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REVIEW ARTICLE

Genetic and genomic resources for improving proso millet (*Panicum miliaceum* L.): a potential crop for food and nutritional security

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Abstract

Proso millet (*Panicum miliaceum* L.) is a food crop domesticated around 10,000 years ago, that has been prevalent throughout ancient civilization. However, use of millets in the diets has drastically been reduced owing to the addition of cereals such as rice and wheat. At present, proso millet is being cultivated and consumed by a marginal population. Irrespective of the lack of popularity, the crop is well known for its climate resilience traits as well as nutritional properties. Noteworthy, the crop is low on glycemic index, gluten-free, possesses good quality protein, vitamins, minerals, and other nutraceutical properties. Being a C_4 panicoid species, proso millet possesses better water-use and nitrogen-use efficiency, thus promising this as an ideal crop for cultivation in the scenario of global climate change. The extent of food insecurity among the ever-growing population, as well as the prevalence of malnutrition and undernutrition among the children, reinstate the requirement of a nutritious diet that millets and other traditionally important crops can address. Given this, the present review enumerates the progress made in genetics, genomics, and other omics of proso millet that would pave way for improvement using biotechnological as well as breeding interventions. Further, this review elaborates a roadmap for improving this crop in the mainstream cropping system.

Keywords Proso millet (*Panicum miliaceum* L.) · Millets · Domestication · Germplasm resources · Nutraceuticals · Climate-resilience

Introduction

Millets are a group of small-grained cereals that flourish on marginal lands, with poor soil conditions, mainly as rainfed crop. The word ‘millet’ is derived from the word ‘mil,’ meaning thousand, attributing to its capacity to produce thousands of grains from a single seed. Hugg Doggett defined small millets as “millets cultivated for their small grains which are borne on short, slender grassy plants” [22]. Finger millet (*Eleusine coracana*), proso millet (*Panicum miliaceum*), kodo millet (*Paspalum scrobiculatum*), foxtail millet (*Setaria italica*), barnyard millet (*Echinochloa* sp.) and little millet (*Panicum sumatrense*) are predominantly cultivated small millets in the world [68, 69]. Teff (*Eragrostis tef*) and fonio (*Digitaria* sp., also called hungry rice) are also small millets, but these crops are prominent in Ethiopia and Western Africa only [22]. Important small millets grown in India are finger millet, kodo millet, foxtail millet, little millet, proso millet, and barnyard millet. Though sorghum and pearl millet are also cultivated, they are regarded as coarse millets, and hence, not included under small millet clades [27, 73]. India is the largest producer of millets in the world, producing 11.64 million tonnes (Fig. 1a). Still, small millet production share is only about 2.4 million tonnes, of which finger millet solely accounts for 1.98 million tonnes as of 2017–2018 [23, 39]. In India, proso millet is cultivated in 0.41 lakh hectares, with an output of 0.22 lakh tonnes [1]. The Indian Council of Agricultural Research, ICAR’s initiative to set up an All India Coordinated Small Millets Improvement Project (AICSMIP) in 1986 helped to coordinate and boost research of these crops in India and explains the increased productivity of small millets over the years (391 kg/Ha in 1986 to 804 kg/Ha in 2018) [39].

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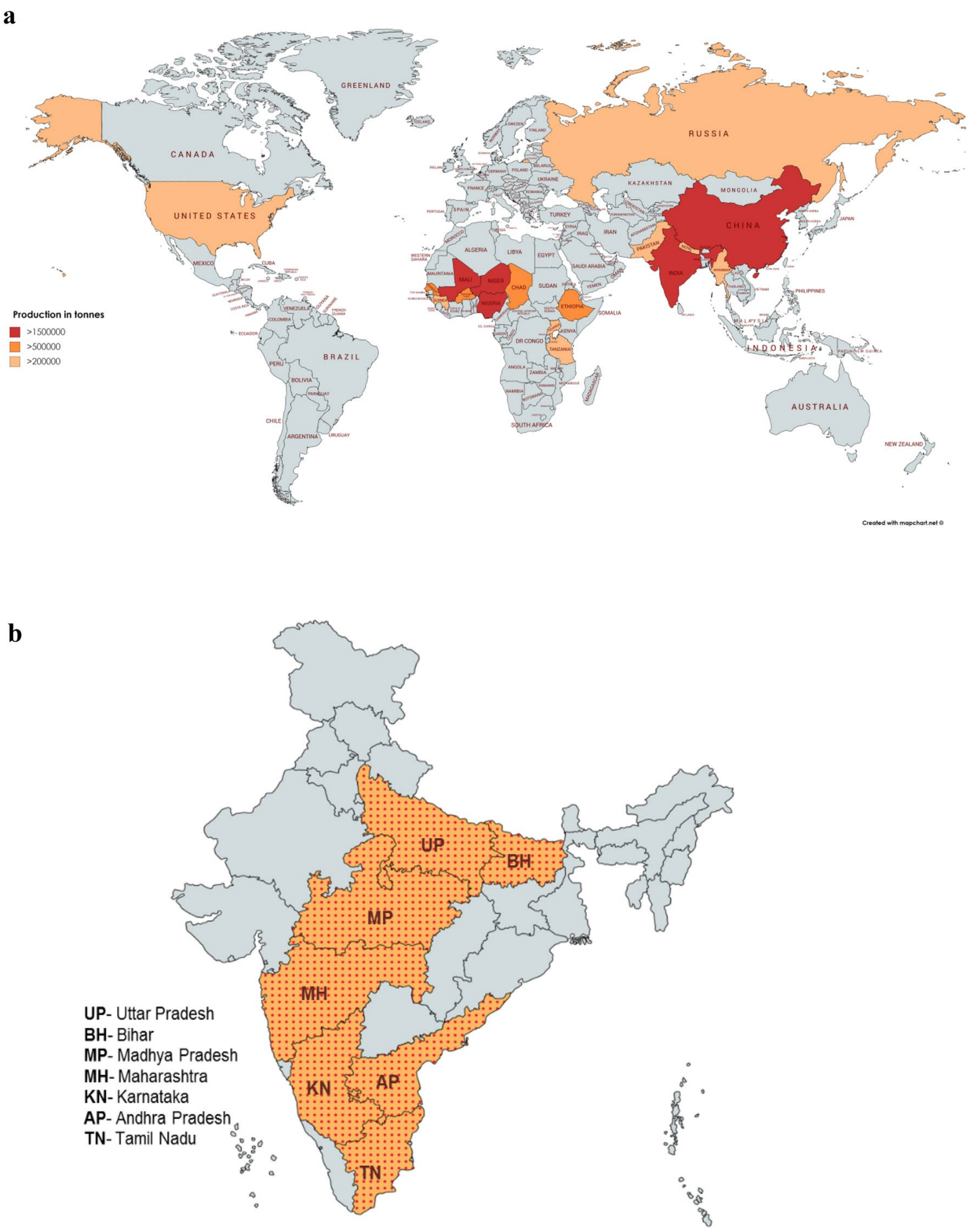


Fig. 1 **a** Global millet production: the major millet producing countries with their production in Metric Tons (MT), categorised as > 1,500,000 tonnes, > 500,000 tonnes and > 200,000 tonnes [23]; **b** major proso millet producing states in India [80]

Proso millet is unique owing to its low water and nutrient requirements, short growing season, and nutritional richness. It can be grown at high altitudes (up to 3500 m in India) and plateaus. It can establish and grow well on varied soil types and low fertile soils [9, 56]. It is presently cultivated in Asia, Australia, North America, Europe, USSR, and Africa. It is consumed as food in parts of Asia and used as bird and livestock feed in developed countries [66, 73, 83]. Proso millet is known by different names like common millet (Japan, Korea), *hog* millet (USA), broomcorn millet

