Theoretical and Applied Genetics (2021) 134:3147–3165
https://doi.org/10.1007/s00122-021-03878-z

REVIEW

Genetic determinants of micronutrient traits in graminaceous crops to combat hidden hunger

P. Sushree Shyamli[1,2] · Sumi Rana[3] · Sandhya Suranjika[1,4] · Mehanathan Muthamilarasan[3] · Ajay Parida[1] · Manoj Prasad[5]

Received: 15 February 2021 / Accepted: 29 May 2021 / Published online: 6 June 2021

Abstract

Key message **Improving the nutritional content of graminaceous crops is imperative to ensure nutritional security, wherein omics approaches play pivotal roles in dissecting this complex trait and contributing to trait improvement.**

Abstract Micronutrients regulate the metabolic processes to ensure the normal functioning of the biological system in all living organisms. Micronutrient deficiency, thereby, can be detrimental that can result in serious health issues. Grains of graminaceous crops serve as an important source of micronutrients to the human population; however, the rise in hidden hunger and malnutrition indicates an insufficiency in meeting the nutritional requirements. Improving the elemental composition and nutritional value of the graminaceous crops using conventional and biotechnological approaches is imperative to address this issue. Identifying the genetic determinants underlying the micronutrient biosynthesis and accumulation is the first step toward achieving this goal. Genetic and genomic dissection of this complex trait has been accomplished in major cereals, and several genes, alleles, and QTLs underlying grain micronutrient content were identified and characterized. However, no comprehensive study has been reported on minor cereals such as small millets, which are rich in micronutrients and other bioactive compounds. A comparative narrative on the reports available in major and minor Graminaceae species will illustrate the knowledge gained from studying the micronutrient traits in major cereals and provides a roadmap for dissecting this trait in other minor species, including millets. In this context, this review explains the progress made in studying micronutrient traits in major cereals and millets using omics approaches. Moreover, it provides insights into deploying integrated omics approaches and strategies for genetic improvement in micronutrient traits in graminaceous crops.

Communicated by Rajeev K. Varshney.

P. Sushree Shyamli and Sumi Rana have contributed equally

✉ Ajay Parida
drajayparida@gmail.com

✉ Manoj Prasad
manoj_prasad@nipgr.ac.in

1 Institute of Life Sciences, NALCO Square, Chandrasekharpur, Bhubaneswar, Odisha 751023, India

2 Regional Centre for Biotechnology, National Capital Region Biotech Science Cluster, Faridabad, Haryana (NCR Delhi) 121001, India

3 Repository of Tomato Genomics Resources, Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, Telangana 500046, India

4 School of Biotechnology, Kalinga Institute of Industrial Technology, Bhubaneswar, Odisha 751024, India

5 National Institute of Plant Genome Research, Aruna Asaf Ali Marg, New Delhi 110067, India

Introduction

Globally, 2 billion people suffer from severe micronutrient deficiencies due to the non-availability of nutritious food (Mayer et al. 2008; White and Broadly 2009). Micronutrients constitute minerals and vitamins that are derived from the diet, as they could not be synthesized in the body (Bouis and Welch 2010). The daily requirement of each micronutrient varies according to the stage of development. An average healthy adult requires 15 mg each of iron (Fe) and zinc (Zn) and 600 µg of vitamin A; however, this requirement is not met through the food consumed by the majority of the population. The present-day food constitutes cereals in significant proportion, and these cereals have high calorific value. Thus, the normal staple diets supply only 2–3 mg of Fe, 7–8 mg of Zn, and traces of vitamin A, which is not sufficient to sustain the human body (https://www.who.int/vmnis/database/en/). These values are projected to decrease due to the poor availability of micronutrients in the frontline

crops like rice and wheat. Furthermore, while micronutrients play a prime role in regulating the metabolic activities in the cells and tissues, their deficiency results in irreparable consequences. Approximately 43% of the children below the age of five are prone to anemia due to Fe deficiency (Stevens et al. 2013). In the case of zinc, 17.3% of the population faces inadequacy in dietary zinc (Wessells and Brown 2012). Similarly, 190 million children at their pre-school age face vitamin A deficiency (Imdad et al. 2017; Visser et al. 2017). Previously, White and Broadly (2005) showed that 340 million children suffer from malnutrition, leading to stunting, wasting, or overweight, which results in disorders like xeropthalmia, skin disorders, and cancers. This has necessitated the biofortification of major cereals through breeding and transgene-based approaches (Garg et al. 2018). Further, studies on the mechanism of micronutrient uptake, the effect of soil fertility, processing techniques, targeted genes for nutrient mobilization, and anti-nutritional factors that hinder the bioavailability of micronutrients were performed (Welch and Graham 2004; Palmgren et al. 2008; Cakmak 2009; de Valenca et al. 2017; Das et al. 2019; Hossain et al. 2019).

Advancements in genetic dissection of micronutrient biosynthesis and accumulation in major cereals had pinpointed the genes, alleles, and QTLs underlying these complex traits. This information was further used in molecular breeding for improving the micronutrient content of the grains. Recently, recombinant DNA and genome editing approaches were also being deployed in major cereals for biofortification (Majumdar et al. 2018). These studies also widened the understanding of the research gaps and limiting factors in attaining the target mineral bioavailability in cereals. Though significant progress has been made in major graminaceous species (like rice, wheat, maize, and sorghum), a class of minor cereals, including small millets, remain underutilized and neglected (Muthamilarasan and Prasad 2021). Small millets constitute eleven millet species that are rich in minerals, vitamins, essential amino acids, and antioxidants (Saleh et al. 2013; Muthamilarasan et al. 2016; Vetriventhan et al. 2020). The micronutrient content of millet grains in comparison with major cereals is provided in Table 1. Despite their nutritional superiority over the major cereals, deciphering the genetic determinants of micronutrient contents in minor millets and exploiting them for the genetic improvement in cultivated varieties with enriched minerals and vitamins remain elusive. However, studies on major cereals provide a roadmap for identifying the genes, alleles, and QTLs underlying micronutrient traits in other graminaceous species. Further deployment of omics tools will enable the manipulation of target genes for improving the nutritional content of minor species per se. Also, it will facilitate the transfer of candidate genes to other major cereals through transgene-based approaches. Thus, identifying the genetic determinants holds the key for such biofortification programs, and given their importance, the present review enumerates the knowledge generated so far in understanding the genetic determinants of micronutrient traits in graminaceous crops using different approaches. It also provides the roadmap for further studies to enhance the nutritional potential of small millets and other graminaceous crops to provide a long-term and sustainable solution to micronutrient deficiency prevalent worldwide.

Current understanding of the mechanism of micronutrient uptake and their manipulation to enhance mineral absorption

The solubilization and mobilization of minerals from the soil to the grains are intricate processes involving different biochemical and molecular components. As the mineral uptake is proportional to the concentration or richness in the soil, agronomic biofortification provided limited success in improving the nutritional status of major cereals (Garg et al. 2018). However, an exogenous supply of minerals resulted in the accumulation of unused elements in the soil, which could be toxic to plant growth at higher

Table 1 Micro- and macronutrient content of millets and cereals

Crop	K	Ca	P	Mg	S	Zn	Fe	B	Mn	Ni
Barnyard millet	2680.1	1188.4	3198.2	1340.5	1080.5	32.7	22.7	5.8	14.45	2.6
Finger millet	3206.6	2707.6	2207.15	1378.35	954.7	22.85	26.8	8	114.65	0.95
Foxtail millet	2992.86	440.04	3939.12	1510.92	1615.56	44.45	18.15	4.45	9.4	2.7
Kodo millet	1817.85	167.3	2599.35	1263.7	1057.25	25.6	12.55	3.55	9.35	1.35
Little millet	1922.1	180.05	3392.5	1432.15	1185.1	26.9	32.65	4.55	9.15	1.85
Pearl millet	2756.75	180	2520.8	920.3	989.25	26.7	25.5	6.85	9.55	1.5
Proso millet	1773.15	98	3165.05	1357.55	1233.7	28.9	21.95	3.2	8.9	1.4
Wheat	3329.45	477.25	3055.2	870	835.35	28.8	19.6	2.85	19.7	0
Rice	1715.65	155.15	2753.75	1071.6	1065.05	33.3	8.15	10.8	5.8	0.85
Sorghum	3390.75	256.2	3105.55	1330.05	848.35	19.3	17.1	0	11.15	0.45

The data show the mean normalized concentrations (ppm) of the elements analyzed using inductively coupled plasma atomic emission spectrometer (ICP-AES)

concentrations. On the other hand, growing the crops in micronutrient deficient soils results in decreased accumulation, affecting the grain nutritional content (Shukla et al. 2016). For example, the uptake of zinc has been severely affected in crops grown in calcareous soils (Gupta et al.2016). Thus, gaining mechanistic insights into the nutrient uptake will provide insights into understanding the nutritional quality of these crops. Nutrient absorption from the soil could be facilitated by the secretion of redox enzymes, chelating compounds, and the association of microbes in the soil. Graminaceous crops absorb the micronutrients by chelation, while non-graminaceous species imbibe the micronutrients by reduction reactions occurring in the rhizosphere. Cereals eventually capture these micronutrients from their rhizosphere, where the nutrients enter the roots by acidification of their plasma membrane (Morrissey and Guerinot 2009). Due to this humification and change in the Eh (redox potential) of soils, the tightly held cations in the soil surface are released toward the roots by a cation exchange reaction (Gaxiola et al. 2007). To stimulate this absorption, plants also release organic acids and phytosiderophores to facilitate the uptake of essential nutrients like Fe and Zn. One such phytosiderophores is mugienic acid that has a higher affinity for absorbing Fe and Zn by chelation. Cereals like rice, wheat, and maize release these compounds into their rhizosphere using a transporter-like TOM 1 (Ishimaru et al. 2006). Rice secretes 2-deoxymugineic acid (2-DMA) while barley releases 3-epihydroxymugineic acid. These compounds effectively form complexes with the available Fe and Zn from the rhizosphere and effects absorption by chelation (Schaaf et al. 2004).

After absorption from the soil, further studies on the ascent of micronutrients under normal and controlled conditions in rice described the role of roots for a continuous uptake under control conditions. Typically, the roots absorb the required nutrients for the growth and development up to the plant establishment phase. After the ripening stage, the sinks depend entirely on their leaves for nutrient translocation (Cakmak and Kutman 2018). Hence, the roots initially transport the nutrients from the soil toward the stem and leaves which are stored as a reserve for distribution to other parts. Thus, identifying the genes involved in this uptake mechanism and exploiting them for enriching the cereal grains using transgenic-based approaches have derived importance (Fig. 1). Biofortification of the mainstream cereals by manipulating such genes involved in the uptake process has increased the Fe uptake up to 3.7-fold in rice (Goto et al. 1999). Improvising the Fe uptake in rice by overexpressing the chelating gene *NAS1* (nicotianamine synthase), iron influx gene *YSL2* (yellow stripe-like2), iron uptake gene *IDS3* (iron-deficiency-specific clone 3), and manipulation of Fe uptake regulating translocators [vacuolar iron transporters *VIT1* and *VIT2*, and hemerythrin motif-containing really interesting new gene (RING)-and zinc-finger protein *HRZ1*] has significantly increased the Fe concentration in the grains (Suzuki et al. 2008; Lee et al. 2012; Ishimaru et al. 2010). The uptake of Fe by reducing Fe^{3+} to Fe^{2+} to

