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Genome-wide association study of trace elements in maize kernels

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Abstract

Maize (*Zea mays* L.), a staple food and significant economic crop, is enriched with riboflavin, micronutrients and other compounds that are beneficial for human health. As emphasis on the nutritional quality of crops increases maize research has expanded to focus on both yield and quality. This study explored the genetic factors influencing micronutrient levels in maize kernels through a comprehensive genome-wide association study (GWAS). We utilized a diverse panel of 244 inbred maize lines and approximately 3 million single nucleotide polymorphisms (SNPs) to investigate the accumulation of essential and trace elements including cadmium (Cd), cobalt (Co), copper (Cu), nickel (Ni), selenium (Se) and zinc (Zn). Our analysis identified 842 quantitative trait loci (QTLs), with 12 QTLs shared across multiple elements and pinpointed 524 potential genes within a 100 kb radius of these QTLs. Notably ZmHMA3 has emerged as a key candidate gene previously reported to influence the Cd accumulation. We highlighted ten pivotal genes associated with trace element transport including those encoding heavy metal ATPases, MYB transcription factors, ABC transporters and other crucial proteins involved in metal handling. Additionally, haplotype analysis revealed that eight inbred lines accumulated relatively high levels of beneficial elements while harmful elements were minimized. These findings elucidate the genetic mechanisms underlying trace element accumulation in maize kernels and provide a foundation for the breeding of nutritionally enhanced maize varieties.

Keywords Maize kernel, Trace element, GWAS, QTLs, Candidate genes, Haplotype analysis

Introduction

Since the onset of the 21st century, trace element deficiency has emerged as a significant global health concern and a phenomenon often described as “hidden hunger” [1–3]. Deficiencies in trace elements in humans can lead to poor growth, intellectual impairment, and increased risk of morbidity and mortality, thus, nutritional imbalances and hidden hunger are pressing global health issues [1, 4].

Various trace elements, including zinc (Zn), iron (Fe), selenium (Se), and copper (Cu), are indispensable for human health and play crucial roles in numerous biological processes [5]. An imbalance in trace element intake whether insufficient or excessive can adversely affect

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human health [5, 6]. For instance Cu is essential for hemoglobin production and maintaining normal blood levels helping to prevent iron deficiency anemia and related diseases. Conversely, excessive Cu can denature hemoglobin, leading to hemolytic anemia and bile excretion dysfunction [6, 7]. Zn is crucial for a variety of transcription factors that regulate metabolism-related genes [8, 9]. Se, which is vital for human thyroid gland metabolism and antioxidant systems, and is also beneficial to the crops [10, 11]. Deficiencies in cobalt (Co) can impair vitamin B12 synthesis and red blood cell regeneration leading to anemia and other serious conditions [12, 13]. Similarly, Nickel (Ni) is essential for the function of various enzymes and the deficiency can result in metabolic disorders and growth retardation [14, 15]. Cadmium (Cd), although important for fat metabolism and insulin regulation, can be toxic in excess causing acute intoxication [16, 17]. In plants, Cu, Co, Ni, and Se participate in photosynthesis and increase photosynthetic efficiency, enhancing plant disease resistance and stress resistance [18–21]. Co can increase nitrogen uptake and utilization [21]. Zinc is an important component of plant enzymes, which can promote the growth of plant [22]. Se, Cd, Co is a non-essential trace element in plants, among which Se is a beneficial trace element, Cd and Co are toxic heavy metals, and excessive amounts of these elements can inhibit photosynthesis [20, 23, 24]. Therefore, the accumulation of trace elements in plants not only affects the growth of plants, but also has a certain impact on humans who use them as food.

Maize (*Zea mays* L.) is a major global food crop that plays a crucial role in ensuring food security and is a primary dietary source of calories, protein and trace elements worldwide [25, 26]. The absorption rates of trace elements such as Zn and Se vary among maize varieties [27, 28]. This highlights the importance of developing high-quality maize varieties with elevated beneficial element content and reduced harmful element content for food security.

Trace elements from the soil are absorbed by plant roots and transported to accumulate in kernels through a complex genetic network regulated by multiple genes [29]. In plants, elements reach the kernels via transporters and metal chelators involving protein families such as the natural resistance-associated macrophage protein (NRAMP), heavy-metal ATPase (HMA), yellow stripe-like (YSL), zinc-regulated transporter (ZRT)/iron-regulated transporter (IRT)-like protein (ZIP), ATP-binding cassette (ABC) transporter, metal tolerance protein (MTP), nicotianamine synthase (NAS) and multidrug and toxic compound extrusion (MATE) [30–33]. Trace elements affect various transcription factors that regulate plant genes, including *WRKY*, *bZIP*, *MYB*, *ERF*, *HSE*, *ARF13*, *SPLs* and *NACs* [32, 34, 35].

Selenium uptake in plants can occur in forms such as selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}), as well as in organic forms such as selenocysteine (SeCys) and selenomethionine (SeMet), with different absorption pathways for each [36–38]. For example selenite is metabolized into organic selenium forms within roots and then transported to kernels primarily via the phloem facilitated by transporters such as the nitrate transporter NRT1.1B, which increases the Se content in kernels [39–41].

