



Improvement of small seed for big nutritional feed

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Abstract Exploding global population, rapid urbanization, salinization of soils, decreasing arable land availability, groundwater resources, and dynamic climatic conditions pose impending damage to our food security by reducing the grain quality and quantity. This issue is further compounded in arid and semi-arid regions due to the shortage of irrigation water and erratic rainfalls. Millets are gluten (a family of proteins)-free and cultivated all over the globe for human consumption, fuel, feed, and fodder. They provide nutritional security for the under- and malnourished. With the deployment of strategies like foliar spray, traditional/marker-assisted breeding, identification of candidate genes for the translocation of important minerals, and genome-editing technologies, it is now tenable to biofortify important millets. Since the bioavailability of iron and zinc has been proven in human trials, the challenge is to make such grains accessible. This review encompasses nutri-

tional benefits, progress made, challenges being encountered, and prospects of enriching millet crops with essential minerals.

Keywords Biofortification · Malnourishment · Millets · Micronutrient deficiency · Translocation of minerals

Introduction

Global food and nutritional security are under threat with an ever-increasing population, changing climatic conditions, and dwindling natural resources. Plants are exposed frequently to both biotic and abiotic stresses due to their sedentary nature. Both these devastating stresses drastically reduce the biomass and final yields. There are 50,000 edible crops (Cheng et al. 2017), but food production and consumption are mostly dominated by rice, wheat, and maize, thus limiting our ability to deal with adverse conditions and nutritional security at the global level. Millets, often called “famine reserves,” are endowed with climate-resilience and acclimatization to a broad range of

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ecological conditions, besides less irrigational requirements, optimal growth, and productivity under arid and semi-arid zones. Among many, pearl millet (Varshney et al. 2017), foxtail millet (Bennetzen et al. 2012; Bhat et al. 2018), finger millet (Hittalmani et al. 2017; Hatakeyama et al. 2018), proso millet (Zou et al. 2019), and teff have been sequenced (Cannarozzi et al. 2014).

People in the Asian continent are deficient in micronutrients like Fe^{2+} , Zn^{2+} , Ca^{2+} , as well as vitamin A. Millets, are a good source of carbohydrates, proteins, fats, fibers, essential amino acids, and minerals such as Ca^{2+} , K^+ , Fe^{2+} , Zn^{2+} , selenium (Se) (Saleh et al. 2013). Some millets accumulate Se (Table 1) which is an essential trace mineral, known to be a constituent in the biosynthesis of a number of selenoproteins and has been considered as an important antioxidant molecule in human health. Selenocysteine has been recognized as the 21st amino acid and performs several enzymatic functions in the human body. Se assists in modulating intracellular redox state via selenium-dependent glutathione peroxidases, hence prevent the risk of cancer and give protection against peroxynitrite (Allan et al. 1999). Foxtail millet accumulates 100.3 μg of Se per Kg seed weight in many Chinese accessions compared to other millets (Table 1). They form staple food, furnish most of the calories and protein in arid and semi-arid tropics, thus providing nutritional security in areas prone to drought and heat stresses. Millets display stress adaptations viz., flowering adjusted to rainfall, increased root length during the drought, accumulation of osmolytes, enhanced antioxidants in little millet (Ajithkumar and Panneerselvam 2014), and low Pi conditions in foxtail millet (Roch et al. 2020). Since millets can provide succor to the subsistence farmers, it is important to surpass the current yield limits, biotic and abiotic stress tolerance too. This review focuses on the nutritional aspects, biofortification, bioavailability, antinutrients, uptake and translocation of minerals, and their role in the accumulation in millet crops.

Millets: productivity, nutrition and medicinal values

Millets rank sixth among cereal grains and are consumed by one-third of the global population. Asia and Africa constitute 97.6% of global production, Africa is the leading producer of millets (Habiyaemye et al. 2017), followed by Asia, Europe, the Americas, and Oceania. The global millet production is about 28.37 million tons from 31.65 million hectares (FAOSTAT 2019). India ranks first in the production of millets with 10.23 million tons, followed by Niger, China, Nigeria, Mali, and Sudan with 3.27, 2.3, 2.0, 1.87, and 1.13 million tons respectively (FAOSTAT 2019).

In India, Assam and Bihar consume the highest amounts of small millets (18.82 kg/hsh/m, and 18.69 kg/hsh/m respectively). Madhya Pradesh has nearly 32.4% of the small millet grown area, followed by Chhattisgarh (19.5%), Uttarakhand (8%), Maharashtra (7.8%), and Gujarat (5.3%). But productivity is high in Uttarakhand (1174 kg/ha), followed by Tamil Nadu (1067 kg/ha) and Gujarat (1056 kg/ha) (Anbukkani et al. 2017). Several millets have high fiber content and can become good components in the diets of patients suffering from obesity, constipation, and gallstones. Millets are rich in a unique type of resistant starch (RS) that has been recognized as a functional fiber that maintains a low glycemic index. There are four types of RS; physically inaccessible starch (type RS1), native granular starch (RS2), retrograded starch (RS3), chemically and thermally modified starch (RS4). In digestive physiology, functional fibre plays an important role. When RS is fermented in the stomach, the metabolic products generated help in the proper functioning of the bowel. RS is similar to soluble, fermentable fiber, and assists in feeding the friendly bacteria in the human gut with an enhanced production of short-chain fatty acid like butyrate. Butyrate plays a key role in gastrointestinal health; helps prevent and treat colon cancer. RS helps with weight loss and beneficial heart health, lowers blood pressure, manages blood sugar levels, insulin sensitivity, and digestive health (Mikulikova et al. 2005). Such information helps in formulating functional food products which can cater to the needs of diverse target groups including marginal farmers. In this review, we considered grain millets, but not *Panicum maximum* (Guinea grass) and *Paspalum notatum* (Bahia grass) since they are used mostly for growing lawns or fodder.

