Review article

Quantitative trait loci and candidate genes for iron and zinc bio-fortification in genetically diverse germplasm of maize (*Zea mays* L): A systematic review
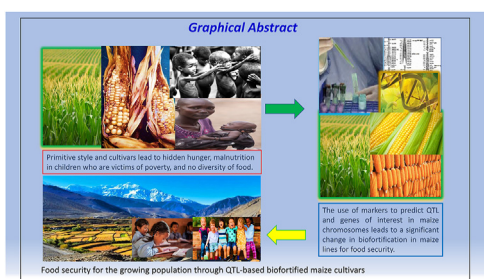
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GRAPHICAL ABSTRACT

ARTICLE INFO

ABSTRACT

Genetically and economically, Maize plays a pivotal role in tackling Iron–Zinc mineral deficiency through the crop's biofortification approach to high-yielding cultivars. The objective of this study is to summarize quantitative trait loci (QTL) is useful for identifying novel genes of interest in diverse germplasm for understanding the exact genetic mechanism for Iron and zinc uptake, deposition, and biosynthesis in *Zea mays L* endosperm. various techniques like Germplasm Genetic Wide Association, QTL meta-analysis, and biparental linkage analysis are used by researchers in diverse germplasm of Maize for the gene of interest marking and are extracted as secondary information through a systematic review of scientific published sources in peer-reviewed sites. A literature review was focused on quantitative trait loci with candidate genes from different families like YS, NRAMP, ferritin, Cation efflux, etc., and cloned four phytase soluble genes which influence the concentration as well as bioavailability of Fe & Zn in the endosperm. More than 30 QTLs with 15-Fe, 17-Zn; 10 Meta QTLS are common and linked with micronutrient concentration as well 17 candidate genes from different families are responsible for the zinc-iron deposition on the endosperm. More than 46 Fe–Zn (20 + 26) SNPs and 22 SNPs (10 + 12) on nine different chromosomes play a significant role in the variation of the mineral value of inbreeds and Double haploid Bi-parental population of *Zea mays L*. In Rice and Maize, five different chromosomes are collinear for the uptake to deposition of these minerals in the endosperm. The success of marker-based biofortification depends upon the nature of germplasm, the gap between flanking marker and targeted genes, the selection of genotypes in each generation, and genotype-environment interaction which are the future area of study. This study can assist the breeders in fast-tracking Fe and Zn biofortification through frequency multiplication of these desired loci of Maize.

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1. Introduction

Malnutrition as hidden hunger due to micronutrient deficiency is a serious health insecurity issue in a changing world due to the rapidly growing population as well as vampire COVID-19 [1,2]. Due to a lack of food diversity, the strong future pillars of every nation are children, expectant women, and others from mostly underdeveloped, developing, and least-developed countries who will be more susceptible to diseases as a result of the lack of food diversity [3, 4]. Zinc and iron are minerals and nutrients that are necessary for a variety of biochemical pathways and are present in over 300 enzymatic reactions as co-factor involved in the formation and breakdown of macromolecules such as polysaccharides, peptides, and triglycerides [5, 6].

Maize biofortification is a necessary foundation for food security and exposing the world's stunning hidden hunger [7]. A report by the Food and Agriculture Organization (FAO) shows that in 2020, about 770 million people are undernourished, which means 160 to 118 million more than in 2014–2021 [8]. The most prevalent regions are Asia and Africa, which have covered 91% of the nutrient deficiency load over 100 [8,9]. The undernutrition prevalence in children is still around 22%, which is down from 32% in 2000 [9]. Data shows that 7.3 billion people in the world consume cereal-based stable food, which supplies 93% of the total calorie supply for daily needs [10, 11]. Micronutrient deficiencies are associated with less consumption of bio-available zinc, iron, and a regular diet due to poverty [12, 13] Maize is the major cereal crop for which the kernel has the potential to accumulate zinc-iron in a more bioavailable concentration, so it acts as an ideal model crop for bio-available nutrition enhancement at the desired level through a transgenic biofortification approach [14, 15].

Our body only needs minute quantities of minerals that our diet provides [15]. These nutrients work together biologically in our bodies in several ways [14]. Maize offers both calories and minerals. The top ten risk factors for diseases like anemia include deficiencies of the key minerals Zn and Fe, which are placed fifth and sixth respectively [16]. The most prevalent of these is an iron deficiency; more than one-third of the world's population experiences iron deficiency-induced anemia, with 80% of cases occurring in developing countries; other conditions include zinc deficiency, which causes diarrhea, skin inflammation, and recurrent infections in humans affects and over 2 billion people globally [16]. Lactating breasts and cultured breast cells showed gene expression of various distinct zinc transporter, indicating the significance of zinc in lactation [17]. Children who lack enough zinc experience several chronic health issues, including growth retardation, loss of weight, higher rates of infectious, and fatalities [18]. Each cell in the body has zinc. The body's immune system and defense mechanisms need to function effectively. Cell division, cell proliferation, wound healing, and the digestion of carbohydrates are all impacted by it. Taste and aroma are other senses that require zinc [19].

