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REVIEW ARTICLE

Achieving nutritional security in India through iron and zinc biofortification in pearl millet (*Pennisetum glaucum* (L.) R. Br.)

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Abstract The health problems caused by iron (Fe) and zinc (Zn) deficiency plague developing and underdeveloped countries. A vegetarian person mainly depends on cereal based diet with low quantity of Fe and Zn. Biofortification is an economical and sustainable approach to challenge the micronutrient malnutrition problem globally. Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is one of the nutri-cereals and mostly grown under hot, dry conditions on infertile soils of low water-holding capacity, where other crops generally fail. It contains anti-nutrient compounds like phytic acid and polyphenols which reduce the mineral bioavailability because of their chelating properties. Biofortification of pearl millet is like a double-edged sword which cuts down the economic burden and simultaneously supplies required nutrition to the poor, offering a great scope for food security as well as nutritional security. With this background, this review focus on biofortification of grain Fe and Zn content in pearl millet. Genetic research on Fe and Zn uptake and accumulation in pearl millet grain is crucial in identifying the 'bottlenecks' in biofortification. The review also reveals the need and strategies for increasing bioavailability of Fe and Zn in humans by increasing promoters and decreasing anti-nutritional factors in pearl millet.

Keywords Biofortification · Fe and Zn · Pearl millet · Nutritional security

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Introduction

Nutritional insecurity is the major menace in the world which needs to be addressed on a priority basis. Globally, it was estimated that at least 50% of children in the age group of 6 months to 5 years and > 2 billion people are facing the deficiency of essential micronutrients and vitamins (Oh et al. 2020). Deficiencies in micronutrients mainly of iron, zinc, iodine, vitamin A and folate have a negative consequence that adds to the burden of food deficiency due to poverty (CDC 2020). Millets can play an important role in addressing the challenge of nutritional insecurity, worldwide. Millets are small-seeded crops like pearl millet (*Pennisetum glaucum*), foxtail millet (*Setaria italica*), finger millet (*Eleusine coracana*), kodo millet (*Paspalum setaceum*) and barnyard millet (*Echinochlo autilis*). Among all millets, pearl millet [*Pennisetum glaucum* (L). R. Br] has many advantages in terms of environmental challenges and nutritional security (Vadez et al. 2012).At present, India is the largest producer of pearl millet in Asia, both in terms of area and production and the second-largest producer of rice and wheat globally (FAO 2020). The production of pearl millet is 8.61 million tonnes in India and productivity is 1243 kg/ha (Directorate of Millets Development 2020; Project Coordinator Review 2020). Despite the fact that food security has been achieved, the major challenge of nutritional security remains unresolved.

Globally, iron and zinc deficiencies are the major problems and various dietary factors influencing the development of iron deficiency anaemia include low dietary iron intake and/or the intake of compounds that decreases its bioavailability. Iron deficiency may also result from a low intake of other essential nutrients, such as vitamin A, E, B_{12}, and folate (WHO 1996). It was estimated that dietary iron (mainly heme iron) contribute 10–15% of total iron intake in meat-eating populations, but, because of its higher and more uniform absorption (estimated at 15–35%), it could contribute $\geq$ 40% of total absorbed iron (Hurrell et al. 2010). Iron absorption is a complex process that reflects not only the iron content in the diet but also the bioavailability of iron. Similar to iron, zinc deficiency is largely related to inadequate intake or absorption of zinc from the diet. Many times people may take sufficient zinc but still face zinc deficiency which is mainly due to a high level of inhibitors like phytate, cadmium etc. in the diet (WHO 1996).

To overcome iron and zinc deficiency, there are many strategies which include dietary diversification, food fortification, external supplementation and biofortification. The most acceptable and reliable approach is biofortification, as it is a highly cost-effective and sustainable approach to enhance the essential micronutrient in staple food grain crops (Rehman et al. 2021). Biofortification is a strategy to increase the bioavailability and the concentration of nutrients in crops through both conventional plant breeding and recombinant DNA technology (genetic engineering) approaches, which reduces the concentration of antinutrients and favours micronutrient absorption (Sperotto et al. 2017). However, like in other cereals, biofortification in pearl millet is still limited by the presence of antinutrients like phytic acid, polyphenols, tannin and oxalates. The present review aims to focus on biofortification, genetic variability and molecular approaches to increase the grain iron and zinc content in pearl millet.

Present scenario and effects of iron and zinc malnutrition

Iron deficiency is the most widespread nutritional problemwhose significance ranks 9th among all the human health risks (Stoltzfus 2003). It mainly affects the physical and mental development of children and also their ability to learn (Talpur et al. 2018). Iron deficiency leads to a serious condition called anaemia, which is also a prime cause of women's death during childbirth (Anuradha et al. 2017). The main causes of iron deficiency include low intake of bioavailable iron, increased iron requirements as a result of rapid growth, pregnancy, menstruation, and excess blood loss caused by pathologic infections (Larocque et al. 2005; Crompton and Nesheim 2002). Among the population of the developing world including India, the most commonly followed diet is vegetarian which largely depend on cereals and legumes and has limited access to fruits, meat, eggs, milk products etc. (Puranik et al. 2017). This low nutrient diet is the main cause of micronutrient deficiency. Micronutrient deficiency mainly affects women and children. An estimated 45% of deaths of children under the age of 5 are linked to micronutrient malnutrition (Gernand et al. 2016). During early infancy, iron requirements are met by small amounts of iron contained in human milk (Joint 2004). After birth, its need increases markedly and reaches approximately 0.7–0.9 mg/day during the remainder of the first year (Joint 2004). Adolescents also have higher iron requirements, especially during periods of growth. The average adults store about 1–3 g of iron in their bodies (Joint 2004). The iron requirement in menstruating women increases to levels above average iron requirements (Abbaspour et al. 2014).

Like iron deficiency, Zinc insufficiency is accompanied by a wide range of physiological problems such as hindrance in brain development, improper growth and augmented vulnerability to infectious diseases like diarrhoea, pneumonia and low birth outcomes in pregnant women (Hambidge and Krebs 2007). It is essential for the proper development of the human body and its deficiency is ranked as the 5th major risk factor for impairment (Anuradha et al. 2017).Its deficiency is particularly prevalent in children under 5 years old as they have a comparatively huge demand for zinc to sustain development (Black et al. 2008). Zinc deficiency is also responsible for the economic burden of disease in developing countries and has a substantial impact on Gross National Product (GDP) through declining productivity, which automatically increases health care costs (Darnton-Hill et al. 2005).The World Bank, in collaboration with the WHO, developed the Disability Adjusted Life Years (DALYs) methodology and introduced DALYs as a measure for the global burden of disease (GBD) (Stein et al. 2009).

Importance of iron and zinc in the human body

Iron is an essential element for most life on earth, including human beings, by participating in a wide variety of metabolic processes, including oxygen transport, electron transport and DNA synthesis (Venkataramani2021). Iron is needed for a number of highly complex processes, e.g. the transportation of oxygen around the body (Vogt et al.

2021). It is required for the production of red blood cells (a process known as haematopoiesis), but it's also part of haemoglobin (that is the pigment of the red blood cells), binding to the oxygen and thus facilitating its transport from the lungs to all cells throughout the body (Duck and Connor 2016). Iron exists in complicated forms with protein (heme protein), as heme compounds (Hb or Mb), heme enzymes and non-heme compounds (flavin-Fe enzymes, transferrin and ferritin). The human body requires iron for the production of heme enzymes and non-heme enzymes that are essential in the transfer of electrons (cytochromes and catalase) and oxidation–reduction reactions (Rodgers and Gilreath 2019). Almost two-third of the iron is found in the Hb, 25% is found in a readily mobilizable iron store and approximately 15% is associated with myoglobin in muscle cells and a variety of enzymes involved in the oxidative metabolism (Trumbo et al. 2001). The average adult male has approximately 1000 mg of iron as stored iron, which is sufficient for about 3 years, while the average woman has only approximately 300 mg, sufficient for about 6 months (UCSF 2020). Men need 28 mg of iron per day; pregnant women need 38 mg per day while lactating and non-pregnant women need 30 mg per day (Kaur 2016).

Zinc has various physiological roles in a biological system and estimated that about 3000 proteins in the human body are zinc-dependent proteins (Krezel and Maret 2016). Its interaction with enzymes and other proteins are necessary for the structural, functional and regulatory processes in the human body.