- (a) *Cenchrus americanus* [(L.) R. Br.] (formerly known as *Pennisetum glaucum* [(L.) R. Br.] (Pearl millet) Pearl millet is an allogamic, diploid species with $2n = 2x = 14$ chromosomes (Table 2), and provides food security to the poor people of Asia and Africa. Nearly, 31 million hectares of land are being utilized for growing pearl millet and 90 million people depend upon it for food and income. It occupies 50% of the market share of global millet production. Its production in India is approximately 9 million tons, from a cultivated area of ~ 8.5 million hectares during the years 2009–2010 to 2013–2014 (Agricultural Statistics, GOI 2014). India is the largest producer with a total production of 9.1 million metric tons from 7.4 million ha. It displays anti-allergic properties and hence can become a part of daily dietary habits for infants, lactating mothers, and convalescents. It has a 600% higher iron content than rice (Passi and Jain 2014), and is a good source

Table 1 Minerals, vitamins and amino acids present in diverse millets

Millet	Minerals (mg/100 g of seed)	Vitamins (mg/100 g seed) (unless otherwise mentioned)	References
Pearl millet	Zn (3.1), Fe (70–180), Ca (10–80), Cu (0.54), Se (5.4 micro grams/100 g seed weight), K (440–442)	Folic acid, thiamine (0.3), riboflavin (1.48), niacin (1.11), pantothenic acid (0.5), biotin (0.64)	Passi and Jain (2014), Malik (2015), Rao et al. (2017) and Kumar et al. (2018)
Finger millet	Zn (36.6), Fe (3.6), Ca (376–515), Cu (0.67), Se (0.5 mg in the variety Pwana), K (408–570)	Thiamine (0.4), riboflavin (0.6), niacin (0.8), pantothenic acid (0.29), biotin (0.88)	Fernandez et al. (2003) and Saleh et al. (2013)
Foxtail millet	Zn (60.6), Fe (2.80), Ca (31), Cu (1.4), Se (100.3 µg/Kg) in Chinese accessions, K (250–400)	Vitamin D, Thiamine (0.6), riboflavin (1.65), niacin (0.55), lysine (2.3–2.9)	Puranik et al. (2017), Rao et al. (2017) and Kumar et al. (2018)
Proso millet	Zn (3.7), Fe (0.8), Ca (14), Cu (1.6), Se (2.70 µg), K (250–320)	Thiamine (0.41), riboflavin (0.28), niacin (4.54), folic acid Essential amino acids like methionine and cysteine, and lecithin (supports neural health)	Chandel et al. (2014), Shankaramurthy and Somannavar (2019) and Das et al. (2019)
Little millet	Zn (3.7), Fe (9.3), Ca (350), Cu (0.34), K (129–370)	Thiamine (0.26), riboflavin (0.05), niacin (1.29), pantothenic acid (0.6), biotin (6.03), rich in methionine, valine, lysine	Chandel et al. (2014), Mbithi-Mwikya et al. (2000) and Himanshu et al. (2018)
Kodo millet	Zn (32.7), Fe (0.5), Ca (27), Cu (0.26), K (144–170)	Thiamine (0.15), riboflavin (2), niacin (0.09), pantothenic acid (0.63), biotin (1.49)	Chandel et al. (2014), Kulkarni and Naik (2000), Rao et al. (2017), Kumar et al. (2018) and Himanshu et al. (2018)
Barnyard millet	Zn (57.45), Fe (6.91), Ca (11) Cu (0.6)	Thiamine (0.33), riboflavin (4.2), niacin (0.1)	Chandel et al. (2014), Rao et al. (2017) and Kumar et al. (2018)
Teff millet	Ca (180), Fe (7.63), Mg (184), P (429), K (427), Na (12), Zn (3.63)	Niacin (3.363), vitamin B6 (0.482), thiamine (0.39), riboflavin (0.27), vitamin K (phylloquinone) (1.9 µg), vitamin A (9 IU), and α-tocopherol (0.08)	Geburu et al. (2020) USDA Food Composition Databases (2017)
Fonio millet	Mg (2.53), Mn (0.03), Zn (0.45), Fe (0.57), Cu (0.05), Na (4.70), Ca (19.70), K (26.53), P (13.42)	Thiamin (1.50), riboflavin (0.22), niacin (1.15)	Sanusi et al. (2019) Barikmo et al. (2007) Zhu (2020)

of antioxidants. Nutrients of pearl millet play a role in the prevention of diabetes, cancer, cardiovascular and neurodegenerative diseases. Thus, the potential exists for harnessing the positive attributes of pearl millet that help in nutritional security.