The total maize production in the world is 1,162 million tons (in 2020) growing at an average annual rate of 3.07% and consumption per day in the world is approximately 49 g, which occupies the third position after wheat (179) and rice (148) [7,20,21] Maize is cultivated on around 236 million hectares in more than 165 countries in the world, so it acts as a powerful energy improvement channel through a transgenic biofortification approach [2, 22]. Biofortification is the emerging technology that is essential for proper marking of which candidate genes are involved in nutrient accumulation in crops [3, 23] Maize is also called the queen of cereals because it has high productivity and nutrient qualities as

well as quantity due to its genetic potential and contributes 39% to global grain production [24]. The goal for Zn in maize was determined at 33 g/g based on this information reviewed by (Dr. Erick Boy's communication). The typical Zn content in maize is between 20 and 25 g/kg [25]. A list of bio-fortified Maize cultivars is shown in Table 1.

Researchers found that 17 candidate genes of the different families are identified by different researchers for iron uptake, mobilization, and storage in a kernel of maize, but in the case of the maize that is grown in water, a total of 47 candidate genes and 39 QTLs play a role in the limitation of iron [26]. The genes that play an important role in the adaptation of maize to different iron-available soils are genes *Naat1, Nas1, Nramp3, Mtk, and Ys1* [27,28].
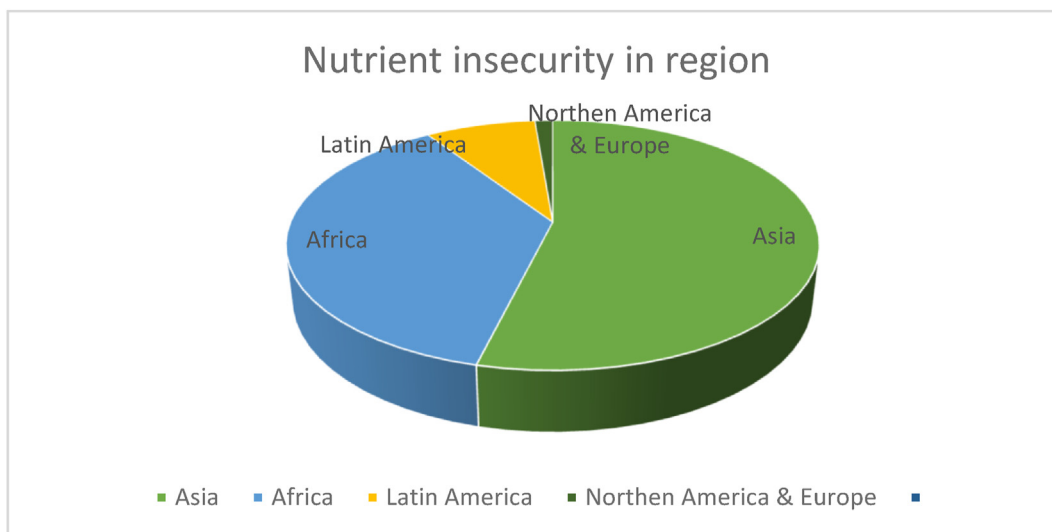
About 30% of world soil exhibits less effective iron for plants, according to a study on soil [26]. RNA interference and Genome editing techniques, which are also employed for transgenic biofortification are the methods used for transgenic approaches in maize [1, 29, 30]. There are certain genetic engineering techniques such as TALENS (transcription activator-like effector nucleases) or CRISPR (clustered regularly interspaced short palindromic repeats) and through the application of these techniques, by using nuclease enzymes, we can create precise deletions, insertions, and substitutions of genomic sequence in a plant reviewed by [23]. Ethyl methane sulphonate (EMS) mutagenesis techniques are used for low phytic acid-containing seed development in maize [17, 31]. Researchers have found that *ZmIRT1, ZmNRAMP6, and ZmHMA* genes show linkage under Zn deficiency in maize, which is the genetic proof and possible bottom line for genomic functional improvement [3, 23] When we talk about the Fe dissemination of maize grain during development, the *ZmYSL2* gene plays a foundational role [32]. All possible alleles are selected through functional markers [23].

Marker-assisted breeding with Meta-analysis is a powerful strategy for the identification of quantitative trait loci with a gene of interest for introgressive biofortification in maize because conventional breeding is a long-run approach, agronomic fortification like micro-organism-based inoculation to rich the desired level of nutrients from the beginning isn't a sustainable type, and transgenic fortification is more controversial and costly due to the diverse germplasm of maize [33, 34, 35]. Microorganism-based inoculum on seed or soil with techniques called 16S rRNA sequencing was used to identify *Bacillus* sp. (ZM20), *Bacillus arya-bhattai* (ZM31 and S10), and *Bacillus subtilis* (ZM63) [36]. This analysis revealed that contaminating Zn-solubilizing strains with corn has the potential to promote growth and could be used as bio-inoculants for biofortification to combat hidden hunger [37]. The QTL analysis approach is one of the potential mechanisms for breeding different maize cultivars with low kernel phytic acid because it slows down the uptake, mobilization, deposition, and bioavailability after processing of essential nutrients and vitamins like zinc and iron in the digestive system of humans [38, 39].