Factors affecting iron and zinc bioavailability

Plant-based foods like grains, beans, vegetables, fruits, nuts, and seeds contain non-heme iron (Patel et al. 2017). Non-heme iron is the major fraction of total dietary iron, its absorption rate may only be 2–20% from vegetable and staple crops such as rice, wheat bran and maize (Abbaspour et al. 2014).Although absorption from the non-heme iron pool constitutes a major fraction of daily iron intake, unlike heme iron, its absorption depends on the presence of a variety of dietary components ingested simultaneously (Fleming 2005). Ascorbic acid and muscle tissue from meat, fish, and poultry (Dasa and Abera 2018) enhance non-heme iron absorption, whereas phytic acid (Armah et al. 2015), polyphenols (Ahmad et al. 2017), and soy (Milman 2020) inhibit non-heme iron absorption. The mixture of foods eaten by humans and the interplay of nutrients and non-nutrients affects non-heme iron absorption in complex ways. Pearl millet contains non-heme iron whose bioavailability is highly influenced by dietary constituents and depends on luminal dietary factors viz. enhancer and/or inhibitors of absorption (Moretti 2017).

Enhancers of iron absorption

Ascorbic acid can counteract the inhibitory effect of phytic acid, polyphenols, and calcium. Organic acid such as citric acid has been reported to potentially enhance iron absorption (Teucher et al. 2004).

Inhibitors of iron absorption

Phytic acid

Phytic acid (myoinositol-1, 2, 3, 4, 5, 6 hexakisphosphate) has a strong chelating ability and readily forms complexes with monovalent and multivalent cations of potassium, calcium, iron, zinc and magnesium, reducing their bioavailability and creating a deficit in their absorption. Cereals contain intrinsic phytase, able to degrade phytic acid, but the conditions required for the degradation to take place are not easily met in common food preparation methods (Koreissi-Dembele et al. 2013). Phytate can be enzymatically degraded before consumption (Hurrell et al. 2003). Sandberg et al. (1996) has suggested that a phytase that is activated at gut pH may be effective in increasing iron absorption when added to a meal just before consumption. The addition of dietary phytase has been shown to enhance iron bioavailability when added to a meal prior to consumption (Troesch et al. 2009).

Polyphenols and tannins

Polyphenols, similarly to phytic acid, can bind with iron and make it unavailable for absorption by inducing its precipitation or reducing its solubility. As polyphenols are a complex and diverse class of chemical compounds (flavonoids, tannic acid), it can be expected that different polyphenols from different foods affect iron absorption differently (Moretti 2017).

Calcium

Calcium is the only dietary component to inhibit both non-heme as well as heme iron absorption (Hallberg et al.1991).

The inadequate dietary intake of Zn (2+) and inhibitors of its absorption are the most common causative factor for its deficiency. Phytate has a strong negative effect on Zn (2+) absorption from composite meals. Cadmium, which is increasing in the environment, also inhibits Zn (2+) absorption (Lonnerdal 2000). The type of protein in a meal also affects Zn (2+) bioavailability. Casein in milk has a negative effect on its absorption (Sandstrom et al. 1983). The calcium content of the diet may, however, affect Zn (2+) absorption from phytate-containing meals. This is because calcium has a tendency to form complexes with phytate and Zn (2+) that are insoluble and consequently have an inhibitory effect on its absorption (Fordyce and Robbins 1987). Amino acids, such as histidine and methionine, and other low-molecular-weight ions, such as EDTA and organic acids (e.g. citrate), are known to have a positive effect on zinc absorption (Lonnerdal 2000). Micronutrient absorption can be increased by reducing the concentration of antinutrients (Sperotto et al. 2017). Several studies have reported the range of natural variability of minerals content in millet grain, but very limited work was done for antinutritional factors (Simwemba et al. 1984). Nowadays, a number of strategies are used to overcome the effects of these food antinutrients, including processing treatments such as milling, soaking, germination, autoclave, microwave, treatment and fermentation (Samtiya et al. 2020).

Physiological processes of metal uptake and translocation in plants

Micronutrient concentrations in plants are controlled by complex homeostatic mechanisms. Iron is slightly soluble under aerobic conditions, high-pH and calcareous soils (Ramzani et al. 2016). The mugineic acid family phytosiderophores (MAs), which are Fe (III)-solubilizing molecules, secreted from Fe-deficient graminaceous plants (Bashir et al. 2006). MAs-Fe (III) is taken up into root cells through the *Yellow Stripe (YS) or Yellow Stripe-Like (YSL) transporters* (Curie et al. 2001). *Yellow stripe 1 (YS1)* is firstly identified from maize and targeted to the plasma membrane. Eighteen putative *Yellow Stripe 1 (YS1)*-like genes (*OsYSLs*) are identified in the rice genome (Koike et al. 2004). MAs have been identified to date, all of which are synthesized through a conserved pathway from S-adenosyl-L-methionine (SAM). This pathway includes three sequential enzymatic reactions mediated by Nicotianamine Synthase (NAS), Nicotianamine Aminotransferase (NAAT), and Deoxymugineic Acid Synthase (DMAS), generating 2-Deoxymugineic Acid (DMA), and the precursor of all other MAs (Barberon et al. 2011). The NAS enzyme is localized on the membrane of the vesicles, whereas NAAT is present within the vesicles, suggesting that these vesicles are the site of MA biosynthesis. The Transporter of Mugineic Acid Family Phytosiderophores 1 (TOM1) from rice, revealing the final piece in the mechanism (Nozoye et al. 2011). After iron is transported to the root endodermis from epidermis, it needs to be transported to the above ground parts of plants through the xylem. The contents of organic acids, such as citrate, malate, and succinate, are elevated in xylem under iron deficient conditions. The transport of citrate and iron to the xylem is mediated by *FRDL1* in rice, which is crucial for iron translocation (Yokosho et al. 2016). Iron is also capable of translocation in xylem in the form of Fe-nicotianamine (NA) and Fe-MAs. NA as a non-protein amino acid is produced from *S*-adenosyl methionine by nicotianamine synthase (NAS) (Bonneau et al. 2016). In rice, NA and 2′-deoxymugineic acid (DMA) are present in xylem exudates. Once the iron reaches the leaves, it must be unloaded to leaf cells from the apoplastic space. NA and DMA are also required for the phloem-based transport (Curie et al. 2009). In rice, *OsYSL2* is likely to be involved in the translocation of Fe (II)-NA to shoots and seeds (Ishimaru et al. 2010). *OsYSL9* is involved in the Fe distribution in developing seeds via Fe(II)-NA and Fe(III)-DMA form (Senoura et al. 2017). Iron mobilization and uptake in higher plants are represented in Fig. 1. Similarly, Zn (2+) is taken by the plants as divalent cation, Zn(2+) or as a Zn- phyto-siderophore complex which assists Zn (2+) to move towards root hairs (Yoneyama et al. 2015). Subsequently, Zn (2+) is radially transported across root layers to be loaded onto the vasculature in the root stele. Zn (2+) is effluxed into the xylem for long distance transport by the *Heavy Metal Transporters* (HMA), which localize to the plasma membrane of the root and shoot vasculature. Zn (2+) are thought to be transported in the phloem as NA chelates and, although the transporters involved in phloem loading and unloading are not fully known, they are thought to include members of the *YSL* group (Ajeesh et al. 2020). The schematic representation of Zn (2+) transport from soil to grain is illustrated in Fig. 2.

Why does pearl millet serve as a model crop for food and nutritional security?

In India, the major crops are wheat, rice, maize, millets and pulses (Nelson et al. 2019). Pearl millet is less popular than wheat and rice except in some Indian states (Fig. 3). It is serving as a staple food crop in Indian states like Uttar Pradesh, Maharashtra, Gujarat and Rajasthan. The inhabitants of such states cultivate it and consume it as the main food (Basavaraj et al. 2010).