- (b) *Eleusine coracana* (Finger millet)
Finger millet or African finger millet or Coracan or Koracan or Ragi is an important cereal crop of temperate and semi-arid climates. It is an allotetraploid ($2n = 4X = 36$) crop (Table 2) and is commonly called finger millet due to finger-like branching on the panicle. Finger millet is the 6th most important cereal crop and its global production is about 4.5 million metric tons. India ranks first in production with 1.2 million metric tons after Africa with 2.5 million metric tons (Kumar et al. 2016). It is an under-utilized crop for many rural areas of poor nations besides being accounted for 85% of millets produced in India (Sakamma et al. 2018). Out of 34,160 finger millet genotypes, India has 22,583
- (c) *Setaria italica* (foxtail millet)
Setaria italica or *Panicum italicum* (Foxtail millet or Italian millet or German millet or Hungarian millet or Green foxtail, or Foxtail bristle-grass or Giant setaria or Dwarf setaria) has originated in China and is an important millet in China and India. Taxonomically, the genus *Setaria* comprises two subspecies namely, *S. italica* and *S. viridis*. Based on phenotypic features, three races viz., Moharia, Maxima, and Indica have been identified (Rao et al. 1987). It is a self-pollinating crop with chromosome number $2n = 18$ (Brutnell et al. 2015) (Table 2). It was domesticated in Northern parts of China nearly 9000–6000 years before the present. *S. italica* and *S. viridis* (wild) have a small genome size (515 Mb),

Table 2 Chromosome number, genotype sequenced, genome sizes, and number of genes in some millets

Name of the millet	Chromosome number	Genotype sequenced	Proximate genome size	Proximate number of genes	References
Pearl millet	2n = 2x = 14 (Diploid)	–	–	–	Martel et al. (1997)
Finger millet	2n = 4x = 36 AABB (Tetraploid)	Tift 23D ₂ B ₁ -P1-P5	1.79 Gb	38,579	Varshney et al. (2017)
		ML-365	1196 Mb	85,243	Hittalmani et al. (2017)
		PR202	1500 Mb	62,348	Hatakeyama et al. (2018)
Foxtail millet	2n = 2x = 18 (Diploid)	–	–	–	Goron and Raizada (2015)
		Yugu I	510 Mb	24,000–29,000	Wanous (1990) Bennetzen et al. (2012)
Proso millet	2n = 4x = 36 (Tetraploid)	Zhang gu	423 Mb	38,801	Bhat et al. (2018)
		–	–	–	Baltensperger (1996)
		Land race	923 Mb	55,930	Zou et al. (2019)
		Accession No. 00000390	887.8 Mb	63,671	Zou et al. (2019)
Little millet	2n = 4x = 36 (Tetraploid)	–	–	–	Kubesova et al. (2010)
		Longmi4	1020.5 Mb	–	Wanous (1990)
Kodo millet	2n = 4x = 40 (Tetraploid)	–	–	–	Burton (1940)
		–	–	–	Hiremath and Dandin (1975)
Indian Barnyard millet	2n = 6x = 54 (Allohexaploid)	Not known	1.91–1.98 Mb	Not known	Jarret et al. (1995)
		–	–	–	Wanous (1990)
		–	2.7 Mb	Not known	Renganathan et al. (2020) Abrahamson et al. (1973)
Japanese Barnyard millet	2n = 6x = 54 (Allohexaploid)	–	–	–	Renganathan et al. (2020)
Teff millet	2n = 4x = 40 (Allotetraploid)	–	–	–	Hundera et al. (2000)
		Tsedey cultivar (DZ-Cr-37)	772 Mb representing 87% of the total tef genome size	Not known	Cannarozzi et al. (2014)
		Land race Dabbi	622 Mb	68,255	VanBuren et al. (2020)
Fonio millet	2n = 4 × = 36 (Allotetraploid)	–	–	–	Adoukonou-Sagbadja et al. (2007)
		Accession number CM05836	893 Mb	57,021	Abrouk et al. (2020)
		Niatia v1.0	760.66 Mb	67,855 (protein coding genes)	Wang et al. (2021)

short life-cycle with a high potential for abiotic stress tolerance. The crop is grown in 26 countries including China, India, Bangladesh, Syria, and Africa, and ranks second in total world production of millets with 6 million tons of food in Europe, and Asia (Nadeem et al. 2018). It contains high Zn that can boost immunity in humans. Regular consumption of foxtail millet helps to fight diseases like osteoporosis and reduce the risk of bone fracture. It regulates cardiac function, Alzheimer's disease, enhances memory, and helps to strengthen muscles (Sharma and Niranjana 2018).

(d) *Panicum miliaceum* (Proso millet)

Proso millet or Broomcorn millet or Hog millet or Hershey millet or Common millet or Russian millet is the first domesticated tetraploid ($2n = 4x = 36$) millet (Hunt et al. 2014) (Table 2). It is cultivated in half a million acres of semi-arid regions of China, India, Nepal, Russia, Ukraine, Belarus, Turkey, Romania, and the United States of America (Yang et al. 2019). The crop was first domesticated before 10,000 BCE in the Northern parts of China, and the estimated genome sequence is around 920 megabases (Zou et al. 2019). It is a gluten-free, warm seasonal crop used as human food, feed for birds, forage, and bioprocessing purposes (Yang et al. 2019), and has significant amounts of essential amino acids.

(e) *Panicum sumatrense* (Little millet)

Panicum sumatrense (Little millet or Indian millet) is grown in both tropical and temperate zones, cultivated in China, India, Malaysia, East Asia, and the Caucasus, and can sustain both waterlogging and drought conditions. It is a tetraploid with a chromosome number of $2n = 4x = 36$ (Table 2) and domesticated in India as a part of tribal agriculture (de Wet et al. 1983). Grains are highly nutritious, used for the consumption of humans, as birdseed, and for alcohol production. It has 350 mg of calcium per 100 g of seed weight which is 8-times higher than wheat (Srilekha et al. 2019). It is used as fodder for cattle and also for alcohol production (Kumar et al. 2016).