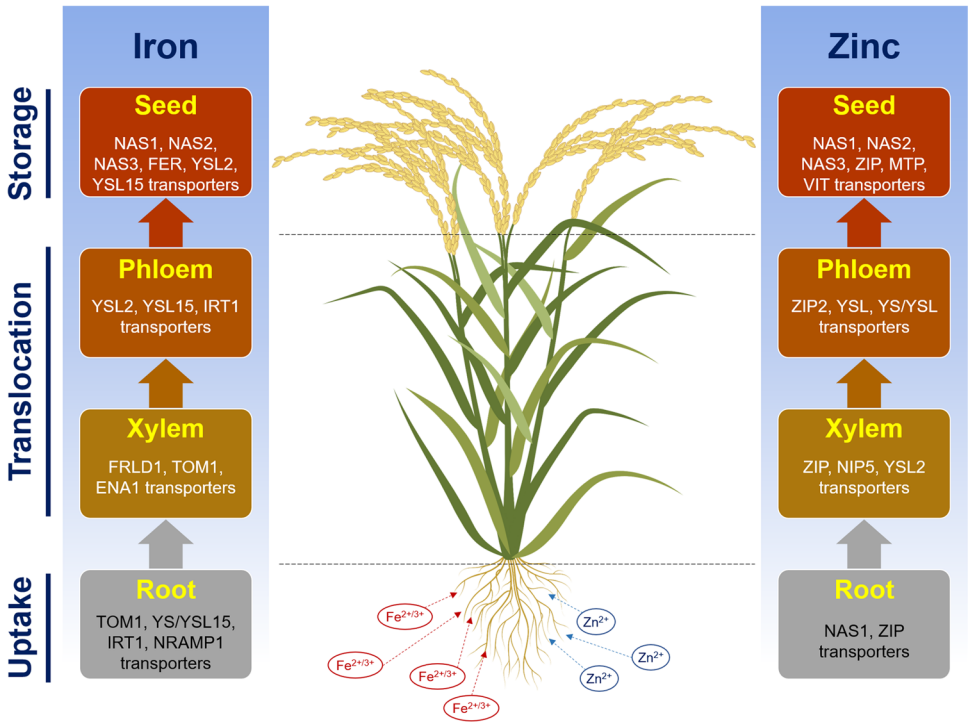

Fig. 1 Genes and transporters underlying uptake, translocation and storage of iron and zinc in graminaceous crops. ZIP: zinc-regulated transporter; ZIP2: zinc-regulated transporter 2; YS: yellow stripe; YSL: yellow stripe-like transporter; MTP: metal transporter protein; ENA 1: efflux transporter of nicotianamine 1; VIT: vacuolar iron transporter; IRT1: iron-regulated transporter 1; NRAMP 1: natural resistance-associated macrophage protein 1; FRDL 1: ferric reductase defective-like 1 transporter; TOM1: transporter of mugineic acid family phytosiderophores 1; FER: ferritin; NAS: nicotianamine synthase. Figure generated using Biorender

improve the solubility enhances the Fe uptake in plants (Kim and Guerinot 2007). A major proportion of the Fe that gets absorbed is accumulated as ferritin in the grains' aleurone layer, which is facilitated by the ferroxidases. These ferroxidases can conserve up to 4500 atoms of iron in their complexes. Manipulating the ferritin genes has proved to be successful in improving grain Fe content in cereals. For example, manipulating soybean ferritin genes with rice globulin promoters in the rice cultivar, Swarna, showed a 3.7-fold increase in Fe content (Vasconcelos et al. 2003; Paul et al. 2012, 2016). This was considered a significant leap in achieving Fe-rich rice to circumvent anemia and related issues in the human population.

Biofortification for Fe content in rice has been successfully achieved by Masuda et al. (2013) through seven transgenic approaches. In the first approach, endosperm-specific expression of Fe storage protein, *ferritin* (*SoyferH1, SoyferH2, Pvferritin*), was performed to achieve a twofold increase in Fe content. In the second approach, nicotianamine synthase genes (*OsNAS1, 2, 3; HvNAS1*) were overexpressed to produce the metal chelator, nicotinamine. The transgenic plants showed a threefold increase in grain Fe content. Enhanced expression of a Fe(II)-nicotianamine transporter gene (*OsYSL2*), in the third approach, increased the Fe content by fourfold in the grains. Approach four had introduced a mugineic acid synthesis gene of barley (*HvIDS3*) in rice to enhance the Fe content in grains by 1.4-fold. The fifth approach was to overexpress the Fe transporters, *OsIRT1* and *OsYSL15*, the sixth approach involved the overexpression of Fe homeostasis-related transcription factor (Iron-related bHLH transcription factor, *OsIRO2*), and the seventh was to knock down the vacuolar Fe transporters (*OsVIT1* and *OsVIT2*), which resulted in increased Fe accumulation in the grains. Combining these approaches had also proven successful in improving the Fe content. For example, approaches one, two, and three were combined to produce Fe-fortified rice, which showed 4—to sixfold high Fe content in the seeds (Masuda et al. 2013).

Agronomic biofortification by incorporating biofertilizers in the soils eventually plays a crucial role in solubilizing the micronutrients for uptake by the plants. Microbiome in the rhizosphere, including terrestrial fungi and bacteria, establishes the essential association with the plants to solubilize the soil nutrients for enabling the plants to take up through symbiotic relationships. This underlines the importance of other external factors like symbionts and fertilizer application in improving nutrient availability. An increase in the acquisition of Fe and Zn was successfully achieved by inoculation of arbuscular mycorrhizae in the rhizosphere (Coccina et al. 2019), ferti-fortification by incorporating increased external soil application (Clemente et al. 2007), foliar application of micronutrients (Cakmak et al. 2010), iron solubilizers like azotobacter and azospirillum (Hussain et al. 2018), and iron- and zinc-coated fertilizers (Kutman et al. 2010). Understanding the mechanism underlying these accessory uptakes and identifying the genes having roles in the processes could also extrude a more significant drift in enhancing the absorption of micronutrients into the plants from the soil.

Conventional plant breeding approaches for enhancing micronutrient contents

Conventional breeding for biofortification has been successful in delivering improved lines to the farmers. However, the success was limited to the crops with excellent genetic diversity, whereas transgene-based approaches were used in the species that had limited genetic diversity, reduced heritability, and linkage drag. Crossing the parent lines with high micronutrient content with the recipient lines with better agronomic traits for several generations develops elite lines with both enhanced micronutrient content and desired agronomic traits. This has been demonstrated in several varieties, including IR6844 (IR 8 × Taichung Native 1) and IR68144-3B-2–2-3 (IR72 X Zawa Bonday), which retained 80% of the Fe content in the polished seeds (Gregorio et al. 2000; Virmani and Ilyas-Ahmed 2008; Has et al. 2005). The Consultative Group for International Agricultural Research (CGIAR), in collaboration with CIAT (International Center for Tropical Agriculture) and IFPRI (International Food Policy Research Institute), established the HarvestPlus initiative, which focuses on breeding for biofortified crop species. The initiative was successful in enhancing Fe, Zn, and vitamin-A in several cultivated species, including rice, wheat, and maize (Bouis and Welch 2010). In addition to deploying breeding to improve the nutritional levels, the approach has also been instrumental in reducing the antinutritional content in grains. For example, marker-assisted backcross breeding developed low phytic acid grains in rice, maize, and wheat, ensuring better bioavailability in the human body after consumption (Virmani and Ilyas-Ahmed 2008; Velu et al. 2014; Jeng et al. 2012). In India, the Indian Council of Agricultural Research (ICAR) and International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) had released several biofortified varieties for cultivation by farmers.

Dissecting micronutrient traits using genetics and genomics approaches

The agronomic and conventional breeding approaches operate without the knowledge of the genes, alleles, and QTLs underlying the desired traits, whereas genetics and genomics had proved successful in identifying the precise molecular determinants, thereby enabling the researchers to manipulate them for trait improvement. In this direction, a considerable amount of work has been done in the previous decades to

identify the genetic determinants underlying grain micronutrient contents (Table 2). Providing direct access to the genes, alleles, and QTLs regulating these traits overcome the limitations in agronomic biofortification and conventional breeding.

Rice

Rice being a major staple cereal necessitates the enhancement of its grain micronutrient content. Lu et al. (2008) initially identified micronutrient QTLs in 241 RILs obtained from a cross between Minghui 63 and Zhenshan 97. The QTLMapper1.0 detected 10 QTLs for micronutrients, of which two major QTLs for Fe (*qFE-1, qFE-9)* and three major QTLs for Ca [qCA-5 (chromosome 5), qCA-9 (chromosome 9), and qCA-4 (chromosome 4)], one minor QTL for Mn (qMN-1, on chromosome 1) and Cu (qCU-2 on chromosome 1), three minor QTLs for Zn (*qZN-5*, *qZN-7*, and *qZN-11*) were detected. The QTLs, qFE-1, qFE-9, qZn-5, qZn-11, and qMN-1 with digenic interactions for micronutrients were also located. This presented the involvement of more genes in enhancing the micronutrient status. Similarly, five QTLs in RILs between Madhurkar and Swarna for Fe and Zn concentrations on chromosomes 1, 3, 5, 7, and 12 were detected by Anuradha et al. (2012). Interestingly, the study showed a co-localization of Fe and Zn QTLs on chromosome 7 which underlined their interrelationship. Further, putative genes for Fe (metal tolerance protein, *OsMTP1;* and *OsYSL1*), zinc (*OsNAS1-2;* acireductone dioxygenase, *OsARD2;* and *OsIRT1*), as well as both Zn and Fe (adenine phosphoribosyltransferase, *OsAPRT;* and *OsNAS3),* were identified from this study, and this could be implied in biofortifying rice for micronutrients. Parallel co-localization in double haploids (DH) was seen in QTLs of Mg/Mn, Mg/P, and Mn/Zn present on the 8th and 9th chromosomes. This describes the desirable selection for combined improvements for micronutrients in cereals. Double haploids were also utilized by Swamy et al. (2018a, b) to identify 59 QTLs and six gene families such as *endogenous ferritin (OsFER), OsNAS, natural resistance-associated macrophage protein* (*OsNRAMP), OsYSL,* and *zinc-induced facilitator-like (OsZIFL)* for Zn content in rice.

Later, Calayugan et al. (2020) assessed rice DH derived from IR05F102 X IR69428 for three different seasons by best linear unbiased estimates (BLUEs) from SNPs. These data were further used for inclusive composite interval mapping to identify 23 QTLs distributed on all the chromosomes except 4, 8, and 11. The dissected QTLs for micronutrients were also found to be associated with eight agronomically significant traits. Among all, two QTLs for Fe (*qFe9.1* and *qFe12.1*) on chromosomes 9 and 12, and 4 QTLs for Zn (*qZn1.1*, *qZn5.1, qZn9.1, and qZn12.1*) on chromosomes 1, 5, 9, and 12, respectively, were notable. Interestingly, the SNP interval 9,809,545–9,819,278 showed co-localization of *qFe9.1* and *qZn9.1* on chromosome 9. This corresponds to the previous studies in understanding the genic linkages and confirms their interrelationship. Further, candidate genes within the QTLs like, *LysM receptor-like kinase 10 (*OsLysM-RLK10*)* and *Receptor-like Cytoplasmic Kinase 276 (*OsRLCK276) (within *qFe9.1*), *SWEET13 (sugars will eventually be exported transporter*), and *OsSWEET13* (within *qFe12.1), ARGOS-like (OsARL1e),* O*sGATA8*, *Sar1b* and *OsGATA14 (*within *qZn1.1)*, and *Os09g0511500* (within *qZn9.1*) were identified. Among these genes, the position of rice *Zrt and Irt-like protein 6 (OsZIP6*) was toward the right of the QTL *qZn5.1*, which was an Fe transporter, also stratifies the association between iron and zinc uptake in crops (Calayugan et al. 2020).

Although several QTLs have been identified, they differ with respect to the confidence level and ambiguous localization in the chromosomes (Fig. 2). Hence, meta-QTL analysis was more preferable to project the consensus QTLs with their exact location. This robust genome-based technique pools and analyzes data that have already been reported to show concordance level of the traits/QTLs. Several research groups have performed meta-QTL analysis, and one such study by Dixit et al. (2019) identified meta-QTLs for Fe and Zn, on chromosomes 3 and 2. The in silico analysis of these QTLs also found few candidate genes, namely *OsFDR3, Auxin-responsive Aux/IAA gene (IAA5), Proton-dependent Oligopeptide Transporter (OsPOT),* and *OsZIP4*. Another meta-QTL analysis linked to Fe and Zn also detected 48 QTLs on chromosome 12 and identified 663 candidate genes, which could be widely used for marker-assisted breeding and biofortification in rice (Raza et al. 2019). These genes and QTLs identified through the meta-QTL analysis could be further employed for downstream studies to characterize and deploy them in trait improvement.