Climate-resilient: At present, the climate is changing and the water level is declining. In the coming days, it would be hard to irrigate crops but pearl millet can be grown in an enormous array of environmental conditions including repeated drought events, heat stress and meager soil fertility (Shivhare and Lata 2017). It can even grow in areas that received less rainfall, Jaisalmer and Hanumangarh, Rajasthan, India from 2006 to 2016 received less than 300 mm of annual rainfall from the average annual rainfall (Yadav et al. 2018). Hence, it provide a source of food security in a drought-prone area. It is cultivated in 26 million hectares in some of the harshest semi-arid tropical environments of South Asia and sub-Sahara Africa (All India Coordinated Research Project on Pearl Millet

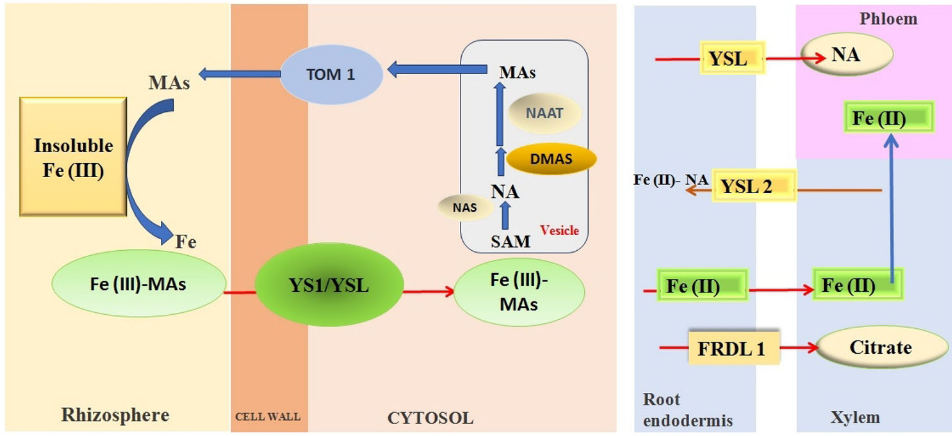
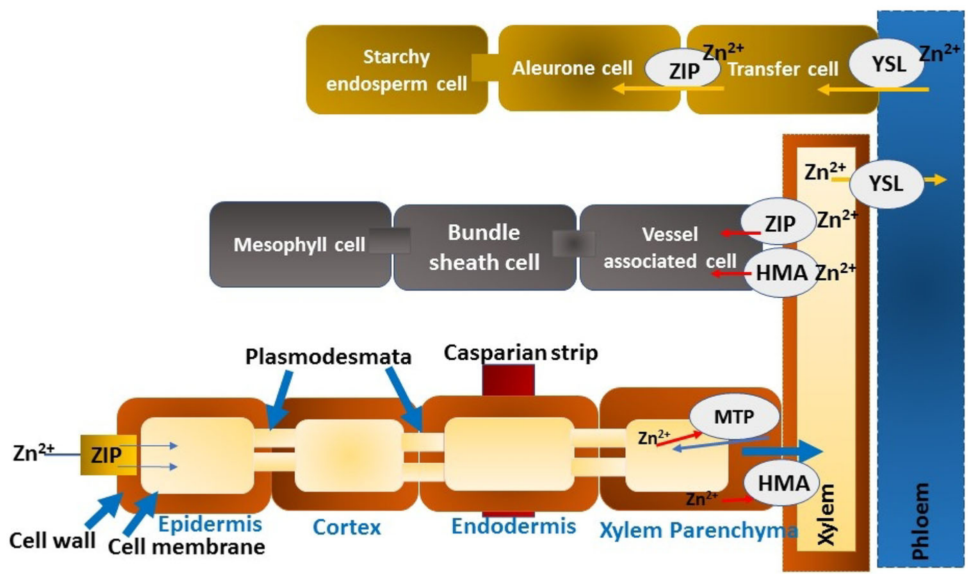


Fig. 1 Schematic representation of Fe acquisition and transport in higher plants Abbreviations: *DMAS* deoxymugineic acid synthase, *MAs* mugineic acid family phytosiderophores, *NA* nicotianamine, *NAAT* nicotianamine amino transferase, *NAS* nicotianamine synthase, *SAM* S-adenosyl-L-methionine, *TOM1* transporter of mugineic acid family phytosiderophores 1, *YS1/YSL* YELLOW STRIPE 1/YELLOW STRIPE 1–like, *FRDL* 1 (Ferric Reductase Defective Like 1)

Fig. 2 Schematic representation of zinc acquisition and its transport from soil to grain Abbreviations: ZIP (ZRT- IRT-like proteins), HMA (heavy metal ATPases), MTP (metal tolerance protein), YSL (yellow-stripe-like) transporters



(AICRP-PM)). Hence, pearl millet is one of the most imperative staple crop in South Asia and sub-Sahara Africa (Stich et al. 2010).

Nutritional value: Pearl millet is a very nutritious cereal compared to other cereals. It is a principal source of protein, vitamins, fat, minerals and micronutrients like Fe (2+ , 3+), Zn (2+), Ca (2+), K (1+) etc. for millions of poor populations where it is cultivated. The total amount of Fe (2+ , 3+) in pearl millet will provide about 60% of the Estimated Average Requirement (EAR). It is highly fibrous (12 g/kg) compared to other food grains. It is also high in fat content (50 mg/kg), mainly unsaturated fatty acids with improved fat digestibility as compared to other food grains, but it has high activities of lipases that result in rapid release of fatty acids, which limits its shelf life. It is having a high amount of vitamin B complex (thiamine, riboflavin and niacin) (Satyavathi 2019). It is also rich in Vitamin A and folic acid than wheat (Satyavathi 2019). Among micronutrients, it is loaded with an abundance of Fe (2+ , 3+) and Zn (2+) content (Satyavathi et al. 2021). Hence, Pearl millet is a highly nutritious cereal whereas bioavailability is low, because of the presence of certain anti-nutritional factors like phytic acid, polyphenols etc. Polyphenols content was found to range from 4.91 to 7.65 g /kg whereas phytic acid content ranged from 3.54 to 8.25 g/kg (Satyavathi et al. 2017). Protein and starch digestibility of pearl millet is low because of the anti-nutrients in grain. The digestibility for protein ranged between 54.2 and 59.2%, whereas 12–18.7 mg maltose released/g for starch (Satyavathi et al. 2017). Recently, pearl millet is getting attention

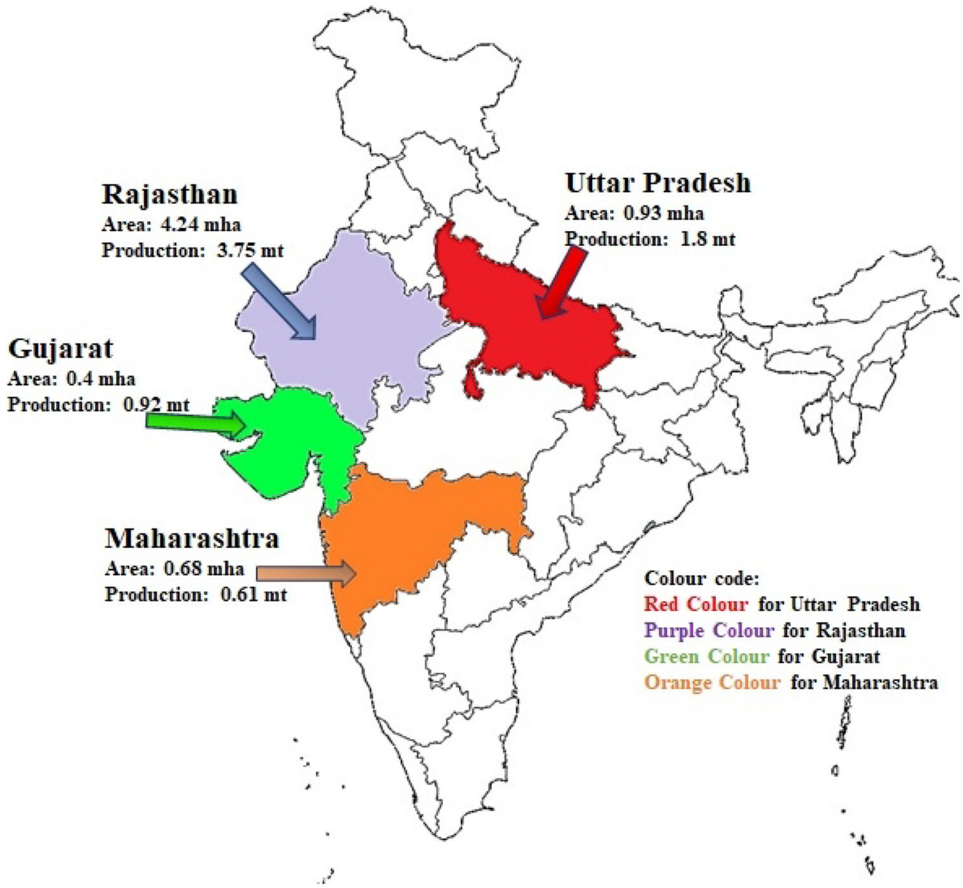


Fig. 3 The map of India presents four major states with cultivation areas and production of pearl millet (Uttar Pradesh, Maharashtra, Gujarat and Rajasthan)

for its good nutritional quality in the medical field (Kaur et al. 2014). Pearl millet is a sustainable cereal with good dietary properties for diabetic patients. It has superior glycemic control over wheat and rice because of the presence of slowly digestible starch (SDS) and resistant starch (RS). The Government of India declared '2018' as "National Year of Millets" to march towards "Malnutrition Free India" by the end of the year 2022. In this regard, Indian Council of Agricultural Research (ICAR), New Delhi, India has established minimum levels of Fe (2+ , 3+) and Zn (2+) to be bred into national varieties of pearl millet. The UN Food and Agriculture Organization (FAO) also decided the year 2023 to be the "International Year of Millets".