(China), *hersey* (Germany), and French white (France) [24, 66]. In India, it is known by different names in different languages like *Panivaragu* (Tamil), *Cheena* (Bengali), *Baragu* (Kannada), *Variga* (Telugu), *Vari* (Marathi), *China Bacharibagmu* (Odia) and *Cheno* (Gujarati) [38]. In India, important states cultivating the crop are Madhya Pradesh, eastern Uttar Pradesh, Bihar, Tamil Nadu, Maharashtra, Andhra Pradesh, and Karnataka [80] (Fig. 1b). Proso millet, being a C_4 crop, possesses better photosynthetic and water use efficiency. It is highly drought tolerant and can come up in arid and semi-arid regions and set seed with 330–350 mm annual rainfall [9, 56]. The grains are a good source of carbohydrates, rich in protein, dietary fiber, vitamins, minerals, and essential amino acids. Proso millet has immense potential in addressing the global concerns of increased demand for food production, water scarcity, and malnutrition. It can also help realize the goal of a sustainable and diversified production system [3, 31]. Despite the benefits of this crop, it has only about 5 million tonnes of production globally, which is only 1% of wheat production [34]. Given these facts, the review attempts to give an overview of proso millet crop, journeying through its domestication, botany, nutritional composition, and genetic improvement. It also proposes the steps to harness the genetic potential of this crop for the future.

Domestication of proso millet

Proso millet is one of the ancient crops to be domesticated. It is believed to be domesticated in China around 10,000 years Before Present (BP) based on the various archaeological evidence and historical records. The millet is believed to have earmarked a transition from hunter-gatherers to millet farmers in Northern China based on traditional wisdom [11, 30]. Interestingly, noodles, a favorite dish across the globe, have also contributed to tracing back the domestication history of proso millet. Noodles sample (dating 4000 years BP) of Late Neolithic China—Lajia archaeological site excavations (Northwestern China), when subjected to phytolith and starch grain analysis, confirmed the presence of *P. miliaceum* and *S. italica* [26, 53, 54]. Barton et al. [10] divided the domestication process in the Dadiwan site of Northwest China into two phases (phase I: non-intensive, 7900–7200 calendar years BP and Phase II: intensive, 5900 years BP) based on stable isotope analysis and carbon dating of bones of animals and humans. Other evidence throwing light into the domestication history of the crop includes macrofossils from Loss plateau site of Dadiwan, China, carbonized grains from Eastern Inner Mongolia, China (Xinglonggou site) and charred grains from Europe [37, 51, 94, 96]. Hunt et al. [35] undertook genetic diversity and phylogeographic studies of proso millet in Eurasia, attempting to understand the archaeobotanical records of the millet discovered from Eastern Europe and the possibility of multiple domestication centers. Their studies had identified one western and one eastern gene pool, but more research efforts and evidence would be required to establish a second domestication center (Eastern Europe or Central Asia).

Taxonomic hierarchy and botany

Proso millet is a tetraploid crop ($2n = 4x = 36$) and belongs to the genus *Panicum*, tribe *Paniceae,* family *Poaceae* and order *Poales* [9, 16, 24]. *Panicum capillare* (witch grass) and *Panicum repens* (Torpedo grass) are weedy forms of *P. miliaceum* that are reported to be contributing to its allotetraploid origin. Still, a wild ancestor of the crop is yet to be identified [34]. The species *Panicum* has two subspecies (*subspp*), i.e*., subspp.ruderale*, which has weedy forms and natural variants and *subspp.miliaceum* that has cultivated types [24, 92]. The subspecies *miliaceum* is further subdivided into five races based on the type of inflorescence, i.e., *miliaceum, patentissimum, contractum*, *compactum*, and *ovatum* [24].

Morphology and reproductive biology

The plant grows to a height of (30–100) cm, with hollow, hairy or glabrous stem, swollen internodes, and a shallow root system (Fig. 2) [9, 41, 87]. It is a short day, short duration (60–90 days) crop [28, 29, 82]. It is tolerant to drought, but sensitive to frost and waterlogged conditions. The crop is harvested at its physiological maturity to avoid shattering of grain. The plant reaches maturity early during drought [8, 30]. It flowers naturally between 10:00 am and 12:00 noon. Bright sunlight and low humidity may advance, and cloudy days may delay flowering. The inflorescence is a drooping panicle and looks like a broom [14, 30] with basipetal opening of florets, i.e., from top to bottom. A single spikelet contains two glumes and two lemmas. The lower lemma has a sterile floret, and upper lemma has a fertile floret [28]. It has three anthers and two feathery stigmas. The anther dehiscence coincides with stigma receptivity and anthers dry within a few minutes of flower opening. At our Millet Breeding station, the anthers appeared bright yellow immediately after extrusion from florets, and within 5–7 min, it dried, turned orange, and then to brown. It may take about 12–15 days for a panicle to complete flowering. Though self-pollination is predominant, more than 10% cross-pollination may occur [65]. These flowering features make it challenging to employ crossing in proso millet without damaging the stigma and without shedding of pollen before emasculation [28]. Nelson et al. [60] report crossing techniques in proso millet using hand emasculation, and cold spray technique for emasculation and crossing is reported by Nandini et al. [61]. Seeds of proso millet are oval 3 mm long, and colour varies from white, golden yellow, orange, red, brown to black [9].

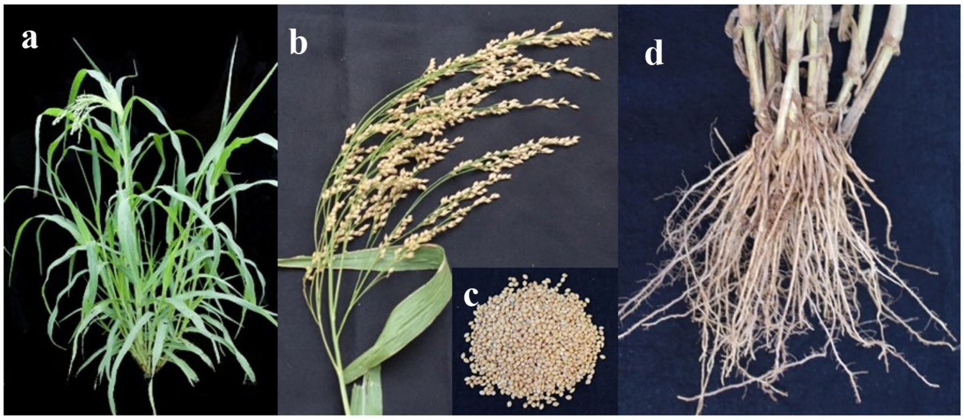


Fig. 2 Proso millet (*Panicum miliaceum* L.): **a** plant morphology, **b** panicle, **c** grains, and **d** root

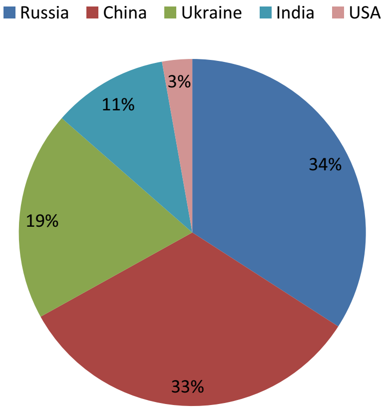


Fig. 3 Per cent share of major countries to proso millet germplasm collection [84]

Among the collections in China, the thousand-grain weight is reported to be between 1.5 and 10 g [88].

