Identification of quantitative trait loci and the exploration of candidate genes for the tolerance to Zn deficiency in maize

Jianqin Xu
China Agricultural University
Xiaoyang Zhu
China Agricultural University
Xiuyi Fu
Beijing Academy of Agriculture and Forestry Sciences
Futong Yu (yuft0328@163.com)

Research article

Keywords: Maize, Zn, Zn deficiency tolerance, QTL

Posted Date: August 13th, 2019

DOI: https://doi.org/10.21203/rs.2.12668/v1

Abstract

Background Zn is essential for plants and Zn deficiency leads to great reduction in quality and quantity of crops. Maize, as one of the most important main staple crops worldwide, is more susceptible to Zn deficiency than any other cereal crops. Therefore, understanding the functional mechanisms in tolerance to Zn deficiency in maize is urgent but is still lacking. In this study, quantitative trait loci (QTL) analysis in K22 and By815 RIL population with high-density bin map was conducted to investigate genetic basis of the mechanisms in maize to tolerate Zn deficiency, subsequently some candidate genes were identified and considered as being associated with Zn metabolisms in plants. Results 21 QTLs were detected and accounted for 5.9% - 16.6% of phenotypic variations. Based on the co-localization in this study and the comparisons with previous studies in different RIL and GWAS populations, 223 candidate genes were identified inside the reduced QTL peak intervals on chromosome 1, 2, 6, 7 and 9. Furthermore, 9 genes detected within the peak bins of valuable genomic regions are suggested to be associated with ions transportation and some redox processes affected by Zn deficiency. Additionally, 5 genes, including ZmIRT1, ZmNRAMP6, ZmEIN2 and ZmHMAs, whose homologous gene have been studied and considered to be responsible for metal cations transportation and ethylene-signaling pathway requiring a transition metal were discovered in 5 loci we mapped. Conclusions 14 target genes identified in 9 loci we mapped in this work were explored to elucidate the potential functions in Zn homeostasis and the direct or indirect effects on mechanisms in Zn deficiency tolerance in maize. It is the first time that ZmIRT1, ZmNRAMP6, ZmHMAs were identified using linkage analysis under Zn deficiency in maize, providing genetic evidence and foundation for further gene functional characterization. Our findings have assisted us untangling the genetic basis of possible mechanisms in response to Zn deficiency in maize.

Background

Zinc (Zn) is an essential micronutrient for plant metabolism, development and productivity. Zn deficiency is the most ubiquitous micronutrient deficiency problem in world crops. Zn deficiency leads to stunted growth, chlorosis of leaves, small leaves and spikelet (ear) sterility (Marschner 1995; Broadley et al. 2007). Moreover, Zn deficiency causes large reduction in quality of crop products and increases susceptibility of plants injured by high light intensity and temperature and infection by certain fungal diseases (Graham 1983; Cakmak 2000). Plant-based foods are significant sources of Zn for humans (Welch and Graham, 2004). In 2011, 1.1 billion people were at risk of zinc (Zn) deficiency respectively due to inadequate dietary supply (Kumssa et al. 2015).

Maize (*Zea mays* L.) is the most important resource for food, feed, and biofuel and is one of the most cultivated crop plants all over the world. In 2014, more than 1.0 billion tons of maize grain was produced globally (FAOSTAT 2014). With a growing world population and need for biofuel, increasing maize grain yield is necessary to meet the market demand. However, the limited cultivable land does not support the approach of increasing maize production by enlarging its planting area. Besides, zinc deficiency leads to large reductions in crop yield (Sadeghzadeh 2013). Nevertheless, maize is the most susceptible cereal crop to Zn deficiency (Alloway 2009). In fact, correction of Zn deficiency via fertilization is not always

successful due to agronomic and economic factors (Hacisalihoglu and Kochian 2003). Therefore, enhancing genetic grain yield potential via breeding efforts and improve the breeding efficiency and effectiveness using molecular tools have become feasible and integral strategies for many maize researchers worldwide (Prasanna et al. 2010; Zhan et al. 2018).

Quantitative trait locus (QTL) analysis provides an effective means of dissecting the genetic basis of complex trait in plants and animals (Zeng 1994; Alonso-Blanco et al. 2009). Marker-assisted selection (MAS) based on QTLs can greatly facilitate crop improvement (Prasanna et al. 2010; Li et al. 2018). Recently, QTL analysis was used to investigate the genetic basis of the mechanism of the tolerance to Zn deficiency in plants, including rice (Gao et al. 2013), wheat (Velu et al. 2017), barley (Lonergan et al. 2009; Sadeghzadeh et al. 2010), Arabidopsis (Ghandilyan et al. 2012). Genc et al. (2009) found that most of the QTLs linked to wheat seedling growth under Zn deficiency were associated with height genes with greater seedling biomass associated with lower Zn concentrations. In rice, four QTLs associated with plant mortality were detected, and only one of those co-localized with one of the four QTLs detected for leaf bronzing, implying that both were under independent genetic control (Wissuwa et al. 2006).