HarvestPlus biofortification program of Pearl millet

HarvestPlus program of the Consultative Group on International Agricultural Research (CGIAR) targeted the crops in order to improve the nutritional background of crop varieties genetically with their desired traits in different countries through a conventional breeding program. HarvestPlus breeding strategies were implemented in India at ICRISAT to improve the Fe (2+ , 3+) and Zn (2+) content in the edible part of pearl millet. It is done in two stages through initial survey and screening to know the baseline and variability present in pearl millet and then to enhance the grain Fe (2+ , 3+) through breeding approaches. The nutritionist team determined the target level of essential micronutrient that is needed to make a measurable effect on the human body (Table 1). They initially look at such variables as bioconversion and bioavailability of ingested nutrients, because micronutrient losses during cooking, storage and other processing are very high. Targets were set for children of 4–6 years old and women of reproductive age (HarvestPlus).The main goal of pearl millet biofortification is to increase Fe (2+ , 3+) (target value: 77 mg/kg) content in grain.

Initial phase of HarvestPlus program (2003–2008)

The first phase started with the screening of pearl millet germplasm accession for a diverse range of Fe (2+ , 3+) and Zn (2+) by ICRISAT and found a range of 30–76 mg/kg of Fe (2+ , 3+)and 25–65 mg/kg of Zn (2+). By using this data target was fixed to enhance the Fe (2+ , 3+) content in pearl millet grains in populations and hybrids (Table 2). Both micronutrients have a high correlation

Table 1 Details of the target level of iron content. *Source*: HarvestPlus https://www.harvestplus.org/sites/default/files/Biofortification_Progress_Briefs_August2014_WEB_2.pdf

Nutrition factors		Nutrient intake at current	Target levels required for optimum growth
Pearl millet consumption,	Women	300 g/day	244 g/day
grams/day (dry weight)	Children	150 g/day	72 g/day
Fe retention (%)		90%	95%
Fe absorption (%)		5%	7–7.5%
Absorbed incremental Fe as % of EAR		60%	60%

EAR estimated average requirement

Table 2 Focussed biofortification program of iron pearl millet by HarvestPlus. *Source*: HarvestPlus,http://www.harvestplus.org/sites/default/files/BiofortificationProgressBriefsAugust2014WEB0.pdf

Crop	Target Country	Baseline (mg/kg)	Target increment (mg/kg)	Target level in crop (mg/kg)	Farmer benefits
Pearl millet	India, secondary countries: West Africa	47	+ 30	77	High yielding, downy mildew resistant, drought tolerant

under additive genetic control. Potential parents and the most promising local germplasm were used to study the G × E interaction for verifying the stable genotypes. With the effort of ICRISAT and the Indian National Agricultural Research System (NARS) partners, a handful of biofortified varieties and hybrids got released by Govt. of India for cultivation as part of Harvestplus program. Rai et al. (2016) screened around 18 open-pollinated varieties (OPVs) and 122 hybrids released and/or commercialized in India. Among OPVs, ICTP 8203 released in 1988 (Fe: 67 mg/kg and Zn: 52 mg/kg) and ICMV 221 in 1993 (Fe: 61 mg/kg and Zn: 45 mg/kg) were found promising for high iron-zinc content. While, among hybrids Ajeet 38, Proagro XL 51, PAC 903 and 86M86 have been developed with Fe (2+ , 3+) content of 55–56 mg/kg and Zn (2+) content of 39–41 mg/kg. These genotypes were selected as a donor for imparting high micronutrients content and used for initial crosses.

Second phase of HarvestPlus program (2009–2013)

The breeding line and germplasm containing more than 90 mg/kg Fe (2+ , 3+) and 60 mg/kg Zn (2+) were validated. Large-scale and high throughput screening of high Fe (2+ , 3+) lines through XRF spectrometry was carried out by ICRISAT, and helped in the development of Fe (2+ , 3+) rich OPV, Dhanshakti. Later, they concentrated on the development of hybrids. Currently, under the HarvestPlus program and other consortium programs, breeders from many institutions around the world are primarily focusing on the development of stable and high grain Fe (2+ , 3+) containing hybrids. The details of high iron varieties and hybrids are shown in Table 3 and variability studies in pearl millet for grain Fe (2+ , 3+) and Zn (2+) are shown in Table 4. Several studies reported improved lines of grain Fe (2+ , 3+) content in pearl millet germplasm beyond the HarvestPlus target level (target increment of Fe: 30 mg/kg). Huge variability existed for micronutrient content among pearl millet lines which were far better than regularly consumed cereals (wheat and rice), even after biofortification efforts (Bouis et al. 2017).

The selection of germplasm lines for improved grain minerals and mapping of associated QTL will help in the selection of donor parents for developing Fe (2+ , 3+)/Zn (2+) rich hybrids or varieties. The proper knowledge of the genetic background of these micronutrients and systematic utilization of pearl millet germplasm in biofortification program is very crucial. Many pilot studies revealed the genetic basis of Fe (2+ , 3+)and Zn (2+)content in pearl millet grain (Rai et al. 2012; Govindaraj et al. 2013; Kanatti et al. 2014). The studies also interpreted the correlation between Fe (2+ , 3+) and Zn (2+), combining ability including both General Combining Ability (GCA) and Specific Combining Ability (SCA) in different crosses, gene action and broad-sense heritability (Table 5). Only a few reports are available on the exploitation of heterosis for Fe (2+ , 3+) and Zn (2+) content as the trait is predominantly governed by additive genetic variance with no better parent heterosis (Velu et al. 2011).

Table 3 Details of high iron varieties/hybrids of pearl millet. *Source*: ICAR- All India Coordinated Research Project on Pearl Millet, http://www.aicpmip.res.in/pearl%20millet%20hybrids%20and%20varieties.pdf

S. nos	Hybrid/variety	Parentage	Bred at	Area of adoption	Salient features	DF	DM	GY (t/ha)	Fe (mg/kg)	Zn (mg/kg)
1	Dhanshakti (ICTP 8203 Fe10-2)	(S. O. 1146 (E) 24.04.2014)	Mahatma Phule Krishi Vidyapeeth (MPKV), Dhule	Maharashtra, Karnataka, AP, Tamil Nadu, Rajasthan, Haryana, MP, Gujarat, UP and Punjab	Early maturing bold, globular, shining slate grey colored seed resistant to downy mildew disease	45	76	2.2	81	43
2	HHB 299(MH 2076)	ICMA 04888 × H 13/0001	Chaudhary Charan Singh Haryana Agricultural University (CCS HAU), Hisar	Rajasthan, Haryana, Gujarat, Punjab, Delhi, Maharashtra and Tamil Nadu	Medium maturing, compact panicle grayish hexagonal-shaped grain and resistant to major diseases	50	81	3.3	73	41
3	AHB 1200 Fe (MH 2072 (AHB 1200)	ICMA 98222 × AUBI 1101	National Agricultural Research Project (NARP), Aurangabad	Rajasthan, Haryana, Gujarat, Punjab, Delhi, Maharashtra and Tamil Nadu, AP and Telangana	Medium maturing, cylindrical panicle resistant to downy mildew and highly responsive to fertilizers	47	78	3.2	77	39
4	AHB 1269Fe (MH 2185)	ICMA 98222A1 × AUBI 1101	National Agricultural Research Project (NARP), Aurangabad	Rajasthan, Haryana, Gujarat, Punjab, UP, Delhi, Maharashtra, Tamil Nadu, AP, Telangana and Karnataka	Medium maturing, long cylindrical type panicle, bold grain and resistant to Major diseases	50	81	3.2	91	43
5	HHB 311	ICMA 02333 × H 14/003	Chaudhary Charan Singh Haryana Agricultural University (CCS HAU), Hisar	Rajasthan, Haryana, Gujarat, MP, Punjab, Delhi, Maharashtra and Tamil Nadu,	Medium maturing, compact panicle having grey colored hexagonal-shaped grain, Highly resistant to downy mildew and other diseases	50	81	3.2	83	39
6	RHB 233(MH 2173)	ICMA 99444 × RIB 15176	Sri Karan Narendra Agriculture University (SKNAU), Jobner	Rajasthan, Haryana, Gujarat, MP, Punjab, Delhi, Maharashtra and Tamil Nadu,	Medium maturing, grey globular shaped grain; Highly resistant to blast and downy mildew diseases	49	80	3.2	83	46
7	RHB 234(MH 2174)	ICMA 02333 × RIB 15177	Sri Karan Narendra Agriculture University (SKNAU), Jobner	Rajasthan, Haryana, Gujarat, MP, Punjab, Delhi, Maharashtra and Tamil Nadu	Medium maturing, conical-shaped compact panicle with grayish colored hexagonal-shaped grain, Highly resistant to downy mildew	49	81	3.2	84	4

