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REVIEW

# Biofortified foxtail millet: towards a more nourishing future

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**Abstract**
Biofortification of staple food crops is an economical and practical way to mitigate micronutrient malnutrition as it predisposes humans to different health maladies. Despite the availability of various methods for biofortification, the biofortified crops, especially millets, could offer a great scope. Foxtail millet has adequate content of minerals, non-starchy polysaccharides, vital amino acids, and proteins, and is regarded as one of the most important nutri-cereals. However, biofortified foxtail millet can potentially alleviate the micronutrient deficiency. Genetic modification to improve the micronutrient content through the available zinc and iron-regulated transporters in foxtail millet can be useful to fine tune the enrichment of micronutrients. The availability of well annotated foxtail millet genome sequence information can facilitate gene mining, transcripts and proteins related to nutritional quality. Combining the insights gained from proteomics, transcriptomics, genomics, and metabolomics might help foxtail millet to become a model system. This article describes the different aspects of biofortification in foxtail millet as the biofortified crop for the present and future.

**Keywords** Foxtail millet · Biofortification · Micronutrients · Macronutrients · Genomics · Proteomics · Metabolomics

## Introduction

Malnutrition, caused by vitamin A, iodine, and/or iron insufficiency, as well as zinc inadequacy, affects the human population including women and children in the world's poorest communities however, pregnant women and children under five years are at a higher risk (Pritwani and Mathur 2015). Considerable progress has been made to reduce the extent of malnutrition, but the deficiencies continue to impinge on the health of women and children (Kennedy 2002). Several sustainable food production strategies and nutri-crop systems are being developed to manage nutritional security in developing countries (Singh and Mondal 2017). The four major staple crops that meet food security are rice, wheat, maize and barley, however biofortified crops such as millets are thought to significantly contribute to nutritional security (Kaur et al. 2019). Millets are mainly produced in the central regions of Africa and Asia. The global scenario of millet production at 28.4 million metric tons with India as the major producer (Fig. 1 A; FAOSTAT 2021). Millets belong to the family Poaceae and vary in shape, size and color. Several types of millets such as; kodo millet (*Paspalum scrobiculatum* L.), pearl millet (*Cenchrus americanus* L. Gaertn & Morrone), barnyard millet (*Echinochloa*

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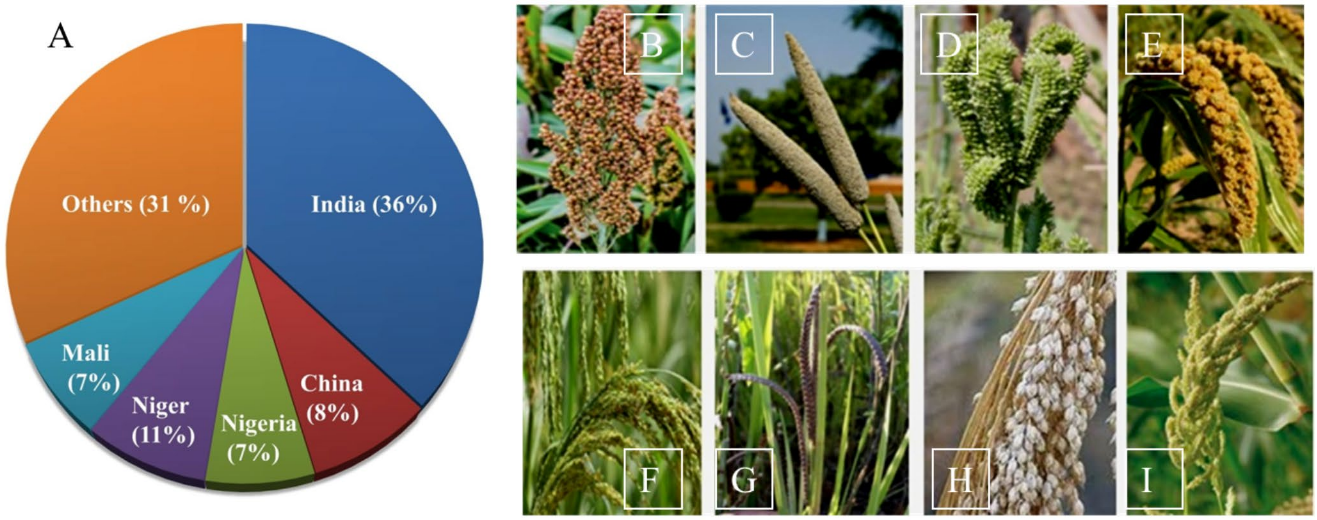