Germplasm resources

Maintaining genetic variability among the cultivated varieties of a crop is essential to have a stable production system. The success of any plant breeding program depends on the variability exhibited by the germplasm. Efforts to collect, characterize, and document germplasm collections from across the globe are essential for crop improvement programs. Work on proso millet has been sidelined due to the focus on major crops. The most extensive collection of proso millet germplasm accessions (8778) is maintained in Russia. Other major gene banks conserving the crop's genetic variability are in China, Ukraine, India, and USA (Fig. 3) [84]. In India, two national institutes All India Coordinated Research Project on Small Millets (AICRP-small millets), National Bureau of Plant Genetic Resources(NBPGR)] and one international institute International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) are involved in plant genetic resource maintenance. AICRP established a separate unit in Bengaluru in 1976[2]. This unit is actively engaged in the collection, evaluation, and documentation of small millet germplasm in India. AICRP also maintains a collection of 920 accessions of Proso millet. NBPGR is the nodal organization in India for the management of plant genetic resources of agri-horticultural crops and has about 994 accessions of proso millet. ICRISAT with 849 accessions is involved in germplasm characterization and evaluation of proso millet. ICRISAT developed a core collection of 106 accessions from 833 proso millet accessions of 30 countries based on 20 qualitative and quantitative traits. Random selection of 10% accessions from each of the 101 clusters was employed to develop the core set [82].

Nutritional and nutraceutical potential of proso millet

Millets are an excellent nutritional competitor to commonly grown cereals as they provide energy, protein, minerals, vitamins, fat and dietary fiber [5]. Proso millet has nutritional composition comparable to or even better than predominant cereals (Table 1) [50]. The millet is a good source of energy, providing 74 g/100 g of edible portion as carbohydrates. The content of crude fiber (5.5 g/100 g flour) is also higher than most cereals and millets. The protein content (10–14 g/100 g) is similar to wheat (11.6 g/100 g) and higher than rice (8.5 g/100 g), but with an enhanced essential amino acid composition (leucine, phenylalanine, methionine) (Fig. 4). The essential amino acid index is reported to be 7% higher than wheat. Leucine contributed highest to proso millet protein complex, and lysine was found limiting. Protein and amino acid content are reported to be significantly influenced by the environment [21, 44]. Puffed proso millet also had comparable protein content to other cereals, better in vitro digestibility to unprocessed grain, and hence has potential for making puffed whole grain snacks [19]. The feeding value of the millet in broiler diets have been studied and found comparable with sorghum. The total metabolizable energy was 4130, 3995, and 4007 kcal/kg in corn, sorghum, and proso millet, respectively. When corn, sorghum and proso millet were given as a feed in broiler diet, there was a significant reduction in body weight gain at the 4-week stage for sorghum and proso millet diet in comparison to corn. But the performance was reported to be improved with methionine and lysine supplementation for both the diets [55].

Proso millet, like other small millets has significant nutraceutical properties and health benefits (Table 2). The dietary proso millet protein increased HDL-cholesterol (High-Density cholesterol) in mice and suggests a positive effect on cholesterol metabolism [63]. It is gluten-free and can be suggested for patients with gluten intolerance who cannot consume other cereals like wheat and barley [5, 64]. The glycemic index of the crop was lower than rice, wheat, and barley. The millet-based products (100% proso millet) gave

Table 1 Nutrients in various cereals versus proso millet (in 100 g edible portion)

Cereal	Protein (g)	Fat (g)	CHO (g)	Crude fiber (g)	Ash (g)
Wheat[a]	11.6	2	71	2	1.6
Brown rice[b]	8.5	2.6	74.8	0.9	1.6
Maize[a]	9.2	4.6	73	2.8	1.2
Sorghum[c]	9.5	2.5	76	2.7	1.6
Pearl millet[a]	11	5	69	2.2	1.9
Foxtail millet (flour)[d]	11.5	2.38	75.2	4.3[f]	0.47
Finger millet[a]	6	1.5	75	3.6	2.6
Kodo millet (dehuskedgrain)[e]	8.3	1.4	65.9	9.0	2.6[a]
Proso millet(flour)[f]	14.1	1.2	74	5.5	0.6

[a][50], [b][42], [c][78], [d][58], [e][20], [f][21]

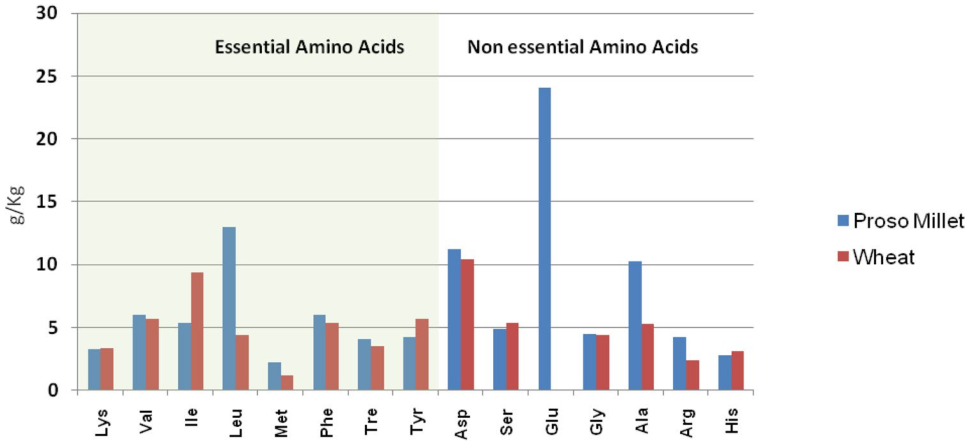


Fig. 4 Essential and non-essential amino acid content: proso millet versus wheat, expressed in g/kg (*Lys* lysine, *Val* valine, *Ile* isoleucine, *Leu* leucine, *Met* methionine, *Phe* phenylalanine, *Tre* threonine, *Tyr* tyrosine, *Asp* aspartic acid, *Ser* serine, *Glu* glutamic acid, *Gly* glycine, *Ala* alanine, *Arg* arginine, *His* histidine) [44]

Table 2 Nutraceutical properties reported in proso millet

	Health benefit	Responsible factor	References
1.	Type-2-diabetes, cardio vascular disease	Low glycemic index	[57]
2.	Obesity	Increase HDL cholesterol (high density lipoprotein) or good cholesterol	[63, 64]
3.	Improve lipid metabolism Improve insulin sensitivity	Increase adiponectin	[64]
4.	Tumor	Tumor necrosis factor (TNFα) down-regulation	[64]
5.	Celiac disease	Absence of gluten protein	[5]
6.	Breast cancer, liver cancer	Anti-proliferative action against MDA breast cancer cell and HepG2 liver cancer cells	[93]
7.	Reduced constipation	High fibre content (13.8%)	[68]
8.	Anti-oxidant and anti-cancerous	Phenolic compounds, flavanoids, proanthocyanin and phytic acid (concentrated in bran)	[13, 21]

glycemic index values of 50–65 (%/g) when it was 70–80 for corn and wheat-based products [57]. Hence, it can be advocated for patients with type-2-diabetes mellitus and cardiovascular diseases (CVD). The vitamins and mineral content in proso millet is also superior [18]. The minerals like Fe, Zn, Mg, Mn, K and P are found to be higher than rice but lower than wheat. Mineral components like Fe, Zn and Ca in germplasm core collection maintained at ICRISAT are reported to be in the range of 41–73 mg/kg, 26–47 mg/kg and 91–241 mg/kg, respectively. The accessions with high levels of multiple nutrients have also been identified, which can be utilized as a nutrient-rich parent source for biofortification programs [86]. The crop is rich in vitamin B1 or thiamin (0.63 mg/100 g edible portion) and riboflavin or vitamin B12 (0.22 mg/100 g) [18]. The tocopherol content in some proso millet varieties was reported to be 3.6–4 mg/100 g [7].

