leads to the production of different forms of food. The extrusion process has many advantages in preparing RTE foods desired in different shapes, sizes, textures and sensory characteristics. Extrusion has also found application in solving malnutrition in developing countries due to its beneficial process. The cooking process employed a high temperature for a short time for processing starchy materials. Advantages of extrusion cooking include low cost, increased productivity, speed and high product quality. The extrusion also assists in product development without waste, increased in vitro protein digestibility, versatility, unique product shapes and energy savings. During the extrusion process, the solubility and structure of the proteins decreased and were interrupted when they were applied at high pressure and temperature (Manjula and Visvanathan 2014).

The extrusion process is widely applied in food industries to prepare breakfast cereals and snacks. The flours are prepared from various plant sources in different forms to produce extruded products like extruded snacks, noodles, macaroni, spaghetti, baby foods and pasta that children and teenagers prefer. Snacks are ready-to-eat products that are very popular and whose demand is increasing among all age groups (Limsangouan et al. 2010; Siddhart 2014). The fortification of extruded products with minerals and vitamins is also used to balance the nutritional composition lost during processing and prevent micronutrient deficiencies. Extruded products can be coated to provide sweet or savoury flavours to attract.

Cold extruded items such as vermicelli, pasta and noodles are mainly produced from durum wheat or refined wheat flour. Those items may also be developed using millets with the same equipment called "cold extruder". The basic material in millet-based cold extruded goods has fine-sized millet semolina (355 μ) mainly to maintain the binding nature resulting in less cooking losses. Since millet grains lack the binding properties and structure firmness of wheat semolina/suji (500 μ), every millet-based cold extruded product is now mixed with wheat semolina/suji (500 μ). To form a wet homogeneous mass, millet extruded products are blended with various ingredients and required moisture levels. Then it is pushed through a pierce plate or dies with a certain form (it changes for pasta, vermicelli and noodles), and its blades trim it to a specified size. As a shelf-life extension and quality of this product, this wet product should now be dried in mechanical dryers. The ideal temperature for the die is 37–40 °C. Cooling water should be provided once this

Fig. 16.4 FM vermicelli

temperature has been reached in order to retain it. Steaming for 20–30 min, particularly for millets-based cold extruded products, improves appearance, taste, colour, texture, appearance and total solids loss. It will also keep the products' structure after cooking without disintegrating, and solids losses are minimal. The starches in the millets should partially gelatinize here, which can be accomplished by heating the application. Transfer the product to steaming shelves right away and dry for 4–5 h at 60 °C. Millet cold extrudates products may be kept for over six months (Fig. 16.4).

16.4.2.6 Baking

The baking process is the most important stage in the production of bread and cookies. The last stage of the bread-making process is the baking in which the dough acquires a light, porous, delicious structure and is also easily digestible under the influence of heat. Modern ovens have been developed on the principle that bread dough is exposed to different temperatures and amounts of steam for some time on trays, pans or conveyor belts. For cookies, the baking process is after the dough-forming step. After the dough is formed into the cookie shape, the baking process begins. The dough is transferred to the conveyor belt and taken to the oven after taking a certain distance and baked. Almost all types of cookies are baked in conveyor ovens. Heat and mass transfer takes place simultaneously in the dough during baking and involves major changes. Bakery products are popular and appealing due to their nutritional, sensory and textural characteristics. Presently, the ICAR–IIMR has manufactured true millet-based cookies and cakes with better texture by using various emulsifiers within limits, which are close to conventional wheat-based cookies. These cookies come under the gluten-free cookies category. Cookie/biscuit-cutting machine, rotary rack oven, cake-filling machine, planetary mixer, commercial biscuit-making line and other machines are used in the millets baking process technology. FM cookies are made by the creaming process and combining leavening agents, flavouring agents, FM flour, and appropriate moisture levels, and then shaping the dough into a soft dough, cutting and baking at 180 °C for 15 min in a pre-heated oven. The smooth and tasty FM chocolate cake is made entirely with FM, eggs, sugar, and oil and with other raising materials at 180 °C for 15 min. This is very healthy and appealing to children groups. Research and development (R&D) on pure FM bread and pizza are still in progress to

Fig. 16.5 FM cookies

Fig. 16.6 FM pizza base

Fig. 16.7 FM cake

attain the trueness of millets in these products. FM grains are packed with gluten in their structure, so it causes some poor sponginess and inferior quality in its products. But still, some of the products with FM from the bakery were possible like cookies, cakes and muffins (Figs. 16.5, 16.6 and 16.7).