**Fig. 1** **A**. Global status of millet production (This is an original diagram constructed by K. P. Ingle for this manuscript and data obtained from (FAOSTAT, 2021); **B-I**: Different millets grown in Asia and Africa (FAOSTAT, 2021) **B**, Sorghum **C**, Pearl millet **D**, Finger millet **E**, Foxtail millet **F**, Little millet **G**, Kodo millet **H**, Proso millet **I**, Barnyard millet

*esculenta* (A. Braun) H. Scholz), finger millet (*Eleusine coracana* (L.), little millet (*Panicum sumatrense* Roth. ex Roem. & Schult.), proso millet (*Panicum miliaceum* L.), foxtail millet (*Setaria italica* (L.) P. Beauv) are indigenous to several countries (Neeraja et al. 2017; Garg et al. 2018; Kumar et al. 2018). Some of the major millets are grown in Asia and Africa (Fig. 1B-I).

Millets are rich in different nutrients, proteins, and various minerals, and almost 80% of millet grains are used as food while 20% is utilized for feed and industry (Shivran 2016; Kumar et al. 2018). Millets are considered as ideal nutrition for newborn children, lactating mothers, convalescents and old. The grains dissipate sugar gradually into the circulatory system and are considered "sans gluten" (Arendt and Dal Bello 2008). There is a high demand for the millets due to rich protein and high fiber content which favored them as dietary nourishment for individuals with cardiovascular sicknesses and diabetes (Arendt and Dal Bello 2008). Flavonoids and phenolic acids in millets have an important function in scavenging free radicals caused by oxidative stress, which has a lowering influence on blood glucose levels (Kunyanga et al. 2012; Muthamilarasan et al. 2016). Pearl millet has iron (Fe) and zinc (Zn) in the range of 5-11.2 and 3- 7.1 g/100 g, respectively (Kulp 2000; Hadimani et al. 2001) with considerable amounts of bioactive substances such as phenols, carotenoids and phenolic acids (Kumar et al. 2018; Zhang et al. 2007). Finger millet has abundant polyphenols (Chandrasekara and Shahidi 2011; Devi et al. 2014), minerals such as calcium, magnesium, and potassium (Devi et al. 2014; Kumar et al. 2018) and elevated levels of amino acids such as lysine and methionine, tryptophan (Bhatt et al. 2011). Barnyard millet has high rough fibre (13.6%) and Fe (186 mg/kg dry matter), while proso millet has the highest protein content (12.5%) (Kumar et al. 2018). Barnyard millet grains have gamma-aminobutyric acid (GABA) and -glucan, which act as cell reinforcing biochemicals which help lower blood lipid levels (Sharma et al. 2016). The dietary fibre level of little millet and kodo millet is high, while little millet has high magnesium concentration (1.1 g/kg dry matter).

Foxtail millet is reported to have been domesticated in China almost 8700 years ago and is regarded as one of the world's oldest crops (Yang et al. 2012; Goron and Raizada 2015). The most extensive collection of foxtail millet germplasm, totaling 27,059 accessions is maintained at the Chinese Academy of Agriculture Science (Diao and Jia 2017) followed by 14,000 foxtail millet germplasm accessions in gene banks of Japan, Korea, the United States of America, Russia, and in other countries, and 1542 foxtail millet germplasm accessions are maintained at the International Crop Research Institute for Semi-Arid Tropics (ICRISAT) (Vinoth and Ravindhran 2017). The genetic diversity of foxtail millet has been explored by using morphological and biochemical indices (Van et al. 2008; Jia et al. 2009; Nirmalakumari and Vetriventhan 2010). The modern foxtail millet has a variability based on waxy and non-waxy grain type which is due to low amylase levels in the grain endosperm, imparting a sticky texture to grain upon cooking (Van et al. 2008). Interestingly, such diversity is seen to set a coincidence of ethnological preferences with the geographical occurrence of these two groups of foxtail millet. For example, some local communities prefer waxy millet phenotype (Van et al. 2008), while the non-waxy grain phenotype is more widely planted and grown in Africa and Eurasia (Kawase et al.