DF days to flowering, *DM* days to maturity, *GY* grain Yield, *Fe* Iron, *Zn* Zinc

Table 4 Iron and zinc variability reported in earlier studies in pearl millet

S. no	Breeding material used	Fe (mg/kg)	Zn (mg/kg)	Author reported
1	Germplasm accessions: two sets of line x tester crosses	30–76	25–65	Govindaraj et al. (2011)
	Set 1: 89 entries (17 parents and 72 hybrids)			
	Set 2: 28 parents and 192 hybrids			
	Set 1 Parents: ICMB 93222, ICMB 98222, ICMB 00888, ICMB 94111, ICMB 93333, ICMB 04777, ICMB 89111, ICMB 97111, IPC 1650, IPC 843, IPC 616, IPC 774, IPC 1307, IPC 1354, IPC 390, IPC 828, IPC 1178			
	Set 2 Parents: 841 B, 843 B, 863 B, ICMB 88006, ICMB 91222, ICMB 92111, ICMB 95333, ICMB 96333, ICMB 99444, ICMB 00999, ICMB 02444, ICMB 03111, ICMB 04222, ICMB 04555, ICMB 04999, IPC 338, IPC 404, IPC 536, IPC 689, IPC 735, IPC 811, IPC 1254, IPC 1268, IPC 1642, ICMR 356, ICMR 06333, ICMR 06888			
2	Set 1: 386 Advance breeding lines,	61–80	41–60	Rai et al. (2012)
	Set 2: 232 Population progenies	52–135	40–92	
3	122 Hybrids ((21 hybrids from 9 public sector research organizations, including ICRISAT; and 101 hybrids from 33 seed companies)	31–61	32–54	Rai et al. (2013)
	HHB 197, ABH 999, Bio 70 GHB 719, Mahodaya 325, RBH 173, HHB 223, RBH 121, GHB 538, N 61, RHB 154, Hi Pearl 51, Shanti, GHB 744, Mukhia, GK 1135, NBH 1188, KBH 261, Bio 8494, 86M 66, Mahodaya 318, PHB 2168, JKBH 1103, NPH 1651, GHB 732, NPH 2798, Bio 8141, HHB 94, MPMH 17, RBH 177, Proagro 9444, HTBH 4201, VBBH 3028, ABH 1, NBH 1717, Bisco 5151, NBBH 411, KBH 11, NPH 3685, Ajeet 37, 86M 01, Mahodaya 331, X 7, KBH 9119, Bio 13, NBH 5464, JKBH 1096, S 368, VBBH 3087, GHB 558, Hi Pearl 138, TEJAS, Biogene 33+ , Navbharat Gavari, Mahodaya 330, Gangotry 67, HTBH 4202, RBTH 10-007, VBH 444, NBBH 908, KH 302, VBH 456, M 64, Nu 306, Kaveri Boss 65, Bisco 5141, B 2301, 86M 88, Solid 78, GK 1104, Euro 1133, MRB 204, NPH 2475, Kaveri Super Boss, Sujlam 68, Hi Pearl 91, VIRAT, Kaneri × 563, MRB 2210, TNBH 0642, KBH 287-36, NBH 4903, GK 1116, B 2195, S 361, KBH 7206, N 68, S 362, Ajeet 35, RBH 1818, Sanjivani 333, Dhamal, Proagro 9450, Nu 310, VBBH 3125, VBBH 3115, PAC 909, JKBH 676, RBTH 10-005, NBH 1024, GK 1090, Hi Pearl 130, KBH 2360, JKBH 1097, HTBH 4203, Navbharat Banas express, Saburi, PAC 931, KBH 7201, Sanjivani 2312, NBH 1134, ICMH 356, RBH 9, HTBH 4204, PAC 982, Shradha, Ajeet 39, XL 51,			
	86M 86, PAC 903, Ajeet 38, Sanjivani 222			
5	Parental lines	34–102	34–84	Govindaraj et al. (2013)
	Set I: 8 B-lines and 9 R-lines			
	Set II: 16 B-lines and 12 R-lines			
	Set I: 8 B-lines (ICMB 89111, ICMB 93222, ICMB 93333, ICMB 94111, ICMB 97111, ICMB 98222, ICMB 00888, ICMB 04777) and 9 R-lines (IPC 390, IPC 616, IPC 774, IPC 828, IPC 843, IPC 1178, IPC 1307, IPC 1354, IPC 1650)			
	Set II: 16 B-lines (841 B, 843 B, 863 B, ICMB 88006, ICMB 91222, ICMB 92111, ICMB 95333, ICMB 96333, ICMB 99444, ICMB 00999, ICMB 02444, ICMB 03111, ICMB 04222, ICMB 04555, ICMB 04888, ICMB 04999) and 12 R-lines (IPC 338, IPC 404, IPC 536, IPC 689, IPC 735, IPC 811, IPC 1254, IPC 1268, IPC 1642, ICMR 356, ICMR 06333, ICMR 06888)			
	Hybrids	33–76	34–70	
	Set I: 72 line × tester hybrids	30–80	31–64	
	Set II: 192 line × tester hybrids			
6	Parental lines: 14 B-lines (: ICMB 88004, ICMB 92111, ICMB 92888, ICMB 93222, ICMB 97111, ICMB 98222, ICMB 02555, ICMB 04888, ICMB 05555, ICMB 07555, ICMB 07777, ICMB 07999, ICMB 08222, ICMB 08333)	30–77 32–82	27–45 29–56	Kanatti et al. (2014)
	14 R-lines (PRP 1, PRP 2, PRP 3, PRP 4, PRP 5, PRP 6, PRP 7, PRP 8, PRP 9, IPC 616, IPC 843, IPC 1178, IPC 1354, IPC 390)			
7	196 Hybrids	26–65	26–48	
8	106 RILs (F_6) + 2 parents (ICMB 841-P3 × 863B-P2) + 4 checks	28–124	29 -120	Kumar et al. (2016)
9	106 RIL population (open-pollinated)	22–77	22–74	
10	130 Association mapping panel + two checks (ICTP 8203Fe and ICMB 98222)	32–112	27–74	Anuradha et al. (2017)

Table 4 continued

S. no	Breeding material used	Fe (mg/kg)	Zn (mg/kg)	Author reported
11	317, F_6-RILs	20–131	18 -110	Kumar et al. (2018)
12	130 genotypes including one open-pollinated check variety, ICTP 8203Fe	27–125	28 -86	Anuradha et al. (2018a)
13	130 Association mapping panel + two checks (Dhanshakti and ICMB 98222)	23 -122	20- 86	Anuradha et al. (2018b)
14	Two parents (PPMI 683 × PPMI 627) + 210 RILs (RIL 1 to RIL 210)	36–130	12–29	Singhal et al. (2018)
15	Two parents (PPMI 683 × PPMI 627) + 210 RILs (RIL 1 to RIL 210)	36–114	20–106	Singhal et al. (2021)

Molecular approaches for iron and zinc content in pearl millet

Detection of genes/major QTL and understanding the molecular basis of grain Fe (2+ , 3+) and Zn (2+) content will facilitate breeding for high Fe (2+ , 3+) and/or Zn (2+) in pearl millet through Marker-Assisted Selection (MAS), Marker-Assisted Backcross Breeding (MABB) and Marker-Assisted Recurrent Selection (MARS).