Based on the premise that the subjected molecule has 28.20 percent phosphorus, PA concentrations of maize ranged from 0.62 to 1.17 g/100 g in different samples. Molar ratios of more than 15:1 and more than 18:1 are exclusively related to zinc uptake inhibition and suboptimal body zinc levels in humans, according to the World Health Organization (WHO) and the International Zinc Nutrition Consultative Group (IZiNCG) [17, 31] When we see the result of transgenic rice, provides 30% of the daily physiological requirement for both Zn–Fe [40, 41] This technique helps in the improvement of micronutrients to the desired level among various cultivars of germplasm [12, 42] It has been found that there is no association between kernel Zn concentration and grain yield, implying

Table 1. Lists Biofortified maize cultivars in the world. These biofortified maize cultivars help to reduce the micronutrient-associated hidden hunger in some countries of Africa with the collaboration of CYMMIT and other domestic research organizations. So this is a recent innovation for tackling micronutrient deficiency.

S.N.	Biofortified maize cultivars	Name of the country	Collaboration with	Superior % of Zn than other varieties.	References
1	ICTA HB-18 and ICTA B-15	Guatemala	CIMMYT- CGIAR- ICTA-Harvest Plus	ICTA HB-18 > 15% ICTA B-15 > 60% Zn > tortillas	[17, 29]
2	BIO-MZN01	Colombia	CIMMYT-Harvest Plus	36% > other	[10, 17, 22]

Nutrient insecurity in region

Northern America
& Europe

Latin America

Africa

Asia

- Asia ■ Africa ■ Latin America ■ Northern America & Europe ■

Figure 1. Percentage share by people for nutrient insecurity in different region of world ([8].This figure shows which region of the world is most susceptible to hidden hunger. Asia is most susceptible region. The scope of bio-fortification for Asian countries is necessity factor. The prevalence of undernourishment for Northern America and Europe is estimated to be less than 2.5 percent.

that Zn can be increased without lowering yield [38]. In contrast, there has been evidence of a negative relationship between grain yield and kernel Zn. The kernel Zn level is related to the kernel Fe concentration in a positive way [43]. Because some of the genes that encode metal transporter proteins transport numerous metals, the strong association between Zn and Fe could be due to the linkage between both the genes affecting the accumulation of these two phytonutrients or (ii) frequently regulated processes such as uptake and translocation in the maize [25, 44]. The condition of hidden hunger in the world is shown in Figure 1.

1.1. Methods

Secondary sources provided us with information on QTL and the biofortification of the gene of interest. The search engines used were "Google Scholar," "Research Gate," and "PubMed." Researchers went through a range of journal papers, book chapters, theses, websites, and other governmental and non-governmental materials to discover more about the exact QTL, or related gene, a mechanism for mineral uptake to deposition on endosperm rather than an embryo. In this study, we summarize the current knowledge of quantitative trait loci (QTL) and the position of candidate genes within the locus, also called gene of interest, which has been identified through analysis as well as multiple studies using diverse germplasm and review of the identified genes correlated with Zn–Fe uptake, sequestration, and accumulation in a kernel of maize endosperm [41, 45, 46]. The combined application of both quantitative trait loci with candidate genes contributes to the pipeline development of

Zn–Fe biofortified crops [47]. Maize biofortification is a necessary foundation for food security and exposing the world's stunning hidden hunger [7].

1.2. Statement of problems

The World Bank estimates that 689 million people, or 9.2 percent of the world's population, live in abject poverty on less than $1.90 a day. More than 4.5 billion people in 94 developing nations receive at least 30% of their daily calories from maize, along with rice and wheat [48]. They include the 900 million underprivileged people who favor maize as a staple food [22]. Approximately to 27percent of worldwide people suffer from iron deficiency anemia (IDA), and over 1.1 billion people are in danger of zinc (Zn) insufficiency [49]. Most of those people are suffered from those problems due to a lack of variation in dietary intake in their food. This huge problem is not solved instantly, but the biofortification of minerals nutrients especially Iron and Zinc through QTLs identification and gene of interest introgression in high-yielding cultivars of maize might be the sustainable way to combat this situation and have huge scope.

2. Role of different family genes to uptake, mobilization, and restoration of zinc and iron in maize kernel endosperm

Certain members of the HMA family genes (heavy metal ATPase) on chromosomes 1 and 9 *(GRMZM2G000219, GRMZM2G151406)* show similar properties to *PAA1* and *RNA1* genes that contribute to Zn and Cu

Table 2. Different chromosomal loci with respective alleles show the additive effect as well phenotypic variation for the accumulation of iron and zinc shown in the table. These are the major chromosome. The colocalization loci range in each chromosome shows phenotypic variation due to various additive effects. the information of target genes within the loci detected in the K22×By815 RIL population. The given data shows that chromosome 1 with co-localization of loci 12.5 to15.7 Mb shows 8.5 to 9.4 percentage phenotypic variation in kernel zinc accumulation with 0.33 additive effect followed by chromosome no 2,9,6 and least one by chromosome 10 for phenotypic variation.