(f) *Paspalum scrobiculatum* (Kodo millet)

Native *Paspalum* or Kodo millet or Ditch millet or Cow grass or Rice grass or Indian crown grass, African bastard millet grass is native to South America and domesticated 3000 years ago in India (de Wet et al. 1983). *Paspalum scrobiculatum* var. *scrobiculatum* is a tetraploid species ($2n = 4x = 40$) and grows in India (Table 2), while *Paspalum scrobiculatum* var. *commersonii* is the wild relative indigenous to Africa (Heuze et al. 2015). In India, it grows in Kerala, Tamil Nadu, Rajasthan, Uttar

Pradesh, and West Bengal, cooked as flour in India and whole grain in Africa and used as animal fodder for cattle, goats, pigs, sheep, and poultry. It has the highest dietary fiber among cereals (Mohamed et al. 2009) and contains lecithin that aids in strengthening the nervous system. A small amount of prussic acid has been found in leaves and kernels. Crude extracts of leaves have antifungal and tranquilizing activities (Mishra et al. 2000).

(g) *Echinochloa esculenta* (Japanese barnyard millet) and *E. frumentaceae* (Indian barnyard millet)

Barnyard millet or Japanese barnyard millet mainly grows in Japan, India, China as food and fodder with a maturation time of around 50–70 days (Hulse et al. 1980). The genus *Echinochloa* is an allohexaploid with $2n = 6x = 54$ chromosomes (Table 2) and has two species, one is *E. esculenta* and another *E. frumentaceae*. This millet is grown in some pockets of India, Pakistan, and Nepal. Barnyard millet has the lowest carbohydrate and the highest crude fiber (13.6 g/100 g) (Saleh et al. 2013). The iron content is more than the daily recommended dietary allowance in men (8.7 mg) and women (14.8 mg) aged between 19 and 50 years. It is a domesticated millet in the semi-arid tropics of Asia and Africa, with 8,000 accessions available in Japan and India. In India, ICRISAT Genebank conserves 749 accessions (Upadhyaya et al. 2014). Japanese barnyard millet contains antioxidative phenolic acids such as N-(p-coumaroyl) serotonin, luteolin and tricetin (flavonoids). While the antioxidative activity of luteolin is equal to that of quercetin, tricetin activity is lower than that of luteolin (Watanabe 1999). Besides serotonins, phytates, phenols, and tannins contribute to the antioxidant activity. Antioxidative molecules keep up human health, slow down the process of aging and maintain proper metabolism.

(h) *Eragrostis tef* (Zucc.) Trotter or teff millet

Eragrostis tef (Zucc.) Trotter or teff is a self-pollinated, warm-season millet. It is an allotetraploid ($2n = 4x = 40$) species (Table 2), originated in the Horn of Africa, and grows in marginal soils under low rain-fed conditions in arid and semi-arid regions (Tadele and Assefa 2012). In Ethiopia, the yields of teff are low with an average of 1.7 tons per hectare (Cochrane and Bekele 2018). It is cultivated in 2.9 million hectares, with an average production of 4.5 million tons (Numan et al. 2021). Teff is a gluten-free, nutraceutical food, rich in carbohydrates (57.27%), protein (20.9%), fat (0.5%), fiber (2.8%), and amino acids (8.15%). Seeds of teff are 22% rich in unsaturated fatty acids (72.46%), with oleic and linolenic acid contents in fairly good amounts

(32.41% and 23.83% respectively). The seed oil contains anti-hyperlipidemic and anti-hyperglycemic activities (El-Alfy et al. 2012). Teff contains flavonoids, saponins, tannins, glycosides, and steroids, and grains have been recommended against celiac disease (triggered by eating gluten), hypertension, anemia, diabetes, and cancer (Akansha and Chauhan 2020).

(i) *Digitaria exilis* (Kippist) Stapf and *Digitaria iburu* Stapf or fonio millet

Digitaria exilis (Kippist) Stapf and *Digitaria iburua* Stapf are known as white and black fonio millets respectively (husks are white and dark brown in colour and hence the names). They are African orphan millets, with a very short life cycle and are often referred to as women's crop (Haq and Ogbe 1995; Small 2015). They are domesticated by West African tribes in marginal lands as staple food dating back to 5 millennia BC (Murdock 1959; Larson et al. 2014). *Digitaria exilis* is an allotetraploid (Table 2), and the number of chromosomes is $2n = 4x = 36$ (Adoukonou-Sagbadja et al. 2007). Seeds of fonio are called the “grain of life” since they have good culinary and nutritional properties. Grains of both *D. exilis* and *D. iburua* are gluten-free, containing 74.4 and 75.6 g of carbohydrates, 7.1 and 8.9 g of protein per 100 g of seed weight, and 7.4 and 6.2 g dietary fiber respectively (Vodouhe et al. 2007). Grains are rich in sulphur containing essential amino acids like methionine and cystine, cure coeliac disease, good for lactating women and also diabetic patients. Abrouk et al. (2020) established high-quality genomic resources including chromosome-scale reference assembly as well as deep re-sequencing of 183 cultivated and wild species which ultimately helps its improvement through molecular breeding.

Millets serve as a natural source of antioxidants in food applications and also as a nutraceutical, abode highly valued mineral elements like Ca^{2+} , K^+ , Fe^{2+} , Zn^{2+} , and Se, besides vitamins, essential fatty acids, amino acids, and antioxidative compounds (Table 2). Cultivation of millets in dry or marginal lands makes them productive and at the same time ensures the security of food and nutrition to the future generations especially under climate-smart agriculture (Chandel et al. 2014; Kumar et al. 2018). Given their health benefits, their daily usage must be promoted for the well-being of society especially in resource-poor areas.