16.4.2.7 Flaking

Flakes are ready-to-eat pure millet-based products with a crispy texture. FM thin flakes are a pack of vital nutrients with improved starch and more protein digestibility and are beneficial to all age groups people. The process of flaking the whole FM grains involves pre-cleaning, grading, soaking, roasting, flaking, drying, sieving and cooling. The roller flaking machine is employed in flaking manufacturing. FM-roasted grains are manually put into the roller flaking machine and flat by pressing both rollers at the same time. The spacing between the rollers has a big influence on the thickness of the flakes, so make sure it is uniform. More clearance of whole grains at the output and less clearance provide crack flakes and deteriorate the quality of flakes. The output of the flake roller machine is sieved (1204 μ) to get superior quality flakes from FM, which are then dried at 60 °C for 3 h. The flakes' final yield is approximately 80–85% for FM. The drying process extends the shelf life of flakes by maintaining the moisture levels to 6–8%. The longevity of the flakes is commonly a challenge. So the addition of some antioxidants and suitable packaging material could help in this issue. Packaging with effective moisture and oxygen barrier qualities can be used to extend its storage period (Alavi et al. 2019). It can be consumed with minimal cooking in the home. These are lightweight and convenient to ship and store. FM flakes can be consumed by adding milk or making *poha* or *chuduwa, jagger*-based *ladoos,* etc. (Fig. 16.8).

16.4.2.8 FM Soup

Recently, the utilization of millets for the preparation of soup was greatly explored. A blend of FM with vegetables delivers a good amount of great nutrients to the human. So, the research has been done by ICAR–IIMR on FM flour with a combination of different vegetables and spices named "FM instant vegetable soup mix". It is made by involving different technologies like milling, sieving, blending and drying technologies. The flour milling industry is a vibrant sector that combines traditional skills with high

Fig. 16.8 FM flakes

technology to produce a wide range of flours. It is the reduction of endosperm to a uniform particle size. This is generally done by the sequence of cleaning, grinding and separation operations. Also drying process is used to remove the moisture to enhance the shelf life with minimal loss of nutrients. Subsequently, it is blended with other adjuncts such as milk powder, sugar and maltodextrin. The mix reconstitutes water to a uniform consistency and boils it for fewer minutes to form soup with a highly desirable aroma and relishing taste. The use of incipient germinated millet enhances its nutrient density. This product is free from food additives, still it has longer shelf life of around 6 months with a good nutritional profile. It is rich in micronutrients like calcium, iron and zinc. This is recommendable for middle age and elderly people (Fig. 16.9).

Fig. 16.9 FM soup

16.4.2.9 Processing Technologies of FM that Will Enhance Nutrients and Their Bio-Availability

Soaking

Soaking is the process of adding distilled water to FM grains until the grains are fully steeped in water and leaving overnight at an ambient temperature of 30–60 °C. After draining the soaked water, the grain will be washed with clean water for removing the foreign material. Then the grain is dried in a hot air oven at 60 °C for 90 min before being ground into *atta* (Banusha and Vasantharuba 2013). Soaking may reduce the availability of anti-nutritional compounds such as phytic acid, while increasing the bioavailability of minerals like zinc (Saleh et al. 2013).

Germination

It is a traditional process in which FM grains are soaked for about 2–24 h and spread on a muslin cloth for up to 24–48 h or incubated at 30° C for 48 h (Shimray et al. 2012). The germination process can be done economically without costly equipment. The germination process has been used for centuries to soften grain structure and improve the nutritional values and concentration of carbohydrates, minerals, vitamins and essential amino acids, thus increasing the functional properties of grains (Mbithi-Mwikya et al. 2000; Chove and Mamiro 2010; Pushparaj and Urooj 2011).