The anti-nutritional factors like phenolic compounds, flavonoids, proanthocyanin, and phytic acid are present in the crop but concentrated mainly in bran layers and will be significantly reduced after processing. Total phenolic content (TPC) reduction due to dehulling was 72% in proso millet, which was higher than most other small millets' values. Proso millet hull contained 50 (µmol ferulic acid equivalent/g defatted meal) hydrogen peroxide scavenging capacity and 104 (µmol Trolox equivalent/g defatted meal) oxygen radical absorption capacity. These components were also found to decrease upon dehulling. These anti-nutritional factors can also become beneficial components if exploited for their antioxidant and anti-cancerous properties. Therefore, unused dehulled portions of the millet grain can be a potential natural source in food supplements [13, 21].

Genetic improvement of proso millet

Agronomic traits

In proso millet, crop improvement programs are focused on improving traits like yield, lodging resistance, non-shattering, early maturity, panicle type, waxiness, etc. Through conventional methods like pure-line selection, pedigree breeding, and backcrossing, improved varieties have been developed in proso millet. China, India, USA, Russia, and Kenya are major countries involved in proso millet breeding [24]. In India, K2 is a variety developed through pure-line selection, which is non-lodging and non-shattering [80]. The varieties TNAU 202 and ATL 1 are high yielding varieties developed through hybridization program. Dawn and Early-bird are early maturity varieties developed from the USA. Russian variety Alba is known to have non-shattering property. 'Plateau' is an amylose free or waxy cultivar developed by the Nebraska Agricultural Experiment Station, USA. Waxy forms of millet are preferred in the food industry for their glutinous nature and beverage industry for their fermentation efficiency. Studies to understand the genetics and inheritance of waxy traits have been carried out. Waxy trait in proso millet is reported to be controlled by duplicate recessive alleles. *GBSSI* gene (with two loci-S, L) mutations are identified to be resulting in waxiness, and the *GBSSI*-S locus is mainly contributing to the trait [25, 36, 70, 74]. Rajput et al. used molecular breeding to identify eighteen quantitative trait loci (QTL) for eight important phenotypic traits like heading date, 100-grain weight, grains per panicle, lodging, peduncle length, plant height, grain shattering, and panicle length. These QTLs can be used for marker-assisted selection.

Abiotic stress tolerance

Proso millet is predominantly cultivated in marginal lands, arid and semi-arid regions. Hence, concerted efforts to understand and improve stress tolerance are essential to enhance yield. The occurrence of drought at ear emergence stage has been found to affect the grain yield and WUE significantly in proso millet. Though the panicles per plant did not decrease, the number of seeds per panicle and seed weight reduced considerably. Hence during limited water conditions for irrigation, the ear emergence stage was found to be the most critical stage [76]. Sabir et al. [71] reported that salt stress significantly affected yield among proso millet accessions studied and also reduced the relative water content (RWC). Out of the eighteen accessions studied, three accessions (008211, 008214, and 008226) that gave more than 50% seed weight over control were categorized as salt-tolerant. Hong Yue et al. [91] identified 22 PmWRKY genes as abiotic stress-responsive genes as they significantly varied under abiotic stress treatments. Transcriptome sequencing identified differentially expressed genes related to stress tolerance, predominantly cold and salt stress. This study was conducted among two genotypes Yumi No. 2 (drought-sensitive) and Yumi No. 3 (Drought and Salt tolerant). *Unigene34608,* which is predicted to code for HSBP1 (Heat Shock Binding Protein 1), was found to upregulate 400-fold in Yumi No. 3 genotype during cold stress treatment. Expression level variation among the genotypes under various stress treatments of *Unigene33484* indicated an osmoregulatory role of the gene. *Unigene35973* was found to have a 100-fold increase in expression during cold and salt stress in Yumi No.3. These results show the role of these unigenes in abiotic stress tolerance and provide scope for further research into understanding the molecular mechanism of stress tolerance [90]. It can be used to develop functional markers for tolerance screening.

Biotic stress tolerance

The diseases reported in the crop are very few, like smut, bacterial stripe, sheath blight, brown spot, melanosis, rust, and blast. Other than the attempts made through conventional breeding to develop improved cultivars, no major research has taken place towards understanding the genetics and genomics behind biotic stresses. Biotic stress is not found to cause considerable economic yield loss in this crop. There are few germplasm sources or cultivars identified as the biotic tolerant source in Proso millet from different countries. In Russia, Ukraine, and China, research on head smut resistance has been carried out [30, 80, 95]. ‘Soyuz’, ‘Sputnik’, ‘Slavjanskoe’ and ‘Quartet’ are smut resistant cultivars available from Russia [4, 24]. Mutation breeding has been employed to develop resistant mutants for smut and melanosis. These mutants were further used in the hybridization program to release a mutant variety ‘Khar’kovskoe’ [84]. Soldatov and Agafonov identified 12 resistant varieties to melanosis from 300 varieties [77]. PRC 1 is a variety from India that offers resistance against *helminthosporium* [24]. Genotypes resistant and moderately resistant to sheath blight have also been reported [40]. Studies towards insect pest tolerance are very limited in proso millet. Insect pests like aphids, wheat stem maggots, thrips, mites, and armyworms are reported to cause a considerable infestation in the USA [43]. Shoot fly infestation is reported in India. Among the Indian varieties, TNAU 145, TNAU 151, TNAU 202, Co (Pv)5, and ATL 1 are reported to be tolerant to shoot fly [24].

Genomics and transcriptomics

Genomic resources in the crop are scarce because of the lack of funding and intensive global research efforts and also due to the complexity of the genome (Fig. 5). One of the earliest reports on the use of genetic resources in the crop includes Random Amplified Polymorphic DNA (RAPD) marker-based study to understand the intra- and inter-specific crop diversity of *Panicum*. RAPD polymorphic markers have also been utilized in the crop to understand the relationship between weed and cultivated biotypes in proso millet in North America [59]. The study identified 97 RAPD markers out of which five markers exhibited consistent polymorphism among the cultivated and weedy plant types [17]. The cDNA clones from leaves of proso millet have been isolated and characterized to understand the gene expression variation. Messenger RNA coding for bundle sheath mitochondrial translocator, 2-oxoglutarate (2-OG)/malate translocator, was found to be higher in leaves when compared to other non-photosynthetic parts indirectly indicating the role of bundle sheath mitochondria in C4 pathway [79].

The informativeness, high reproducibility, and cost-efficiency of simple sequence repeats (SSRs) provided a quantum leap in genetic marker development and utilization. SSR markers and other genomic tools in proso millet have been developed by using genomic resources in related