Genome-wide association studies (GWAS) have been instrumental in identifying genetic variations associated with quantitative traits in plants, leading to the identification of critical loci and candidate genes related to mineral element regulation in species like rice and maize [42–49]. Such studies have revealed significant associations between genetic variants and trace metal ion accumulation in maize, contributing to the understanding of the regulatory networks involved.

In this study, we determined six trace element contents of 244 maize inbred lines and the GWAS was performed with 3 million single nucleotide polymorphisms (SNPs) covering the whole genome. Significant SNPs associated with trace elements were identified by GWAS, and key candidate genes were screened to provide a new reference for further understanding of the genetic mechanisms. Several excellent germplasm resources with higher beneficial trace elements and lower harmful metal elements have been identified by analyzing the genetic variation in trace elements in maize kernels. This study aimed to reveal the genetic mechanism underlying trace element accumulation in maize kernels and provide conditions for breeding new varieties of maize with high nutritional quality.

Results

Phenotypic analysis of trace elements

Descriptive statistical analysis was performed on the trace element content data from the populations in the two environments (Table 1; Fig. S1), and the 6 trace element contents in kernels from Jiangmen were higher than those in kernels from Hainan. The average contents of Cd, Co, Cu, Ni, Se, and Zn in the kernels from Hainan were 0.0145, 0.0318, 2.1368, 0.612, 0.0456 and 15.9197 mg/kg, respectively (Table 1). The average contents of the 6 trace elements in Jiangmen_1 and Jiangmen_2 were 0.0382/0.0311, 0.1996/0.23, 5.0306/4.3635, 5.4285/6.4784, 0.0736/0.1406, and 26.0946/26.6259, respectively (Table 1). The phenotypic variation coefficient of the 6 trace elements in the population was large, indicating that there was wide phenotypic diversity (Table 1). The 6 trace element contents showed skewed or incomplete normal distributions (Table 1; Fig. 1). In addition, the Pearson correlation showed that there was a significant correlation between Ni and Co, and a significant

Table 1 The contents of Cd, Co, Cu, Ni, Se and Zn in the maize kernels of Hainan (HN), Jiangmen_1 (JM1) and Jiangmen_2 (JM2)

Trait	Env	Mean	SD	CV	Skewness	Kurtosis
Cd	HN	0.0145	0.0141	97.09%	1.94	3.89
	JM1	0.0382	0.0258	67.53%	1.78	5.95
	JM2	0.0311	0.0237	76.31%	2.37	9.22
Co	HN	0.0318	0.0297	93.34%	5.50	38.57
	JM1	0.1996	0.1416	70.95%	1.65	3.56
	JM2	0.2300	0.1594	69.32%	2.21	6.96
Cu	HN	2.1368	1.2108	56.66%	4.46	38.39
	JM1	5.0306	3.9224	77.97%	4.83	32.80
	JM2	4.3635	2.2718	52.06%	2.68	13.98
Ni	HN	0.6120	0.3573	58.39%	4.92	37.99
	JM1	5.4285	3.7522	69.12%	1.72	3.84
	JM2	6.4784	4.8970	75.59%	2.58	9.62
Se	HN	0.0456	0.0149	32.63%	0.04	-0.11
	JM1	0.0736	0.0546	74.18%	1.04	0.10
	JM2	0.1406	0.1141	81.15%	1.68	2.56
Zn	HN	15.9197	3.7936	23.83%	0.34	-0.46
	JM1	26.0946	15.5152	59.46%	2.96	12.20
	JM2	26.6259	6.2173	23.35%	0.86	3.14