Few reports indicate that molecular studies are already underway to identify the genetic basis of micronutrients in pearl millet but very limited studies are done in molecular aspects in this crop (Table 6). Singhal et al. (2021) reported 22 QTLs for grain Fe (2+ , 3+) and Zn (2+), of which 14 were for Fe (2+ , 3+) and 8 were for Zn (2+) and also observed the huge variation among RILs. The observed phenotypic variance (R^2) explained by different QTLs for grain Fe (2+ , 3+) and Zn (2+) content ranged from 2.85 (*QGFe.E3.2014–2016_Q3*) to 19.66% (*QGFe.E1.2014–2016_Q3*) and from 2.93 (*QGZn.E3.2014–2016_Q3*) to 25.95% (*QGZn.E1.2014–2016_Q1*), respectively. They also identified the candidate genes inside the QTLs such as *Ferritin gene*, *Al^{3+} transporter*, *K^+ transporter*, *Zn^{2+} transporter* and *Mg^{2+} transporter*.

The major effective Fe (2+ , 3+) and Zn (2+) content QTL were reported by Kumar et al. (2018) using DArT and SSRs markers through the construction of a linkage map in RIL population. They reported that the grain Fe (2+ , 3+) and Zn (2+) content in the RILs ranged from 20–131 mg/kg and 18–110 mg/kg, respectively which indicated a wide range for QTL mapping. A total of 19 QTLs were identified for grain Fe (2+ , 3+) and Zn (2+) content, out of 19, 11 for Fe (2+ , 3+) and 8 for Zn (2+) were found. They further claim the reported QTL may be useful in marker-assisted selection breeding programs, genomic selection and population improvement programs for pearl millet as the phenotypic variance explained by different QTL varied from 9.0 to 31.9% for Fe (2+ , 3+) and 9.4 to 30.4% for Zn (2+).

Anuradha et al. (2017) studied association mapping for grain Fe (2+ , 3+) and Zn (2+) content using 130 pearl millet lines, genotyped with 320 DNA markers (250 SSRs and 70 genic markers) and identified three markers on trait-associated regions. Of the three associated SSRs, *XPSMP 2261* was recognized in intergenic location on pseudo-molecule 5 and *Xipes0810* was observed on pseudo-molecule 3. They further stressed upon that the marker, *Xipes0810* may be useful in delineating high and low Fe (2+ , 3+) and Zn (2+) genotypes.

Kumar et al. (2016) identified the QTL for grain Fe(2+ , 3+) and Zn(2+) content using 106 RILs and 305 markers (96 SSR + 208 DArT) in pearl millet. They reported that grain mineral content in selfed seeds ranged from 28.4 to 124.0 mg/kg for Fe (2+ , 3+) and 28.7 to 119.8 mg/kg for Zn (2+). Grain Fe (2+ , 3+) and Zn (2+) content ranges from 22.4 to 77.4 and 21.9 to 73.7 mg/kg respectively in open-pollinated seeds. The phenotypic variation of identified QTL for Fe (2+ , 3+) and Zn (2+) on LG 3 was 19% and 36%, respectively. Similarly, two QTL for Fe (2+ , 3+) on LG 3 and LG 5 and two QTL for Zn (2+) on LG 3 and LG 7 were also reported for open-pollinated seeds with 16% and 42% phenotypic variance, respectively. They are of the opinion that QTL identified on LG3 will assist in marker-assisted selection.

Table 5 Review of the genetics of grain iron (Fe) and zinc (Zn) content in pearl millet

Author	Objective	Material	Fe Range (mg/kg)	Zn range (mg/kg)	Correlation between Fe and Zn	Correlation of Fe and Zn with other traits	Gene action/ associated marker/s or QTL	GCA and SCA	Heterosis		Genetic variability, G × E interaction (GEI)
									Fe	Zn	
Rai et al. 2012	a) Variability for Fe and Zn content b) Character association c) Genetics of micronutrients in grain	a)386 advanced breeding lines b)232 early-generation progenies	a)18–97 b)52–135	a) 22–69 b) 40–92	Highly significant positive	a) Negative correlations between the Fe content &grain yield in 3 of the 6 trials (r = -0.39 to − 0.58) b) No correlation between the Zn content and grain yield	Additive gene action	GCA, highly significant ($p < 0.01$)	–	–	–
Govindaraj et al. (2013)	a) Combining ability b) Genetic variability and c) Heterosis for grain Fe and Zn	Two sets of hybrids and their parental lines Set 1 Set 2 Parental lines	33–76 30–80 34–102	34–70 31–64 34–84	Highly significant ($p < 0.01$) and positive correlation in parental lines ($r = 0.90$ in the set I trial and $r = 0.86$ in the set II trial)	–	Additive gene action	Highly significant ($p < 0.01$) &positive correlation between the GCA of Fe & Zn content in parental lines ($r = 0.89$ in the set I trial and $r = 0.78$ in the set II trial)	1) No better parent heterosis 2)set I trial: 55 out of the 72 hybrids had significant ($p < 0.01$) mid parent heterosis (MPH), all in the negative direction 3) 93 hybrids had significant ($p < 0.01$) MPH in the set II trial, of which 91 were in the negative direction	Zn content of 37 hybrids in the set I trial& 82 hybrids in the set II trial had significant ($p <$ 0.01) MPH, and all were in the negative direction	1) Hybrid × environment (H × E) interaction was significant ($p < 0.05$) in the set I trial 2)In the set II trial, this interaction effect was highly significant ($p < 0.01$)

Table 5 continued

Author	Objective	Material	Fe Range (mg/kg)	Zn range (mg/kg)	Correlation between Fe and Zn	Correlation of Fe and Zn with other traits	Gene action/ associated marker/s or QTL	GCA and SCA	Heterosis		Genetic variability, G × E interaction (GEI)
									Fe	Zn	
Kanatti et al. (2014)	a) Genetic variability b) heterosis for Fe and Zn content	14 maintainer lines (B lines) as female parents (F), 14 restorer lines (R lines) as male parents (M), and 196 hybrids produced by F × M crosses	Parent: 32–82 and hybrid: 26–65	Parent: 26–65 and hybrid: 26–48	Highly significant and high positive correlations between the Fe and Zn densities	a) Highly significant and moderate positive correlation with grain weight (r = 0.61) in hybrids b) Negative and weak correlation with grain yield (r = 0.23)	Additive genetic control	Significant GCA for Fe and Zn content	Fe content among the hybrids varied from 25.8 to 64.5 mg/kg, but none of the hybrids showed significant better-parent heterosis		H × E were highly significant for all four traits, Fe content, Zn content,1000 grain weight and grain yield
Satyavathi et al. (2015)	Stability of newly developed inbred lines, derived from a RIL population for mapping high grain iron and zinc content	Twelve promising pearl millet lines	35–98	40–81	A strong correlation between grain Fe and Zn content, overall correlation r = 0.74 ($p < 0.01$)	–	–	–	–		The GEI was highly significant. first two principal components (PC) explained 77.14% & 85.31% of the total G x E variation for grain Fe and Zn contents respectively
Kumar et al. (2016)	a) Correlations b) Mapping quantitative trait loci controlling high iron and zinc content in self and open-pollinated grains of pearl millet	Recombinant inbred line (RIL)	a) Fe-Self 28–124 b) Fe-OP 22–77	a) Zn-Self 29–120 b) Zn-OP 22–74	A very strong significantly positive association between selfed [Fe] and [Zn], while correlation was moderate for trait pairs, [Fe] and Zn-OP, Fe-OP and Zn-OP, and [Zn] and Fe-OP		a) QTLs for Fe and Zn content on LG 3 with phenotypic variation 19% and 36% respectively b) In open-pollinated seeds- two QTLs for grain Fe content on LG 3 and 5, and two QTLs for grain Zn content on LG3 & 7. The total phenotypic variance for Fe and Zn was 16 and 42% respectively				G × E interactions were significant (at $P < 0.01$) for all observed traits across environments except for Fe-OP, which was Significant at $P < 0.05$