S. N	Respective chromosome	Chromosome range/ co-localization of loci	Major alleles interval	Phenotypic variation	Additive effect	References
1	1	12.5–15.7 Mb	qKB-RDW1-2, qKBSDW1-1	8.5%–9.4%	0.33	[76]
2	2	7.7–8.8 Mb	(qKB-PH2-1 + qKB-SDW2-2)	9.2%–12%	0.13	[77]
3	6	150.7–152.3 Mb	qKB-PH6-1 & qKB-ZnSc6-1	5.5%–9.2%	(0.15–0.19)	
4	9	28.0–89.4 Mb	qKB-ZnSc9-1 & qKB-RDW9-2	5.2%–9.8%	(0.16–0.17)	[76]
5	9	100.9–107.3 Mb	qKB-PH9-1 & qKB-ZnSc9-2	5.2%–9.8%	(0.16–0.17)	[50]
6	10	qKB-ZnSc10-1 (QTL)	PZE110104601 to SYN19780	5.2%	(0.15–0.19)	[10, 13]

Table 3. Genetic variability for kernel Zn concentration in different studies. These are the different cultivars that have average Zn in their endosperm. These are the germplasm that is studied to date.

S.N.	Range Zn (mg/g)	Sorts of Germplasm	No of germplasm	Place of evaluation	References.
1	12.9–57	Landraces and Improve Genotypes.	1,814	Zimbabwe & Mexico	[81]
2	11.65–95.6	Inbred lines	109	Nigeria	[29]
4	16.5–20.5	Varieties	28	Nigeria	[82]
5	16.5–24.6	Varieties	49	Nigeria	[82]
6	16–23.6	Hybrid	28	Croatia	[45]
6	19.4–24.6	Varieties	20	Nigeria	[83]
7	18.1–29.8	Inbreeds	14	Zimbabwe	[83]
8	15.0–47.0	Core accessions	400	-	[10]
9	14.0–45.0	Inbreeds	310	Nigeria	[10, 84]
10	13.4–46.4	Inbreeds	25.	India	[85]
11	16.4–28.6	F4 families of Hybrids	294	Croatia	[86]
12	17.6–49.1	Inbreds and Hybrids	49	India	[87]
13	21.9–40.9	Landraces	31	India	[83]
14	19.3–30.9	Hybrids	42	Mexico & Ethiopia	[88]
15	15.1–53.0	Inbreds and landraces	30	India	[85]
16	4–63	Inbreed	2009	Brazil	[89]
18	3.8–35.8	Inbreds and landraces	81	India	[85]
19	12.6–39.4	QPM inbreds	46	India	[90]
20	5.4–30.8	-	50	India	[90]
21	19.4–32.6	Improved genotypes	48	India	[90]
22	20.0–53.0	Inbreeds	24.	Nigeria	[90]
23	17.1–43.8	Inbreeds	923	Mexico	[91]
24	25.75–42.	mixed	30	India	[85]
25	39–54	Diverse	67	India	[85]

transport in maize plants, but the exact mechanism is not fully understood [50, 51]. ZIP gene discovery using the Arabidopsis AtZIP1 (*AT3G12750)* gene in a blast search against the corn genome; discovered a maize gene, GRMZM2G045849, with a distinctive ZIP domain and a 54 percent resemblance to AT3G12750 [17]. This ZIP domain is responsible for the transport of Zn metal ions in plants. A record in yeast mutants shows that *AtIRT3* plays a role in the translocation of both Zn and Fe [17, 52]. The OsZIP4 gene is stimulated in transgenic rice plants, and Zn is primarily stored in the roots as opposed to the shoots [53]. OsZIP4 is a Zn transporter that concentrates in the roots, vascular bundles of leaves, and phloem cells of stems. Its constitutive expression modifies how Zn is distributed in rice [3]. Iron and Zinc concentrations in the leaves, flowers, and seeds of tobacco plants are elevated when the barley NAS gene HvNAS1 is overexpressed [54]. A high-density bin map was used to

analyze quantitative trait loci (QTL) in K22 and By815 RIL populations [50]. These loci were discovered on chromosomes 1, 2, 3, 5, 6, 7, 9, and 10, and explained 5.2 to 16.6% of phenotypic variation for zinc. On chromosomes 6, 9, and 10, four QTLs for Zn score were found (qkb-ZnSc6-1, QB-ZnSc9-1, qKB-ZnSc9-2, qKB-ZnSc10-1). At these four mapped loci, alleles from By815, the Zn-efficient parent, displayed increasing effects (0.15–0.19) on the Zn score [22, 23]. Together, *SYN11817 and PZE-106098680* bordered qKB-ZnSc6-1 on chromosome 6, and together they explained 9.2% of the variation [22]. On chromosome 9, the genes qKB-ZnSc9-1 and qKB-ZnSc9-2 were discovered in the PZE-109025227-PZE109051633 intervals [12, 23, 50, 55].

(*GRMZM2G064382*) The 6.03-chromosome bin-*ZmZIP5* gene is important for zinc accumulation early in grain development [38, 43, 56]. But in the case of overexpression, it increases the concentration of Fe in the root and seed parts of the transgenic Arabidopsis plant, indicating that the exact mechanism of the regulation is still unclear in maize [12, 57]. Another family gene, ZRT, IRT-like protein, NRAMP (The natural resistance-associated macrophage protein), demonstrates the linkage between HMA and ZIP genes locus (qKB-R/S7-2) the leading gene (*GRMZM2G149040*) *bZIP58* as transcription factor under low Zn on natural phenotypic variants line of maize [23]. The gene locus has an additive effect with iron accumulation genes and is distributed across 21 genomic regions associated with Zn deficiency, each of which deals with a different phenotypic variation [10]. Different chromosomal loci with respective alleles show the additive effect, as well as a phenotypic variation for the accumulation of iron and zinc, which are shown in Table 2.