Maize

In maize, Brunson and Quackenbush (1962) showed the variability in the germplasm to facilitate the breeding for provitamin A. Studies on the structural differences between α- and β- carotene revealed the role of *lycopene epsilon cyclase (lcyE)* locus (Harjes et al. 2008), and also, methodologies to improve carotenoid contents by incorporating *beta-carotene hydroxylase 1 (crtRB1)* and *lcyE* in maize were suggested (Yan et al. 2010). Later, in CIMMYT, GWAS for carotenoid content with 380 inbred maize lines identified 476,000 SNPs and their validation identified genes like *crtRB1* and *nonheme di-iron β-ring hydroxylase* (*HYD5* and *HYD1*), which had a crucial role in improving Vitamin A in maize. Moreover, genes like *carotenoid cleavage dioxygenases (CCD1), Geranylgeranyl pyrophosphate synthase 2 (GGPS2), 1-deoxy-d-xylulose*

Table 2 Genetic determinants identified in graminaceous crops having roles in micronutrient traits

Crops/Cultivars	Micronutrient traits	Genetic determinants	Reference
Rice			
Zhenshan 97 X Minghui 63 (RILs)	Ca, Cu, Fe, Mn, Zn	qC-4, qCA-5, qCA-9, qCU-2, qFE-1, qFE-9, qMN-1, qZN-5, qZN-7, qZN-11	Lu et al. 2008
Madhurkar X Swarna (RILs)	Fe, Zn	Fe*: OsMTP1*, *OsYSL1*; Zn*: OsNAS1-2, OsARD2, OsIRT1*; Fe & Zn*: APRT, OsNAS3, Heavy metal ion transport and OsN-RAMP1*	Anuradha et al. 2012
Chunjiang 06 X TN1 (DHs)	Ca, Fe, Mn, Zn, P,K, Mg	32 QTLs (24 novel QTLs)	Du et al. 2013
PSBRc82 x IR69428; PSBRc82 x Joryeongbyeo (DHs)	As, B, Ca, Co, Cu, Fe,K, Mg, Mn, Mo, Na, P, Zn	59 QTLs; Zn: *OsFER, OsNAS, OsNRAMP, OsYSL* and *OsZIF*L	Swamy et al. 2018a
O. sativa cv Swarna and O.nivara (2 accessions) (BC_2F_3)	Fe, Zn	qZn12.1, qFe8.2, qFe3.1 and qFe2.1; 16 metal homeostasis gene	Swamy et al. 2018b
IR05F102 x IR69428	Fe, Zn	*qFe9.1*, *qFe12.1, qZn1.1*, *qZn5.1, qZn9.1 and qZn12.1; OsLysM-RLK10 and OsRLCK276, OsS-WEET13, OsARL1e, OsGATA8, Sar1b, OsZIP6,Os09g0511500*	Calayugan et al. 2020
Meta-QTL analysis	Fe, Zn	*OsFDR3, AsIAA5, OsPOT and OsZIP4*	Dixit et al. 2019
Meta-QTL analysis	Fe, Zn	*48* mQTLs and 663 candidate genes	Raza et al. 2019
Maize			
26 tropical population	Provitamin A	*CrtB1 and LcyE* polymorphism	Babu et al. 2013
380 inbred lines	Carotenoid	*crtRB1, HYD5, HYD1, CCD1, GGPS2, SXS1 and ZEP1*	Suwarno et al. 2015
Maize genome sequence	Fe, Zn	48 genes (*ZIP*, *NRAMP*, *YS*, *CE*, *ferritin f*amily)	Sharma and Chauhan 2008
B73 X Mo17 (RILs)	Fe	3 QTLs	Lung et al. 2011
Mu6 x SDM; Mo17 X SDM ($F_{2:3}$ Population)	Fe, Zn	31 QTLs	Qin et al. 2012
178 X P53 ($F_{2:3}$ Population)	Fe, Zn	10 mQTLs and 5 QTLs	Jin et al. 2013
CIMMYT and partner germplasm	Fe, Zn	Fe: 26 SNPs Zn: 20 SNPs	Hindu et al. 2018
Barley			
Clipper X Sahara 3771 (DHs)	Zn	*SZnR1* marker (MFLP); *Xbcd175, Xpsr108, XksuF15* (RFLP) and *vrs1* (morphological marker)	Sadeghzadeh et al. 2010, 2015)
Clipper X Sahara 3771 (DHs)	Zn	5 QTLs	Lonergan et al. 2009
298 landraces	Zn	*Zn-qtl-6H_SCRI_RS_10655*	Mamo et al. 2014
Hordeum vulgare ssp. vulgare cv. Scarlett and Hordeum vulgare ssp. Spontaneum (ILs)	Fe, Zn	41 QTLs and *Zinc transporter gene 8*	Reuscher et al. 2016
336 spring barley cultivars	Ba, Cu, Fe, K, Mg, Mn, Na, S, Si, Zn, Ca	45 QTLs (Zn: *Zn-1H-21.97, Zn-2H-87.34*, and *E-5H-44.99*)	Gyawali et al. 2017
180 lines (ICARDA)	Cu, Fe, Mn, Zn	43 MTAs, *MTP5*	Detterbeck et al. 2019
Sorghum			
KS115 X Macia (RILs)	Carotenoid	5 QTLs for β-carotene	Fernandez et al. 2008
407 lines (SAP)	Element content	Zn:*Sobic.007G064900*, Mn:*Sobic.003G349200*, Fe:*Sobic.001G213400*, Mg: *Sobic.001G443900*	Shakoor et al. 2016
296 B X PVK 801 (RILs)	Fe, Zn	–	Phuke et al. 2017

Table 2 (continued)

Crops/Cultivars	Micronutrient traits	Genetic determinants	Reference
403 accessions	Carotenoid	*β*-carotene:14 SNPs (*CYP97A, PDS*, *GGPPS*) Zeaxanthin: 38 SNPs (*MDS, ZDS, DXR, ZEP)*	Cruet-Burgo et al. 2020
Small millets			
Little millet	Fe	*FRO2*	Chandel et al. 2017
Finger millet (113 genotypes)	Ca	9 Anchored SSR markers	Kumar et al. 2015b

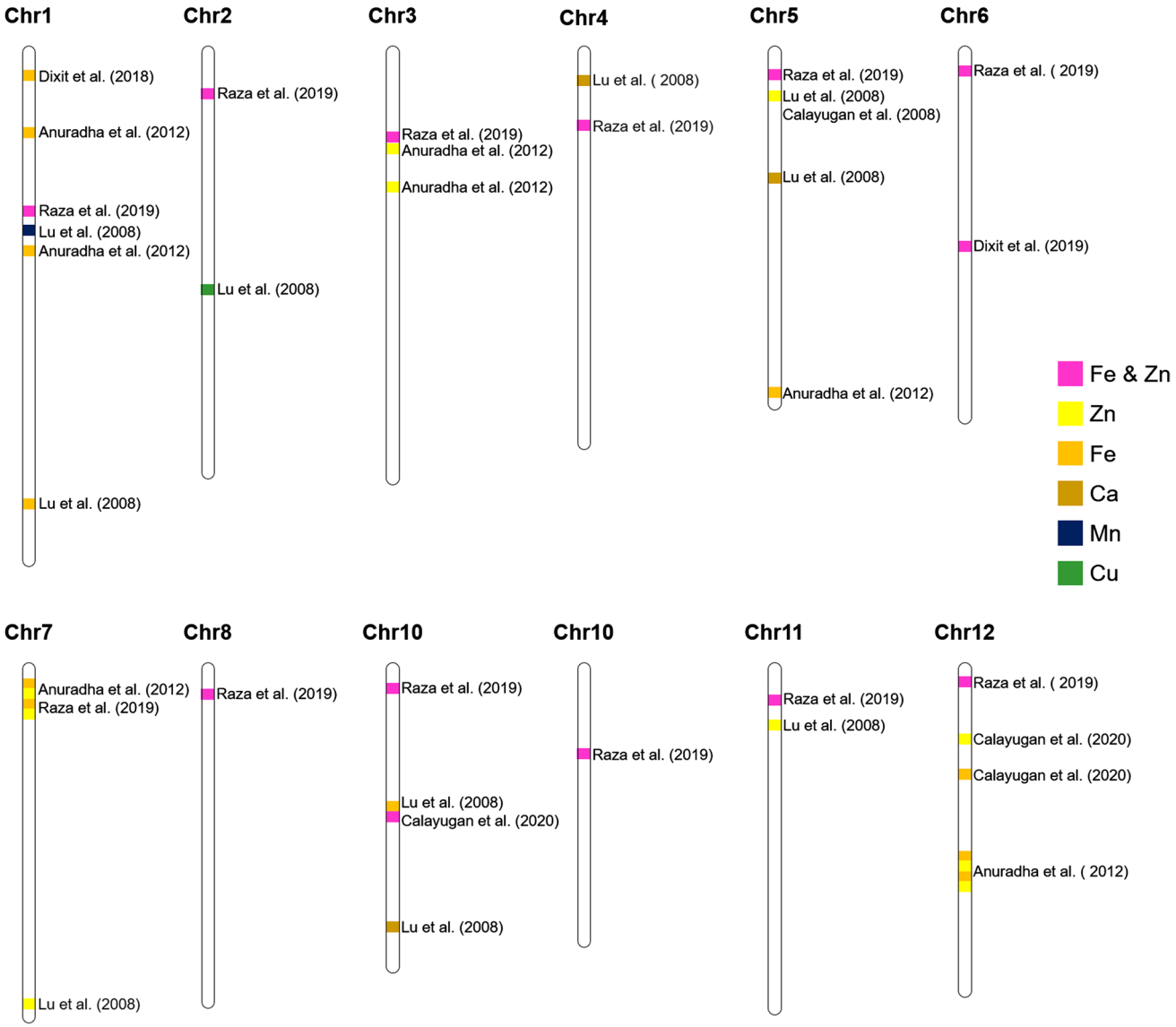

Fig. 2 Physical map of rice showing the locations of QTLs identified for micronutrient traits by different studies. The vertical bars represent chromosomes and the QTL for each micronutrient is mapped in colored blocks. Also, the reference to the respective study has been provided to the right of each box

5-phosphate synthase (DXS1), and *Zeaxanthin epoxidase (ZEP1)* were found to have a putative role in enhancing the total carotenoid content (Suwarno et al. 2015). Earlier, Aluru et al. (2008) had demonstrated that overexpressing bacterial phytoene synthase gene, *crtB,* and *crtI* with super *γ*-zein promotor increased the carotenoid (especially

β-carotenoid) accumulation by 34-fold, for four consecutive generations, and could help develop genetic stocks. Recently, Natesan et al. (2020) have also developed introgression lines with 7.3-fold higher *β*-carotene using crtRB1 3'TE as a genic marker from HP467-15, UMI 1200, and UMI 1230. Interestingly, Gautam et al. (2010) had also explained that *β*-carotene has a significant role in increasing the bio-accessibility of other minerals. Thus, improving *β*-carotene could consequently enrich maize with other essential minerals.

QTLs for different micronutrients in maize revealed markers for Zn and Fe. Forty-eight genes associated with Fe and Zn in maize were classified into families, namely ferritin (1), cation efflux (1), ZIP (13), natural resistance-associated macrophage protein (NRAMP, 16 genes), and yellow stripe (17) gene family. This also identified 34 SSRs from 28 putative genes for Fe/Zn transporters, which was amplified in 124 inbred lines. Several SNPs were seated in the exons of candidate genes like ZmZIP11 and ZmZIP7. One SNP in ZmZIP11 exhibited polymorphism among the inbreds that could be used as a genic marker in molecular breeding programs (Sharma and Chauhan 2008). Later, Lungaho et al. (2011) had identified 3 small QTLs for grain Fe content using linear unbiased predictors (BLUPs) among RILs of B73 and Mo17. Molecular breeding using these small QTLs would prove to be a burdensome task. Later, Qin et al. (2012) had published data of a linkage map for Fe and Zn in Mo17, Mu6, and SDM inbred lines that progressed the QTL studies in maize for micronutrients. Here, the QTL identification and analysis were performed using inclusive composite interval mapping and identified 31 QTLs for Zn and Fe. Also, Jin et al. (2013) had performed both QTL analysis (178 × P53) and meta-analysis for QTLs (previous QTL research) associated with Fe and Zn and identified ten mQTLs and 5 QTLs using BioMercator2.1. Out of these ten mQTLs, eight were associated with both Fe and Zn, which could be used to explain their correlation in simultaneous improvements.

In addition to QTLs, GWAS was performed by Hindu et al. (2017) to identify the genomic regions associated with Fe and Zn in 923 inbred lines. This revealed 347,765 SNPs across varying environmental conditions. Out of all the SNPs, 46 SNPs were called for Fe and Zinc, of which 26 were for Fe and 20 for Zn. These associated SNPs could be used to assist the molecular approaches in identifying target donors from germplasm. Alternatively, transgenic approaches to enhance the Fe and Zn uptake by overexpressing the phytase gene (Drakakaki et al. 2005), and RNAi of *ZmZIP5* for the uptake of zinc (Li et al. 2019) are other alternative methods to reduce the antinutritional factors like phytic acid in maize for enhancing the micronutrient bioavailability.

Barley

Genetic determinants for micronutrients and vitamin A in barley were also identified using different QTL, GWAS, and GBS approaches. Biparental mapping (Clipper and Sahara 3771) was used extensively to identify QTLs for Zn in barley by Sadeghzadeh et al. (2010, 2015) wherein they had identified QTLs for Zn on chromosome 2HS. Subsequently in 2015, they detected extra QTLs on the chromosomes 2HL, 3H, and 4H. Like rice and maize, the double haploid population in barley (Clipper and Sahara 3771) was used by Lonergan et al. (2009) to execute the association study using Map Manager QTX and QGene 4.0. They located 5 QTLs for grain Zn content on three chromosomes, namely 1H, 2H, and 5H (Lonergan et al. 2009). Following this, Mamo et al. (2014) experimented with a marker-linked association in 298 landraces of barley and identified a single QTL, *Zn-qtl-6H_SCRI_RS_10655,* on 6H chromosome. Later, critical QTLs such as IL157 on 1H and IL146 on 4H were dissected from introgression libraries designed for Fe and Zn (Reuscher et al. 2016).