Env: environment; SD: standard deviation; CV: coefficient of variation

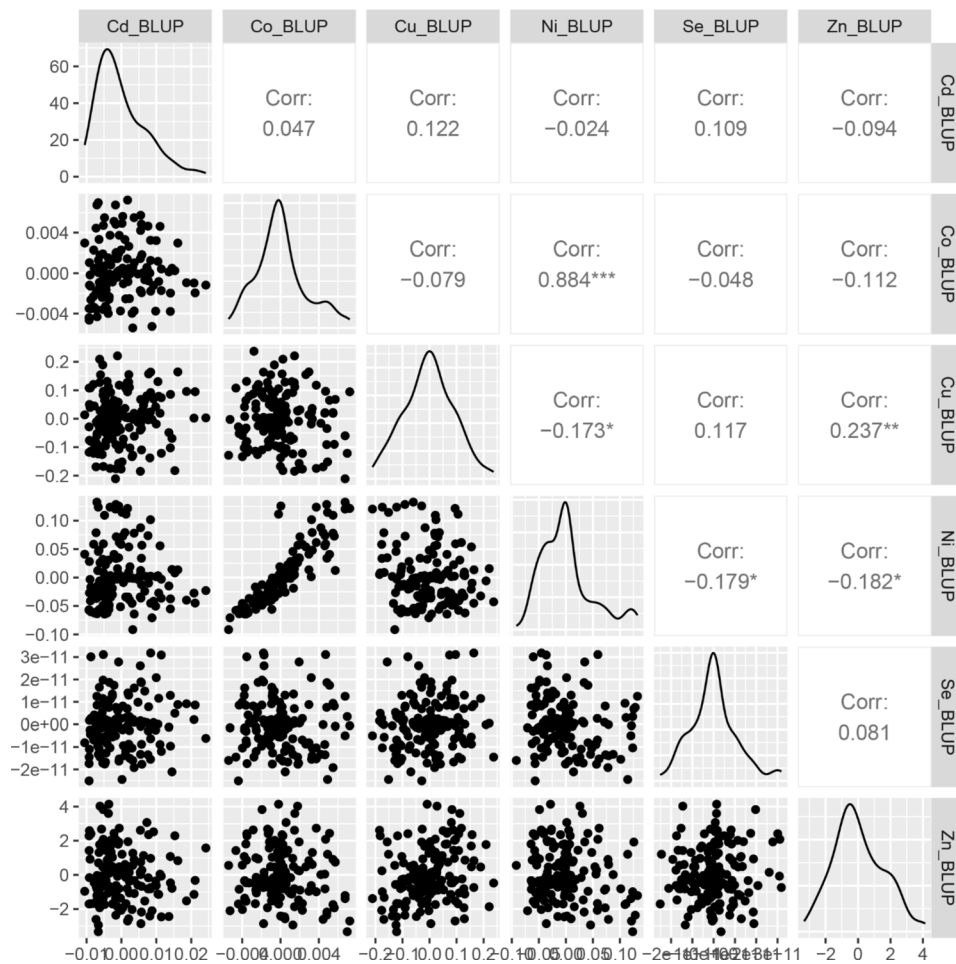


Fig. 1 Correlation analysis and frequency distributions of Cd, Co, Cu, Ni, Se and Zn. Asterisks represent a significant difference. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

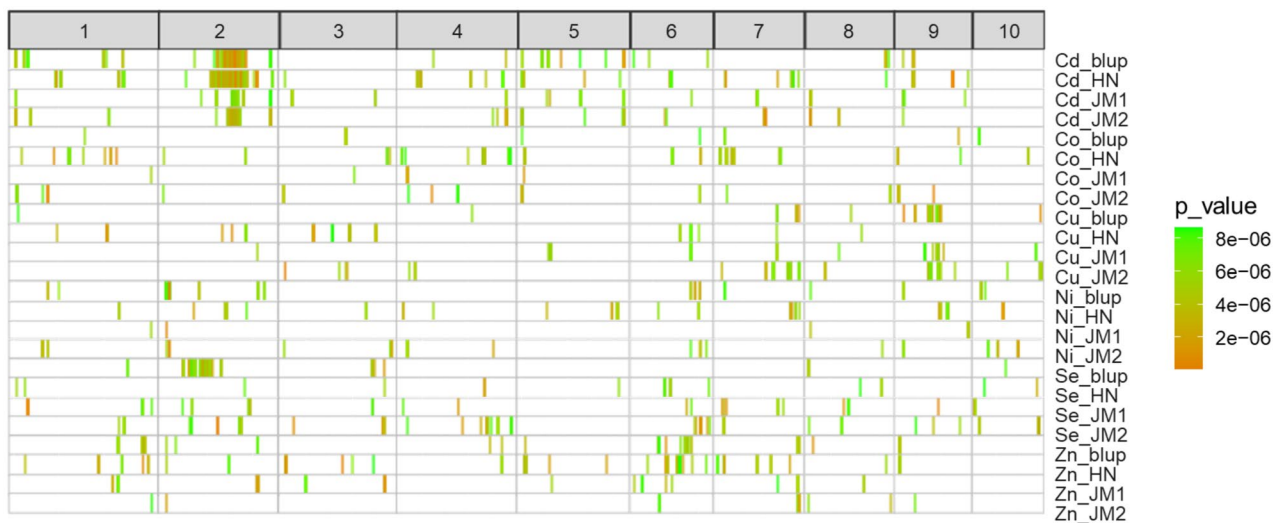


Fig. 2 Distribution of the significant SNPs on chromosomes. The vertical line represents the position of the SNP on chromosome, and the color represents significance

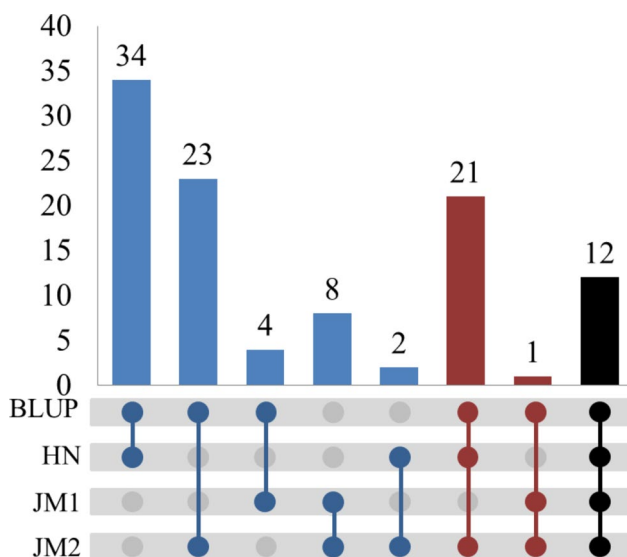


Fig. 3 Number of significant QTLs of the six traits co-located by BLUP and multiple environments. HN: Hainan, JM1: Jiangmen_1, JM2: Jiangmen_2

negative correlation existed between Ni and Se (Fig. 1; Fig. S2), indicating that the accumulation of trace elements was affected by the environment.