Biofortification of minerals in millets

Stunting, wasting, and being underweight/overweight are some of the indicators of malnutrition globally in children. Nearly half of the children in Asian continent are affected by wasting live. Similarly, half of the countries have experienced overweight (UNICEF/WHO/The World Bank 2021). Fe and Zn deficiencies are widespread and affect 2 billion people on the global scale (Webb et al. 2018). Among African populations, the risk for micronutrient deficiencies is 54% for Ca^{2+} , 40% for Zn, 28% for Se, 19% for iodine, and 5% for Fe (Joy et al. 2014). Fe deficiency can lead to severe microcytic anemia and impaired immune function (Bailey et al. 2015). These circumstances necessitate addressing the problem of malnutrition immediately by producing staple food crops like millets with enriched minerals and also their accessibility to the mal/undernourished. Biofortification is an economically feasible food-based strategy to address the problem of “hidden hunger” by bringing nutrient-dense crops to the doorsteps of the under- and malnourished in resource-poor areas (Bouis et al. 2011). The Harvest Plus-Consultative Group for International Agricultural Research (CGIAR) Micronutrients project's Biofortification Challenge Program (BCP) has targeted seven major staple crops with three important micronutrients like Fe, Zn, and vitamin A (Welch and Graham 2004). Fe and Zn deficiencies are the most significant, impacting more than three billion people worldwide specifically from underdeveloped countries (Chasapis et al. 2012; Webb et al. 2018; Kramer and Allen 2015). Pearl millet outperforms rice and wheat in terms of nutritional values like fibre, Ca^{2+} , Fe^{2+} , Zn^{2+} , phosphorous (P), and potassium (K^+) contents (Saleh et al. 2013). They grow and perform well under dry, semi-arid, and drought-prone environments. Millets have been chosen as an attractive option for genetic improvement of biofortification since they form as staple sources of calories/energy in resource-poor countries of Africa and Asia, mainly in rural areas (Vijayakumari et al. 2003; Manwaring et al. 2016).

Strategies to improve biofortification and exploitation of natural genetic variation

To develop biofortified crops, (a) screening the genetic resources for high and low micronutrient contents (b) supplementation of minerals as organic supplements/manure into the soil (c) foliar application (d) traditional plant breeding methods, and speed breeding, (e) molecular breeding including high-throughput genomics, phenotyping, and (f) transgenic/genome-editing approaches (g) improved uptake and transport of micronutrients, nitrogen,

phosphorous, and photoassimilates to the grain filling site (Fig. 1) are the options that can accelerate micronutrient densities in millet grains (Bouis and Welch 2010; Manwaring et al. 2016). In pearl millet, variability existed for Fe and Zn were 35–116 mg/kg, 21–80 mg/kg respectively while protein varied from 6 to 18% (Pujar et al. 2020). Similarly, Zn concentrations in finger millet were assessed and the content ranged from 10 to 86 µg/g weight of grains among the 319 genotypes (Yamunarani et al. 2016). Their results indicate the success of screening and breeding programs for high Fe and Zn utilizing genetic variability. So, germplasm for all millets must be screened first for genetic diversity in grain nutrients especially Fe²⁺, Zn²⁺, Se, Ca²⁺, copper and iodine, and then such a natural variation must be exploited by breeding/speed breeding methods. Using breeding strategies, pearl millet, and finger millet hybrid lines with high Fe (70–75 mg/kg), Zn (35–40 mg/kg) densities have been evolved in India in the background of high yielding character (Govindaraj et al. 2019). Variability in the mineral content exists in the wealth of natural germplasm, but foxtail, kodo, barnyard, teff, and fonio millets did not receive much attention for biofortification though they are being consumed by many in Asia and Africa. To achieve effective biofortification of Fe²⁺, Zn²⁺, Se, and any other micronutrients, molecular breeding, genetic engineering, genome editing coupled with novel agronomic and edaphic management strategies are essential. Such a game plan is imperative for genetic gains.

Biofortification of Ca²⁺, Se, and iodine

Besides Fe and Zn, Ca²⁺ is another important nutrient that provides vascular and muscular contractions, nerve signal transmissions, and fights against osteoporosis. It is essential for protection against breast, colorectal, and prostate cancers (Puranik et al. 2017) and for reduction of adipose tissue, body weight, and diabetes (Parikh and Yanovski 2003; Oei et al. 2013). They assessed some of the challenges associated with Ca²⁺ biofortification in finger millet, it being a rich source. But we still do not have complete knowledge about the potential risks of improving Ca²⁺ levels in millets and weigh it out against the potential benefits and associated changes in the antinutrients which need to be taken care of. There is a need to find out about consumer acceptability coupled with sensory satisfaction with enhanced levels of Ca²⁺ and micronutrients in the millets. Furthermore, market avenues for such special grains with improved nutritional quality (varieties and hybrids) do not exist now, therefore, Governmental organizations and private seed companies need to devise strategies for marketing to address malnutrition (Yadav et al. 2021). Consumption of biofortified pearl millet improved the cognitive outcomes in adolescents with an increase in the bioavailability of Fe and Zn in studies conducted in few states of India (Kodkany et al. 2013), but not from other millets. Similarly, the focus so far has been on improving Fe²⁺ and Zn²⁺, but not other micronutrients like Se, Cu²⁺, Mn²⁺, and iodine. Se is an indispensable element and its deficiency is a key problem globally.