Broader studies with 45 QTLs for 13 micronutrients [Ba (2), Ca (2), Cu (4), Fe (11), K (2), Mg (3), Mn (6), Na (4), S (3), Si (5), and Zn (3)] by GWAS (TASSEL) projected three significant QTLs for Zn viz, *Zn-1H-21.97, Zn-2H-87.34*, and *E-5H-44.99* (Gyawali et al. 2017). Besides, population structure analysis across three regions with DArT™ markers and BLUEs presented 43 MTAs for micronutrients, viz. 15 for Cu, 6 for Fe, 9 for Mn, and 13 for Zn. The MTAs associated with Zn were present on the chromosome 2H (2H|bPb9754) near the 2 *YSL* (yellow stripe-like) genes (*AtYSL2* and *YSL9*). MTAs associated with Cu had the highest association with the marker 2H|bPb4040. The pleiotropic effects among these micronutrient QTLs were between Zn, Cu, and Mn, which was linked to the marker bPb4909 (1H), Zn and Cu were linked to markers bPb9754 and bPb4040 (2H), and Zn and Fe were linked to bPb8836 (6H). This pleiotropism has to be further phenotypically evaluated to describe the morphological effects in enhancing the Fe and Zn content in barley. Finally, the validation of these QTLs established *MTP5* as one of the candidate genes for Zn accumulation in barley (Detterbeck et al. 2019).

Sorghum

One of the significant problems in sorghum is the low bioavailability of micronutrients and vitamin A. The genetic variation in the level of carotenoids in sorghum grains was used for QTL and GWAS mapping. Fernandez et al. (2008) initially developed a RIL population with 85 SSRs to associate QTLs for carotenoids relating to their endosperm color. Nine putative genes for endosperm color, with *Phytoene synthase 3 (Psy3*) as a major contributor, were detected with a co-localization

of a carotenoid Ccd1a gene. CIM among the QTLs revealed significant co-localization of QTLs for endosperm color with carotenoids. *Phytoene desaturase (Pds)* and *Psy3* genes showed association with β- carotene and zeaxanthin, while six genes, Crtrb1 (5%), *Ccd1a, Vp14, Lcye, Psy1,* and *Psy 3* (1%), showed significant association with lutein content. The elemental genotypic and phenotypic variation studied with the GBS data across three locations with Sorghum Association Panel was available from Morris et al. (2013), and these data exhibited a moderate heritability for Cu (45%), which was higher than the heritability of Ca, Co, S, P, Se, Mo, Mn, Fe, Zn, and K (< 30%). GWAS in these accessions identified several potential candidates, namely Sobic.007G064900 (for Zn content) was homologous to *AtZIP5*, Sobic.003G349200 (for Mn content) was homologous to *AtMTP11,* Sobic.001G213400 locus (for Fe content), and Sobic.001G443900 locus (for Mg content) which has been annotated as *Peptide transporter* (PTR2). Ionomics approaches in these genes were later suggested for dissecting the genomics of these genes (Shakoor et al. 2016). Later, Phuke et al. (2017), with 336 RILs, projected the negative association of Fe and Zn with yield, and their positive association with 100 seed weight suggested a selection pressure for bolder seeds in biofortification of sorghum. They also observed a higher environmental influence for Fe than Zn.

Another GWAS for carotenoids in sorghum revealed 14 SNPs for *β*-carotene from which only three were located in the proximity of major genes, viz. *cytochrome P450 97A (CYP97A)* (chromosome 2), *PDS* (chromosome 6), and *geranylgeranyl diphosphate synthase* (*GGPPS*; chromosome 2). For zeaxanthin, overall, 38 SNPs were identified with 12 association regions, out of which 4 showed presence near the priori gene candidates, and they were MDS (chromosome 4), ZDS (chromosome 2), DXR (chromosome 3), and ZEP (chromosome 6). For lutein, no significant SNPs were detected. This study indicated a profound association between the SNPs and the Zeaxanthin content as *zeaxanthin epoxidase (ZEP)* and *2-C-methyl-D-erythritol-2,4-cyclodiphosphate synthase (MDS)* genes were associated with zeaxanthin. They proposed that *ZEP* could have a pertinent role in controlling carotenoid content in sorghum and could be further utilized in increasing the provitamin A content in sorghum. Development of RILs and ILs is in progress to validate the markers (Cruet-Burgo et al. 2020). Probably, marker assisted selection using the identified markers could aid in fast forwarding the bio-fortification process in sorghum.

Genetics and genomics of micronutrient traits in small millets

Minor millets are hidden reserves for several traits, and they are highly nutritious than mainstream cereals. They possess the desirable glycemic index and are gluten-free in nature. Each millet has its distinctive features, which are yet to be explored (Muthamilarasan and Prasad, 2021). Among them, little millet exhibit abundance in micronutrients with a considerably higher iron content. Studies portrayed ferric chelate reductase (FRO2) in metal uptake of plants, which was characterized in little millet using next-generation sequencing with the rice ortholog, *OsFRO2*. The amplified *FRO2* (2.7 kb) gene from little millet (RLM-37 genotype) showed a significant similarity with the sequence of *OsFRO2*. The protein sequence of *FRO2* incorporated the domains like NOX_Duox_Like_FAD_NADP and ferric reductase and showed fascinatingly higher similarity in 3D structure of both little millet *FRO2* and *OsFRO2*. This study recommended that desirable alleles and micronutrient associated gene orthologues in millets could be identified using the already reported genes in staple crops (Chandel et al. 2017). Previously, Kumar et al. (2015) had evaluated 113 genotypes of finger millet which is known for its Ca content which is of about 450 mg per 100 g. Molecular screening of this germplasm with 23 anchored-SSR primers designed based on Ca transporters and sensors revealed polymorphism for 14 markers, and they contributed to almost 83 alleles. Dendrogram analysis divided the accessions into 7 clusters, and the association studies with TASSEL and STRUCTURE identified nine markers linked with the Ca content, which could be validated in the near future (Kumar et al. 2015). The studies in small millets are still in its budding state, and several efforts are ongoing to bring these in the mainstream research.

Transcriptomic approaches to understand micronutrient traits in major cereals

At present, the NCBI SRA database contains easily accessible RNA-seq data of more than 35,250 samples of rice, and also, Gene Expression Omnibus includes a massive amount of transcriptome profile data achieved from microarrays (GEO: https://www.ncbi.nlm.nih.gov/geo/). Several transcriptomic studies have been reported in rice and mostly based on stress. There are fewer reports in micronutrient content. In one of the studies by Zheng et al. (2009), microarray and transcriptomic analysis were performed to understand the antagonistic interaction of Phosphorous (P) and Iron (Fe). To decipher the complex nature of the rice transcriptome, Lu et al. (2010) used the rice cv. *Indica* and *Japonica* to develop the whole genome transcriptome profiles. Successively, Dong et al. (2018) grew the rice with and without minerals, and used root samples to generate the entire transcriptome RNA seq data. They explained the role of Ser/Arg proteins in the regulation of mineral nutrient homeostasis. Similarly, Sperotto et al. (2012) performed the expression profiling of 25 genes associated with metals

and their homologs, out of which nine (OsNAC5, OsFRO1, OsNRAMP1,7 and 8, OsYSL6,8 and 4, and OsNAS1) were related to Fe and Zn uptake. Recently, Ren et al. (2019) analyzed large-scale RNA-seq data and identified 1584 novel peptides, which further improvised the annotation of the rice genome.

Messias et al. (2014) performed the expression analysis for the carotenoid-related genes among 22 landraces of corn (kernel) by RT-qPCR in the hybrid 30F53 at different developmental stages and amplified the genes *ZmCCD1, CYP97C, HYD3,* and *PSY1*. The results showed that the PSY1 expression was high at 16 to 22 days after pollination (DAP). Whereas both *CYP97C* and *HYD3* exhibited a peculiar expression pattern wherein an increase in expression was observed at 10 DAP, it decreased at 13–16 DAP, followed by a sharp increase at 19 DAP. Inclusive to all these findings, there was a correlation in the expression of *HYD3* with *PSY1* and *CYP97C*. However, *ZmCCD1* showed a negative correlation with the content of carotenoids. Also, it explained that the conversion to lutein and zeaxanthin from *β*-carotene could be determined by the expression analysis of *CYP97C* and *PSY1* (Messias et al. 2014). Elucidation of elemental accumulation and variation in grains can be made easier with transcriptomic studies. For instance, in barley (cv Golden promise), Tauris et al. (2009) isolated the cells embryo, aleurone, endosperm, and the transfer cell tissues using Laser-assisted microdissection followed by an RNA extraction. Affymetrix-based microarray (22 K Barley GeneChip) was used to achieve tissue gene expression profile. This explained the abundance of 25 genes related to metal homeostasis, which were further categorized into several gene families like HMA (heavy metal ATpase), ZIP (Zrt-, Irt-like proteins), *NRAMP* (natural resistance-associated macrophage proteins), CAX (cation exchanger), VIT1 (vacuolar iron transporter), CDF (cation diffusion facilitator), ZIF1 (zinc-induced facilitator1), NAAT (nicotianamine aminotransferase) metallothionein, NAS (nicotianamine synthase), and YSL (yellow stripe-like). Using this gene expression data, a zinc trafficking path in barley grains to the maturing seed from the phloem was framed. *HvYSL9* was expressed in all other tissues except for the embryo and is expected to have a metal transport role in cells (Tauris et al. 2009). Similarly, Detterbeck et al. (2019) studied the comparative transcriptome of barley lines with low and high Zn content. They reported 26 differentially expressed genes (DEGs), among which 19 could not be annotated. Further, data search revealed the presence of homeostasis genes, which were earlier reported by Tauris et al. (2009), and they were PCS (phytochelatin synthase), ZIFL (zinc-induced facilitator-like), and PME (pectin methylesterase). Concurrently, they also proposed that MTP and YSL transporters could have a significant role in Zn content in barley grains (Detterbeck et al. 2019).

Transcriptomic studies in small millets to understand micronutrient traits

The sequence information for all the species of small millets is unavailable, and they are yet to be disseminated for utilization, except for foxtail millet. Hence, the identified transcriptomes in small millets serve as a desirable approach in targeting the key genes in millets and cereals. Finger millet is rich in Ca content and has a variable concentration of Ca in plant tissues due to its differential gene expression. For the first time, Singh et al. (2014) conducted a transcriptome-wide investigation for Ca sensor genes in GPHCPB-45(GP-45) and GPHCPB-1 (GP-1) genotypes of the finger millet showing a considerable difference in their Ca content (about 100 mg). The tissues were collected for RNA isolation from four different spike developmental stages like spike emergence (S1), pollination (S2), dough (S3), and maturation (S4) and were subjected to transcriptome sequencing using Illumina HiSeq 2000 platform. De novo assembly of the transcriptome data against CDS of calcium sensor genes from rice was performed. They identified 82 distinctive calcium sensor genes, which were categorized into eight families [calmodulin (CaM) and calmodulin like (CaML), calcineurin B-like protein (CBLs), calcium-dependent and CaM-independent protein kinases (CDPKs), CaM-dependent protein kinases (CaMK), phosphoenolpyruvate carboxylase kinase-related kinases (PEPRKs), CDPK-related protein kinases (CRKs), Ca^{2+}-/CaM-dependent protein kinases (CCaMK), and CBL-interacting protein kinases (CIPKs)]. Out of these, 24 genes in GP-45 (high-Ca content) and 11 genes in GP-1 (low-Ca content) showed upregulated expression (Singh et al. 2014), and these genes could be further validated and used for Ca biofortification.