Table 5 continued

Author	Objective	Material	Fe Range (mg/kg)	Zn range (mg/kg)	Correlation between Fe and Zn	Correlation of Fe and Zn with other traits	Gene action/ associated marker/s or QTL	GCA and SCA	Heterosis		Genetic variability, G × E interaction (GEI)
									Fe	Zn	
Anuradha et al. (2017)	a) Character association b) G × E interaction in different agro-climatic zones of India	40 Pearl millet genotypes and one check variety (Dhanshakti, G30)	29–115	16–76	Highly associated (r = 0.8, $p < 0.01$)	Negative correlation with grain yield	–	–	–	–	Genotypes (G), Environmental effects (E) and GEI effects were highly significant ($P < 0.01$) for grain Fe and Zn content
Anuradha et al. (2017)	a) Association mapping of QTLs for Fe and Zn	130lines (B lines, R lines and advanced breeding lines) + two checks, (ICTP 8203Fe and ICMB 98222)	32–112	27–74	–	–	*Xpsmp2261* (13.34% R^2value), *Xipes 0180* (11.40% R^2-value) and *Xipes 0096* (11.38% R^2-value) were associated with grain Fe and Zn content	–	–	–	–
Kumar et al. (2018)	a) Mapping grain iron and zinc content QTLs in an iniadi-derived immortal population of pearl millet	317, F_6-RILs	20–131	18–110	Genotypic (9.4**) and phenotypic (9.3**) associations between Fe and Zn were very strong and significantly positive	–	Three QTLs for Fe and Zn were mapped, one on LG1 and two on LG7	–	–	–	–
Anuradha et al. (2018)	a) Association of agronomic traits and micronutrients in pearl millet	130 lines including one open-pollinated check variety, ICTP 8203Fe	27–125	28–86	Grain Fe is highly associated (genotypic correlation) with Zn	Grain Fe was correlated with total phosphorus and Mn but not correlated with grain yield	Additive gene action	–	–	–	–

Table 5 continued

Author	Objective	Material	Fe Range (mg/kg)	Zn range (mg/kg)	Correlation between Fe and Zn	Correlation of Fe and Zn with other traits	Gene action/ associated marker/s or QTL	GCA and SCA	Heterosis		Genetic variability, G × E interaction (GEI)
									Fe	Zn	
Anuradha et al. (2018)	Genetic variability for grain yield and micronutrients in the arid zone of India	130 pearl millet genotypes including two check varieties, viz., Dhanshakti and ICMB 98222	23–122	20–86	Phenotypic and genotypic correlation between Fe and Zn content was highly significant and was in positive direction	Grain Fe and Zn had a non-significant correlation with grain yield	Additive gene action	–	–	–	–

GCA general combining ability, *SCA* specific combining ability, *QTL* quantitative trait locus, *G* × *E interaction* genotype-environment interaction

Major challenges and way forward

To achieve the iron and zinc rich pearl millet, it should be capable of extracting Fe (2+ , 3+) and Zn (2+) from the soil and storing it in grain. The physiological basis for Fe (2+ , 3+) and Zn (2+) efficiency and their accumulation controlling processes in the edible part of pearl millet is not properly understood (Manwaring et al. 2016). In some crops, micronutrient fertilizers e.g. Zn^{2+}, Ni^{2+}, I, Co^{2+}, Mo^{2+}, and Se^{-2}, have special effects on their deposition in the edible part of the plant (Bouis and Welch 2010). Whereas some other micronutrients fertilizers have a trace effect on the amount of the micronutrient accumulation, applied to soils (Dimkpa and Bindraban 2016). This is particularly a fact in the case of Fe (2 + , 3 +) as it has limited phloem sap mobility (Bouis and Welch 2010). Hence, not only the fertilizers applied to soil but also the efficiency of a plant to take up micronutrients from soil, translocation and finally stored in the edible portion is required for increasing grain Fe(2+ , 3+) and Zn (2+)-content in pearl millet. Hence, more studies should be aimed to know the factors responsible for the accumulation of more Fe (2+ , 3+) and Zn (2+) in grains of pearl millet.

To develop the high grain yielding, Fe (2+ , 3+) and Zn (2+) dense pearl millet, breeders should have Fe (2+ , 3+) and Zn (2+) rich lines and access to molecular labs with skilled labour to identify Fe (2+ , 3+) and Zn (2+) efficient lines in early segregating generations. In this area, the improvement is very limited and the development of highly nutritious varieties is a challenge.

The bioavailability of a nutrient is defined as the proportion of the ingested micronutrient that is absorbed and used for normal body functions (Gharibzahedi and Jafari 2017). Many factors could affect this process, but the prominent ones are the original profile of food, processing of food, and digestion efficiency. Nutritional composition is the mix of macronutrients and micronutrients within a product, but their absorption efficiency in the body depends on the ability of food to be digested easily. Food that is absorbed easily maintains or improves health and energy status by providing appropriate macro-and micronutrients in balanced form and is thus called healthy food. Neglected or underused crops such as minor millets and pseudo-cereals were part of the common diet of ancient cultures but slowly, after the Green Revolution, the higher availability and accessibility of rice, wheat, and maize overtook these neglected crops and started providing $> 60\%$ of the calorific intake through these three crops only, thus starting to create a nutrient-imbalanced diet (Rodríguez et al. 2020). Hurrell and Egli (2010) reported improved iron absorption with the phytate-to-iron ratio of $< 1:1$ and preferably $< 0.4:1$ in plain cereal and legume-based meals, or $< 6:1$ in composite meals including vegetables with

Table 6 Review of various Quantitative trait locus (QTLs) and association mapping studies carried out in reference to grain Iron (Fe) and Zinc (Zn) content in pearl millet

Authors	Objective	Material	No. of lines evaluated	Fe range (mg/kg)	Zn range (mg/kg)	No. of markers used	No. of associated marker/s and QTLs name or linkage group (LG)/positions (cM)	Flanking markers	Phenotypic variation explained (PVE)
Kumar et al. (2016)	Mapping quantitative trait loci controlling high iron and zinc content in self and open-pollinated grains of pearl millet	F_6: Recombinant inbred line (RIL) derived between ICMB 841-P3 (Low Fe-Zn) × 863B-P2 (High Fe-Zn)	144	a) Fe-Self 28.4–124 b) Fe-OP 22.4–77.4	a) Zn-Self 28.7–119.8 b) Zn- OP 21.9–73.7	96 SSRs; 208 DArT	a) In selfed seed, a single co-localized QTLs for Fe and Zn content on LG 3 (3/110) b) In open-pollinated (OP) seeds-two QTLs for grain Fe content on LG 2 (2/30) and 5 (5/118), and two QTLs for grain Zn content on LG 3 (3/110) and 7 (7/96)	a) Fe and Zn: *Xpsmp2214-Xipes0142* b) OP Fe: *Xpsmp322-Xipes181* *Pgpb11029-Pgpb8456* OP Zn: *Xpsmp2214-Xipes0142* *Xpsmp2040-Pgpb10727*	Fe: 19% Zn:36% OP Fe:18.1%, 18.3% OP Zn: 50.1%, 19.7%
Anuradha et al. (2017)	Genome-wide Association mapping of QTLs for Fe and Zn	A diverse inbred panel consists of B lines, R Lines and advanced breeding lines	130	32.3–111.9	26.6–73.7	250 SSRs and 17 genic markers	SSR markers were associated with both grain Fe & Zn content were present on LG 3, LG 5 and LG 7	*Xipes 0180* (*aspartic proteinase gene*) *Xpsmp2261* (intergenic region) *Xipes 0096* (dropped)	11.40%, 13.34% and 11.38% R^2 value respectively
Kumar et al. (2018)	Mapping grain iron and zinc content QTLs in an iniadi-derived immortal population of pearl millet	F_6- RILs derived between (ICMS 8511-S1-17–2-1–1-B-P03: Low Fe-Zn × AIMP 92901-S1-183–2-2-B-08): High Fe-Zn	317	20–131	18–110	177 DArTs and 19 SSRs	Eleven QTLs were for Fe and eight were for Zn. Three co mapped QTLs for Fe and Zn were observed, one on LG1(1/54) and two on LG7 Fe: (7/86, 7/108) Zn: (7/82,7/112)	Fe-Zn: *pgpb10531-pgpb9130* Fe: *pgpb8427-pgpb13221* *pgpb11938-pgpb8987* Zn: *Xipes198-pgpb8427* *pgpb12329-pgpb9721*	Fe: 31.9% Zn:30.4% Fe: 12.2% 12.5% Zn: 10.2% 10.9%
Pujar et al. (2020)	Genome-wide association study uncovers genomic regions associated with grain iron, zinc and protein content in pearl millet	A diverse inbred panel consists of B lines, R lines and advanced breeding lines	281	32–120	19–87	58,719 SNP	18 MTAs (Marker-Trait Associations) were associated with Fe, 43 with Zn Among which 4 MTA were present on Chr-4, 5 and 7. co-segregated for Fe and Zn	*Pgl04_64673688* (unknown function) *Pgl05_135500493* (*Glycosyl transferase, family 1*) *Pgl05_144482656* (unknown function) and *Pgl07_101483782* *(Pentatricopeptide repeat)*	5.71%, 6.43%, 5.18% and 5.91 R^2 value respectively