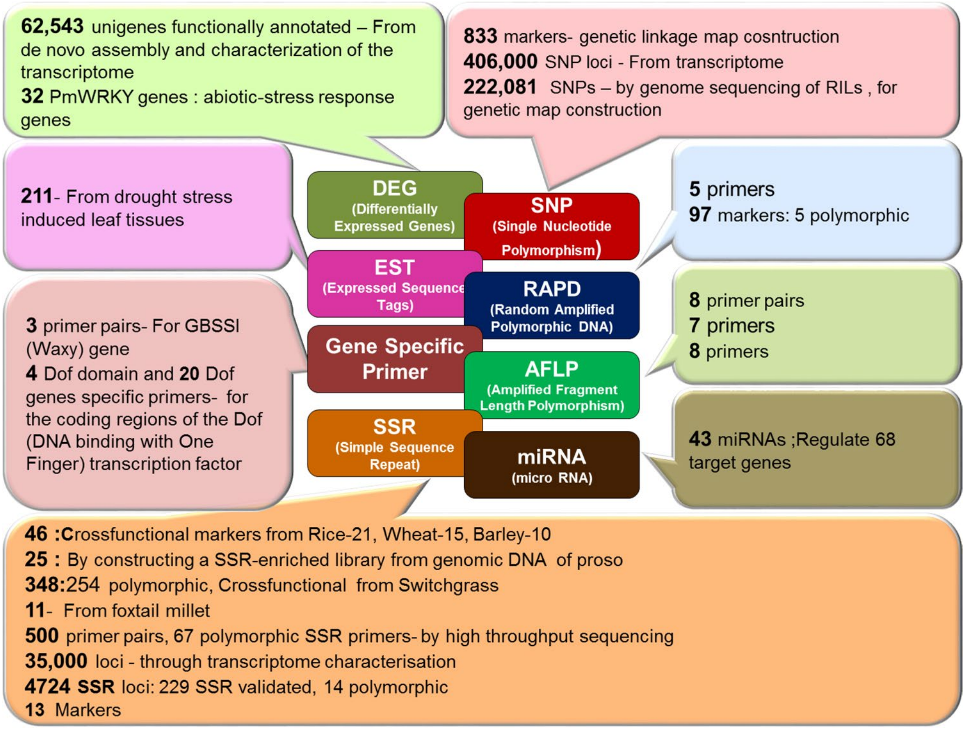


Fig. 5 Details of molecular markers reported in proso millet [6, 15, 17, 32, 33, 45–49, 52, 59, 62, 67, 72, 81, 84, 89–91, 97]

grass species. The cross-functionality of SSR markers developed in other important crop species like rice, wheat, and barley have been exploited to some extent in proso millet [33]. Rajput et al. [66] attempted cross-amplification of 548 switchgrass SSR markers on proso millet genotypes, out of which 339 amplified successfully implying their transferability. Apart from these, polymorphic SSR markers (25 markers) have been developed and characterized from the genomic DNA of proso millet with an average allele size of 4.4 per locus [15]. Rajput et al. [67] also developed the first genetic linkage map in the crop from RIL population (recombinant inbred lines) using GBS-SNP markers (genotype-by-sequencing).

Transcriptome of proso millet was assembled and characterized by Yue et al. [90]. The transcriptome of two proso millet genotypes Yumi No. 2 and Yumi No. 3, were sequenced to identify unigenes, SSRs, differentially expressed genes (DEG), etc. About 35,216 SSRs and 406,000 SSR loci were identified with trinucleotide repeats being the most abundant type. Out of the various types of repeat units, A/T was found to be the most frequent type. AG/CT was the most common dinucleotide type repeat unit, which contrasted with the AC/GT type reported in soybean, maize, rice, wheat, and barley. The study identified 113,643 unigenes, out of which 62,543 were functionally annotated. The gene homology to monocots revealed a 43.68% homology to *sorghum bicolor*. The study reported 292 differentially expressed genes in proso millet. The unigenes, *34608* was found to be associated with cold stress*, 33484* with the osmoregulatory role, and *35973* with cold stress. These unigene were observed in Yumi No.3 (drought and salt-tolerant) and showed consistent patterns with RNA-seq analysis.

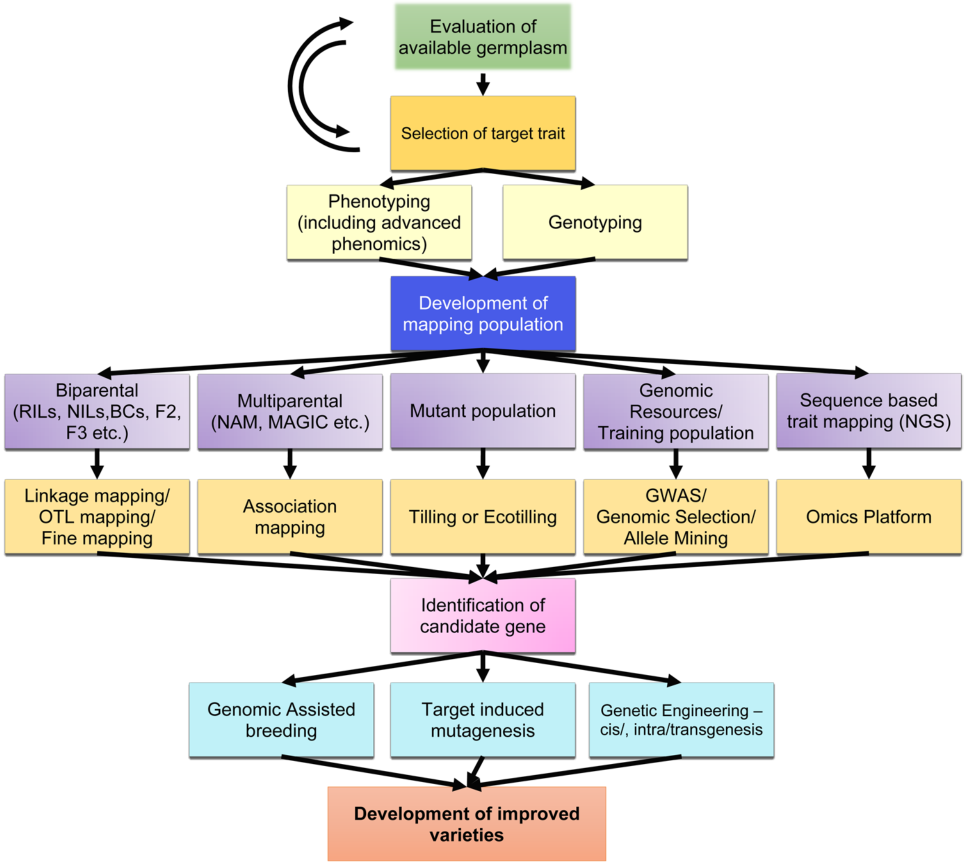
Chloroplast DNA is highly conserved mainly due to the maternal inheritance pattern and can be utilized for evolutionary studies, species identification, etc. The chloroplast genome of proso millet was independently sequenced and published by Cao et al. [12] and Nie et al. [62]. Cao et al. reported the chloroplast genome size as 139,929 bp with 38.6% GC content. The study annotated 132 genes, including 84 protein-coding genes. The studies by Nie et al. reported the typical quadripartite structure for the chloroplast genome with one long single copy region (LSC), one small single-copy region (SSC), and two inverted repeats (IR). It was reported to be 139,826 bp in size [62]. The sequences were annotated, and 108 genes were identified in the chloroplast genome. Seventy-six protein-coding, twenty-eight transfer RNA genes, and four ribosomal RNA genes were also identified. The protein-coding genes were mostly genes coding for ribosomal proteins, photosystem I, photosystem II and genes for ATP synthase. Comparative analysis was carried out between proso millet and five panicoidae subfamily chloroplast genomes (sugarcane, foxtail millet, sorghum, switchgrass, and maize). The gene structure, content, and organization were highly conserved among them. The regions *rpoC2, ndhB, trnL, rpl22, rpl23, psbK* and *matK* were found to be highly divergent among the six species. These regions can be used as markers for species identification and phylogenetic studies. In addition to this, 13 SSR markers were also reported that can be used for panicoideae species identification. RNA editing sites are conserved evolutionarily, and closely related species share more editing sites and proso millet share highest RNA editing sites with switchgrass (*Panicum virgatum*).

Recently, whole genome sequencing and assembly of proso millet has been accomplished by Zou et al. [97], generating enormous molecular data. The genome length was determined to be 923 Mb with 55,930 protein-coding genes, 339 microRNA genes, 1420 transfer RNAs, 1640 ribosomal RNAs, and 2302 small nuclear RNAs. The repetitive sequences constituted about 58.2% of the genome, of which 92.1% were transposable elements (TE). The most common TE was retrotransposons. The individuals from F_6 generation of RIL population were sequenced to construct a genetic map with 18 linkage groups. The studies using homologous gene pairs threw insights into the evolutionary history of the crop and suggested that tetraploidisation might have occurred 5.8 million years ago. They also compared the proso millet genome with already sequenced six grass species (rice, wheat, maize, sorghum, foxtail millet, and purple false broom) and identified a close relationship between proso millet and foxtail millet. Comparative genomic studies further found that only 4.2% of gene families were unique to proso millet, whereas 74.5% was shared among the five grasses, including foxtail millet, maize, sorghum, and rice.