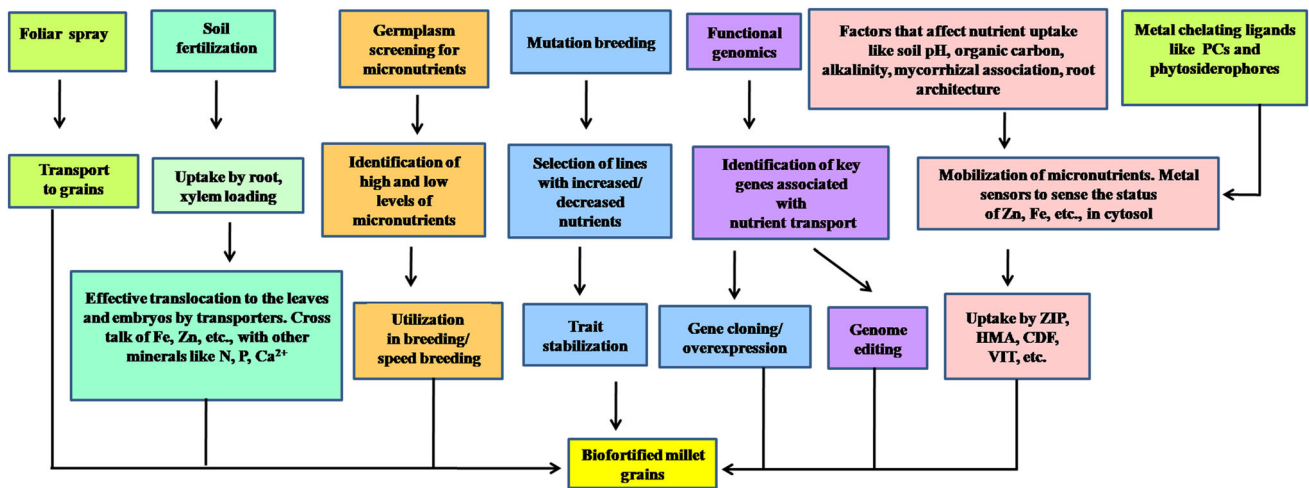


Fig. 1 Strategies that affect accumulation of micronutrients in millets. Diverse strategies like screening the germplasm for high micronutrient content, foliar spray, soil fertilization, traditional breeding/molecular breeding methods, and genetic engineering

approaches help biofortification of millet grains. Ca²⁺—calcium, CDF—cation diffusion facilitator, Fe—iron, HMA—heavy metal ATPase, N—nitrogen, P—phosphorous, PCs—phytochelatin, VIT—vacuolar iron transporter, Zn—zinc, ZIP—zinc-iron-permease

Millets accumulate Se; therefore, they are a rich source of Se nutrition to the consumers. Foliar application of sodium selenite (Na_2SeO_3) in foxtail millet has been investigated by Liang et al. (2020) which has resulted in 9.8-fold enhancement in selenomethionine and selenocysteine with a concurrent increase in K^+ and Fe content. The results infer Se-inducible proteins in foxtail millet that could be useful Se-enriched millets in the years to come. To biofortify Se in millets, nanosized biofortification, and supplementation with multiple micronutrients are recommended (Schiavon et al. 2020). Breeding strategies using high Se lines must be adapted as an alternative approach. Since Fe^{2+} , Zn^{2+} , and Se are an immune boosters, if biofortified food can be used against Corona virus disease or not needs further investigations. Another important micronutrient that is essential to humans is iodine (I), associated with thyroid hormone synthesis, which in turn control metabolism and brain development during pregnancy. Based on the available literature, it appears that the best method for improving Se and iodine is foliar fertilization with Se(VI) as a source (Izydorczyk et al. 2021). The experiments conducted thus far indicate that iodosalicylates and iodobenzoates are ideal for the fortification of iodine. So, millets can be biofortified for iodine and used for promoting human health.

Antinutrients and bioavailability of micronutrients

On the flip side, antinutrients like phytates, polyphenols, protease inhibitors, and tannins limit the mineral accessibility in humans by conjugating multivalent cations like Fe^{2+} , Zn^{2+} , Ca^{2+} , Mg^{2+} , and K^+ (AbdelRahman et al. 2005). Phytate, tannins, oxalates, non-starch polysaccharide glucans affect the digestibility and decrease the bioavailability of Fe, Zn and others. To overcome such issues, strategies like soaking the seeds, autoclaving, debranning, and supplementation of enzymes are being deployed (Tharifkhan et al. 2021). Further, the germplasm of millets has been underutilized, but core collections representing the genetic diversity need to be screened for low levels of antinutrients (Kumar et al. 2016). So, strategies need to be designed specifically to reduce antinutrients like phytate on one hand and to improve the bioavailability of minerals on the other in minor millets. The ICRISAT biofortification program is pursuing a goal by supporting existing high-Fe hybrid parents and advanced breeding lines acquired from the mainstream breeding strategy to generate high-yielding, high-Fe hybrids. De Moura et al. (2014) conducted human trials with staple food crops biofortified by breeding methods. Fe-biofortification interventions among women and children have significantly improved the Fe status, hemoglobin

levels, and serum ferritin concentrations. These experiments indicate that iron-biofortification is efficacious in improving Fe status in the target group who are Fe-deficient. The same groups have also carried out trials using biofortified vitamin A staple crops. The results suggest a positive impact on the functional outcome (De Moura et al. 2014). However, challenges exist in sustainable interventional trials that need to be undertaken on a larger scale among target populations, especially in the resource-poor countries. The experiments also reveal that the bioavailability of Fe and Zn is not a barrier if biofortified millets have to be fed to the target groups.