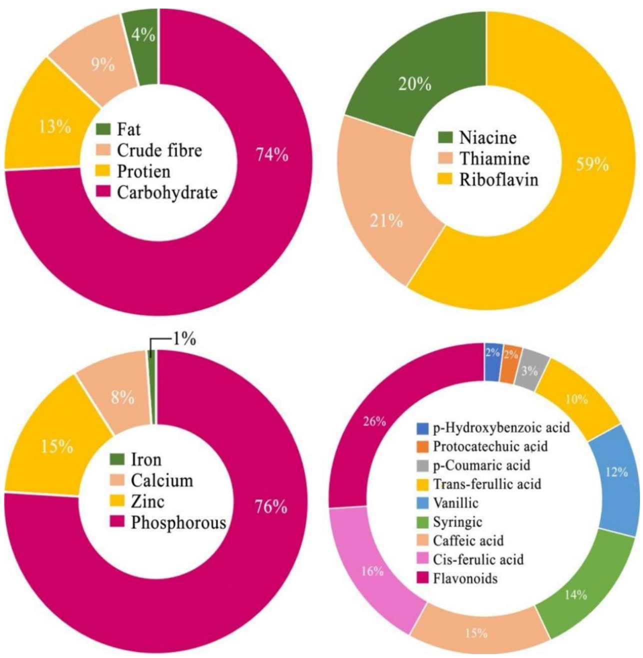



**Fig. 2** Diagrammatic representation of the nutritive value of foxtail millet. (This is an original diagram constructed by K. P. Ingle for this manuscript and data obtained from (Neeraja et al. 2017; Kumar et al. 2018)

2005). To date, two complete reference genome sequence data have been generated in genotypes Yugu1 and Zhang Gu (Wang et al. 2012; Lata et al. 2013).

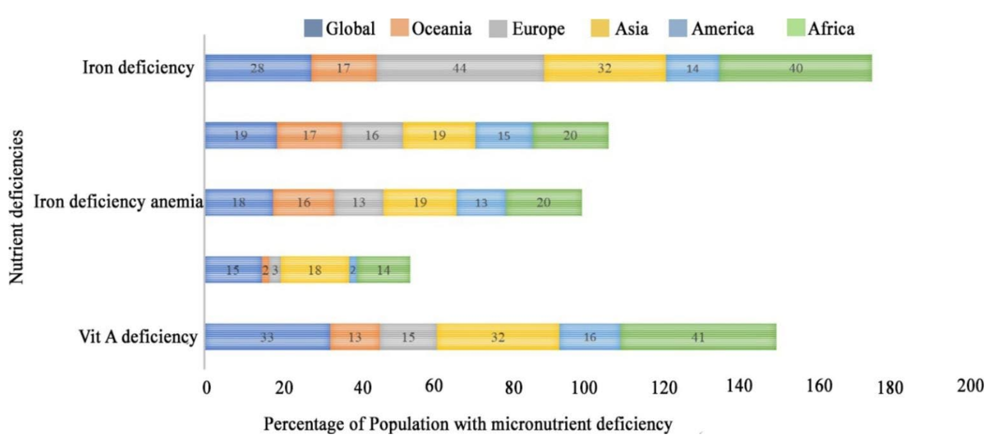
Foxtail millet has high content of minerals, non-starchy polysaccharides, vital amino acids, and proteins, and hence it is regarded as one of the world's most important nutri-cereals (Gowda et al. 2022). The main carbohydrate present in foxtail millet is starch and it contributes up to 60% of dry weight. Amylose makes up 25% and amylopectin, up to 75%. The linear structure of amylose and the amylopectin's branched structure contribute to the millet's unique nutritive quality. The major amino acids present in foxtail millet are methionine, valine and lysine. The grain composition of foxtail millet has high protein (14–16%), fat (5–8%) and minerals as compared to cereals (Thathola et al. 2011; Ravindran 1992). Further, digestible protein also has majority of the essential amino acids compared to major cereal crops such as rice and wheat (Zhang et al. 2007). Nutritional superiority of foxtail millet grain is also shown by more edible fiber content (2.5 fold) and the bran has 9.4% crude oil containing 66.5% linoleic and 13.0% oleic acid (Liang et al. 2010; Black et al. 2013).

Foxtail millet is extensively used as an energy source for the children, diabetic patients and pregnant and nursing women (Pasricha et al. 2021). Health benefits are represented by its effects in reducing serum lipids, blood glucose and glycosylated hemoglobin in patients with type 2 diabetes (Thathola et al. 2011). The mineral content of foxtail millet ranges from 1.7 to 4.3 g/100 g dry weight. The calcium, iron, phosphorus, and zinc concentrations in foxtail millet are 31, 3.5, 300 and 60.6 mg/100 g dry weight, respectively. Thiamine, niacin and riboflavin are present in foxtail millet and their concentration is 0.60, 0.55, 1.65 mg/100 dry weight, respectively. A higher concentration of vitamins and minerals than other cereals makes foxtail millet an easy and cheap substitute to tackle nutritional adversity (Neeraja et al. 2017). Besides macro and micronutrients, foxtail millet possesses important phenolic acids, flavonoids, and tannins, known for their antioxidant, anti-mutagenic, antiviral, and anti-inflammatory effects (Neeraja et al. 2017). A detailed presentation of different phenolic compounds along with vitamins, macro and micronutrients is given in Fig. 2.