Road map for genetic and genomic improvement

The genetic and genomic improvement in proso millet lags much behind most cereals. There is a need for an extensive evaluation of germplasm to identify target traits or donors for traits like biotic and abiotic stress tolerance, non-lodging, non-shattering, yield, compact panicle, bold grains, genetic male sterility, etc. Phenotyping, coupled with genotyping, is to be followed in order to develop mapping populations. Mapping populations can further be mined for their traits using modern molecular tools like QTL mapping, fine mapping (dense genetic linkage map construction), association mapping, tilling, eco-tilling techniques, GWAS (Genome-Wide Association Studies), genomic selection, etc. The advancements in next-generation sequencing (NGS) opens an array of omics platforms like full genome sequencing, RNA-seq analysis, etc. that can aid in developing genetic and functional markers for the identification of candidate genes [85]. These platforms can also assist in understanding the genetics and inheritance of complex traits. The draft

Fig. 6 Roadmap for genetic and genomic improvement in proso millet



genome being sequenced in proso millet can be utilized to develop molecular markers. SNP markers offer higher polymorphism in comparison to SSR and can be very useful in a self-pollinated crop like proso millet and can also be used for true F1 identification [75]. The identified genes from the donors can be introgressed into popular cultivars using modern breeding methods like genomics assisted breeding (Marker Assisted Selection, Marker-Assisted Back Cross, haplotype breeding, Speed breeding, etc.) and transgenic approaches. Functional markers and gene introgression for traits like genetic male sterility, non-lodging, etc. can also be attempted from the model grass species, foxtail millet in which genetic dissection of traits are occurring at a faster pace. Genome editing tools like targeted mutagenesis, cisgenesis, intragenesis, RNA silencing, etc. can also be used to alter the gene sequences to improve the cultivars. These tools can also be used to enhance the bioavailability of nutrients in proso millet by altering their biosynthetic pathways [75]. The result of all these concerted research efforts would fill in the research gaps and deliver competitive improved cultivars (Fig. 6).

Conclusion and future perspectives

Despite the potential advantages of the crop, proso millet has lost its prominence in competition with major cereals like rice, wheat, and maize. In the present context of changing climate, the dearth of natural resources, and increasing malnutrition, there is a need to revitalize hardy and nutritious crops like proso millet. The research gaps in the crop have to be identified and addressed with the cooperation from national, international organizations, state agricultural universities, non-governmental organizations, and other stakeholders. Development of high yielding improved varieties and improvements in post-harvest handling and processing technologies are quintessential for the production to expand. The technological intervention has to happen concurrently in all small millet crops, promoting a diversified nutrient-rich consumption pattern. This can create a substantial market for small millets and would stimulate production to meet this demand. Product diversification and value addition is another aspect that can strengthen the market for the crop. Proso millet can be cultivated on poor and marginal lands with minimum inputs. Government interventions through policy support and subsidies can encourage farmers to take up cultivation.

All these efforts in collaboration can realize the full potential of the crop and rekindle its importance.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. AICRP on Small Millets. Improved production technology for proso millet. GKVK, Bengaluru; 2017–2018.
2. AICRPSM. Introduction. 2020. http://www.aicrpsm.res.in/About%20Us/Introduction/Introduction.pdf. Accessed 24 May 2020.
3. Alexandratos N, Bruinsma J. World agriculture towards 2030/2050: the 2012 revision. 2012.
4. Al-Khayri JM, Jain SM, Johnson DV. Advances in plant breeding strategies: agronomic, abiotic and biotic stress traits. Berlin: Springer; 2016.
5. Amadou I, Gounga ME, Le G-W. Millets: Nutritional composition, some health benefits and processing-A review. Emir J Food Agric 2013;25(7):501–8
6. Araki M, Numaoka A, Kawase M, Fukunaga K. Origin of waxy common millet, *Panicum miliaceum* L., Japan. Genet Resour Crop Evol. 2012;59(7):1303–8.
7. Asharani V, Jayadeep A, Malleshi N. Natural antioxidants in edible flours of selected small millets. Int J Food Prop. 2010;13(1):41–50.
8. Baltensperger D, Lyon DJ, Anderson R, Holman T, Stymieste C, Shanahan J, et al. EC95-137 producing and marketing proso millet in the high plains. Lincoln: Historical Materials from University of Nebraska-Lincoln Extension; 1995. p. 709.
9. Baltensperger DD. Progress with proso, pearl and other millets. In: Trends in new crops and new uses. 2002:100–3.
10. Barton L, Newsome SD, Chen F-H, Wang H, Guilderson TP, Bettinger RL. Agricultural origins and the isotopic identity of domestication in northern China. Proc Natl Acad Sci. 2009;106(14):5523–8.
11. Bettinger RL, Barton L, Morgan C, Chen F, Wang H, Guilderson TP, et al. The transition to agriculture at Dadiwan, People's Republic of China. Curr Anthropol. 2010;51(5):703–14.
12. Cao X, Wang J, Wang H, Liu S, Chen L, Tian X, et al. The complete chloroplast genome of *Panicum miliaceum*. Mitochondrial DNA Part B. 2017;2(1):43–5.
13. Chandrasekara A, Naczk M, Shahidi F. Effect of processing on the antioxidant activity of millet grains. Food Chem. 2012;133(1):1–9.
14. Changmei S, Dorothy J. Millet-the frugal grain. Int J Sci Res Rev. 2014;3(4):75–90.
15. Cho Y-I, Chung J-W, Lee G-A, Ma K-H, Dixit A, Gwag J-G, et al. Development and characterization of twenty-five new polymorphic microsatellite markers in proso millet (*Panicum miliaceum* L.). Genes Genomics. 2010;32(3):267–73.
16. Christenhusz MJ, Byng JW. The number of known plants species in the world and its annual increase. Phytotaxa. 2016;261(3):201–17.
17. Colosi JC, Schaal BA. Wild proso millet (*Panicum miliaceum*) is genetically variable and distinct from crop varieties of proso millet. Weed Sci. 1997;45(4):509–18.
18. Das S, Khound R, Santra M, Santra DK. Beyond bird feed: proso millet for human health and environment. Agriculture. 2019;9(3):64.
19. Delost-Lewis K, Lorenz K, Tribelhorn R. Puffing quality of experimental varieties of proso millets (*Panicum miliaceum*). Cereal Chem. 1992;69(4):359–65.
20. Deshpande S, Mohapatra D, Tripathi M, Sadvatha R. Kodo millet: nutritional value and utilization in Indian foods. J Grain Proc Storage. 2015;2(2):16–23.
21. Devisetti R, Yadahally SN, Bhattacharya S. Nutrients and antinutrients in foxtail and proso millet milled fractions: evaluation of their flour functionality. LWT Food Sci Technol. 2014;59(2):889–95.
22. Doggett H. Small millets—a selective overview. New Delhi: Small Millets in Global Agriculture Oxford and Ibh Publishing; 1989. p. 3–17.
23. FAOSTAT. 2020. http://www.fao.org/faostat/en/#data/QC. Accessed 25 May 2020.
24. Gomeshe S. Proso millet, *Panicum miliaceum* (L.): genetic improvement and research needs. In: Patil JV, editor. Millets and sorghum biology and genetic improvement. Hoboken: Wiley; 2016. p. 150–79.
25. Graybosch RA, Baltensperger D. Evaluation of the waxy endosperm trait in proso millet (*Panicum miliaceum*). Plant Breed. 2009;128(1):70–3.
26. Guedes JDA. Millets, rice, social complexity, and the spread of agriculture to the Chengdu Plain and Southwest China. Rice. 2011;4(3–4):104–13.
27. Gupta A. Improvement of millets and pseudo-cereals for rainfed agriculture in hill region. Sustainable production from agricultural watersheds in North West Himalaya, Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, Uttaranchal, India. 2006:163–74.
28. Gupta A, Sood S, Agrawal PK, Bhatt JC. Floral biology and pollination system in small millets. Eur J Plant Sci Biotechnol. 2011;6:81–6.
29. Gupta H, Gupta A. Agro-biodiversity in the food crops of North Western Himalayas. Agrobiodivers Crop Genetic Resourc Conserv. 2007;1:139–46.
30. Habiyaremye C, Matanguihan JB, D'AlpoimGuedes J, Ganjyal GM, Whiteman MR, Kidwell KK, et al. Proso millet (*Panicum miliaceum* L.) and its potential for cultivation in the Pacific Northwest, US: a review. Front Plant Sci. 2017;7:1961.
31. Hoogeveen J, Faurès J-M, Peiser L, Burke J, de Giesen NV. GlobWat—a global water balance model to assess water use in irrigated agriculture. Hydrol Earth Syst Sci. 2015;12(1):801–38.
32. Hou S, Sun Z, Li Y, Wang Y, Ling H, Xing G, et al. Transcriptomic analysis, genic SSR development, and genetic diversity of proso millet (*Panicum miliaceum*; Poaceae). Appl Plant Sci. 2017;5(7):1600137.
33. Hu X, Wang J, Lu P, Zhang H. Assessment of genetic diversity in broomcorn millet (*Panicum miliaceum* L.) using SSR markers. J Genet Genom. 2009;36(8):491–500.
34. Hunt HV, Badakshi F, Romanova O, Howe CJ, Jones MK, Heslop-Harrison JP. Reticulate evolution in Panicum (Poaceae): the origin of tetraploid broomcorn millet, *P. miliaceum*. J Exp Bot. 2014;65(12):3165–75.
35. Hunt HV, Campana MG, Lawes MC, Park YJ, Bower MA, Howe CJ, et al. Genetic diversity and phylogeography of broomcorn millet (*Panicum miliaceum* L.) across Eurasia. Mol Ecol. 2011;20(22):4756–71.
36. Hunt HV, Moots HM, Graybosch RA, Jones H, Parker M, Romanova O, et al. Waxy phenotype evolution in the