Similarly, in another study, the investigators identified Ca-related genes using rice data from MPSS and Affymetrix gene expression. The identified genes (*CAX1, TPC1, CaMK2, CaMK2, calmodulin, tubulin,* and *14–3-3*) were amplified and cloned with the vector pGEM-T Easy (Promega) and sequenced by Applied Biosystems 370. The Ca content determination revealed a considerable variation during the reproductive and vegetative stages. It was found that the flag leaf tissues accumulated more Ca with respect to the spike. The accession GPHCPB-45 had higher Ca than GPHCPB-1 with a difference of almost 100 mg. Also, the expression of the *two-pore channel* (TPC1) and CAX1 was higher in the spike of GPHCPB-45 could be the probable reason of higher Ca content. Even CAM showed a higher expression pattern similar to Ca^{2+} ATPases in GPHCPB-45. Interestingly, a contrasting Ca accumulation pattern was observed in both the genotypes wherein GPHCPB-45 had a higher accumulation of Ca in seed, whereas GPHCPB-1 showed a higher accumulation of Ca in flag leaf. Hence, this differential expression could be validated to understand the

uptake of Ca. The study also proposed that the CAX1 gene could be a putative gene in enhancing the seed calcium content. Moreover, a construct containing both CAX and CaM gene would help in the biofortification of grains and cereals (Mirza et al. 2014). Recently, Kokane et al. (2018) investigated the role of the protein CIPK with respect to CAXs and Ca^{2+} translocation during grain filling in cv. GP-1 and GP-45. Their differential gene expression revealed that CaM was highly expressed in the GP-45 seeds, suggesting that it might have a potential role in Ca^{2+} movement and interacts with Ca^{2+} ATPase. Moreover, CAX1 showed higher abundance in vegetative tissues and in developing spikes, whereas CAX3 was highly expressed in spikes. Hence, they suggested that Ca^{2+} translocation and accumulation might be controlled by tripartite interactions (Kokane et al. 2018). Despite these studies, the transcriptomes of several other millets are yet to be studied to understand the differentially expressed genes during grain filling and maturation stages that have role in biosynthesis and accumulation of micronutrients in the grains.

Proteomics and metabolomics studies in major cereals and small millets

The proteome profiling of cereals for their micronutrients began from Gayen et al. (2016), who compared the nutritional content of transgenic rice with *Xa21* (bacterial blight resistant) against the wild type using 2D gel electrophoresis and observed no significant variation in their nutrient profile. Most of the proteomics study in rice is predominantly analyzed for its stress response rather than its nutritional content. Metabolomics of cooked grains from 10 rice varieties using UPLC-MS for around 3097 metabolites revealed a key variation based on race (*aus, indica* and *japonica*). They also explained that the genes associated with the biosynthesis pathway showed variation in SNP. For tocopherol and phenolics, the gene γ-TMT was associated with vitamin E content in rice (Heuberger et al. 2010). Calingacion et al. (2012) studied three waxy rice cultivars (TSN1, HNN, and KNL) by integrating genome-wide genotyping with metabolomics (H-NMR, derivatized GC–MS, ICP-MS, and headspace GC–MS). SNP association and metabolic profile revealed that the metabolic profile for each rice variety was unique and showed relevance to nutritional content as the geographical origin of crop plays a significant role in plant metabolite profile. Parallelly, Hu et al. (2015) performed metabolite profiling of the rice cv. *japonica* and *indica* to dissect 121 seed metabolites that showed a correlation of phenotype with the metabolites which were dependent on the geographical location of rice origin.

Being natural and common pigment of grains, carotenoids are widely studied in metabolomics studies. CYMMIT and HarvestPlus have come together to fortify corn with provitamin A using breeding strategies. Worldwide, carotenoid characterization has been performed using targeted metabolomics. For instance, Kuhnen et al. (2011) assessed the carotenoid content in 26 landraces of maize (white, variegated, orange, purple, and yellow) using HPLC–UV-VIS to characterize the carotenoids, from which they found a significant amount of lutein and zeaxanthin, with traces of α-, β- carotene, and β-cryptoxanthin. Uarrota et al. (2014) characterized the carotene content in eight landraces, viz. MPA, Roxo, Roxa, and Palha, which had a higher accumulation of carotenoids like α-carotene, *cis* and *trans*-β-carotene and also β-cryptoxanthin. They detected the presence of non-provitamin A like lutein and zeaxanthin. Later, Messias et al. (2014) studied the carotenoid biosynthesis pathway and its catabolism across 22 landraces of maize (yellow, white, and orange). They used HPLC–DAD to characterize the carotenoids to differentiate α- and β-carotene and α- and β-cryptoxanthin. Non-provitamin A like, lutein and zeaxanthin were also characterized to be higher in MC3 and MC14. Similarly, in another study, varieties of sweetcorn, viz. Jingtian 3 and 5, and varieties of waxy corn, viz. Jingtianzihuanuo 2, Jingnuo 8 and Suyunuo 11, were collected and found that α-cryptoxanthin (provitamin A), lutein, and zeaxanthin were the more significant carotenoids present in corn varieties (Song et al. 2015).

Beyond these approaches, thirty-five orange and twenty-six white maize landrace procured from different areas of Malawi revealed lutein to be the most abundant carotenoid followed by zeaxanthin, β-carotene, and β-cryptoxanthin in the orange landraces. Their analysis suggested that orange maize could be a provitamin A natural source (Hwang et al. 2016). Succeeding them, four maize landraces were evaluated for their carotenoid content and compared to commercial lines. The local landraces (Nano di Verni) contained higher amounts of β-carotene and β- cryptoxanthin than lutein (Capocchi et al. 2017). There are many reports on characterizing carotenoids in maize germplasms to increase provitamin A content by breeding or genome editing strategies. Zn could be bound to high molecular weight protein in the grains, and this was studied by Dionisio (2018). They conducted a proteomics study on Zn binding proteins in the grains of barley using three different techniques viz. SDS-PAGE (protein separation), PVDF (blotting), and DTZ stain. Later, they identified the Zn binding proteins like 7S globulins, β and γ hordeins, dehydrins and LEA, and prolamin family members, which would help in biofortification programs. They also suggested that these proteins might have a role in binding other cationic elements, which could be validated with further investigations (Dionisio 2018). Expanding the proteomic and metabolomic studies to other underutilized graminaceous crop species will widen the understanding of micronutrient biosynthesis and accumulation in the

grains, which could further be exploited using multi-omics approaches for enhancing the contents.

Phenomics studies to understand micronutrient traits in graminaceous crops

There are various high throughput tools available for phenotyping in rice from its leaves (Micol et al. 2009), rice panicle (Ikeda et al. 2010), the morphology of roots (Zhu et al. 2011), biomass of shoot (Golzarian et al. 2011), grain yield (Duan et al. 2015) and photosynthetic integrity (Bauriegel et al. 2011). Techniques like visible-light imaging, near-infrared imaging, hyperspectral imaging, fluorescence imaging, digital X-ray radiography, and X-ray computed tomography are the major photonic-based strategies used in phenomics (Yang et al. 2013). Unfortunately, there is no technique yet that could help in phenotyping the micronutrient traits. Several phenotyping facilities are present to determine the morphological phenotypes of maize, but these do not help understand the micronutrient content. Hence, most of the phenomics studies in maize are based on metabolite analysis, as discussed earlier. In barley, Long et al. (2017) developed RNAi lines of *HvIRT1* that showed a 5% decrease in the gene expression under Mn supplementation. The plants were grown in alkaline soil depriving the plant for Mn, and their phenotype was monitored. The RNAi lines showed less biomass in the shoot with chlorosis symptoms, whereas the wild type grew properly with increased biomass. Upon exposure to radiolabeled Mn, the RNAi lines showed reduced Mn uptake than the wild type. Interestingly, the RNAi lines showed a twofold higher accumulation of Mn in the root apoplast, which might be the probable reason that Mn could not be loaded to stele, xylem, and pericycle cells. Hence, this proposed the role of *the IRT1 gene in barley for higher Mn accumulatio*n (Long et al. 2017). To study the decorticated carotenoid content in sorghum grains, Fernandez et al. (2008) analyzed the grain endosperms by color using Konica Minolta colorimeter CR-300, and out of the three (a, b, and c) output spectrums, b was selected, which infers the role of the intensity of the yellow endosperm color. These studies necessitate the importance of phenomics for studying the molecular determinants of grain micronutrients.

Integrated omics approaches and strategies for genetic improvement in micronutrient traits

Genetic engineering has been an indispensable tool for manipulating the genome to increase the nutrient content (Table 3). *Agrobacterium*-mediated transformation was used to transfer the ferritin gene from soybean to rice to enhance Fe content (Goto et al. 1999; Sivaprakash et al. 2006). Later, Ye et al. (2000) incorporated the biosynthetic pathway for β- carotene in rice endosperm to enhance the provitamin A. Similarly, Lee et al. (2009) used nicotianamine synthase gene activation to biofortify rice with Fe. Interestingly, Singh et al. (2017) developed transgenic rice lines with enhanced Fe, Zn, and β-carotene content by incorporating three genes in one locus, namely nicotianamine synthase 1 (*AtNAS1*) from Arabidopsis, bacterial carotene desaturase (*CRTI*), ferritin (*PvFerritin* from bean), and phytoene synthase (*ZmPSY*) from maize (Singh et al. 2017). Earlier, Colmsee et al. (2012) had developed the OPTIMAS—Data Warehouse (DW) for maize, and it compiles data generated from all the omics techniques, including experimental and analytical data from various research. The import tool of the OPTIMAS-DW was based on Javascript that aided in uploading the data from the experiments performed. For easy accessibility of the data, the tool supports various file formats that can further analyze and visualize datasets (Colmsee et al. 2012). In order to study the reproducibility of different profiling techniques, Zeng et al. (2014) selected three tissues to perform metabolomics and microarray studies from 11 traditional varieties of maize. They compared the data from both the studies and suggested that microarray data could be hindered by the variant genomic sequences with unknown functions (Zeng et al. 2014).

Several transgenic maize varieties have been produced for disease resistance, but only a few are made to enhance micronutrient trait. One such study was performed by overexpression of bacterial phytoene synthase gene, *crtB,* and *crtI* in maize using super γ-zein promotor that increased the carotenoid (especially β-carotenoid) accumulation by about 34-fold, and the trait was reproduced for four generations. They have used a biolistic approach for transforming maize zygotic embryos with two plasmid constructs, viz. pBAR184 + pRBS + pRIS and pBAR184 + pRB + pRI, wherein pRB contained *crtB* gene and pRI contained *crtI* gene. The transgenic lines showed varying carotenoid content in each generation and individual kernels from a particular year. This could be the result of epigenetic factors or germplasm that were selected for transformation. They also concluded that the enhanced β-carotene content shows a higher correlation with the increased abundance of lycopene β-cyclase gene (Aluru et al. 2008).

Tiong et al. (2014) developed transgenic barley lines overexpressing *HvZIP7* gene and observed that it was highly induced in leaves and roots with higher accumulation of Zn during deficiency of Zn with no alteration in the Fe, Mn, Cu, and Cd content. However, the study does not explain the accumulation of Zn in the grain (Tiong et al. 2014). Earlier, Podar et al. (2012) have observed that the HvMTP1 helps in transporting Zn to the vacuole when expressed in yeast and proposed a hypothesis where they have mentioned that overexpressing *HvMTP1* with D-hordein (endosperm specific promotor) could enhance the endosperm Zn content. Later, they expressed this *HvMTP1* gene along with D-hordein

Table 3 Transgenic crops developed with enhanced micronutrient traits using *Agrobacterium*-mediated or biolistic approach