Mineral uptake and translocation for improved accumulation

For the grains to accumulate micronutrients, transportation of micronutrients and photosynthates from the leaf to the grain filling site is highly crucial. Studies on Zn translocation in 12 finger millet genotypes revealed wide variation in root uptake and transport depending upon the genotypes (Yamunarani et al. 2016). This clearly underlies the importance of variation for uptake and translocation of minerals and also the opportunities to improve grain Zn nutrition using breeding programs. Therefore, natural genetic diversity for Fe and Zn uptake in millets needs to be screened and identified for subsequent utilization. Conditions such as soil redox potential and pH, influence mineral nutrient availability. Fe is rapidly oxidized in soils at higher pH levels, resulting in insoluble ferric oxides. In contrast, at lower pH, ferric Fe is liberated from the oxide, making it accessible for root uptake via the action of a ferric chelate reductase (Marschner and Rimmington 1988). Calcium, calmodulin, and other signal components involved in the signaling networks, and transport of micronutrients and calcium (CAX transporters) to the grain filling site are shown in the Fig. 2. Zhao and McGrath (2009) noticed that Fe and Zn transporters also transport toxic metals like that of Cd. AtIRT1, a divalent metal transporter of the ZIP family, transports Fe into root epidermal cells along with Zn, Mn, Cu, and Ni micronutrients (Korshunova et al. 1999; Krishna et al. 2020). Fe flows into the xylem with the help of citrate complex and subsequently transports into the phloem using nicotianamine (NA) and YELLOW STRIPE-LIKE (YSL) transporters (Morrissey and Guerinot 2009) (Fig. 2). NA transporters play a key role in metal homeostasis and help in the movement of Fe in and out of the phloem via YSL transporters. Since they bind with Cu^{2+} , Ni^{2+} , Co^{2+} , Zn^{2+} , and Mn^{2+} (Curie et al. 2009), their role as transporters of multiple nutrients must be recognized. Hence, improving Fe content in millets, may concomitantly improve the

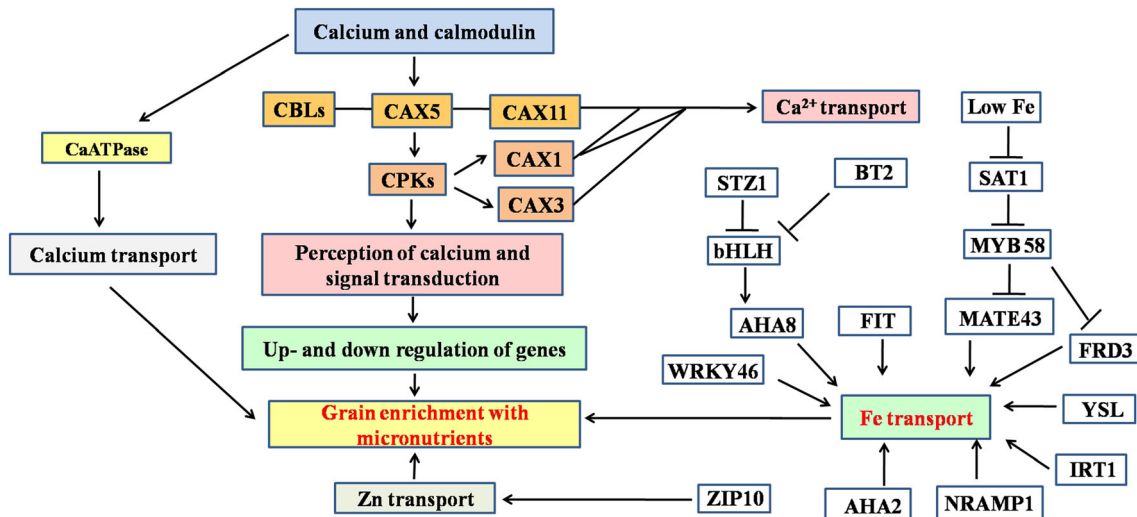


Fig. 2 Calcium, calmodulin and other signal components involved in the signaling networks, and transport of micronutrients to the grain filling site. AHA—plasma membrane H⁺ ATPase, BT—E3 ligase, bHLH—basic helix-loop-helix, CaATPase—calcium ATPase, CAX—cation/H⁺ transporter, CBL—calcineurin B-like protein, CPK—calcium-dependent protein kinase, FIT—FER-like iron deficiency-induced transcription factor, FRD—FERRIC REDUCTASE

DEFECTIVE, IRT—iron regulated transporter, MATE—multidrug and toxin efflux, MYB—myeloblastosis transcription factor, NRAMP—natural resistance-assisted macrophages protein, SAT—symbiotic ammonium transporter, SIZ1—SUMOE3 ligase, WRKY—transcription factors with a motif WRKYGQK at the N-terminus, YSL—yellow stripe 1-like transporter, ZIP—zinc-iron-permease

concentrations of other minor nutrients like Cu²⁺, Ni²⁺, Co²⁺, and Mn²⁺. Zn²⁺ is an essential micronutrient and is available in water soluble form with + 2 oxidation state. Zn²⁺ and Fe²⁺ uptake always compete for NA transporters. Zn²⁺ transport is mediated by a family of ZIP transporters (Fig. 2) from the epidermal cell to xylem via a symplastic pathway and move towards the phloem (Olsen and Palmgren 2014). Besides aiding Zn uptake, zinc-regulated, iron-regulated transporter-like protein (ZIP) family members like *IRT₁* and *IRT₂* also help in Cd uptake (Guerinot 2000), hence caution must be exercised. Se is a vital micronutrient for living organisms as it performs a role in a number of physiological and metabolic processes. Plants are the primary dietary source of Se for humans, since many species may metabolize and accumulate organic Se in edible portions that can be ingested in native or processed forms. Various research findings have explored for Se biofortification of plants in order to develop Se-enriched products and induce Se production (D’Amato et al. 2020). Plant-based Se biofortification has many advantages over the direct Se supplementation as an inorganic form of Se [selenide (Se²⁻), selenite (SeO₂-3SeO₃²⁻), and selenate (SeO₂-4SeO₄²⁻)] is converted to organic form (SeMet and SeCys). While selenite uptake is via phosphate transporters (Li et al. 2008), selenate is transported through sulfate transporters like *SULTR1* and *SULTR1;2* (Takahashi and Saito 2008). Se biofortified plants exhibit high levels of minerals and antioxidants (D’Amato et al. 2020). Since transporters are known, efforts must now aim at improving their translocation and accumulation in the grains of