The scanning of maize genomic sequence shows that 48 candidate genes can be engaged in iron and zinc accumulation in the grain of maize but no mechanism of that genes is not fully expressed [31, 58] Among them, 13 genes belong to the ZIP family which are *ZmZIP1 to ZmZIP12* (zinc-iron regulated transporter proteins), 16 genes belong to NRAMP (Natural Resistance-associated Macrophage Protein), 17 genes from the YS (yellow strip) family, and one from the CE (cation efflux) + ferritin family are acknowledged through the occurrence of characteristic signature sequence in respective gene families [17, 56, 59]. Similarly, in 28 candidate genes, around 34 Di, Tri, and Tetra-nucleotides are present in introns, exons, 3'UTRs, and 5'UTRs regions of maize as iron and zinc transporters [60]. Leaf had the highest levels of *ZmZIP5, ZmZIP11, ZmZIP1, and ZmZIP10* expression, whereas developing inflorescence, silk, and husk displayed extremely low levels of expression [59]. Quality protein maize has a comparatively higher amount of Zn in endosperm because the presence of the O2 locus inhibits the synthesis of zein proteins, which increases the Zn binding proteins like albumins, globulins, glutelins [13, 61, 62], etc. Genetic variability for kernel Zn concentration in different studies is shown in Table 3.

3. Rice-maize, gene-orthologs for metal transportation and deposition in mature seeds

Meta QTLS are the programs used for Genetic analysis of the different QTLs which governed the specific Quantitative traits called complex traits through the different-Biochemical pathway [63]. Nine M-QTLs

Table 4. Rice-maize gene- orthologs for UTS of Fe–Zn (Collinear with pleiotropic effects). U=Uptake, T = Translocation, and S= Sequestration. A GWAS (genetic-wide association study) was conducted to identify the quantitative trait loci responsible for Fe and Zn biofortification in maize. Major pathway-level analysis of different genes and their respective products is taken as a reference (Owens et al., 2014).

S.N.	Chromosome of maize	Co-linear chromosome of rice	Rice gene	Maize Orthologs	References
1	9 (9.06–9.07)	3	*OsTOM1*	*GRMZM2G063306/ZmTOM1/mMQTL3*	[54, 75]
2	2	4	*OsYSL6*	*GRMZM2G085833/mMQTL2.1*	[3, 74]
3	10	4	*OsYSL16*	*GRMZM2G026391/mMQTL10*	[70]
4	2	7	*OsNRAMP1/rMQTL7.1*	*GRMZM2G366919/mMQTL2.1*	[16, 17]
5	2	7	*OsHMA3*	*GRMZM2G175576/mMQTL2.1*	[12, 78]
6	3	1	*OsIR O 2*	*GRMZM2G057413/mMQTL3*	[79, 80]

Table 5. GWAS (genetic wide association survey) for quantitative trait loci responsible for Fe and Zn biofortification in maize. These are the major QTL on different chromosome bins which plays role in limitation in Iron and Zinc endosperm concentration in Bi-Parental populations.

S.N.	Minerals	GWAS-identified SNPs	QTL bin/chromosome.	QTL Interval	Physical position (bp)	Annotation	References.
1	Zn	S1_275780297	1.25	-	275,780,297	Protein-coding	[10]
2	Zn	S1_279913529	1.27	-	279,913,529	Zinc ion binding and ZOS8- 04—CH2 2 zinc finger protein	[10]
3	Zn	S3_40522792	3.04	umc1504–umc1386a	40,522,792	PPR repeat-containing protein	[17, 22]
4	Zn	S4_161165956	4.06	bnlg1621a–dupssr16	161,165,956	-	[12, 75]
5	Zn	S5_100070727	5.04	umc1110–bnlg1208	100,070,727	Ice binding, Homo-isothermy,	[1, 64]
6	Zn	S5_68423957	5.68	-	68,423,957	Glycosyltransferase protein	[10]
7	Zn	S5_71718466	5.71	-	71,718,466	Translation initiation factor (eIF3 subunit)	[10]
8	Zn	S9_151265550	9.06–9.07	umc1310–bnlg128	151,265,550	-	[10, 38]
9	Zn	S9_151265550	9.07	dupssr29–bnlg619	151,265,550	-	[64]
1	Fe	S2_19265861	2.07	umc1542–umc1042	19,265,861	Pectin-esterase activity	Reviewed by [64]
2	Fe	S3_186200393	3.04–3.06	mmp144a–umc1266	186,200,393	Regulation of transcription and pentatricopeptide	Reviewed by [2]
3	Fe	S4_236412442	4.23		236,412,442	-	[10]
4	Fe	S5_5104719	5.51	rz87, RZ87	167,189,737	-	[2, 22]
5	Fe	S7_174289806	7.17		174,289,806	AMP-activated protein kinase beta-2 subunit protein	[64]
6	Fe	S8_164741044		ZM0825	164,741,044	Serine/threonine-protein kinase	[10, 17]
7	Fe	S8_164741133	8.06	ZM0825	164,741,133	Serine -protein kinase	Reviewed by [92]
8	Fe	S8_164750150		ZM0825	164,750,150	Isoprenoid biosynthetic process	Reviewed by [44]
9	Fe	S8_167013673	8.06	-	167,013,673	Starch binding domain-containing protein	[10]
10	Fe	S8_167086477	8.06	-	167,086,477	nsp1-like C-terminal region family protein	[10]
11	Fe	S8_167089929	8.06	-	167,089,929	nsp1-like C-terminal region family protein	[10]
12	Fe	S8_172006274	8.07	-	172,006,274	Protein-coding	[10]