Crop	Micronutrient	Target gene	Promoter	Transformation method	Micronutrient content in control plants	Micronutrient content in transgenic lines	Reference
Rice	Fe	*SoyferH1*	*GluB-1*	*Agrobacterium* mediated	14.3 µg/g seed	35.9–38 µg/g seed	Goto et al. 1999
	Fe	*PyFerritin, fgMT*	*Gt-1*	*Agrobacterium* mediated	9.99–10.65 µg/g seed	11.53–22.07 µg/g seed	Lucca et al. 2002
	Fe	*SoyferH-1*	*GluB-1*, *Glb-1*	*Agrobacterium* mediated	11.2 ± 1.8 µg/g seed	15.1–15.7 µg/g seed	Qu et al. 2005
	Fe	*OsYSL2*	*OsSUT1*	*Agrobacterium* mediated	-	4.4 fold higher in polished rice	Ishimaru et al. 2010
	Fe	*OsNAS1, OsNAS2, OsNAS3*	*CaMV- 35S promoter*	*Agrobacterium* mediated	23.3 µg/g seed	38.8 µg/g seed, 42.8 µg/g seed, 35.6 µg/g seed	Johnson et al. 2011
	Cu, Fe, Zn	*OsNAS3*	maize ubiquitin promoter	*Agrobacterium* mediated	1.2(Cu), 10 (Fe), 20 (Zn) µg/g seed	2 (Cu), 34 (Fe),42 (Zn) µg/g seed	Lee et al. 2009
	Fe	*Pvferritin, Afphytase, AtNAS, pmi*	*GluB-1, 35S promoter*	*Agrobacterium* mediated	-	sixfold increase in Fe content	Wirth et al. 2009
	Fe, Zn	*Osfer2*	*OsGluA2*	Biolistic method	7 (Fe), 20 (Zn) µg/g seed	11–15.9 (Fe), 27–30.75 (Zn) µg/g seed	Paul et al. 2012
	β-carotene	*psy* (daffodil), *crtI* (bacteria)	*Gt-1* and *CaMV-35S*	*Agrobacterium* mediated	–	–	Ye et al. 2000
	β-carotene	*psy* and β-*lcy* (Daffodil), *crtI* (Erwinia)	*GluB-1, CaMV-35S promoter*	*Agrobacterium* mediated	–	1.6 µg/g dry rice endosperm	Beyer et al. 2002
	β-carotene	*Zmpsy crtI* (Erwinia)	*Glu01, Ubi-1*	*Agrobacterium* mediated	1.6 µg/g dry rice endosperm	37 µg/g dry rice endosperm	Paine et al. 2005
	Fe, Zn, *β*-carotene	*AtNAS1, PaCRTI, ZmPSY, PvFerritin*	*CaMV- 35S, OsGLUTELIN1, OsGLOBULIN*	*Agrobacterium* mediated	5.9 (Fe), 32 (Zn) µg/g of seed, No β-carotene	6.1–9.1(Fe), 36.7–38.7 (Zn), 1.57–2.69 (*β*-carotene) µg/g seed	Singh et al. 2017
Maize	*β*-carotene	*crtB and crtl* (bacterial)	*γ*-zein	Biolistic method	–	34 fold increase in endosperm	Aluru et al. 2008
Barley	Zn	*HvZIP7*	Double *CaMV35S*	*Agrobacterium* mediated	70 mg/ kg of seed	110–120 mg/kg seed	Tiong et al. 2014
Sorghum	*β*-Carotene	*ZmPST-1* and *CRT-1 (Erwinia uredovora), LPA-1, AtDXS, HvHGGT*	*α*-Kafirin, *β*-kafirin, *γ*-zein	*Agrobacterium* mediated	0.9 – 1.5 µg/g *β*-Carotene	3 – 14 µg/g seed	Lipkie et al. 2013

in the Golden Promise cultivar of barley and studied the gene expression. RT-qPCR aided in the selection of lines with transformed homozygous plants (2-homozygous out of 3). To research and compare the gene abundance, they used Golden rice as control, GFP (with CaMV35S promoter) expressing homozygous line and the two homozygous transformed lines. The RT-qPCR revealed that both the transformed lines had a higher expression level of *HvMTP1* transcript than the GFP-expressing line and control, post 21 DAP. The transformed plants were cultivated until maturity in Zinc devoid soil. The grains were harvested at maturity, and the Zn content was measured using ICP-OES for all three transformed lines. Higher accumulation of Zn was observed in the grains of transformed homozygous plants than the transformed heterozygous, control, and GFP expression plant. The homozygous transformed lines also showed a higher accumulation of Cu, Fe, and Mn. They again performed the same experiment by adding Zn in soil. Interestingly, all the plants showed an average increment of 103% Zn accumulation compared to the plants grown in Zn devoid

soil. Surprisingly, there was no fluctuation in the Fe content in both conditions. They also confirmed that the *HvMTP1* is expressed in the grain endosperm using three different techniques of DTZ (Zn-specific stain), ICP-OES, and fluorescence analysis with Synchrotron X-ray (Menguer et al. 2017). In barley, loading of Mn in the grain could be very fast during early developmental stages of grain due to the increased expression of *HvIRT1. S*urprisingly, Long et al. (2017) also observed an increase in expression of *HvIRT1* (35-fold) in the root. They developed *HvIRT1* RNAi lines of barley and observed that the RNAi lines showed diminished photosynthesis function due to low Mn content. In rice and *Arabidopsis thaliana,* it has already been reported that *IRT1* functions in the regulation of Fe, but in this study, no such role for Fe was observed (Long et al. 2017). Hence, this study could help understand the evolution of the *IRT1* gene.

Sorghum, although a staple food, lacks essential micronutrients like Fe, Zn, and β-carotene. Lipkie et al. (2013) developed transgenic lines to increase the bioavailability β-Carotene in sorghum. Transgenic sorghum lines were generated using the *Agrobacterium*-mediated transformation strategy in the immature embryo of sorghum TX430. The transgenic were developed using three construct, viz. (i) ABS168 contained golden rice genes (Paine et al. 2005), but the sorghum promoters of α-Kafirin with *ZmPST-1* and β-kafirin with *CRT-1* gene from *Erwinia uredovora* with selectable marker *PMI* gene, (ii) ABS188 contained all genes from the previous vector along with *LPA-1* gene (Low phytic acid) (Increased Zn and Fe bioavailability) and (iii) ABS203 contained and extra *AtDXS* gene (for increased isoprenoid) + γ-zein promoter, *HvHGGT* gene + α-Kafirin along with the genes in the vector ABS168. The homozygous transgenic plants were achieved with multiple self-pollination. They have harvested transgenic sorghum grains followed by a porridge preparation, which was used for carotenoid determination. The carotenoid determination using HPLC–DAD plus YMC C30 column showed the presence of several carotenoids like zeaxanthin, lutein, α- and β-cryptoxanthin, *all-trans-β*-carotene and *cis-β*-carotene isomers, and provitamin A. One of the transgenic sorghums, Homo188-A, showed the highest bioavailability of provitamin A. On the other hand, no major effect was observed in the transgenic lines containing the *LPA-1, HGGT, and DXS* (Lipkie et al. 2013).

Similarly, in another study, three vector constructs were made using combinations of genes like *Pantoea ananatis CRTI*, *ZmPSY1*, *HvHGGT*, *E. coli* PMT *AtDXS* that were cloned in pSBI super-binary vector. The genotype TX430 was grown in greenhouse, and the immature embryo was harvested for *Agrobacterium*-mediated transformation. The selected transgenic lines were selfed to achieve 3:1 transgenic to non-transgenic seeds, which was further confirmed with qPCR. Finally, to achieve 100% homozygous seeds, the plants were self-pollinated. The carotene content of the seeds was analyzed using HPLC. The PSY1 protein accumulation was determined using LC–MS/MS, which showed a higher accumulation of all-*trans* β-carotene in the transgenic seeds (visibly orange in color). Unfortunately, at room temperature, during seed storage, the β-carotene is degraded due to oxidation. Therefore, they also co-expressed the HGGT (homogentisate geranylgeranyl transferase) gene, positively affecting tocopherol and tocotrienol biosynthesis. Vitamin E is a known antioxidant and hence reduced the oxidative degradation of β-carotene significantly. Thus, Che et al. (2016) successfully developed transgenic sorghum lines with increased Vitamin A content. Despite these reports and success stories in major cereals, no attempt has been made till now in genetic engineering or genome editing of small millet species to understand or improve the grain micronutrient content.

Roadmap to improve micronutrient content and availability in graminaceous species

Micronutrient availability in plants is a crucial content in ensuring food security to the people. Owing to these, several genes and QTLs associated with micronutrient traits have been identified and discussed in the previous sections. These findings had a significant impact on increasing the bioavailability of micronutrients in cereals (Fig. 3). The QTLs, alleles and genes could be effectively introgressed for improving the micronutrient status of the ruling crop varieties and the parents of the elite hybrids. This would satisfy the final utility of the consumers. Commercializing these cultivars at an affordable cost to the underprivileged society would further reduce the nutritional hindrances existing across the world.

In addition, there have been several reports where genome editing has benefitted grass family members like in maize; zinc finger nucleases (ZFNs) were used to insert *PAT* gene cassettes into the endogenous maize *ZmIPK1* gene along with alterations in inositol phosphate profile for the development of maize seeds (Shukla et al. 2009). Similarly, in rice, ZFNs were used for identification of safe domains for introduction of genes in rice plants to aid in gene stacking and gene integration (Cantos et al. 2014). Modern techniques of genome editing like TALENs (Transcription Activator-Like Effector Nucleases) and CRISPR are also exploited in improving crops like maize, rice, wheat, etc. TALEN-based mutagenesis was employed in sabotaging bacterial blight susceptibility gene, SWEET14, to develop blight-resistant rice (Li et al. 2012). Identical studies have reported that powdery mildew resistance in wheat was developed by knocking out three homologs of MLO gene (Jung and Altpeter 2016). TALENs were also employed for improving agronomic traits, as in case of maize, the GLOSSY phenotype was enhanced by removing GL2 genes (Kannan et al.

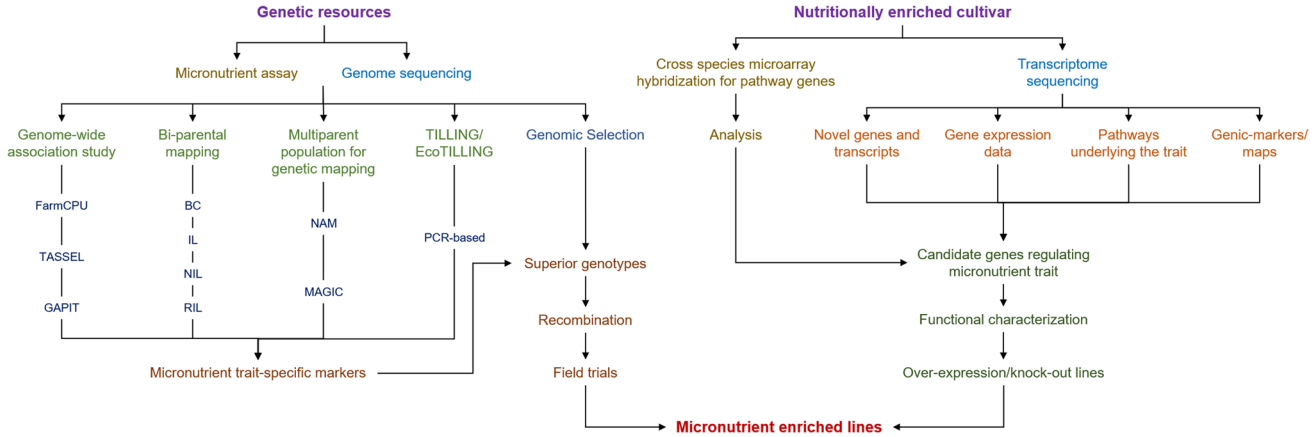

Fig. 3 Schematic representation of current and future strategies for micronutrient trait improvement in graminaceous species

2017). However, nowadays CRISPR Cas technique is most prevalent for approaches like site-directed mutagenesis using embryo bombardment in plants like maize and wheat (Svitashev et al. 2015; Liang et al. 2017). In fact, the CRISPR Cas technique has been optimized in monocots by deploying hexaploid wheat (*Triticum aestivum)* as a template (Liang et al. 2017). Several transgenic approaches are in progress to attain higher nutritional levels while inculcating advanced genome editing techniques to develop superior cultivars in a short breeding cycle. The prospectus of biofortification in cereals should also be widened in the future by identifying desirable genomic regions from small millets as they are closely related to the major cereals. In small millets, nutritional content could be improved by exploiting the knowledge of micronutrient associated biochemical pathways and their regulation using comparative genomics approaches and genome editing. This substantiates and calls for the proportional focus on exploring the nutritionally rich small millets in the near future.

Conclusions

The importance of micronutrients in the grains of cereal crops has been well recognized, and several focused studies were performed to identify the genes, alleles, and QTLs underlying this complex trait. In addition, breeding and biotechnological approaches are already being deployed for the genetic improvement in major cereals, as evident from the literature; however, a portion of graminaceous crops labeled as 'small millets' remains understudied. Though these crops are well known for their superior grain quality traits, including elemental composition, they are not much explored. The development of genomic resources is the first step to proceed with any study to delineate the genes or genomic regions that regulate the complex traits. To achieve this, next-generation sequencing technology should be used to sequencing the genomes and transcriptomes for identifying the genes and pathways and develop large-scale genome-wide molecular markers that are useful for genotyping purposes. The availability of germplasm resources in global repositories should be exploited for this purpose for extensive phenotyping of micronutrient traits. Advanced approaches like GWAS could be effectively used to pinpoint the marker-trait associations as the QTLs underlying micronutrient traits in these crops. Once identified, the information could be further used to functionally characterize the genes and corresponding gene families in millet species and to establish their role in regulating micronutrient content in the grains. These data can be further used to genetically enhance the micronutrient traits in millets per se, and also, comparative genomics could facilitate the implementation of this information for genetic improvement in other graminaceous species.

Acknowledgements The authors' work in the area of molecular genetics and genomics is supported by the Department of Biotechnology, Govt. of India, India. The authors also thank Dr. Lydia Pramitha for critically reading the manuscript. P Sushree Shyamli acknowledges the fellowship from DST-INSPIRE, Ministry of Science and Technology, Government of India.

Author's contribution AP and MP conceived the review, outlined the contents, and supervised the entire study. PSS, SR, and SS wrote the first draft. PSS and SR prepared the tables and figures. MM critically revised the work, addressed the reviewers' comments, and finalized the manuscript.