Malting

FM has good malting characteristics such as resistance to fungal infection, elaboration of alpha and beta-amylase during germination and development of highly desirable aroma as well as taste on kilning the malt. It qualifies as an ideal raw material for malt foods. Besides, the millet malt will be a good source of sulphur, amino acids and calcium. The malting process involves soaking, germination, drying, de-rooting and kilning. Although these unit operations influence the quality of malt, the germination process is the single most important step because the hydrolytic enzymes developed during germination cause *in vivo* biotransformation and lead to improved

nutritional rates and textural features. Normally, the synthesis of some of the vitamins, enhancing the minerals' bioavailability and lowering the water-holding capacity of the millet starch occurs due to germination. Soaking the millet for about 8–12 h is needed to increase its moisture content by about 30%. While soaking, it is highly desirable to change the soak water once or twice, discard the leachates to free it from the carbon-di-oxide formed, and prevent excessive growth of microorganisms. The soaked grains are germinated on a moist cloth. During germination, it is essential to mix and overturn the grains to dissipate the heat developed and provide good aeration to the sprouts. Normally, germination up to 48 h is desirable, but the germination period is reduced to 24–36 h during summer. Then, the sprouts are dried either in sun or mechanically devices. In the case of mechanical drying, the air temperature should not exceed 75 °C to avoid the parboiling effect which hardens the grains and affects milling as well as food qualities. Sun drying the sprouts for 5–6 h will dehydrate them to a 12–14% moisture level. Subsequently, the roots and shoots from the dried sprouts are separated either by gentle brushing or using a fruit pulper or rice huller because they are cellulosic tissues that affect the malt's taste characteristics.

The de-rooted malt is kilned or cured by toasting at about 70 °C by exposing it to hot air or in a conventional toasting pan or rotary heaters. The protonotary heater imparts a better aroma with desirable qualities due to uniform exposure to heat. The malt is an RTE product and needs further processing for various food uses. Conventionally, the malt is pulverized and sieved through the nylon or muslin cloth to prepare the malt flour free from the husk. The malt is milled, and the whole meal is suspended in excess water for separating the starchy portion settled in the bottom for further drying. The above methodology has a drawback, as the yield of the malt flour is hardly 35–40%. In addition, the soluble nutrients from the malt, such as amino acids, free sugars, vitamins and minerals, are lost along with the discarded water.

The dry malt milling process involves mixing the malt with 5–7% additional water to wet the grains' surface, pulverizing and sieving the same to separate the seed coat as coarse material. Normally, the yield of good quality husk-free flour prepared following this process is about 65% on a malted grain basis.

Malt flour is a good source of nutrients besides serving as a source of amylases and hence, it is also termed "Amylase rich food" (ARF). It can be mixed with powdered sugar, milk powder and flavouring agents such as cardamom to use as a milk-based beverage, which is popularly sold as "FM *malt*" in southern India. Since malt flour contains hardly 3–5% protein, it can be blended with vegetable or animal protein sources such as grain legumes, milk powder, egg powder, etc., to prepare supplementary nutritious foods for children. Nowadays, about 5% FM malt is invariably blended with the energy food to improve its texture. This food is produced in bulk and supplied to the weaning children. The process for the preparation of weaning food based on malted millet (two parts) blended with malted green gram (one part) was developed, and the food is popularly termed "malted weaning food". Controlled child feeding trials on the malted weaning food have shown its superior nutritional and textural qualities compared to several proprietary weaning foods. The food on reconstitution with water and heating to boiling forms a nutrient-dense slurry (low bulk) and under comparable consistency. This contains twice the amount of nutrients than that of the roller dried weaning foods. The malt flour as a substitute for maltodextrin can be blended with milk and spray dried to prepare the infant foods also. The special feature of the malt flour to form nutrient-dense free-flowing slurry has been utilized towards the development of enteral foods. For this purpose, the malt flour is blended with other ingredients such as milk powder, sugar, soya flour, legume flours and vegetable oils, and the blend is fortified with essential vitamins and minerals. The enteral foods prepared using the millet malt were found to be cost-effective and clinically efficient in improving the nutritional status of patients and reducing the hospitalization period.

Fermentation

Fermentation is a processing technology widely used on FM where the raw material is the medium for the growth of microorganisms. FM ferments at room temperature for 24–72 h depending on the product or beverages intended to be produced (Blandino et al. 2003). The fermentation process is now used in commercial industries to provide value-added products that meet the need of the urban population. This process inhibits spoilage and pathogenic microorganisms to improve the balance of amino acids, sensory quality characteristics and nutritional value of the grains. Fermentation also provides health benefits by reducing anti-nutritional compounds like amylase inhibitor, phytic acid and tannins in cereal grains. The researchers noted that each FM fermentation product is associated with specific microorganisms.