were created from forty QTLs relevant to zinc content observed in four investigations [12, 64] Two of the nine were found in two genomic areas on chromosome 2, one of which may be significant for kernel Zn. These areas could be useful for map-based cloning and fine mapping. Four MQTL linked to Zn deposition in maize and rice have been identified [13, 17, 31] Certain rice genes are correlated with metal transport protein-encoding genes which are collinear between rice and maize and play a pleiotropic role in uptake, transportation, as well as sequestration of Fe & Zn. These are listed below in Table 4.

4. Regulation and deposition of iron and zinc in maize endosperm

A point that we have to know is that, in general, the sources of zinc in the maize kernel are derived from the remobilization of senescence leaves from the R2 to R4 stage, so a delay of senescence might affect the mineral content of grains [40]. Downregulation of NA-B1 is the transcription factor that decreases the iron and zinc concentration due to less nutrient re-mobilization from vegetative tissues [26, 54]). The remobilized zinc is stored in the embryo, endosperm, etc., but the major concern is to store more zinc in the endosperm rather than the embryo because the embryo-stored nutrients are less bio-available [65]. Naturally, 90% of zinc is present in the embryo (Zn-phytate), the rest of the endosperm (N-containing ligand), and other parts of grains [17, 40]. The maize lpa1-1 and lpa2-1 lines, which contain the LPA-1 (Low Phytic Acid containing Gene.) gene, act as coding proteins of multidrug resistance protein (MRP) ATP-binding cassette (ABC) transporters, which might play a role in the phytic acid accumulation and transport rather than biosynthesis in maize [1, 2, 40] and produce 60% less phytic acid in seed [66, 67]. But this type of seed shows a 23% and 30% reduction in seed dry weight and germination [31, 66]. GWAS studied for iron and zinc limitation in Inbreeds and BI-parental population is shown in Tables 5 and 6.

Table 6. GWAS identified Genes and SNPs for kernel Zn and Fe concentration with respective gene & ID in inbreeds population of Maize. This table data is extracted from [10]. This table helps to mark and study the QTL on the different lines of maize for the improvement of endosperm mineral nutrients Iron and zin in the future. In at least one bi-parental group, SNPs were observed to have a significant impact on the trait variance (P 0.01, R2 0.05).

Traits	Chromosome	No. of SNPs	SNPs Lists/Markers	Name of the gene.
			S1_291258791	*ZmPP23*
	1	3	S1_22439336	*GRMZM2G077127*
			S1_22439335	*GRMZM2G077127*
	2	2	S2_224173983	*GRMZM2G173377*
			S2_225529232	*GRMZM2G458075*
	3	1	S3_204998729	*ZmSTR2*
			S4_843777	*ZmGRAS59*
	4	3	S4_843764	*ZmGRAS59*
			S4_161165956	*GRMZM2G406737*
	5	2	S5_2521372	*GRMZM2G160064*
			S5_2521368	*GRMZM2G160064*
Zn	7	4	S7_7277849	*GRMZM2G107309*
			S7_7065627	*GRMZM2G398996*
			S7_7277737	*GRMZM2G107309*
			S7_7277776	*GRMZM2G107309*
	8	3	S8_80619983	*GRMZM2G311974*
			S8_149148541	*GRMZM2G075058*
			S8_125472630	*GRMZM2G048200*
	9	1	S9_136389202	*GRMZM2G489070*
	10	1	S10_54119964	*GRMZM2G073371*

(continued on next page)

Table 6 (*continued*)

Traits	Chromosome	No. of SNPs	SNPs Lists/Markers	Name of the gene.
Traits	**Chromosome**	**No of SNPs**	**SNPs**	**Name of Gene**
			S1_64238426	*GRMZM2G147698*
	1	6	S1_275647011	*GRMZM2G131629*
			S1_64238509	*GRMZM2G147698*
			S1_81549746	*GRMZM2G302373*
			S1_3741991	*GRMZM2G030567*
			S1_81549744	*GRMZM2G302373*
			S3_102989836	*GRMZM5G837123*
	3	3	S3_224631400	*GRMZM2G034684*
			S3_186200393	*GRMZM2G312201*
Fe	4	1	S4_230884639	*GRMZM2G080056*
			S5_187810664	*GRMZM2G461034*
			S5_14804657	*GRMZM2G010146*
	5	4	S5_5104719	*GRMZM2G016756*
			S5_207514452	*GRMZM2G036418*
	7	1	S7_168921933	*GRMZM2G026991*
			S8_164741044	*GRMZM2G162329*
			S8_167086477	*GRMZM2G162329*
	8	6	S8_125472630	*GRMZM2G048200*
			S8_67381104	*GRMZM2G027546*
			S8_93112257	*AC206644.4_FGT003*
			S9_136390177	*GRMZM2G406737*
	9	3	S9_136389202	*GRMZM2G489070*
			S9_139191545	*GRMZM2G305027*
			S10_54119964	*GRMZM2G073371*
	10	3	S10_136070835	*GRMZM2G152076*
			S10_81182541	*GRMZM2G123410*