allotetraploid cereal broomcorn millet: mutations at the GBSSI locus in their functional and phylogenetic context. Mol Biol Evol. 2013;30(1):109–22.
37. Hunt HV, Vander Linden M, Liu X, Motuzaite-Matuzeviciute G, Colledge S, Jones MK. Millets across Eurasia: chronology and context of early records of the genera Panicum and Setaria from archaeological sites in the Old World. Veg Hist Archaeobot. 2008;17(1):5.
38. IIMR. http://www.millets.res.in/millets_info.php. Accessed 10 April 2020.
39. Indiastat. Agriculture-data. https://www.indiastat.com/agriculture-data/2/agricultural-production/225/small-millets/968772/stats.aspx. Accessed 21 May 2020.
40. Jain A, Amrita T. Evaluation of promising genotypes of proso millet against banded leaf and sheath blight disease caused by *Rhizoctonia solani* Kuhn. Mysore J Agric Sci. 2013;47(3):648–50.
41. Joel JA, et al. A fundamentals of crop botany: directorate of open and distance learning. Coimbatore: TNAU; 2006.
42. Juliano B. The chemical basis of rice grain quality. Chemical aspects of rice grain quality. 1979:69–90.
43. Kalaisekar A, Padmaja P, Bhagwat V, Patil J. Insect pests of millets: systematics, bionomics, and management. Cambridge: Academic Press; 2016.
44. Kalinova J, Moudry J. Content and quality of protein in proso millet (*Panicum miliaceum* L.) varieties. Plant Foods Hum Nutr. 2006;61(1):43.
45. Karam D, Westra P, Niessen SJ, Ward SM, Figueiredo JEF. Assessment of silver-stained AFLP markers for studying DNA polymorphism in proso millet (*Panicum miliaceum* L.). Braz J Bot. 2006;29(4):609–15.
46. Karam D, Westra P, Nissen S, Ward S, Figueiredo J. Genetic diversity among proso millet (*Panicum miliaceum*) biotypes assessed by AFLP technique. Plantadaninha. 2004;22(2):167–74.
47. Kumari K, Muthamilarasan M, Misra G, Gupta S, Subramanian A, Parida SK, et al. Development of eSSR-markers in Setariaitalica and their applicability in studying genetic diversity, cross-transferability and comparative mapping in millet and non-millet species. PLoS ONE. 2013;8(6):e67742.
48. Kushwaha H, Jillo KW, Singh VK, Kumar A, Yadav D. Assessment of genetic diversity among cereals and millets based on PCR amplification using Dof (DNA binding with One Finger) transcription factor gene-specific primers. Plant Syst Evol. 2015;301(2):833–40.
49. Lágler R, Gyulai G, Humphreys M, Szabó Z, Horváth L, Bittsánszky A, et al. Morphological and molecular analysis of common millet (*P. miliaceum*) cultivars compared to an aDNA sample from the 15th century (Hungary). Euphytica. 2005;146(1–2):77–85.
50. Léder I. Sorghum and millets. In: Cultivated plants, primarily as food sources. 2004;1:66–84.
51. Liu C, Kong Z, Lang S. Plant remains at the Dadiwan site and a discussion of human adaptation to the environment. Zhongyuan-Wenwu. 2004;4:26–30.
52. Liu M, Xu Y, He J, Zhang S, Wang Y, Lu P. Genetic diversity and population structure of broomcorn millet (*Panicum miliaceum* L.) cultivars and landraces in China based on microsatellite markers. Int J Mol Sci. 2016;17(3):370.
53. Lu H, Yang X, Ye M, Liu K-B, Xia Z, Ren X, et al. Millet noodles in late Neolithic China. Nature. 2005;437(7061):967–8.
54. Lu H, Zhang J, Liu K-B, Wu N, Li Y, Zhou K, et al. Earliest domestication of common millet (*Panicum miliaceum*) in East Asia extended to 10,000 years ago. Proc Natl Acad Sci. 2009;106(18):7367–72.
55. Luis E, Sullivan T, Nelson L. Nutritional value of proso millets, sorghum grains, and corn in turkey starter diets. Poult Sci. 1982;61(2):321–6.
56. Lyon DJ, Burgener PA, DeBoer K. EC08-137 producing and marketing proso millet in the great plains. 2008.
57. McSweeney M. Proso millet as an ingredient in foods common to North Americans 2014.
58. Mohamed T, Issoufou A, Zhou H. Antioxidant activity of fractionated foxtail millet protein hydrolysate. Int Food Res J. 2012;19(1):207.
59. M'ribu H, Hilu K. Detection of interspecific and intraspecific variation in Panicum millets through random amplified polymorphic DNA. Theor Appl Genet. 1994;88(3–4):412–6.
60. Nandini C, Bhat S. Modified crossing (SMUASB) method for artificial hybridization in proso millet (*Panicum miliaceum* L.) and Little millet (*Panicum sumatrense*). Electron J Plant Breed. 2019;10(3):1161–70.
61. Nelson L. Technique for crossing proso millet 1. Crop Sci. 1984;24(1):205–6.
62. Nie X, Zhao X, Wang S, Zhang T, Li C, Liu H, et al. Complete chloroplast genome sequence of Broomcorn Millet (*Panicum miliaceum* L.) and comparative analysis with other Panicoideae species. Agronomy. 2018;8(9):159.
63. Nishizawa N, Fudamoto Y. The elevation of plasma concentration of high-density lipoprotein cholesterol in mice fed with protein from proso millet. Biosci Biotechnol Biochem. 1995;59(2):333–5.
64. Park K-O, Ito Y, Nagasawa T, Choi M-R, Nishizawa N. Effects of dietary Korean proso-millet protein on plasma adiponectin, HDL cholesterol, insulin levels, and gene expression in obese type 2 diabetic mice. Biosci Biotechnol Biochem. 2008;72(11):2918–25.
65. POPOV G. The importance of cross pollination in plant breeding. Liet. zemdirb.mohsl.tyrimo inst.darbai. 1970;14:23–30.
66. Rajput SG, Plyler-Harveson T, Santra DK. Development and characterization of SSR markers in proso millet based on switchgrass genomics. Am J Plant Sci 2014;5(1):175–86.
67. Rajput SG, Santra DK, Schnable J. Mapping QTLs for morpho-agronomic traits in proso millet (*Panicum miliaceum* L.). Mol Breed. 2016;36(4):37.
68. Rao BR, Nagasampige MH, Ravikiran M. Evaluation of nutraceutical properties of selected small millets. J Pharm Bioallied Sci. 2011;3(2):277.
69. Rao M. The small millets: their importance, present status and outlook. Small Millets. 1989.
70. Rose DJ, Santra DK. Proso millet (*Panicum miliaceum* L.) fermentation for fuel ethanol production. Ind Crops Prod. 2013;43:602–5.
71. Sabir P, Ashraf M, Akram N. Accession variation for salt tolerance in proso millet (*Panicum miliaceum* L.) using leaf proline content and activities of some key antioxidant enzymes. J Agron Crop Sci. 2011;197(5):340–7.
72. Saha D, Gowda MC, Arya L, Verma M, Bansal KC. Genetic and genomic resources of small millets. Crit Rev Plant Sci. 2016;35(1):56–79.
73. Sampath T, Razvi S, Singh D, Bondale K. Small millets in Indian agriculture. In: Small millets in global agriculture. 1989:33–44.
74. Santra D, Heyduck R, Baltensperger D, Graybosch R, Nelson L, Frickel G, et al. Registration of 'Plateau'waxy (amylose-free) proso millet. J Plant Regist. 2015;9(1):41–3.
75. Santra DK, Khound R, Das S. Proso millet (*Panicum miliaceum* L.) breeding: progress, challenges and opportunities. Advances in plant breeding strategies: cereals. Berlin: Springer; 2019. p. 223–57.
76. Seghatoleslami M, Kafi M, Majidi E. Effect of drought stress at different growth stages on yield and water use efficiency of five proso millet (*Panicum miliaceum* L.) genotypes. Pak J Bot. 2008;40(4):1427–32.
77. Soldatov A, Agafonov N. Susceptibility of Panicum millet to melanosis in western Kazakhstan. Trudy po Prikladnoĭ Botanike, Genetike i Selektsii. 1980;69(1):64–9.