Genome wide association study of trace elements

The Q+K model was used for GWAS of Cd, Co, Cu, Ni, Se, and Zn in Hainan, Jiangmen_1 and Jiangmen_2, and the QQ plots showed that the model was more reliable (Fig. 2; Fig. S3; Fig. S4). The SNPs significantly associated with trace elements were also identified. There were 5621, 198 94, 90, 22, and 248 SNPs of Cd, Co, Cu, Ni, Se, and Zn identified in Hainan, respectively, and 176/2343, 9/51, 57/304, 9/53, 53/363 and 63/16 SNPs identified in Jiangmen_1 and Jiangmen_2, respectively (Table S1). The

three sets of environmental phenotypic data were analyzed to obtain BLUP values, and the GWAS was performed. A total of 5653, 16, 107, 37, 423 and 155 SNPs were associated with Cd, Co, Cu, Ni, Se, and Zn, respectively (Table S1). Among the four GWASs, the numbers of colocalized SNPs of Cd, Cu, Zn, Co, Ni and Se (HN, JM_1, JM_2, and BLUP) were 4312, 2, 24, 10, 4 and 17, respectively (Table S1).

By integrating the SNPs into QTLs, a total of 392 QTLs were identified in the Hainan environment, and 248, 42, 16, 27, 14 and 47 QTLs were obtained for Cd, Co, Cu, Ni, Se, and Zn, respectively. A total of 108 and 230 QTLs were detected in JM1 and JM2, respectively. A total of 280 QTLs were obtained by GWAS of BLUP values (Table S2). A total of 842 QTLs were detected in the three environments and BLUP, with 386, 69, 81, 68, 152 and 97 QTLs related to Cd, Co, Cu, Ni, Se, and Zn, respectively (Table S2). Among them, 71 QTLs were detected under two different GWAS conditions, 22 were detected three times, and 12 were detected four times (Fig. 3; Table S3). There are twelve QTLs were colocalized with two elements (Table 2).

Candidate genes analysis

A total of 524 genes with functional annotations were retrieved by searching for candidate genes within 100 kb upstream and downstream of the QTLs, and 265, 25, 43, 52, 65, and 91 candidate genes were associated with Cd, Co, Cu, Ni, Se, and Zn, respectively, among which 17 were colocalized by two elements (Table S4). *Zm00001d005190* (*ZmHMA3*) is a candidate gene, that is a member of the heavy metal transporting ATPase family and is a key gene known by pioneers to regulate Cd transport and accumulation. Eight significant SNPs were

Table 2 The list of significant SNPs identified by two traits

Chr	Start	End	Leading_SNP	p_value	Trait
1	64,016,376	64,018,035	rs1_64016798	1.19E-06	Ni_JM2, Co_JM2
1	75,404,173	75,405,451	rs1_75405451	2.39E-07	Co_JM2, Ni_JM2
1	303,918,107	303,930,067	rs1_303918107	5.23E-06	Co_JM1, Ni_JM1
2	131,043,498	131,079,502	rs2_131078172	3.63E-06	Cd; Se
2	154,731,467	154,840,465	rs2_154798305	8.81E-15	Cd_HN, Cu_HN
2	170,888,718	171,061,555	rs2_170962473	3.10E-06	Cd_JM2, Se_JM2
5	89,563	118,638	rs5_97292	4.77E-07	Cd_JM2, Co_JM2
5	678,984	686,462	rs5_686462	5.39E-06	Co; Cd
6	151,975,407	151,989,702	rs6_151989702	7.67E-07	Ni; Co
7	16,847,839	16,847,858	rs7_16847839	6.60E-06	Co; Ni
7	107,040,266	107,064,221	rs7_107040266	3.08E-07	Cd_JM2, Cu_JM2
7	161,580,762	161,620,521	rs7_161608728	1.82E-06	Cd_HN, Ni_HN

Chr: chromosome, SNP: single nucleotide polymorphism, HN: Hainan, JM1: Jiangmen_1, JM2: Jiangmen_2

Table 3 Information on the key candidate genes

GeneID	Chr	Start	End	Annotation	SNP_ID	Traits
Zm00001d005062	2	156,883,161	156,887,322	vacuolar-sorting receptor 1	2_156981993_156982679	Cd
Zm00001d005189	2	163,016,710	163,020,248	cadmium/zinc-transporting ATPase HMA4	2_162859166_164123078	Cd
Zm00001d012521	8	176,021,482	176,024,817	cation/H(+) antiporter 15	8_176060449_176060449	Cd
Zm00001d018500	5	221,871,850	221,876,358	ABC transporter G family member 28	5_221842724_221842724	Cd
Zm00001d030738	1	156,958,973	156,962,027	mybr42-MYB-related-transcription factor 42	1_157056534_157056534	Co
Zm00001d009492	8	67,174,771	67,176,756	ABC transporter G family member 5	8_67161011_67161011	Cu
Zm00001d032644	1	232,981,371	233,003,696	vacuolar protein sorting-associated protein 52 A	1_232939399_232939704	Ni
Zm00001d004136	2	85,736,310	85,737,686	Glutathione S-transferase family protein	2_85818793_85878621	Se
Zm00001d043240	3	192,831,277	192,837,465	Sulfite exporter TauE/SafE family protein	3_192861869_192886833	Se
Zm00001d002343	2	10,631,062	10,633,353	Zip3-zinc-regulated, iron-regulated transporter-like protein3	2_10656770_10656770	Zn
Zm00001d035918	6	60,271,741	60,272,826	transcription factor MYB1	6_60327518_60327518	Zn