millet. Attempts must also be made to generate Se biofortified millets that are cost effective and accessible to the malnourished. Iodine is translocated as either iodide or iodate (Mackowiak and Grossl 1999). Both iodosalicylates and iodobenzoates have been found as excellent sources of iodine for the biofortification of *Solanum lycopersicum* (Halka et al. 2019). They studied the uptake of inorganic and organic sources of iodine and found that they are transported to tomato fruits. Among the compounds tested for transport, 2-iodobenzoic acid (2-IBeA) has been efficiently transferred to fruits in the form of iodine. Importantly, it has been found to accumulate in the soluble portions of cells. Inorganic and organic compounds applied to tomato plants have affected not only the expression of the *HMT* gene (encodes halide ion methyltransferase), but also *SAMT* and *S3H* genes (encode salicylic acid carboxyl methyltransferase, and salicylic acid 3-hydroxylase) (Halka et al. 2019). However, these experiments need to be carried out in millets and if they can accumulate iodine, the complications that arise from thyroid during pregnancy can be resolved to a large extent. Importantly, any disruption due to drought and heat stress conditions can impair the uptake and translocation of mineral nutrients and subsequently the nutritional quality of the millet grains. Hence, we should make an endeavour to immediately address the challenges of grain nutritional quality in millets especially in the wake of climate change through traditional and molecular breeding approaches.

Scope exists therefore for the introduction of several candidate genes that alleviate abiotic stresses and at the

same time improve nutritional quality especially grain Fe, Zn, and Se contents. Gaddameedi et al. (2020) studied heterosis and combining ability for grain Fe and Zn concentrations in *Sorghum bicolor*. Mahendrakar et al. (2020) identified 29 candidate genes related to Fe and Zn metabolism in pearl millet which exhibited tissue and developmental stage-specific patterns. These studies provide an insight into the cloning and overexpression of these genes for enhanced production of grain Fe and Zn and their use in breeding programs.

Gene knockout studies and CRISPR gene editing

Studies on CRISPR-Cas9 technology in millets and their improvement are scanty. But, CRISPR-Cas has been effectively employed in several cereal crops and therefore has the potential to boost the grain quality of small seed millets (Fiaz et al. 2019). Since the genome sequences of some of the millets are available, it is possible to identify the target genes for improving productivity alongside mineral nutrients. Genes implicated in higher yields, and grain nutritional qualities have been identified in major cereals, hence, their homologs can be detected in millets. Such genes can be exploited for the improvement of millets using CRISPR-Cas9 technology.

Role of non-coding RNA (ncRNA) regulations into biofortification

Given the limitation of studies on next-generation sequencing in lieu of trait-specific nutrition, there is always a need for strengthening the genetic and genomics screening approaches for better breeding efficiency traits (Vetriventhan et al. 2020). As the genetic variation is best seen in ncRNAs, identifying the differentially expressed genes (DEGs) plays an indispensable role in signaling mechanisms (Budak et al. 2020). Among the ncRNAs, microRNAs (miRNAs) are known to regulate the stress responses and also known to maintain nutritional homeostasis (Paul et al. 2015). The process of regulating the expression of transporters and nutrient homeostasis not only allows uptake of minerals but also ensures easy mobilization across the plant lumen. Studies have crept in towards understanding the regulatory mechanisms delineating the crop biofortification and they give dissipating results if the work is augmented on the trait-specific processes. Metal tolerance proteins are one such type of proteins associated at the subcellular level encoding proteins in the wheat genome, as most of the classes of proteins have Zn transporter/cis-regulatory domains or motifs. Several intergenic ncRNAs were discovered with their

expression known in the aleurone layer of grains. As many of the millets are not annotated, they may have lncRNAs which could prove to be involved in biofortification. These can, however be better understood using amplicon-based targeted genome sequencing approaches. With an increased cereal Fe/Zn content in wheat being studied to expedite the better breeding processes, efforts on seedling transcriptome analysis have given a big hope with several key transcripts associated with phyto siderophore biosynthesis and mineral uptake. It will be very insightful to see if any of these miRNA-DEGs would exhibit greater accumulation when there is a significant number of genes that are noticed in tandem. As more and more such whole seedling genome sequencing efforts come in, the biofortification-based ncRNA repertoire will hopefully allow us to have an enhanced understanding.

Conclusions and road ahead

Millets provide food security and prevent malnutrition during pandemics and also under dynamic climatic conditions. They grow easily even under harsh environments and are naturally tolerant to biotic and abiotic stresses. Millets are more nutritious than rice, wheat, and maize with better antioxidative capacity. Supplementation of minerals through fertilizers or foliar sprays must be deployed to enhance the content of mineral nutrition in millet grains. Further, their bioefficacy and bioavailability must be carefully undertaken in the target groups. Uptake and translocation are other important realms for which natural genetic diversity must be identified and exploited in all millets. Utilization of genetic resources, and genome sequences for the identification of candidate genes and their exploitation for the mineral nutrition improvement using CRISPR-Cas9 paves the way for food, feed, and nutritional security in the years to come.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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