In Rice, chromosome number 3 contains a special *RIN O 1* gene that codes for phytic acid biosynthesis by considering this gene breeder uses the Oleosin18 promoter instead of CaMV35S promoters to suppress the phytate level in grain through an RNA immediate grain-specific silencing mechanism [40, 68] Similarly, in maize, silencing of the inositol phosphate kinase (*ZmIpk)* gene decreases the biosynthesis of phytate [2, 69]. A decrease in phytate levels in maize grains increases the amount of Fe and Zn in the endosperm by 1.5 to 2-fold [47]. To boost phytase expression in maize, genetic engineering was utilized to transfer the *Aspergillus niger* phytase gene. In maize seeds, overexpression of the A. niger phytase gene (phyA2) leads to a 5,000% increase in phytase enzymatic activity and a 30% decrease in seed phytate content [17, 40]. The embryo-specific globin 1 promoter was used to introduce the phyA2 gene. The use of largely recessive genes in crop genetic manipulation for nutritional improvement is commonly described. The alleles that control maize's low phytate content are also recessive [56]. In Table 7, phytase-activating genes are extracted from microorganisms.

The expression of HMA and YSLs (yellow stripe-like transporter) genes *(ZmYSL2)* on vascular tissue acts as a candidate gene for transporting Zn from root to shoot [3, 65, 70]. ZmYSL2 encodes a plasma membrane-localized metal-nicotianamine (NA) transporter and was discovered to be expressed mostly in developing endosperm [65]. ZmYSL2 had seven exons and six introns in its genomic sequence and produced a transcript with a 2208-bp coding sequence. *OsYSL2 and ZmYS1* are involved in metal-chelator absorption and transport [65]. *ZmYSL2* expression was gradually raised from 4 DAP during kernel development, demonstrated a high level of expression during the loading process, and peaked at nearly 20–24 DAP [70]. Zinc is absorbed from soil solution in the form of Zn^{++} via chelating mechanisms by nicotinamides (*ZmNAS5), ZmZIP*-3,4,5,7, and 8 as a chelating agent and distributed on young growing tissue as up-regulation + nodulin-like protein-coding genes play a role in a downregulation in maize plant [6]. Similarly, plasma membrane transporters from the ZIP family-*like AtIRT3 play* a role in zinc loading in the xylem [3, 17, 40, 57].In general, Gramineous family plants use a magnetic acid chelation mechanism (Phyto siderophores) to convert insoluble Fe^{+++} to Fe^{++}, but uptake and transport are handled by YS family genes such as *ZmNAS1-3, ZmNAAT1, ZmDMAS1, and others* [71]. In rice, Throughout the 12 rice chromosomes, more than 90 Fe QTLs have been discovered. Of them, 25 contained Fe-related genes either close to or within the QTL, and 17 were noted as stable [3]. Other genes involved in iron homeostasis include *NAS3, MTN, Aconitase 1, ACO1, DEP, IDI4, FDH1, and VIT1* [26] [28]. six candidate genes were proposed to be associated with Fe deficiency tolerance in maize, including *ZmYS1, ZmYS3, ZmVIT, ZmBTS, ZmILR3, and ZmFIT* but other plants have *mEIN2, ZmTOM2, ZmNRAMP1, and ZmEIL1* genes with this 6 gens plays role in Fe homeostasis mechanism [71]. Researchers were able to find novel putative candidate genes such *as Ferredoxin 1, putative ferredoxin PETF, MTP4, and MTP8,* which had not previously been thought to be important for efficient Fe homeostasis under higher and lower Fe levels [26]. Moreover, researchers characterized candidate gene expression and provided insight into putative trans-acting regulatory oversight on candidate genes, particularly for *DMAS1, NAS3, NAS1, FDH1, IDI2, IDI4, MTK, AOC1, DEP, AOC1, FER1, IDEF1, IR O 2, MIT1, NAAT1, NAS1, NRAMP3, ST1, NRAMP3, ST1, YS1* [26]. These seventeen genes are crucial for maintaining Fe homeostasis in both Fe deficient and sufficient conditions for the inbreeds line [26]. This study also performed a selective sweep on the genes *Naat1, Nas1, Nramp3, Mtk, and Ys1,* which suggests that these genes may be important for maize's rapid adaptation to new environments with varying Fe availability [27].