found in *ZmHMA3*, three of which were located on the exon of *ZmHMA3*.

Eleven key candidate genes were identified by gene function annotations and previous studies; among them were 4 candidate genes related to Cd. A QTL consisting of 6 SNPs was identified by Cd on chr2. This QTL was located 95 kb from *Zm00001d005062* and encodes a vacuolar-sorting receptor 1 (VSR) protein that is closely linked to plant root growth and the absorption of ion elements. Furthermore, *s2_163018473* was distributed in the exon of heavy metal ATPase 4 (*Zm00001d005189*, *HMA4*). *Zm00001d012521* (*Chx17*) is located 35.6 kb from *rs8_176060449* and belongs to the cation/H+exchanger (CAX) family. *Zm00001d018500* is a member of the ABC transporter family (Table 3). The key candidate gene associated with Co is *Zm00001d030738* (*Mybr42*), which encodes a MYB-related transcription factor 42. *Zm00001d009492* is also an ABC transporter G family member and is a candidate gene associated with Cu. *Zm00001d032644* encodes the vacuolar protein sorting-associated protein 52 A, which is a candidate gene related to Ni (Table 3). Two key candidate genes related to Se, *Zm00001d004136* and *Zm00001d043240* encode the glutathione S-transferase family protein and sulfite exporter TauE/SafE

family protein, respectively. Two candidate genes associated with Zn content, *Zm00001d002343* (*Zip3*), which encodes a zinc/iron-regulated transporter - like protein 3, and *Zm00001d035918* (*Myb1*), which is a MYB transcription factor (Table 3).

Haplotype analysis

The Hap2 haplotype of *Zm00001d005062* had a significantly higher Cd content than other haplotypes; this was an inferior haplotype that included 45 resources (Fig. 4A; Table S5). The Cd content in Hap3 of *HMA4* was significantly lower than that in the other haplotypes, and Hap3 contained 20 resources (Fig. 4B; Table S5). In addition, we analyzed the haplotypes of key genes associated with Cd, including *ZmHMA3*. The results showed that the Cd content was significantly lower in Hap3 than in the other haplotypes. Hap3 was considered an excellent haplotype, and there were 21 resources of this haplotype (Fig. 4C; Table S5). According to the colocalized QTL data, the key gene *Zm00001d038225* was colocalized with both Co and Ni. The haplotype analysis of *Zm00001d038225* showed that the contents of Co and Ni in Hap3 were significantly lower than those in the other haplotypes (Fig. 4D, E). Therefore, Hap3 was an excellent haplotype, and contained 23 resources in the germplasm resource (Table