Declarations

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

References

Aluru M, Xu Y, Guo R et al (2008) Generation of transgenic maize with enhanced provitamin A content. J Exp Bot 59:3551–3562

Anuradha K, Agarwal S, Rao YV et al (2012) Mapping QTLs and candidate genes for iron and zinc concentrations in unpolished rice of Madhukar × Swarna RILs. Gene 508:233–240

Babu R, Palacios N (2013) Validation of the effects of molecular marker polymorphisms in LcyE and CrtRB1 on provitamin A concentrations for 26 tropical maize populations. Theor Appl Genet 126:389–399

Bauriegel E, Giebel A, Herppich WB (2011) Hyperspectral and chlorophyll fluorescence imaging to analyse the Impact of Fusarium culmorum on the photosynthetic Integrity of Infected) Wheat Ears. Sensors 11:3765–3779

Beyer P, Al-Babili S, Ye X et al (2002) Golden Rice: introducing the beta-carotene biosynthesis pathway into rice endosperm by genetic engineering to defeat vitamin A deficiency. J Nutr 132:506S-510S

Bouis HE, Welch RM (2010) Biofortification: a sustainable agriculture strategy for reducing micronutrient malnutrition in the globle south. Crop Sci 50:S20–S32

Brunson AM, Quackenbush FW (1962) breeding corn with high Provitamin A in the grain1. Crop Sci 2:344–347

Cakmak I (2009) Enrichment of fertilizers with zinc: an excellent investment for humanity and crop production in India. J Trace Elem Med Biol 23:281–289

Cakmak I, Kutman UÁ (2018) Agronomic biofortification of cereals with zinc: a review. Eur J Soil Sci 69:172–180

Cakmak I, Pfeiffer WH, McClafferty B (2010) Biofortification of durum wheat with zinc and iron. Cereal Chem 87:10–20

Calayugan MIC, Formantes AK, Amparado A et al (2020) Genetic analysis of agronomic traits and grain iron and zinc concentrations in a doubled haploid population of Rice (Oryza sativa L.). Sci Rep 10:2283

Calingacion M, Boualaphanh C, Daygon V et al (2012) A genomics and multi-platform metabolomics approach to identify new traits of rice quality in traditional and improved varieties. Metabolomics 8:771–783

Cantos C, Francisco P, Trijatmiko KR et al (2014) Identification of acœsafe harbora loci in Indica rice genome by harnessing the property of zinc-finger nucleases to induce DNA damage and repair. Front Plant Sci. https://doi.org/10.3389/fpls.2014.00302

Capocchi A, Bottega S, Spanò C et al (2017) Phytochemicals and antioxidant capacity in four Italian traditional maize (Zea mays L.) varieties. Int J Food Sci Nutr 68:515–524

Chandel G, Dubey M, Gupta S et al (2017) Identification and characterization of a grain micronutrient- related OsFRO2 rice gene ortholog from micronutrient-rich little millet (Panicum sumatrense). 3 Biotech 7:80

Che P, Zhao Z, Glassman K et al (2016) Elevated vitamin E content improves all- trans β -carotene accumulation and stability in biofortified sorghum. Proc Natl Acad Sci U S A 113(11040–5):113

Clemente R, De La Fuente C, Moral R et al (2007) Changes in microbial biomass parameters of a heavy metal-contaminated calcareous soil during a field remediation experiment. J Environ Qual 36:1137–1144

Coccina A, Cavagnaro TR, Pellegrino E et al (2019) The mycorrhizal pathway of zinc uptake contributes to zinc accumulation in barley and wheat grain. BMC Plant Biol 19:1–14

Colmsee C, Mascher M, Czauderna T et al (2012) OPTIMAS-DW: a comprehensive transcriptomics, metabolomics, ionomics, proteomics and phenomics data resource for maize. BMC Plant Biol 29(12):245

Cruet-burgos C, Cox S, Hu Z et al (2020) Advancing provitamin A biofortification in sorghum: genome-wide association studies of grain carotenoids in global germplasm. Plant Genome 13:e20013

Das S, Chaki AK, Hossain A (2019) Breeding and agronomic approaches for the biofortification of zinc in wheat (Triticum aestivum L.) to combat zinc deficiency in millions of a population: a Bangladesh perspective. Acta Agrobot 72:1770

de Valenca AW, Bake A, Brouwer ID et al (2017) Agronomic biofortification of crops to fight hidden hunger in sub-Saharan Africa. Glob Food Sec 12:8–14

Detterbeck A, Nagel M, Rensch S et al (2019) The search for candidate genes associated with natural variation of grain Zn accumulation in barley. Biochem J 476:1889–1909

Dionisio G (2018) Enrichment and identification of the most abundant zinc binding proteins in developing Barley grains by Zinc-IMAC capture and Nano LC-MS / MS. Proteomes 6:3

Dixit S, Singh UM, Ragavendran A et al (2019) Identification of genomic region(s) responsible for high iron and zinc content in rice. Sci Rep 9:8136

Dong C, He F, Berkowitz O et al (2018) Alternative splicing plays a critical role in maintaining mineral nutrient homeostasis in Rice (Oryza sativa). Plant Cell 30:2267–2285

Drakakaki G, Marcel S, Glahn RP et al (2005) Endosperm-specific co-expression of recombinant soybean ferritin and Aspergillus phytase in maize results in significant increases in the levels of bioavailable iron. Plant Mol Biol 59:869–880

Du J, Zeng D, Wang B et al (2013) Environmental effects on mineral accumulation in rice grains and identification of ecological specific QTLs. Environ Geochem Health 35:161–170

Duan P, Ni S, Wang J et al (2015) Regulation of OsGRF4 by OsmiR396 controls grain size and yield in rice. Nat Plants 2:15203

Fernandez MGS, Hamblin MT, Li L et al (2008) Quantitative trait Loci analysis of endosperm color and carotenoid content in Sorghum grain. Crop Sci 48:1732–1743

Garg M, Sharma N, Sharma S, et al (2018) Biofortified Crops Generated by Breeding, Agronomy, and Transgenic Approaches Are Improving Lives of Millions of People around the World. Front Nutr 5:12

Gautam S, Platel K, Srinivasan K (2010) Influence of β-carotene-rich vegetables on the bioaccessibility of zinc and iron from food grains. Food Chem 122:668–672

Gaxiola RA, Palmgren MG, Schumacher K (2007) Plant proton pumps. FEBS Lett 581:2204–2214

Gayen D, Paul S, Sarkar SN et al (2016) Comparative nutritional compositions and proteomics analysis of transgenic Xa21 rice seeds compared to conventional rice. Food Chem 203:301–307

Golzarian MR, Frick RA, Rajendran K et al (2011) Accurate inference of shoot biomass from high-throughput images of cereal plants. Plant Methods 7:2

Goto F, Yoshihara T, Shigemoto N et al (1999) Iron fortification of rice seed by the soybean ferritin gene. Nat Biotechnol 17:282–286

Gregorio GB, Senadhira D, Htut H et al (2000) Breeding for trace mineral density in rice. Food Nutr Bull 21:382–386

Gupta N, Ram H, Kumar B (2016) Mechanism of Zinc absorption in plants: uptake, transport, translocation and accumulation. Rev Environ Sci Biotechnol 15(1):89–109

Gyawali S, Otte M, Chao S et al (2017) Genome wide association studies (GWAS) of element contents in grain with a special focus on zinc and iron in a world collection of barley (Hordeum vulgare L.). J Cereal Sci 77:266–227

Haas JD, Beard JL, Murray-Kolb LE et al (2005) Ironbiofortified rice improves the iron stores of nonanemic Filipino women. J Nutr 135:2823–2830

Harjes CE, Rocheford TR, Bai L et al (2008) Natural genetic variation in lycopene epsilon cyclase tapped for maize biofortification. Science 319:330–333

Heuberger S, Ellers-Kirk C, Tabashnik BE et al (2010) Pollen- and seed-mediated transgene flow in commercial cotton seed production fields. PLoS ONE 5:e14128

Hindu V, Palacios-Rojas N, Babu R et al (2018) Identification and validation of genomic regions influencing kernel zinc and iron in maize. Theor Appl Genet 131:1443–1457

Hossain A, Mottaleb KA, Farhad M et al (2019) Mitigating the twin problems of malnutrition and wheat blast by one wheat variety, "BARI Gom 33", in Bangladesh. Acta Agrobot 72:1775

Hu Y, Wu Y, Li Q et al (2015) Solution structure of yeast Rpn9: insights into proteasome lid assembly. J Biol Chem 290:6878–6889

Hussain A, Zahir ZA, Asgha HN, et al (2018) Zinc solubilizing bacteria for zinc biofortification in cereals: a step toward sustainable nutritional security. In Meena V. (eds) Role of Rhizospheric Microbes in Soil. Springer, Singapore. https://doi.org/10.1007/978-981-13-0044-8_7

Hwang T, Ndolo VU, Katundu M et al (2016) Provitamin A potential of landrace orange maize variety (Zea mays L.) grown in different geographical locations of central Malawi. Food Chem 196:1315–1324

Ikeda S, Okubo T, Anda H et al (2010) Community- and genome-based views of plant-associated bacteria: Plant-bacterial interactions in Soybean and Rice. Plant Cell Physiol 51:1398–1410

Imdad A, Mayo-Wilson E, Herzer K et al (2017) Vitamin A supplementation for preventing morbidity and mortality in children from six months to five years of age. Cochrane Database Syst Rev 3:CD008524

Ishimaru Y, Masuda H, Bashir K et al (2010) Rice metalnicotianamine transporter, OsYSL2, is required for the long-distance transport of iron and manganese. Plant J 62:379–390

Ishimaru Y, Suzuki M, Tsukamoto T et al (2006) Riceplants take up iron as an Fe3+ -phytosiderophore and as Fe2+. Plant J 45:335–346

Jeng TL, Lin YW, Wang CS et al (2012) Comparisons and selection of rice mutants with high iron and zinc contents in their polished grains that were mutated from the indica type cultivar IR64. J Food Compos Anal 28:149–154

Jin T, Zhou J, Chen J et al (2013) The genetic architecture of zinc and iron content in maize grains as revealed by QTL mapping and meta-analysis. Breed Sci 324:317–324

Johnson AAT, Kyriacou B, Callahan DL et al (2011) Constitutive overexpression of the OsNAS gene family reveals single-gene strategies for effective iron- and zinc-biofortification of rice endosperm. PLoS ONE 6:e24476

Jung JH, Altpeter F (2016) TALEN mediated targeted mutagenesis of the caffeic acid O-methyltransferase in highly polyploid sugarcane improves cell wall composition for production of bioethanol. Plant Mol Biol 92:131–142

Kannan B, Jung JH, Moxley GW et al (2017) TALEN-mediated targeted mutagenesis of more than 100 COMT copies/alleles in highly polyploid sugarcane improves saccharification efficiency without compromising biomass yield. Plant Biotechnol J 16(4):856–866

Kim SA, Guerinot ML (2007) Mining iron: iron uptake and transport in plants. FEBS Lett 581(12):2273–2280

Kokane SB, Pathak RK, Singh M et al (2018) The role of tripartite interaction of calcium sensors and transporters in the accumulation of calcium in finger millet grain. Biol Plant 62:325–334

Kuhnen S, Menel M, Campestrini LH et al (2011) Carotenoid and anthocyanin contents of grains of Brazilian maize landraces. J Sci Food Agric 91:1548–1553

Kumar A, Yadav S, Panwar P et al (2015) Identification of anchored simple sequence repeat markers associated with calcium content in finger millet (Eleusine coracana). Proc Natl Acad Sci India B Biol Sci 85:311–317

Kutman UB, Yildiz B, Ozturk L et al (2010) Biofortification of durum wheat with zinc through soil and foliar applications of nitrogen. Cereal Chem 87:1–9

Lee S, Jeon US, Lee SJ et al (2009) Iron fortification of rice seeds through activation of the nicotianamine synthase gene. Proc Natl Acad Sci USA 106:22014–22019

Lee S, Kim Y-S, Jeon US et al (2012) Activation of rice nicotianamine synthase 2 (OsNAS2) enhances iron availability for biofortification. Mol Cells 33:269–275

Li S, Liu X, Zhou X et al (2019) Improving zinc and iron accumulation in maize grains using the zinc and iron transporter ZmZIP5. Plant Cell Physiol 60:2077–2085

Li T, Liu B, Spalding MH et al (2012) High-efficiency TALEN-based gene editing produces disease-resistant rice. Nat Biotechnol 30:390–392

Liang Z, Chen K, Li T et al (2017) Efficient DNA-free genome editing of bread wheat using CRISPR/Cas9 ribonucleoprotein complexes. Nat Commun 8:14261