## Micronutrients and biofortification

Micronutrients are mainly composed of vitamins and minerals and they are vital for human growth and development. It is estimated that malnutrition-associated mortality in children accounts for 3.1 million deaths, of which 1.1 million are due to micronutrient deficiencies (Black et al. 2008; Brown et al. 2001). The percentage of population with selected micronutrient deficiencies is represented (Fig. 3). The deficiency of micronutrients results in serious illness,

**Fig. 3** Percentage of population with selected micronutrient deficiencies. Adopted from Global Hunger Index -The Challenge of hidden hunger. (modified from (Lockyer et al. 2018))



weakened immune system, malnutrition and underdevelopment. This problem has been identified as a serious and increasing problem in both developing and underdeveloped countries. One of the key causes impacting children, premenopausal women, and adults in low- and middle-income nations is iron deficiency anemia (Brown et al. 2001). Iron, folate and vitamin $B_{12}$ deficiency results in anemia while there is also coassociation of the deficiency with other ailments such as lower learning ability, memory and neuropsychological behavior among children (Bailey et al. 2015). Iron also plays a crucial function in hemoglobin, myoglobin, enzymes, and cytochromes and is necessary for oxygen transport and cellular respiration (Bailey et al. 2015). Unlike iron, zinc does not experience a decline in blood levels in the event of a severe defficiency. Children often have a severe type of zinc deficiency. The diets of people in South Asia, South East Asia, and Africa appear to contain relatively little zinc (Bailey et al. 2015; Lockyer et al. 2018). As zinc interacts with 925 proteins in humans, the symptoms of zinc deficiency may be multiple and indiscriminate, which makes it extremely difficult to diagnose in humans. Deficiency of Vitamin A is associated with childhood mortality and morbidity in the developing nations, particularly in Africa and Southeast Asia (Hodge and Taylor 2022).

Supplementation and fortification are relatively the sustainable approaches that can tackle the problem of micronutrient deficiency. Short-term and long-term strategies have been identified considering the importance and relevance of micronutrient deficiency worldwide (Ruel and Levin 2001). The "Short term strategies" involve the addition of additional food or nutrients in the form of capsules, pills, or syrups to a high-risk population. Considerable success has been made by using the vitamin A and zinc supplementation methods to manage micronutrient deficiencies (Black et al. 2008). One of the success stories includes the vitamin A Global Initiative by the World Health Organization in 1998, leading to the prevention of an estimated 1.25 million deaths in 40 countries (Ruel and Levin 2001). The long-term strategies include food-based strategies to enable increased intake and bioavailability of micronutrients which can be accomplished through boosting the production, availability, and consumption of micronutrient-rich foods, as well as the bioavailability of micronutrients in the diet, trace mineral and vitamin concentrations, and absorption boosters. Currently, the options to improve nutritional conditions include, dietary diversification, which is referred to as consuming a variety of foods such as fruits, vegetables and livestock products rich in micronutrients can tackle the micronutrient deficiency (Mannar and Sankar 2004).

## Phytobiofortification and biotechnological interventions

Phytobiofortification is an innovative platform for delivering the nutrient density to improve the nutrition and livelihood of vulnerable population (Neeraja et al. 2017). Biofortification is utilized to promote mineral transport from roots to tissues, as well as mineral mobilization from soils to roots and mineral absorption in the body (Pérez-Massot et al. 2013). Different techniques of genetic engineering including genetic transformation using Agrobacterium sp., particle gun, genome editing is now used for improving crop plants for various traits such as stress tolerance, yield, plant architecture and nutritional quality (Gantait et al. 2022). Genetic engineering approach for increasing the required micronutrients is facilitated by the expression of genes for the regulation of metal homeostasis and carrier proteins that serve to increase the micronutrient content, bioavailability and greater productivity (Garg et al. 2018). In this regard, the transgenic approach of using transporters has become a good option for the higher uptake of nutrients (Shewmaker et al. 1999; Pérez-Massot et al. 2013). Genetic modification of various crops to improve their micronutrient content has been reported by several researchers (Nadeem et al. 2018, 2020). Genes for phytoene synthase (PSY), carotene desaturase, and lycopene β-cyclase for nutrients, ferritin and nicotinamide synthase for minerals, egg whites for basic amino corrosive, and Δ6 desaturase for basic unsaturated fats are the available target candidates for biofortification research (Fig. 4) (Ravindran 1992). Successful examples include high unsaturated fat soybean, vitamin A fortified golden rice, iron and zinc content in pearl millet, high lysine maize, high provitamin A and high iron and provitamin A cassava (Bouis and Welch 2010; Jaiswal et al. 2022). Furthermore, research needs to be prioritized on inducing genetic variability for the higher synthesis of micronutrients, their tissue redistribution and expanding biochemical pathways in palatable tissues (Yang et al. 2012; Garg et al. 2018).