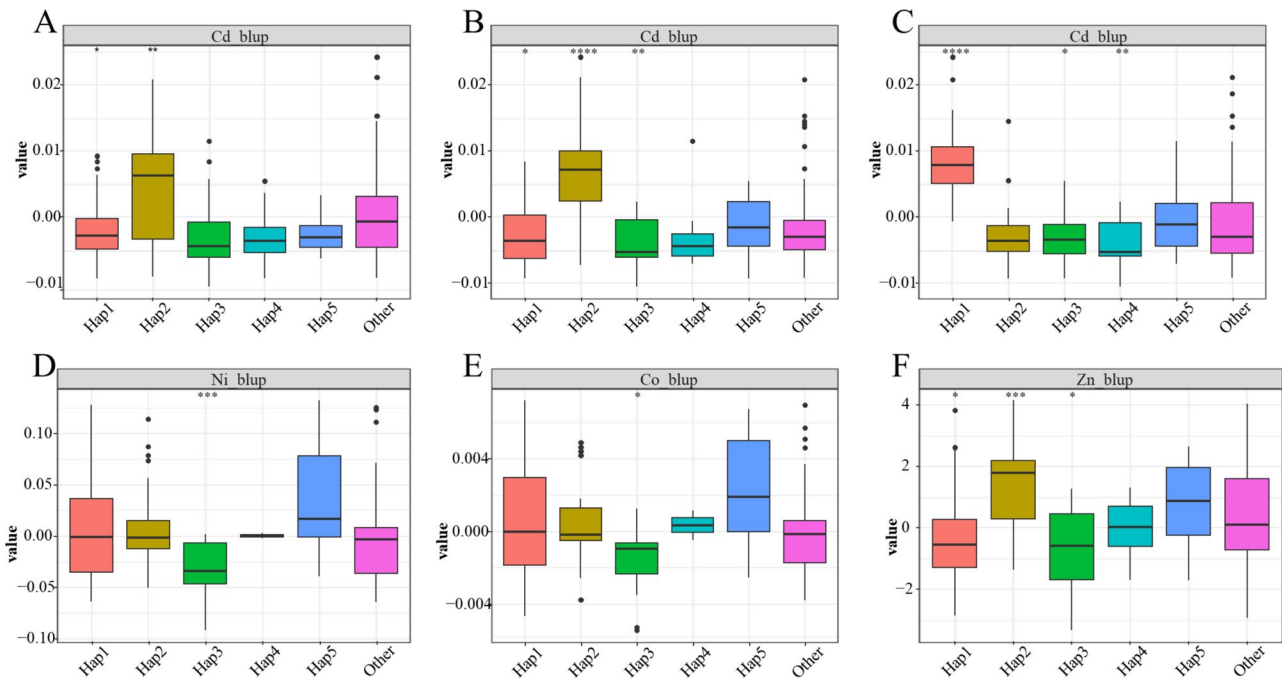


Fig. 4 Haplotype analysis of candidate genes. **A:** *Zm00001d005062*, **B:** *HMA4* (*Zm00001d005189*), **C:** *ZmHMA3* (*Zm00001d005190*), **D:** *Zm00001d038225*, **E:** *Zm00001d0038225*, **F:** *Nas5* (*Zm00001d022557*). The asterisk represents a significant difference. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

S5). *Nas5* is located approximately 130 kb away from rs7_180095813 and is associated with Zn. Hap2 of *Nas5* contained 36 resources, and the Zn content was significantly higher than that of the other haplotypes (Fig. 4F; Table S5). The haplotype with higher Zn content was considered an excellent haplotype. By haplotype analysis, three excellent germplasm resources (CAU280, CAU41 and CAU510) with lower Co, Ni and higher Zn content were obtained; and five excellent germplasm resources (CAU238, CAU318, CAU390, CAU440 and CAU91) with lower Cd and higher Zn content were obtained (Table S6).

Discussion

Phenotypic variation of the 6 trace elements in very different environments

Although many studies have been conducted on trace elements in plants, the genes that regulate trace elements in maize remain largely undetermined. In this study we detected 6 trace elements in 244 maize inbred lines and found that they had rich genetic diversity in this population (Table 1; Fig. S1). These results indicate that this population is an excellent resource for increasing the genetic diversity of maize germplasm and contains a large number of favorable haplotypes, which are necessary for successful detection of the genetic basis of target traits. Pearson correlation analysis revealed significant differences in the contents of trace elements among the different environments (Fig. 1) which may be due to differences in the content of mineral elements in soil and

climate differences in different regions. Previous studies have shown that the amount of fertilizer applied to soil is significantly related to the accumulation of trace elements in plants [29, 50]. The content of trace elements in maize kernels is influenced by both the environment and heredity. Correlation analysis of the different elements revealed that Co, Ni, Cu and Zn were significantly correlated (Fig. S2). Co and Ni are toxic heavy metals, Cu and Zn are essential trace elements which means that the uptake and transport of these two related metal elements in maize kernels may be regulated by the same pathway [30, 51]. Ni is negatively correlated with Zn, Cu and Se, and the uptake of toxic heavy metals by plants may have an antagonistic effect on the uptake of beneficial trace elements, which is in accordance with the results for Se and arsenic (As) [52]. These results showed that there was little correlation between the uptake of various elements in plants, and it was necessary to select a certain element or two antagonistic elements, beneficial and harmful elements, as specific traits to improve and cultivate new varieties.

The QTLs identified by 6 trace elements in maize kernels

A total of 386 QTLs were associated with Cd (Table S2), accounting for 45.8% of the total number of QTLs. More than 5,000 strong and dense SNPs associated with Cd were identified on chr2: 149–171 Mb (Fig. 2; Table S1). The results for *ZmHMA3* and *HMA4* in this region, are consistent with the findings of previous studies, indicating the reliability of the results of this study [53].

Co is a beneficial element for plant growth [12]. However, the mechanism underlying its tolerance and accumulation in maize is still poorly understood. Similarly, little is known about Ni and Cu in maize [30]. In this study, we identified new QTLs associated with these trace elements that were not found in previous studies. The uptake, transport and accumulation of trace elements in plants are complex processes regulated by multiple genes. This study provides a new scientific reference for studies on the genetic basis of trace elements in maize. Among the QTLs associated with Zn, three QTLs located at 180 Mb on chr7 were consistent with previous studies, and new QTLs were also identified (Table S1; Table S2) [54].

Se is an essential trace element for plants and a beneficial element for humans [10]. Se and heavy metals are antagonistic and can combine with heavy metals such as Cd and Ni to form selenomeric protein complexes that are expelled from the body [55]. In this study, we not only identified new QTLs and key genes associated with Se, but also identified QTLs associated with Se and Cd (Table 2). Therefore, this study provides not only a basis for breeding Se-rich maize, but also new ideas for cultivating maize varieties with low concentrations of harmful heavy metal elements [52, 56].