Table 6 continued

Authors	Objective	Material	No. of lines evaluated	Fe range (mg/kg)	Zn range (mg/kg)	No. of markers used	No. of associated marker/s and QTLs name or linkage group (LG)/positions (cM)	Flanking markers	Phenotypic variation explained (PVE)
Mahendrakar et al. (2020)	Discovery and validation of candidate genes for grain iron and zinc metabolism in pearl millet [*Pennisetum glaucum* (L.) R. Br.]	bi-parental RIL mapping population AIMP 92,901 (high grain Fe and Zn), and ICMS 8511 (low grain Fe and Zn)	RIL	–	–	–	–	GFeC and GZnC—*PglZIP, PglNRAMP and PglFER gene families* GFeC—*Ferritin-like gene, PglFER1*	–
Singhal et al. (2021)	Multi-environment quantitative trait loci mapping for grain iron and zinc content using bi-parental recombinant inbred line mapping population in pearl millet	F_6- RILs derived between (PPMI 683 × PPMI 627)	210	36–114 mg/Kg	20–106 mg/Kg	SSRs	a) Grain Fe- four significant common QTL (QFe2.1), (QFe3.1), (QFe5.1) and (QFe 7.1) b) Grain Zn-one common QTL at all locations on LG 3(QZn 3.2)	a) QTL1- *Ppmsb001–Ppmsb002* QTL2- *IPES0142–IPES0180* QTL3- *IPES0157–IPES0093* QTL4- *IPES0206–IPES0015* b) *IPES0142–IPES0180*	a) QTL1-18.34, 17.98, and 15.98% on LG 2 QTL2- 14.99, 16.26, and 14.8% on LG 3 QTL3-19.66, 18.83, and 16.85% on LG 5, QTL4- 15.66, 16.61, and 14.56% on LG 7at Delhi, Dharwad and Jodhpur respectively b)14.39, 16.07, and 22.26% Delhi, Dharwad and Jodhpur respectively

SSRs simple sequence repeat, *Fe-Self* (Fe content of self-pollinated grains), *Fe-OP* (Fe content of open-pollinated grains), *DArT* diversity arrays technology, *SNP* (single nucleotide polymorphism) added enhancers like ascorbic acid and meat. In the case of zinc, its absorption in a diet based on unrefined cereals was estimated at 18–28% when the phytate/zinc ratio was > 18. Another study revealed that feeding young children with biofortified iron and zinc in pearl millet as a major food, fulfilled the dietary requirement for these micronutrients (Kodkany et al. 2013). Tako et al. (2015) evaluated pearl millet with high Fe (2+ , 3+) content enriched with high absorbable Fe (2+ , 3+) but its absorption is limited because of high polyphenols concentration. The objective of this study was to compare the capacity of biofortified iron and conventional pearl millet lines. Pearl millet lines with low iron ("DG-9444") (26 µg/g) and high iron (ICTP-8203 Fe) (85 µg/g) were investigated, and the high iron line exhibited greater absorbable iron after an in vitro comparison. The presence of high polyphenolic content and phytic acid that inhibits iron absorption was indicated by a low in vitro value. This study concludes that a high iron diet can transfer more absorbable Fe (2+ , 3+) by increased Hb and these observations are beneficial as these polyphenols compounds can be modified during breeding in order to improve the bioavailability of dietary iron. Sihag et al. (2016) studied the in vivo bioavailability of iron by weaning food developed from iron and vitamin A fortified pearl millet and also examined the effect of vitamin A on iron bioavailability in males Wistar albino rats. They provided an iron + vitamin A fortified diet to anaemic rats and found increased iron bioavailability, and liver iron returned to normal levels after 30 days, indicating a promoter role of vitamin A in intestinal iron absorption.

An increase in micronutrient contents in the pearl millet is an initial step in making it a rich source of nutrients for humans. Micronutrients in pearl millet are less bioavailable to humans due to the presence of anti-nutrients in plants that hinder the absorption of these micronutrients in human body (Bouis and Welch 2010). In general, pearl millet grains have very less bioavailability of Fe (2+ , 3+) and Zn (2+) i.e. ~ 5% of the total Fe (2+ , 3+) and ~ 25% of the total Zn (2+) present in the grain is thought to be bioavailable. By using a biofortification strategy, it is possible to greatly improve the availability of Fe (2+ , 3+) (~ 5 to 20%) and Zn (2+) genetically. Phytate and certain poly-phenolics present naturally in pearl millet act as antinutrients that reduce Fe (2+ , 3+) and Zn (2+) bioavailability. The high Fe (2+ , 3+) food can deliver more absorbable Fe (2+ , 3+) as evidenced by the amplified Hb (Bouis and Welch 2010). But some Fe (2+ , 3+) fortified varieties are also have elevated polyphenolic content, which reduces Fe (2+ , 3+) bioavailability. So the polyphenols represent prospective targets which can perhaps be modified during the breeding to improve dietary Fe (2+ , 3+) bioavailability. Hence, the anti-nutrients profiles must be carefully evaluated to further improve the nutritional benefits of this crop.

Nutritionists must have accessible resources like Fe (2+ , 3+) and Zn (2+) rich pearl millet lines, strategies to calculate the bio-accessibility and previous assessment of the efficacy of biofortified crop with Fe (2+ , 3+) and Zn (2+). Studies to know about the factors responsible for increasing/decreasing bioavailability of Fe (2+ , 3+) and Zn (2+) especially while using biofortified pearl millet is essential for driving off hidden hunger.

By virtue of its benefiting features and ecological sustainable concerns to health, a conventional but less acknowledged crop such as pearl millet provides a good option for the biofortification program. A chief priority from the breeder's point of view is to utilize genetic diversity in pearl millet gene pools for high Fe (2+ , 3+) and Zn (2+) content. Further, these variations can be used for advance next-generation sequencing technology that generates more markers for characterization of genomics assisted breeding and marker-trait associations. After the implementation of such approaches, it will be simpler to find the genetic profile of these traits in other millets by comparative mapping. Other agronomic traits that govern Fe (2+ , 3+) and Zn (2+) content and related markers that are tightly linked to governing genes can be an efficient approach to develop pearl millet varieties with high Fe (2+ , 3+) and Zn (2+) content by conventional or modern breeding strategies and transformation methods. For instance, before arbitrarily improving grain Fe (2+ , 3+) and Zn (2+) content, the future should aim for improvement in the efficiency of mobilization, transportation and storage of Fe (2+ , 3+) and Zn (2+) content in more biologically available forms in plant.

Conclusion

To march towards "Micronutrient malnutrition free India" millets to be included in daily diet which is a rich source of micronutrients. Among all micronutrients, Fe (2+ , 3+) and Zn (2+) which plays a vital role in hidden hunger are more densely packed in the grains of pearl millet. Further biofortification of pearl millet with Fe (2+ , 3+) and Zn (2+) can address the mania very quickly. Seven biofortified varieties of pearl millet have been released in India since 2013. Their fast multiplication and extension in farmer fields is the need of the hour to alleviate the malnutrition among society. In a way, soil or foliar supplementation with micronutrient fertilizer is a practical option but not sustainable and economically feasible for resource-poor farmers particularly in semi-arid and arid regions where pearl millet is a prime crop. Hence, biofortification in pearl millet comes out to be a popular, cost-effective and long-lasting approach among underdeveloped and developing nations. Many previous studies indicated a large and useful genetic variation for grain Fe (2+ , 3+) and Zn (2+) content in pearl millet which can be exploited intensively under pearl millet biofortification program. Also, a significant and relevant relationship between grain Fe (2+ , 3+) and Zn (2+) content with other agronomic traits have been revealed in many studies. Exploiting the germplasm variability HarvestPlus program with the help of ICRISAT targeted pearl millet for biofortification by screening the germplasm and they also released cultivars for further breeding purposes. In future, designing breeding programs for making micronutrients available at needed proportion, more attention should be paid to the association between Fe (2+ , 3+) and Zn (2+) content, with the increase in promoters and reduction in anti-nutritional elements which hinder micronutrient bioavailability.

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Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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