78. Subramanian V, Metta V. Sorghum grain for poultry feed. Technical and institution options for sorghum grain mold management proc international consultation. In: Chandrasher A, Bandyopadhayi R and Hall AJ (eds) International crop research for the semiarid tropics (ICRISAT) patacheru. 2000:502–4.
79. Taniguchi M, Sugiyama T. Isolation, characterization and expression of cDNA clones encoding a mitochondrial malate translocator from *Panicum miliaceum* L. Plant Mol Biol. 1996;30(1):51–64.
80. TNAU agritech portal. http://agritech.tnau.ac.in/agriculture/minormillets_panivaragu.html. Accessed 9 April 2020.
81. Trivedi A, Arya L, Verma M, Verma S, Tyagi R, Hemantaranjan A. Genetic variability in proso millet [*Panicum miliaceum*] germplasm of Central Himalayan Region based on morpho-physiological traits and molecular markers. Acta Plant. 2015;37(2):23.
82. Upadhyaya H, Sharma S, Gowda C, Reddy VG, Singh S. Developing proso millet (*Panicum miliaceum* L.) core collection using geographic and morpho-agronomic data. Crop Pasture Sci. 2011;62(5):383–9.
83. Upadhyaya HD, Vetriventhan M. Underutilized climate-smart nutrient rich small millets for food and nutritional security. 2018. 109–120.
84. Upadhyaya HD, Vetriventhan M, Dwivedi SL, Pattanashetti SK, Singh SK. Proso, barnyard, little, and kodo millets. In: Genetic and genomic resources for grain cereals improvement. Elsevier; 2016. p. 321–43.
85. Varshney RK, Sinha P, Singh VK, Kumar A, Zhang Q, Bennetzen JL. 5Gs for crop genetic improvement. Curr Opin Plant Biol. 2020;13:1–7.
86. Vetriventhan M, Upadhyaya HD. Diversity and trait-specific sources for productivity and nutritional traits in the global proso millet (*Panicum miliaceum* L.) germplasm collection. Crop J. 2018;6(5):451–63.
87. Vilas ATVB, Kannababu N, Elangovan M, Umakanth AVRK, et al. Millet seed technology: seed production, quality control & legal compliance. Hyderabad: Society for Millets Research and ICAR-Indian Institute of Millets Research; 2015.
88. Wang R, Hunt HV, Qiao Z, Wang L, Han Y. Diversity and cultivation of broomcorn millet (*Panicum miliaceum* L.) in China: a review. Econ Bot. 2016;70(3):332–42.
89. Wu Y, Du J, Wang X, Fang X, Shan W, Liang Z. Computational prediction and experimental verification of miRNAs in *Panicum miliaceum* L. Sci China Life Sci. 2012;55(9):807–17.
90. Yue H, Wang L, Liu H, Yue W, Du X, Song W, et al. De novo assembly and characterization of the transcriptome of broomcorn millet (*Panicum miliaceum* L.) for gene discovery and marker development. Front Plant Sci. 2016;7:1083.
91. Yue H, Wang M, Liu S, Du X, Song W, Nie X. Transcriptome-wide identification and expression profiles of the WRKY transcription factor family in Broomcorn millet (*Panicum miliaceum* L.). BMC Genom. 2016;17(1):343.
92. Zhang J, Lu H, Liu M, Diao X, Shao K, Wu N. Phytolith analysis for differentiating between broomcorn millet (*Panicum miliaceum*) and its weed/feral type (*Panicum ruderale*). Sci Rep. 2018;8(1):1–9.
93. Zhang L, Liu R, Niu W. Phytochemical and antiproliferative activity of proso millet. PLoS ONE. 2014;9(8):e104058.
94. Zhao Z. Flotation results from the Xinglonggou site, North China and the problem of dryland agricultural origins. Antiquities of Eastern Asia. 2005:188–99.
95. Zhou Y, Qu Y, Zhu M, Liu J, Wang Y, Song H, et al. Genetic diversity and virulence variation of Sporisorium destruens isolates and evaluation of broomcorn millet for resistance to head smut. Euphytica. 2016;211(1):59–70.
96. Zohary D, Hopf M, Weiss E. Domestication of Plants in the Old World: the origin and spread of domesticated plants in Southwest Asia, Europe, and the Mediterranean Basin. Oxford University Press on Demand; 2012.
97. Zou C, Li L, Miki D, Li D, Tang Q, Xiao L, et al. The genome of broomcorn millet. Nat Commun. 2019;10(1):1–11.