16.4.3 Nutritional Evaluation of FM Products

Indian Institute of Millets Research in collaboration with the National Institute of Nutrition has conducted clinical studies to establish an authentic database for claiming the nutritional merits of millets. In its study on the impact of sorghum diet on school children, it concluded that a millet-based diet helps the growth of children with improved haemoglobin, serum folic acid, albumin, retinol-binding protein (RBP), ferritin, calcium and iron. In another study on the effect of sorghum diet on glycosylated haemoglobin and lipid profile in diabetes patients, it was concluded that there is a significant decrease in glycosylated haemoglobin and fasting glucose levels. However, the FM is rich in calcium, dietary fibre and other minerals. An authentic data for proving their impact on curing various diseases is vital for effective marketing and fast consumer adoption.

NINs are also done on the glycemic index of different millets products including FM plain *roti*, *roti* with *curry* and *roti* with *dal*. It was observed that the glycemic index of the FM recipe was observed to be in the 70–75% range. In vitro and in vivo (animal) studies indicated the lowering of blood glucose and cholesterol, anti-ulcerative and wound healing properties, etc., of FM. However, appropriate intervention or randomized clinical trials are lacking on these health effects. Glycemic index (GI) studies on FM preparations indicate low to high values, but most of the studies were conducted with outdated methodology. Hence, appropriate glycemic index testing of FM preparations and short- and long-term human intervention trials may be helpful to establish evidence-based health benefits. However, an authentic data for proving their impact on curing various diseases is vital for effective marketing and fast consumer adoption.

16.4.4 Entrepreneurship Development

For any invention to adopt by people, some effective channels for disseminating those technologies to people are required. When it comes to innovations in the value addition of food items, these channels would be the entrepreneurs who can adopt the technologies and take them closure to consumers by offering them a wide range of alternatives. More than 2000+ entrepreneurship development (ED) programmes on millets cultivation, processing and marketing of millet-based products were jointly organized by ITC and ICAR–IIMR with active participation from institutes like PJTSAU, NIN, and College of Home Science, Maharashtra agricultural universities (MAU). The machinery of standardized millet products was demonstrated to the farmers. Around 6000+ rural women and another 7000+ SHGs, farmers and urban entrepreneurs are trained in the value addition of millets. ICAR–IIMR is continuously organizing the below capacity-building programmes to reach out to entrepreneurs, aspirants, academicians, housewives, students etc.

1. Cooking with millets: A monthly training programme to teach the preparation of various healthy as well as recipes with millets.
2. Start-up ignition: A monthly training programme to sensitize aspiring entrepreneurs

about emerging business opportunities across the entire millets value chain.
3. Technology entrepreneurship development programmes (TEDP): A six-week-long training programme covering the entire millets value chain and business opportunities, sponsored by the Department of Science & Technology (DST), Government of India (GoI).
4. Technology dissemination and training: It is an exclusive training programme for entrepreneurs on licensed technologies for making them skill-ready to commence their commercial production.
5. Training programmes for universities and institutes: Active training of students and aspiring entrepreneurs on external platforms such as international and national programmes organized by universities, R&D institutes, incubators, etc.

16.4.5 Promotion and Popularization

Promotion is the main intervention for ensuring reaching the advancements in value addition and aiding in consumer adoption. Several stakeholders such as aggressively undertaken by ICAR–IIMR led consortium on awareness of nutritional merits of millets covering 360° communication strategies. For the promotion of FM products, various stakeholders including consumers, nutritionists, doctors, dieticians, chefs etc. were sensitized by ICAR–IIMR.

Simultaneously outsourced the event managers for the popularization of millet products (360° communication, brand designing logo, etc., with below the line (BTL) and above the line (ATL) strategies implemented) in urban markets and new age media. Massive awareness is created on FM as health and nutri food through marathons, cyclathons, cooking competitions, roadshows (300+), wet sampling counters in public parks, malls, institutes, etc. in Hyderabad and in local, national and international exhibitions. Also imparted awareness about millets among 60,000+ consumers through fabricated *millet rath* in Pune, Bangalore, Jabalpur, Chennai, Coimbatore, New Delhi, etc. Rural consumer drive was undertaken by ITC rural choupalhaats to sensitize the convenience and nutritional aspects. ICAR–IIMR organized marathons and cyclothans in the Hyderabad city, around 2000 runners participated in the event which had run distances of 5, 10, 21 and 32 km and these programmes will be continued every year. Recently, promotions through electronic media such as virtual conferences, F-M radio, social media campaigning etc. are being undertaken.