## Foxtail millet and biofortification

The potential for biofortification of foxtail millet is immense and it can be achieved through conventional breeding, genetic modification and agronomic approaches for increasing the nutrient level in the grains or by increasing the availability of the nutrients by decreasing the anti-nutrient content (Bouis and Welch 2010; Vinoth and Ravindhran 2017). Agronomic methods that apply various minerals as fertilizers are not regarded economical as they require additional costs and management. In foxtail millet, Liang et al. (2020) evaluated the foliar spraying of sodium

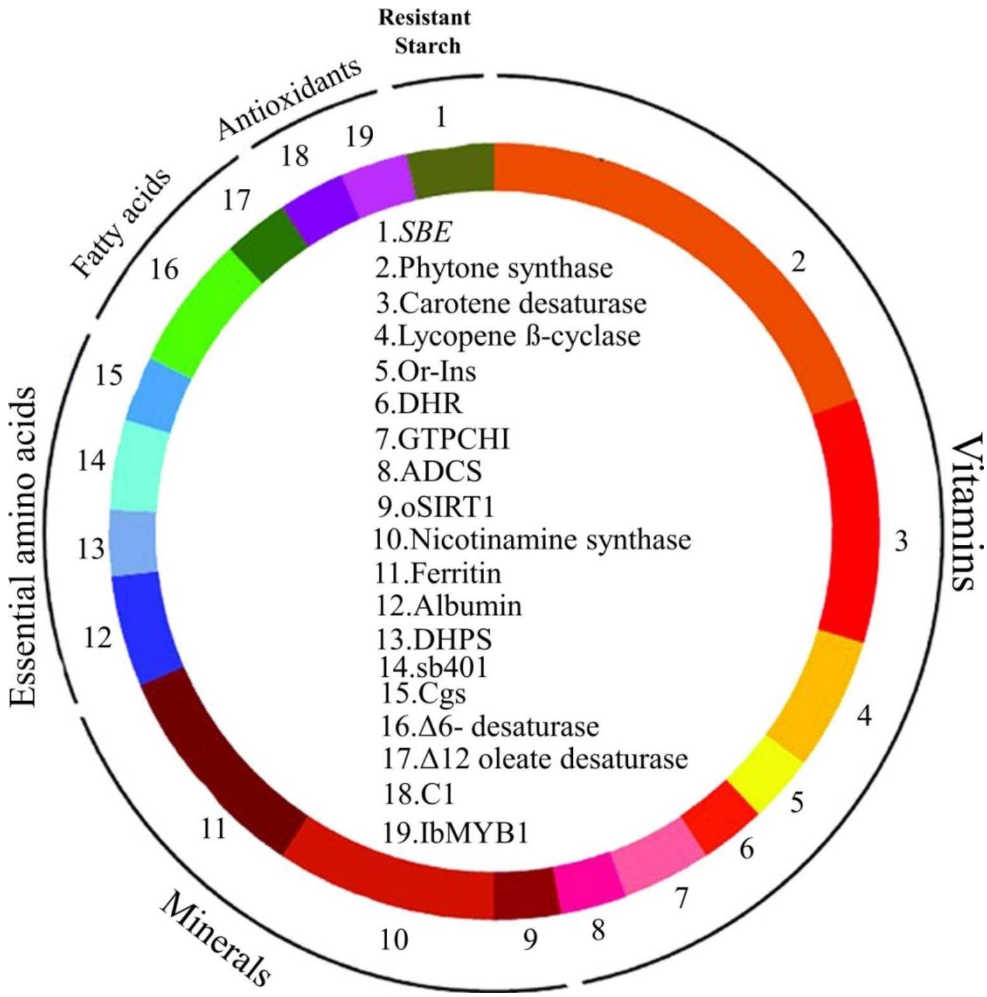



**Fig. 4** Application of different gene resources for biofortification using transgenic approach. [This figure is constructed by K. P. Ingle for this manuscript and data obtained and modified(Hirschi 2009)]