Lipkie TE, De MFF, Zhao Z et al (2013) Bioaccessibility of carotenoids from transgenic Provitamin A Bioforti fi ed Sorghum. J Agric Food Chem 61:5764–5771

Lonergan PF, Pallotta MA, Lorimer M, Paull JG, Barker SJ, Graham RD (2009) Multiple genetic loci for zinc uptake and distribution in barley (Hordeum vulgare). New Phytol 184:168–179

Lu K, Li L, Zheng X, Zhang Z, Mou T, Hu Z (2008) Quantitative trait loci controlling Cu, Ca, Zn, Mn and Fe content in rice grains. J Genet 87:305–310

Lu T, Lu G, Fan D et al (2010) Function annotation of rice transcriptome at single nucleotide resolution by rna-seq. Genome Res 20:1238–1249

Long L, Persson DP, Duan F et al (2017) The iron-regulated transporter 1 plays an essential role in uptake, translocation and grain-loading of manganese, but not iron, in barley. New Phytol 217:1640–1653

Lucca P, Hurrell R, Potrykus I (2002) Fighting iron deficiency anemia with iron-rich rice. J Am Coll Nutr 21:184S-190S

Lung MG, Mwaniki AM, Szalma SJ et al (2011) Genetic and physiological analysis of iron Biofortification in Maize. PLoS ONE 6:e20429

Majumdar S, Chakraborty B, Kundu R (2018) Comparative analysis of cadmium-induced stress responses by the aromatic and non-aromatic rice genotypes of West Bengal. Environ Sci Pollut Res 25:18451–18461

Mamo B, Barber B, Steffenson B (2014) Genome-wide association mapping of zinc and iron concentration in barley landraces from Ethiopia and Eritrea. J Cereal Sci 60:497

Masuda H, Aung MS, Nishizawa NK (2013) Iron biofortification of rice using different transgenic approaches. Rice (NY) 6:40

Mayer JE, Pfeiffer WH, Beyer P (2008) Biofortified crops to alleviate micronutrient malnutrition. Curr Opin Plant Biol 11:166–170

Menguer PK, Vincent T, Miller AJ et al (2017) Improving zinc accumulation in barley endosperm using HvMTP1, a transition Improving zinc accumulation in cereal endosperm using HvMTP1, a transition metal transporter. Plant Biotechnol J 16:63–71

Messias S, Galli V, Delmar S (2014) Carotenoid biosynthetic and catabolic pathways: gene expression and carotenoid content in grains of Maize Landraces. Nutrients 6(2):546–563

Micol JL (2009) Leaf development: Time to turn over a new leaf? Curr Opin in Plant Biol 12:9–16

Mirza N, Taj G, Arora S et al (2014) Transcriptional expression analysis of genes involved in regulation of calcium translocation and storage in finger millet (Eleusine coracana L. Gartn.). Gene 550:171–179

Morrissey J, Guerinot ML (2009) Iron uptake and transport in plants: the good, the bad, and the ionome. Chem Rev 109(10):4553–4567

Muthamilarasan M, Dhaka A, Yadav R et al (2016) Exploration of millet models for developing nutrient rich graminaceous crops. Plant Sci 242:89–97

Muthamilarasan M, Prasad M (2021) Small millets for enduring food security amidst pandemics. Trends Plant Sci 26:33–40

Natesan S, Duraisamy T, Pukalenthy B (2020) Enhancing β -carotene concentration in parental lines of CO6 Maize hybrid through marker-assisted backcross breeding (MABB). Front Nutr 7:1–12

Paine J, Shipton C, Chaggar S et al (2005) Improving the nutritional value of Golden Rice through increased provitamin A content. Nat Biotechnol 23:482–487

Palmgren MG, Clemens S, Williams LE et al (2008) Zinc biofortification of cereals: problems and solutions. Trends Plant Sci 13:464–473

Paul JW, Qi Y (2016) CRISPR/Cas9 for plant genome editing: accomplishments, problems and prospects. Plant Cell Rep 35:1417–1427

Paul S, Ali N, Gayen D et al (2012) Molecular breeding of Osfer2 gene to increase iron nutrition in rice grain. GM Crops Food 3:310–316

Phuke RM, Anuradha K, Radhika K et al (2017) Genetic variability, genotype × environment interaction, correlation, and GGE Biplot analysis for grain iron and zinc concentration and other agronomic traits in RIL population of Sorghum (Sorghum bicolor L. Moench). Front Plant Sci 5:712

Podar D, Scherer J, Noordally Z et al (2012) Metal selectivity determinants in a family of transition metal transporters. J Biol Chem 287:3185–3196

Qin H, Cai Y, Liu Z et al (2012) Identification of QTL for zinc and iron concentration in maize kernel and cob. Euphytica 187:345–358

Qu Q, Yoshihara T, Ooyama A et al (2005) Iron accumulation does not parallel the high expression level of ferritin in transgenic rice seeds. Planta 222:225–233

Raza Q, Riaz A, Sabar M, Muhammad R (2019) Meta-analysis of grain iron and zinc associated QTLs identified hotspot chromosomal regions and positional candidate genes for breeding biofortified rice. Plant Sci 288:110214

Ren Z, Qi D, Pugh N et al (2019) Improvements to the rice genome annotation through large-scale analysis of RNA-Seq and proteomics data sets. Mol Cell Proteomics 18:86–98

Reuscher S, Kolter A, Hoffmann A et al (2016) Quantitative trait Loci and inter-organ partitioning for essential metal and toxic analogue accumulation in Barley. PLoS ONE 11:e0153392

Sadeghzadeh B, Rengel Z, Li C (2015) Quantitative trait Loci (QTL) of seed Zn accumulation in barley population clipper x sahara. J Plant Nutr 38:1672–1684

Sadeghzadeh AD, Mohammad H, Kashi A, Ahmed A, Khoshnood A (2010) Genetic variability of some agronomic traits in the Iranian Fenugreek landraces under drought stress and non-stress conditions. Afr J Plant Sci 4:12-20

Saleh ASM, Zhang Q, Chen J et al (2013) Millet Grains: nutritional quality, processing, and potential health benefits. Compr Rev Food Sci Food Safety 12:281–295

Schaaf G, Ludewig U, Erenoglu BE et al (2004) ZmYS1 functions as a proton-coupled symporter for phytosiderophore- and nicotianamine-chelated metals. J Biol Chem 279:9091–9096

Shakoor N, Ziegler G, Dilkes BP et al (2016) Integration of experiments across diverse environments identifies the genetic determinants of variation in sorghum bicolor seed element composition. Plant Physiol 170:1989–1998

Sharma A, Chauhan RS (2008) Identification of candidate gene-based markers (SNPs and SSRs) in the zinc and iron transport sequence of maize (Zea mays L.). Current Sci 95:1051–1059

Shukla AK, Behera SK, Lenka NK et al (2016) Spatial variability of soil micronutrients in the intensively cultivated Trans-Gangetic Plains of India. Soil Tillage Res 163:282–289

Shukla K, Doyon Y, Miller JC et al (2009) Precise genome modification in the crop species Zea mays using zinc finger nucleases. Nature 459:437–443

Singh SP, Gruissem W, Bhullar NK (2017) Single genetic locus improvement of iron, zinc and β-carotene content in rice grains. Sci Rep 7:6883

Singh UM, Chandra M, Shankhdhar SC et al (2014) Transcriptome wide identification and validation of calcium sensor gene family in the developing spikes of finger millet genotypes for elucidating its role in grain calcium accumulation. PLoS ONE 9:e103963

Sivaprakash K, Krishnan S, And DSK, Parida A (2006) Tissue-specific histochemical localization of iron and ferritin gene expression in transgenic indica rice Pusa Basmati (Oryza sativa L.). J Genet 85:157–160

Song J, Li D, He M et al (2015) Comparison of carotenoid composition in immature and mature grains of corn (Zea Mays L) Varieties comparison of carotenoid composition in immature and mature grains of corn (Zea Mays L.) varieties. Int J Food Prop 19:351–358

Sperotto RA, Vasconcelos MW, Grusak MA et al (2012) Effects of different Fe supplies on mineral partitioning and remobilization during the reproductive development of rice (Oryza sativa L.). Rice 5:27

Stevens GA, Finucane MM, De-Regil LM et al (2013) Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995–2011: a systematic analysis of population-representative data. Lancet Glob Health 1:e16-25

Suwarno WB, Pixley KV, Palacios-Rojas N et al (2015) Genome-wide association analysis reveals new targets for carotenoid biofortification in maize. Theor Appl Genet 128:851–864

Suzuki M, Morikawa KC, Nakanishi H et al (2008) Transgenic rice lines that include barley genes have increased tolerance to low iron availability in a calcareous paddy soil. Soil Sci & Plant Nutr 54(1):77–85

Svitashev S, Young JK, Schwartz C et al (2015) Targeted mutagenesis, precise gene editing, and site-specific gene insertion in maize using Cas9 and guide RNA. Plant Physiol 169:931–945

Swamy BPM, Descalsota GIL, Nha CT et al (2018a) Identification of genomic regions associated with agronomic and biofortification traits in DH populations of rice. PLoS ONE 13:1–20

Swamy BPM, Kaladhar K, Anuradha K et al (2018b) QTL Analysis for grain iron and zinc concentrations in Two O. nivara derived backcross populations. Rice Sci 25:197–207

Tauris B, Borg S, Gregersen PL et al (2009) A roadmap for zinc trafficking in the developing barley grain based on laser capture microdissection and gene expression profiling. J Exp Bot 60:1333–1347

Tiong J, Mcdonald GK, Genc Y et al (2014) HvZIP7 mediates zinc accumulation in barley (Hordeum vulgare) at moderately high zinc supply. New Phytol 201(1):131–143

Uarrota VG, Kobe S, Kuhnen S et al (2014) Biochemical Profile of leaf, silk and grain samples of eight Maize Landraces (Zea mays L.) cultivated in two low-input agricultural systems. J Food Biochem 38:551–562

Vasconcelos M, Datta K, Oliva N et al (2003) Enhanced iron and zinc accumulation in transgenic rice with the ferritin gene. Plant Sci 164:371–378

Velu G, Ortiz-Monasterio I, Cakmak I et al (2014) Biofortification strategies to increase grain zinc and iron concentrations in wheat. J Cereal Sci 59:365–372

Vetriventhan M, Azevedo VC, Upadhyaya HD, et al (2020) Genetic and genomic resources, and breeding for accelerating improvement of small millets: current status and future interventions. Nucleus 63:217–239

Virmani SS, Ilyas-Ahmed M (2008). Rice breeding for sustainable production. In: Kang MS, Priyadarshan P (eds) Breeding Major Food Staples. Oxford, UK: Blackwell Publishing Ltd https://doi.org/10.1002/9780470376447.ch6

Visser ME, Durao S, Sinclair D et al (2017) Micronutrient supplementation in adults with HIV infection. Cochrane Database Syst Rev 5:003650

Welch RM, Graham RD (2004) Breeding for micronutrients in staple food crops from a human nutrition perspective. J Exp Bot 55:353–364

Wessells KR, Brown KH (2012) Estimating the global prevalence of zinc deficiency: results based on zinc availability in national food supplies and the prevalence of stunting. PLoS ONE 7:e50568

White PJ, Broadley MR (2005) Biofortifying crops with essential mineral elements. Trends Plant Sci 10:586–593

White PJ, Broadley MR (2009) Biofortification of crops with seven mineral elements often lacking in human diets - iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytol 182:49–84

Wirth J, Poletti S, Aeschlimann B et al (2009) Rice endosperm iron biofortification by targeted and synergistic action of nicotianamine synthase and ferritin. Plant Biot J7(7):631–644

Yan J, Kandianis CB, Harjes CE et al (2010) Rare genetic variation at Zea mays crtRB1 increases β -carotene in maize grain. Nat Publ Gr 42:322–327

Yang Wu, Bin T, Heyong H et al (2013) The study of zinc sulphide scintillator for fast neutron radiography. Phys Procedia 43:205–215

Ye X, Al-Babili S, Klöti A et al (2000) Engineering the provitamin A (beta-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science 14(287):303–305

Zeng W, Hazebroek J, Beatty M et al (2014) Analytical method evaluation and discovery of variation within maize varieties in the context of food safety: transcript pro fi ling and metabolomics. J Agric Food Chem 62:2997–3009

Zheng L, Huang F, Narsai R et al (2009) Physiological and transcriptome analysis of iron and phosphorus interaction in rice seedlings. Plant Physiol 151:262–274

Zhu J, Ingram PA, Benfey PN et al (2011) From lab to field, new approaches to phenotyping root system architecture. Curr Opin Plant Biol 14:310–317