16.4.6 Commercialization of FM-Based Products

The pilot commercialization of millets products has started with the launch of ICAR–IIMR's own brand, "*eatrite ragi*", with the tag line "*eat millets–stay healthy*". In this regard, five formats of business plans are commercialized for FM products evolved under their relative merit assessed in terms of farmer's share in the consumer rupee. Suitable packaging, labelling, marketing and pricing strategies are adopted for targeting them to urban markets at the national level. Thus interventions made it possible to provide convenient options for consumers among FM foods. Launching the first-ever millet brand has inspired hundreds of entrepreneurs and manifested as 400+ start-ups across India.

Some FM-based products commercialized under *eatrite* brand are given in Figs. 16.10, 16.11 and 16.12.

16.4.7 Policy Sensitization

To get the attention of the policymakers with regard to FM, the ICAR–IIMR, in collaboration with the Directorate of Millets Development (DMD), Jaipur, and the National Institute of Rural Development (NIRD), Hyderabad, conducted a national seminar on millets in November 2010. The seminar was ultimately followed by Brainstorming Session in which a task force on millets promotion was set up. Consequently,

Fig. 16.10 FM flakes *eatrite* brand

Fig. 16.11 FM cookies *eatrite* brand

Fig. 16.12 FM soup mix

the Initiative for nutritional and food security through intensive millets promotion (INSIMP) policy with an outlay of Rs. 300 crore under Rashtriya Krishi Vikas Yogana (RKVK) was launched by the Department of Agriculture Co-operation (DAC) with IIMR as the Centre of Excellence (CoE) for disseminating processing technologies to around 200 processing clusters that were set up under the scheme across the country. The CoE at ICAR–IIMR is now in a full swing disseminating the technologies developed to people from across the country. Three pilot mid-day meal scheme studies with the inclusion of millets diet are initiated in three states of Maharashtra, Karnataka and Andhra Pradesh by the DAC under the technical guidance of ICAR–IIMR and the government is actively contemplating mainstreaming millets in public-funded welfare programmes targeting various groups. This project developed a model for PCS for millet foods.

Several state governments such as Odisha, Karnataka, Telangana, Andhra Pradesh and Maharashtra have streamlined their efforts to increase millets consumption through inclusion in PDS, ICDS, etc. Several R&D institutes such as ICMR–IIMR, CFTRI, etc. and NGOs are working with state governments in piloting millet-based foods, especially FM *malt*, *laddu,* etc. Under the Pradhan Mantri Formalization of Micro Food Processing Enterprises (PMFME) scheme launched by the Ministry of Food Processing Industries (MOFPI), 17 districts from 11 states have opted the millets as their commodity for developing them as

the millet-processing hubs. ICAR–IIMR is the technical partner for the implementation of the policy and is mentoring two millet-based common incubation facilities under the policy.

The decades of efforts on the millet value chain have further resulted in gazetting millets as nutri-cereals and celebration of the national year of millets in 2018. Recently, India sponsored a proposal for declaring 2023 as the International Year of Millets which was supported by 70 + countries, and the United Nations General Assembly (UNGA) passed the resolution on 4 March 2021. These all fast-moving policy interventions are showcasing that the policy advocacy part of the value chain framework is exercised sufficiently.

16.4.8 Incubator and Related Services

For any innovation, the last mile connectivity with the end consumers is essential for the creation of demand and sustenance of the whole value chain. Start-ups and private companies are such links in the value chain, and the number of private players in the market is a direct proxy for the volume of marketing and awareness creation. Hence, the ICAR–IIMR has come up with an incubation centre "Nutrihub" in 2016 to nurture the millet start-ups so that these millet products reach end consumers. With support from DST, RKVY—Remunerative Approaches for Agriculture and Allied Sectors Rejuvenation (RAFTAAR) and Agri Business Incubator (ABI), a unique ecosystem of incubation system with physical cabins processes machines, R&D laboratories and other infrastructure facilities for the start-ups. Mentoring network is built by top institutions like the National Institution for Transforming India (NITI AAYOG), NIN, CFTRI, venture capitalists etc., who would help these start-ups grow with their advisory. Nutrihub is strengthened by RKVY, through which the start-ups can be facilitated with the funding support of up to Rs. 25 lakhs as grant-in-aid. To date, ICAR–IIMR has incubated and handhold more than 170+ start-ups, which offer a networking opportunity for the start-ups to create strategic partnerships amongst themselves. Some start-up brands manufacturing and marketing FM products are supported by Nutrihub and ICAR–IIMR and are given in Table 16.1.