selenite ($Na_2SeO_3$), which led to a 9.8-fold rise in selenomethionine and selenocysteine with a concurrent increase in potassium and iron content. The findings suggest that foxtail millet has Se-inducible proteins that may prove valuable in Se-enriched millets. Nanoscale biofortification and supplementation with numerous micronutrients are advised for biofortifying Se in millets (Schiavon et al. 2020). If the desirable trait is not available in the germplasm, genetic modification technology is used to introduce desirable traits from different plant or non-plant sources. The advantages of this method are that multiple genes of interest can be incorporated and targeted expression in tissues can be achieved (Vinoth and Ravindhran 2017). More recently, engineering of membrane bound nutrient transporters has been viewed to play a key role in the biofortification of crops (Krishna et al. 2022).

Foxtail millet has desirable attributes of drought tolerance, pest resistance and the crop are enriched with micro and macronutrients. The development of a successful core collection depends on the proper sampling of phenotypic associations which are linked to the co-adaption of gene complexes (Reddy and Vijayaraghavan 1995). In this regard, phenotypic correlations are important for initial characterization by trait identification (Jia et al. 2009). Molecular and morphological markers have been used to identify genetic variability for nutritional traits in foxtail millet (Trivedi et al. 2018). Microsatellites, known as simple sequence repeats or SSRs, are responsible for maintaining their high number of polymorphism and high variation levels. Molecular evaluation of 30 foxtail millet accessions, led to exploring of untapped genetic diversity of foxtail millet in the Himalayan region for variability in nutritional traits such as dietary fiber, starch, protein and amino acid content. In the Indian context, there are also new biofortified varieties (SiA 3088: 129 ppm, SiA 3142 & TNAU-186) developed for high iron (> 129ppm) which have shown promise for use in the HarvestPlus program (Singh 2017). High iron foxtail millet varieties can find greater utility in the biofortification programs for alleviating iron deficiency among the preschool children, non- pregnant, non-lactating women of reproductive age (Andersson et al. 2017).

## Nutrient transporters of foxtail millet

Plants are endowed with several metal transporters, which play a crucial role in the uptake of metal ions to maintain metal homeostasis. One of the essential nutrients, nitrate, is transported by the high-affinity transport system (NRT2.1) and NRT1.1. In foxtail millet, both SiNRT1.11 and SiNRT1.12 are up regulated under conditions of nitrogen limitation (Ceasar et al. 2017; Nadeem et al. 2020), and up regulation of SiPHT1.1, SiPHT1.2, and SiPHT1.4 has been observed in roots for better inorganic phosphate (Pi) uptake (Ceasar et al. 2017; Alagarasan et al. 2017). Also, downregulation of SiPHT1;2 affected the Pi uptake in foxtail millet seedlings (Ceasar et al. 2017). The zinc and iron-regulated transporter-like Proteins (ZIP) are majorly involved in the acquisition of zinc and iron (Krishna et al. 2022). In rice, OsZIP4, OsZIP5 and OsZIP8 are functionally validated as zinc transporters, whereas in foxtail millet, SiZIP genes have been shown to be Zn and or Fe transporters engaged in divalent metal ion absorption, transport, and storage (Ortiz et al. 1998). These studies suggest the crucial role of transporters, including the ZIP genes and their regulation in foxtail millet may be useful for enriching the micronutrients content. Membrane transporters for Zn / Fe which are associated with grain filling have been shown to be good candidates for genetic improvement (Krishna et al. 2022; Ramegowda et al. 2013) achieved higher Zn accumulation in finger millet grain through the transfer of rice zinc transporter OsZIP1. In wheat, a vacuolar iron transporter gene ***(TaVIT2)*** under the control of endosperm-specific promoter led to 2-fold higher iron in the grain (Connorton et al. 2017; Boonyaves et al. 2017) reported increased iron levels in grain (10.46 µg/g dry weight) by transgenic expression of a metal transporter, nicotianamine synthase. In rice, knockout of zinc transporter OsZIP9 in rice showed reduced Zn levels in grain and other tissues suggesting crucial role of the gene in Zn uptake (Yang et al. 2020).