Besides than nutrient uptake and mobilization mechanism, it is necessary to prioritize the solubilization of bio-unavailable zinc-iron so certain *Bacillus* species of bacterial strains like *Bacillus sp.* (ZM20), *Bacillus subtilis* (ZM63), and *Bacillus Aryabhata* (ZM31 and S10) also plays a major role in the solubilization of the insoluble source of zinc which helps to increase the Zinc–Iron concentration in grain [36, 72, 73].

5. Meta QTLS for iron and zinc content in maze grain

MQTL1 on chromosome bin 2.04 to 2.07 at 424.41 CM position account for the 9.15% mean phenotypic variation of the 2 QTL for the

Table 7. Major cereal transgenic plants over-expressing phytase gene and their phytase activities. These genes are cloned tested genes in poultry and other livestock feed for the micronutrient bioavailable analysis. Where FTU/kg: phytase unit per kg, U/kg, unit per kg.

S.N.	Type of phytase gene and source	Promoter	Signal Sequence	Phytase activity	Reference
1	*Phys- A. niger*	Rice glutelin-1 seed-specific promoter	Murine immunoglobulin leader peptide sequence	2,900 U/kg	[17]
2	, *Phy2A- A. niger*	Embryo-specific globulin- 1 (Glb) promoter	Synthetic barley α- amylase signal peptide sequence	2,200 U/kg	[85, 93]
3	*PhyA- A. niger*	Endosperm-specific promoter	Murine immunoglobulin leader peptide sequence	20.67 U/kg	[67, 94]
4	*appA- E. coli*	a-amylase gene (*aAmy8*) in rice	α *Amy8* signal peptide sequence in rice.	16,500 FTU/kg in Maize and 2,500 U/kg in Rice.	[95]

iron and phosphorous content in maize kernel [74]. MQTL2 to 5 plays role in the variation of iron and zinc content by influencing 14 QTLS with the highest mean phenotypic variation of 22.59 and least mean phenotypic variation of 8.75 [75]. The chromosomal position of these 3 MQTLS is 2.07,2.08 and 3.04 to 3.06. MQTLs 6 are responsible for the Zn content by influencing 2 QTLs with a mean phenotypic variation of around 28.30 [64]. MQTL7 to 10 also influence both traits accounting for mean phenotypic variation in order 17.23, 9.40, 11.47, 14.85 through influencing more than 7 initial QTLS respectively. The position of these MQTLS on the chromosome bins are 5.04, 9.01, 9.06–9.07, and 10.04 [64].

6. Conclusion

Crop biofortification is the most possible approach to overcome the critical micronutrient deficiency, especially Zn and Fe, in developing countries, because poor people, who are victims of hidden hunger, cannot afford to diversify their food. The consumption pattern of maize in the world, like Asia, Africa, America, etc., is widespread. Therefore, crop biofortification in maize is the foundation for overcoming nutrient deficiency in the world. The goal for Zn in maize was determined at 33 g/g based on this information. The typical Zn content in maize is between 20 and 25 g/kg. This study reveals that HMA family genes (heavy metal ATPase) on chromosomes 1 and 9, which are *GRMZM2G000219 and GRMZM2G151406* respectively, show similar properties to *PAA1 and RNA1* genes that contributed to Cu transport in maize. Under zinc-deficient conditions, *ZmIRT1 (GRMZM2G118821), ZmNRAMP6 (GRMZM2G028036), ZmEIN2, and ZmHMA7-9* play critical roles in the up-regulation (shoot) and homeostasis of Zn in maize plants. The expression of the HMA and YSL (yellow stripe-like transporter) genes *(ZmYSL2)* on vascular tissue acts as a candidate gene for transporting Zn from root to shoot. The gramineous family plant uses a mugineic acid chelation mechanism (Phyto siderophores) to convert unsoluble Fe^{+++} to Fe^{++}, but uptake and transport are handled by YS family genes such as *(ZmNAS1-3, ZmNAAT1, ZmDMAS1)*, and so on. Researchers characterized candidate gene expression and provided insight into putative trans-acting regulatory oversight on candidate genes, particularly for *DMAS1, NAS3, NAS1, FDH1, IDI2, IDI4, MTK, AOC1, DEP, AOC1, FER1, IDEF1, IR O 2, MIT1, NAAT1, NAS1, NRAMP3, ST1, NRAMP3, ST1, YS*H are plays role in Fe deficient and sufficient condition for the homeostasis on endosperm of Maize. However, the exact genetic mechanism for the uptake, mobilization, and deposition of iron and zinc in the endosperm is fragmentary because the accumulation and deposition of seed nutrients in the kernel of maize is a complex polygenic phenomenon. More ever to understand the endogenous pathway regulation for which gene products for which steps and how long is required in the expression of nutrient-related tissues, advanced research on various germplasm of maize is necessary through genetic engineering, the study of QTL through marker-assisted breeding, and Genetic Wide Association Studies. . Several studies show a positive correlation of several genes for fixation of concentration of iron and zinc in endosperm but other traits like oil and starch content might have a reverse relationship which needs to further attention. The information provided here will help future studies focused on the gene of interest introgression through marker-assisted breeding, molecular breeding, genetic engineering, and bioinformatics.

Declarations

Author contribution statement

Bikas Basnet: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Shovit Khanal: Analyzed and interpreted the data; Wrote the paper.

Data availability statement

All the data are included in the manuscript.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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