In addition, there were some colocalized QTLs. Rs2_10959451 identified by BLUP, Jiangmen_1, and Jiangmen_2, was associated with Ni. Therefore, this key locus is related to the Ni content in maize and can be used as a key site for breeding new maize varieties with lower levels of the harmful element Ni. Six QTLs were colocalized by Ni and Co (Table 2), which was consistent with the results of the element correlation analysis. In previous studies, the transport of Ni and Co in plants was shown to be regulated by the same transporter, such as IRON REGULATED1/Ferroportin1 (IREG1/FPN1) and IREG2/FPN2 have been identified in *Arabidopsis thaliana*, which are believed to transport Co and Ni within vacuoles [57]. In rice, ARG1 is an ABC transporter that transports Ni²⁺ and Co²⁺ in chloroplasts [58]. The QTLs colocalized with the two elements identified in this study provide new insights into the study of the specific transport of Co and Ni transporters.

Two QTLs colocalized with Cd and Se (Table 2) Se can reduce the accumulation of Cd in plants effectively and alleviate Cd poisoning. After the addition of Se to hydroponic wheat the genes involved in Cd uptake are down-regulated, resulting in a decrease in Cd accumulation [56]. In addition, Cd, Cu, Co, and Ni colocalized with QTLs, and they are metallic elements. Many metal transporters are involved in the transport of various metal elements [30, 33]. IRT1 transports Fe²⁺/Mn²⁺/Zn²⁺/Co²⁺/Ni²⁺ to the plasma membrane [57]. AtMGT1 is a plasma membrane protein that can transport Mg²⁺/Co²⁺/Ni²⁺

[59]. Therefore, the colocalization QTLs obtained in this study are reasonable.

Key candidate genes for cultivating/developing new maize varieties with higher beneficial elements and lower harmful elements

We identified 11 key candidate genes in this study, among which were two genes encoding the ATP-binding cassette transporter (ABC) protein: *Zm00001d018500* and *Zm00001d009492* (Table 3). The ABC transporter superfamily has many functions and transports a wide range of substrates, such as heavy metal ions and lipids in plants [60]. In maize, the ABC transporter AtPDR8 is an efflux pump for Cd conjugates at the plasma membrane involved in heavy metal resistance [61]. In barley, transcriptome analysis revealed that ABC transporters actively participate in the detoxification of Cu and revealed the mechanism underlying Cu tolerance [62]. However, the ABC transporter is not involved in Cu transport in maize. Therefore, the regulation of Cu transport by ABC transporter in maize was first reported in this study. *Zm00001d005062* and *Zm00001d032644* are vacuolar sorting receptors and vacuolar protein sorting associated proteins, respectively (Table 3). Vacuoles also play an important role in ion transport [63]. *OsHMA3* is considered the primary determinant of Cd accumulation in rice grains, and plays a role in the vacuolar membrane [64]. *ZmHMA3* is involved in mediating the vacuolar sequestration of Cd and affects its transport from roots to stems [53]. Cation/H(+) antiporters (CAXs) are a particular class of vacuolar transporters involved in Ca²⁺, Mg²⁺ and Mn²⁺ transport [65]. StCAX1/4 transport proteins have a unique tolerance to Cd stress in potatoes. In poplar, CAX family genes are involved in Cd stress responses [34]. *Zm00001d012521* encodes a CAX transport protein that is associated with Cd, and this is a discovery in maize. The proteins encoded by *Zm00001d005189* (*HMA4*) and *Zm00001d002343* (*Zip3*) are typical metal ion transport proteins in plants (Table 3) [32, 33]. *Zm00001d030738* (*Myb42*) and *Zm00001d030738* (*Myb1*) are specifically expressed genes. *Myb42* is expressed in the primary roots and grains, while *Myb1* is only expressed in the roots (Table 3). The accumulated trace elements in the grains are taken up by the roots from the soil and then transported to the grains. The genes involved in ion uptake and transport are also regulated by transcription factors [32, 34].

In addition we identified two key candidate genes associated with Se (Table 3). *Zm00001d004136* encodes a glutathione S-transferase protein. The process of selenite assimilation is influenced by glutathione. Ren et al. identified 29 glutathione S-transferases after tea plants were treated with selenite [66]. *Zm00001d043240* encodes a sulfite exporter, the TauE/SafE family protein, which is

a sulfate transporter. Sulfate transporters are involved in selenite absorption in plants [36]. The *Arabidopsis thaliana* mutant deficient in sulfate transport *sel1-9* has a T-DNA insertion in the *Sultr1;2* promoter and is a selenite-resistant mutant [67]. Therefore, the key candidate genes identified in this study provide genetic resources for improving trace element accumulation in maize kernels and can promote the breeding and development of new varieties based on endogenous genetic variation and genome editing.