## Transgenic foxtail millet

Nutritional quality of foxtail millet can also be improved via genetic engineering (Ceasar and Ignacimuthu 2009). The first agrobacterium-mediated foxtail millet transformation was developed by Liu et al. (2005). This method yielded a 6.6% transformation frequency. Until recently, all published reports on foxtail millet have relied on the transgenic methods of Wang et al. (2011) or Liu et al. (2005). Furthermore, both these methods employed undeveloped inflorescence as beginning explants and have a poor transformation efficacy (5.5%). In a novel finding, Ceasar et al. (2017) studied the possibility of employing shoot apices for transformation and achieved higher transformation efficiency of 9%. However, the development of chimera transgenic plants is a shortcoming of employing shoot apices (Ceasar et al. 2017). In recent reports, a simple and robust agrobacterium-mediated transformation method developed in foxtail millet with 27% transformation efficiency (Sood et al. 2020), that may speed up forward and reverse genetic investigations in foxtail millet.

The transgenic approaches for vitamin production in plants have been made possible through metabolic engineering. Golden rice is an example that has been genetically engineered to produce provitamin A (Paine et al. 2005). In order to permit commercial production of genetically modified (GM) crops, regulatory challenges are being solved utilizing scientific data. GM millets with increased vitamin levels can offer good scope for biofortification (Vinoth and Ravindhran 2017). Further, the application of CRISPR/Cas9 mutagenesis in foxtail millet modified the phytoene desaturase (PDS) gene was accomplished using protoplast transfection in foxtail millet (Lin et al. 2018). In a recent study, Liang et al. (2022) have developed single gene and multigene knockouts and single base substitution by CRISPR/Cas9 method in *Setaria italica* and isolated a herbicide mutant by cytosine base editing to target the SiALS (acetolactate synthase) gene. Further utilization of genome editing tools like CRISPR/Cas could help to improve nutrients in foxtail and other millets (Ceasar 2022). This method could be further utilized for editing the target genes to improve crop nutritional quality.

## Genomics

With the rapid advancement of genome sequencing in recent years, the majority of crops now have high resolution genetic linkage maps and genomic sequencing information (Varshney et al. 2021). Furthermore, the sequencing data enables the mapping of sequence variation related to traits of interest, as well as the creation of molecular tools for crop improvement using genomics. Recently, genome-wide association studies have been applied for understanding the genetic regulation of natural variation in foxtail millet for certain agronomic features (Zhang et al. 2012; Jia et al. 2013). Marker-assisted selection has helped to speed up the conventional breeding process. Genotyping diverse foxtail millet germplasm using high throughput resequencing will make it easier to develop novel genetic markers to map important traits. The population genetics of diverse foxtail millet germplasm has been studied using SNPs and SSRs (Jia et al. 2013; Wang et al. 2012). A foxtail millet haplotype map has been built based on 0.85 million SNPs discovered in 916 cultivar genomes throughout the world, as well as 512 quantitative trait loci (QTL) (Jia et al. 2013). For effective crop breeding using marker-assisted selection, it is critical to identify important QTLs (Wang et al. 2017). Also, the identification of markers such as SNPs and InDels, associated with nutritional factors will interpret information on possible genes driving these variables. Because millets have good cross-genera transferability, use of molecular breeding or genetic engineering to introduce nutrient-linked genes into other cereals may become feasible. Transcriptome data on changes in gene expression of storage compound associated genes (Jayaraman et al. 2008) can be looked into for the selection of genes of pathways involved in the biosynthesis of nutritional compounds for calcium accumulation.

The foxtail millet was the first millet crop to have its whole genome sequenced, and it has the smallest genome (423–510 Mb), serving as a paradigm for C4 crop species. Also, the disclosure of draft genome sequences of Yugu1 and Zhang gu, cultivars of foxtail millets has progressed studies for its further improvement (Bennetzen et al. 2012; Zhang et al. 2012). Shi-Li-Xiang, a foxtail millet waxy landrace, was resequenced utilizing Solexa sequencing technology and the Genome Analyzer II to investigate the nucleotide alterations spanning agronomic trait-related genes. InDels, SVs and SNPs were discovered using alignment with reference genomes. Re-sequencing yielded novel markers that aided in the genome mapping of starch synthase, which encodes the GBSS 1 peptide. The GBSS 1 gene was sequenced and transposable elements were discovered, confirming its waxy nature (Bai et al. 2013). Advances in genome editing and use of programmable site-specific nucleases can be useful to genetically alter non-waxy elite cultivars into desirable waxy types (Vinoth and Ravindhran 2017) and will accelerate improvement of biofortified foxtail millet and other millets.