16.4.9 Genomics and Nano Biotechnology Approaches for Searching and Creating Values in FM

India is the chief producer of FM and ranks fourth in the world. In order to harness the potential of FM, there is a need for a new vision of deriving innovations in agriculture. Now it's time when agriculture should not rely on the productivity of different agricultural commodities only, i.e. primary agriculture, but relies on the conversion of agri-resources into value-added products mainly food products, i.e. secondary agriculture, as well as isolation of novel molecules, genes, proteins and metabolites for enhancing the quality of life through practising tertiary agriculture (Sharma et al. 2017; Kumar et al. 2021). There is tremendous scope for innovation through value addition which can be achieved in four different ways:

16.4.9.1 Searching Value

Characterization of FM using various conventional and molecular approaches for identifying the hidden values.

16.4.9.2 Adding Value

Process of changing or transforming a product from its original state to a more valuable state desired.

16.4.9.3 Creating Value

Occurs with actual or perceived value to a customer for a superior product or service.

Table 16.1 ICAR–IIMR supported start-ups (FM-based products)

S. no.	Name of the product	Name of the products	Images
1	Fountainhead Foods Pvt. Ltd	1. FM flakes 2. Multi millet biscuits	 
2	Ridgeland Industries Pvt Ltd	1. FM crisps 2. FM choco balls	 
3	Nutrisnax	1. FM energy bar 2. FM munchies 3. FM ladoo	  

(continued)

Table 16.1 (continued)

S. no.	Name of the product	Name of the products	Images
4	Millenova foods	1. FM beetroot crisps 2. Millet carrot crisps	
5	Coastal Foods	1. FM flakes	
6	Nutrimagic	1. FM cookies	

(continued)

Table 16.1 (continued)

S. no.	Name of the product	Name of the products	Images
7	Indian Sisters Kitchen	1. FM cashew biscuits	
8	Panchmanyam	1. FM *Ladoo*	

(continued)

Table 16.1 (continued)

S. no.	Name of the product	Name of the products	Images
9	Sattva Life Foods	Multi millet pancake mix	
10	Lippia	Multi millet *Rawa*	

16.4.9.4 Capturing Value

Changing the distribution of value in the food/fibre production chain.

In such endeavours, not only agri-processing but also frontier sciences of nano-bio-information technologies can play a pivotal role in order to gain newer knowledge about the hidden values in FM and also the development of processes and value-added products based on scientific approaches. With the advent of deciphering the genome sequence of FM, ample opportunities now exist to explore a new dimension of frontier sciences as given below:

- **Biotechnology**: Genomics, gene technology, genetic engineering and genome editing and other *OMICS* approaches for defining complex agriculturally important traits, gene discovery, marker-assisted breeding and transgenic approaches for the development of superior crops and value-added products.
- **Bioinformatics**: Development of user-friendly and customizable databases of FM, decision support system in the management of agriculture, assembly, annotation, identification and characterization of gene families and molecular networks.
- **Nanotechnology**: Nano-diagnostics for plant disease surveillance, spark plasma sintering (SPS), food safety and quality measures, nano-formulations for delivery of agricultural inputs and human nutrients.
- **Agri-processing**: Research and development for the transformation of FM into value-added products such as functional foods, nutraceuticals etc. as well as the transfer of technology for agri-business development to improve income generation and food security.

16.5 Summary

As the millet value chain model is successful under the National Agricultural Innovation Project (NAIP), it can be replicated in other parts of the world, where millets are the main crop. For future sustainability, policymakers and concern departments should take the initiative to motivate and encourage the millets farmers to go for commercialization by creating demand through the inclusion of millets in PDS, ICDS, etc. and a nationwide campaign of FM could be flagged as health-promoting foods. The value chain of FM will secure the economic condition of dryland farmers and widen the country's food security. As health benefits, it will be a chief component in the present and near future, as millets can control various modern lifestyle diseases. Above all these, the consumers were now offered a diverse range of alternatives with the taste and convenience on par with finer cereals.

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