Haplotype analysis was used to select germplasm resources

Good germplasm resources are the basis of maize variety improvement and genetic breeding. Exploring and utilizing maize germplasm resources through various means is important for variety improvement and genetic breeding [68]. There are abundant variations in the contents of trace elements in germplasm resources, which meets the requirements of haplotype analysis [69, 70]. In this study, we used haplotype analysis to select excellent germplasm resources. Haplotype analysis of *ZmHMA3* and *HMA4* not only revealed excellent germplasm resources with lower Cd content, but also revealed resources with higher Cd content, (Fig. 4). The resources with excellent haplotypes of these two genes were almost identical (Table S5), likely because the two genes have similar functions and act simultaneously in the same regulatory pathway. The haplotype analysis of *Zm00001d005062* revealed that the germplasm resources with higher Cd content (Fig. 4; Table S5). Cd is a poisonous heavy metal, and its enrichment is harmful to both plants and humans. Therefore these results offer new insights into the breeding of new maize varieties with lower Cd content. *Zm00001d022557* is a *nicotianamine synthase 5* (*Nas5*) gene that encodes a class II NAS purportedly involved in the synthesis of the metal ion chelator nicotianamine, which has also been shown to be associated with Zn in previous studies [54]. The maize germplasm resources with higher Zn content were identified through haplotype analysis of *Nas5*. *Zm00001d038225* was associated with both Ni and Co (Fig. 4; Table 2) and haplotype analysis revealed that the same haplotype had significantly lower levels of these two elements than did the other haplotypes (Fig. 4), which provides an important reference for improving multiple traits at the same time. These excellent haplotypes could be useful for molecular breeding of maize in the future.

Materials and methods

Plant materials and field experiments

This study utilized a natural population of 244 inbred maize lines provided by the laboratory of Professor Lai Jinsheng at China Agricultural University [71]. This collection includes crucial genetic resources such as Huang

C, Chang7-2, B73, and Mo17. The experimental crops were cultivated at two locations: Ledong, Hainan (HN, 18.75°N, 109.18°E) in 2020, and Jiangmen, Guangdong (JM, 22.35°N, 113.05°E) in 2021. The two different areas planted in Jiangmen called Jiangmen_1 and Jiangmen_2. The plants were arranged in single rows with a spacing of 25 cm × 60 cm. Each inbred line was self-pollinated, and upon reaching maturity, the plants were harvested individually and preserved for further analysis.

Trace element contents detected in maize kernels

The ears were harvested from different environments and the maize kernels were thrashed by hand. The kernels were subsequently dried in an oven at 80 °C for 3 days and ground into powder. Five grams of each sample was weighed, added to 5 ml nitric acid, incubated for 1 h or overnight, digested with 65% nitric acid in an Anton Paar Multiwave PRO microwave for 45 min, and left to set for 1 h with a temperature gradient of 120–180 °C. It was then heated at 100 °C for 30 min, diluted with deionized water to 10 ml, and mixed for subsequent use. Inductively coupled plasma mass spectrometry (ICP-MS) was used to analyze the Cd, Co, Cu, Se, Ni, and Zn contents in maize kernels. Three technical replicates were performed for each digestion.

Statistical analysis of phenotypic and genotypic data

IBM SPSS Statistics 19 was used to calculate the average, range variation, standard deviation, skewness, kurtosis and variation coefficient of the trace elements in inbred lines from different environments and to perform variance analysis. Origin was used to construct images for each element, and the GGally package of the R language was used to analyze the Pearson correlation coefficient [72]. The best linear unbiased prediction (BLUP) value of trace elements was calculated using the R package lme4, and environmental variables, genotype variables, and repetitions were all taken as random effects. $Y = \mu + a * env + b * geno + e$, and Y is the phenotypic value, environment variable (*env*) and genotype variable (*geno*) are random effects, a is the environmental effect, b is the genotype effect, that is the breeding value [73].

Genome-wide association study

The whole genome sequencing was performed for the natural population used in this study and 3 million SNPs were obtained. The Q+K mixed linear model in the mixed line model (MLM) was used to reduce false positives for GWAS via gemma 0.98.3 [74]. The 100 kb distance was taken as the LD distance by Plink [75]. Admixture structure software was used to analyze the population (v1.3.0) [76]. A total of 106,009 SNPs were obtained by plink, parameter $r^2 > 0.2$, window size=50 and step size=50, so the SNPs significantly associated

with trace element-related traits were identified by the significance threshold of association ($p \leq 1 \times 10^{-5}$). Manhattan plots and quantile - quantile (QQ) plots were created using the CMplot package in R [77]. Significant SNPs were intervened into QTLs [44, 45, 78].

Prediction of candidate genes

All candidate genes within 100 kb upstream and downstream of each significant locus were downloaded from the maize database MaizeGDB (<https://www.maizegdb.org>) based on the B73 AGPv4 reference genome. Candidate genes were annotated and analyzed by NCBI (<https://www.ncbi.nlm.nih.gov>) and MaizeGDB, which were screened according to gene function annotation.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12870-024-05419-4>.

Supplementary Material 1
Supplementary Material 2
Supplementary Material 3
Supplementary Material 4
Supplementary Material 5
Supplementary Material 6
Supplementary Material 7

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Author contributions

Y.Q. and X.L. designed the research; W.C., X.Z. and X.L. analyzed data; W.C. wrote the paper; C.L. and H.C. carried out the experiments. Z.C. and Q.W. reviewed the manuscript. All authors have read and approved the manuscript.

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Data availability

All relevant data are within the manuscript and its additional files.

Declarations

Ethics approval and consent to participate

All experimental studies on plants complied with relevant institutional, national, and international guidelines and legislation.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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