## Proteomics

Information on the composition and quality characteristics of foxtail millet must be evaluated in order to generate value-added and functional protein in foods. The seeds of foxtail millet have health-promoting effects due to their exceptional protein composition, which has important amino acids in abundance. In foxtail seeds, setarins make up roughly 60% of the total protein composition, with less disulfide cross-linked proteins than other crops. Protein fractionation methods can help researchers learn more about the nature of foxtail millet proteins (Sachdev et al. 2021). WD40 proteins have been discovered to play an important role in protein-protein interactions by acting as scaffolding molecules and therefore aiding the proteins' optimal performance. (Mishra et al. 2014). Within the WD40 repetitions, these proteins include 16 conserved amino acids known as the "DWD box" (Angers et al. 2006; Hua et al. 2011). The FT-NIR has been used to assess the protein, amino acid, carbohydrate, and lipid contents of the foxtail millet (Chen et al. 2013). Using computational methods, 16 prolamin encoding genes known as setarins were recently characterized in foxtail millet and it has been shown that setarin genes' sequence alignment with other grains and millets indicated the least similarity, suggesting uniqueness in increased protein quality (Muthamilarasan and Prasad 2016). The authors also overexpressed setarin genes in growing spikes for functional verification in seed protein accumulation (Muthamilarasan and Prasad 2016). Such functional genomics studies might pave way for the development of high protein foxtail millet cultivars.

## Metabolomics

Metabolites are hypothesised to serve as a connection between the genome and the phenotype of the organism, and the metabolomic studies provide a metabolic atlas of the plant's physiological status (Chen et al. 2016). A metabolite study in millet and rice revealed species-specific accumulation of secondary metabolites such as flavonoids (Li et al. 2018). The Ultra Performance Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry equipment was used to identify 116 flavonoid metabolites in foxtail millet, of which 33 flavonoid metabolites were found to be substantially different between high and low eating quality types. These findings demonstrate the diversity in flavonoid accumulation in foxtail millet for breeding for high-flavonoid foxtail millet varieties (Zhang et al. 2021). An LC-MS-based metabolic profile study revealed variations in metabolite accumulation among 150 foxtail millet accessions from India and China. Cyanidin 3-O-glucoside and quercetin O-acetylhexside were found at 43.55 Mb on chromosome 5 and 26.9 Mb on chromosome 7, respectively, based on the mGWAS study, and two Lc genes were identified as candidate genes. This is the first study to use mGWAS in foxtail millet, making way for more research into exploring metabolomic diversity, especially for nutritionally relevant flavonoids (Wei et al. 2021).

## Conclusions and future perspectives

Micronutrient deficiency is considered to have a profound effect on global health. In this regard, millets are exceptionally nutritious crops that can be exploited extensively in the regions of Asia and Africa to overcome the nutrient deficiency. Biofortification is a highly sustainable and cost-effective way to mitigate micronutrient deficiency. However, more evidence will have to be generated on the impact of different strategies on health, morbidity, mortality, adverse effects, composition, use and delivery. The relationship between bioavailability, absorption of micronutrients and outcome requires urgent focus for long-term solutions of nutritional security. With desirable grain characteristics and significant levels of essential amino acids, minerals, and nutrients, foxtail millet is in favor of good acceptance to suit the needs of biofortification strategies. It is becoming the nutri-cereal crop since it is high in nutrients, essential amino acids, non-starchy polysaccharides, and proteins. It has nutritional properties equivalent to or greater than other crops, with high protein, minerals, essential amino acids, carbohydrates, flavonoids and vitamins. The bioavailability of nutrients can be improved by reducing antinutrients or by using innovative transgenic and genome editing tools. Understanding the functions of different transporters in nutrient absorption, translocation, and storage may aid in positioning macro- and micronutrients in millets' edible parts. Thus, biofortification programmes created to combat malnutrition can benefit from using foxtail millet. Further, the understanding of primary and secondary metabolism in foxtail can be useful to fine tune metabolic pathways to achieve high flavonoids accumulating foxtail millet genotypes for biofortification purpose. The availability of highly efficient genome editing systems with single, multiple genes associated with nutritional quality should contribute to fostering biofortification research in foxtail millet. Furthermore, biofortified millets offer a great scope in creating low-cost, protein-rich functional food items for improved nutritional security.

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## Declarations

**Conflict of interest** The authors have no relevant financial or non-financial interests to disclose.

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