As for maize, much more studies were focus on the molecular basis of biofortification for mineral elements, especially iron and zinc, in maize grain, rather than genetic mechanism of the tolerance to Zn deficiency for the whole plants. Šimić et al. (2012) used the ratios as bioavailability traits, and found that three QTLs for Fe/P, Zn/P, and Mg/P were co-localized on chromosome 3, coinciding with SSR marker bnlg1456 which was close to previously identified phytase genes (*ZM phys1* and *phys2*). Based on meta-QTL analysis in rice and maize reported by Jin et al. (2015), three MQTL-containing candidate genes in maize were detected and two maize orthologs of rice, *GRMZM2G366919* and *GRMZM2G178190*, were characterized as NRAMP genes likely responsible for the natural variation in maize grain zinc and iron concentration. Combing single environment analysis with multiple environment trial (MET) QTL analysis in six environments, Zhang et al. (2017) found five candidate genes for the target traits were identified in the intervals detected by meta-QTLs in the previous study.

Up to now, the maize genome has been thoroughly sequenced and assembled. However, systematic analysis of genes responsible for Zn metabolism in maize is still limited. The first as well as the only one gene family has been cloned and described as being associated with Zn transport in maize is ZIP family (the zinc-regulated transporter, iron-regulated transporter protein). In maize, *ZmZIP* genes encode functional Zn or Fe transporters that may be responsible for the uptake, translocation, detoxification and storage of divalent metal ion in plant cells (Li et al. 2013). In transgenic *Arabidopsis*, *ZmIRT1* and *ZmZIP3* are characterized to function as metal transporters with different ion selectivities, and *ZmIRT1* may stimulate endogenous Fe uptake mechanisms, possibly facilitating metal uptake and homeostasis (Li et al. 2015a). So far, except for ZIP family, specific functions in some other genes associated with Zn homeostasis in maize have not yet been studied, such as HMA (heavy metal transporting P-type ATPase) and CDF (the cation diffusion facilitator family).

There is little genetic evidence on the mechanistic basis of Zn deficiency tolerance in maize. Here, K22 and By815 RIL population were utilized to investigate the genetic basis in the tolerance to Zn deficiency in maize. By the identification of QTL co-localization in different traits and the comparison between the loci detected in this work with the QTLs in our previous studies mapped by different populations, some valuable genomic regions were selected with the high-density bin map. Target genes explaining potential causes were mined by the combination of some Zn metabolism-related genes which have been characterized in other plants and some genes unknown for the functions located in the genomic regions refined by peak bins, providing genetic evidence and foundation for further gene functional characterization.

Results

Phenotypic variation in the tolerance to Zn deficiency in maize

Regardless of treatments, all the traits associated with the tolerance to Zn deficiency showed significant difference between K22 and By815 (Table 1, Figure 1). Shoot and root dry weights in the -Zn and -Zn/CK treatments of By815 were more than three times higher than that of K22. R/S ratios in the -Zn treatment of By815 were significantly lower than that of K22. The Best Linear Unbiased Prediction (BLUP) values indicated that the means of the RIL population for all the traits were between K22 and By815 (Figure 1). Besides, there were large variations among inbred lines and the coefficients of variation for all the traits ranged from 14.0% to 55.2%. A normal distribution was observed in each trait, suggesting that the alleles responsible for enhancement in tolerance to Zn deficiency reside in K22 and By815. The variance analysis results revealed that highly significant effects on all the traits were due to genotype and environments except for Zn score (Table 2). Broad-sense heritability of each trait under different conditions was higher than 85%, indicating that much of phenotypic variations in the RIL population were genetically controlled.

Identification of QTLs for each traits

On the basis of a linkage map of 1670.4 cM, twenty-one QTLs were detected in the RIL population at an empirical threshold logarithm of odds (LOD) value of 2.9 estimated by 1000 permutation tests. These loci were distributed among 21 genomic regions on chromosome 1, 2, 3, 5, 6, 7, 9 and 10, explaining 5.2% - 16.6% of phenotypic variation (Figure 2). The QTL interval averaged 9.9 Mb (5.4 cM) ranging from 0.4 to 64.6 Mb (2.0 - 13.6 cM). A total of 21 QTLs were identified to be associated with the traits: four QTLs for Zn score, four QTLs for plant height, four QTLs for shoot dry weights, six QTLs for root dry weights and 3 QTLs for R/S ratio (Table 3).

Zn score Four QTLs (*qKB-ZnSc6-1*, *qKB-ZnSc9-1*, *qKB-ZnSc9-2*, *qKB-ZnSc10-1*) for Zn score were detected on chromosome 6, 9 and 10. Alleles from By815, the Zn-efficient parent, had increasing effects (0.15 - 0.19) on Zn score at these four mapped loci. *qKB-ZnSc6-1* located on chromosome 6 was flanked by

SYN11817 and PZE-106098680, explaining 9.2% of phenotypic variation. *qKB-ZnSc9-1*and *qKB-ZnSc9-2* were detected on chromosome 9 in the intervals of PZE-109025227 - PZE-109051633 and PZE-109059409 - PZE-109064132, respectively. These two loci explained 6.3 - 7.7% of phenotypic variations with additive effects of 0.16 - 0.17. *qKB-ZnSc10-1* was located in the genomic region between bin PZE-110104601 and SYN19780, explaining 5.2% of phenotypic variation.

Plant height Four QTLs (*qKB-PH1-1*, *qKB-PH2-1*, *qKB-PH6-1* and *qKB-PH9-1*) controlling plant height were determined on chromosome 1, 2, 6 and 9 with additive effects of By815 alleles except for *qKB-PH1-1*. *qKB-PH1-1* was mapped in the genomic region of PZE-101205031 ~ PZE-101209438, explaining 10.1% of phenotypic variation. The second largest QTL *qKB-PH2-1*, located in the interval of PZE-102017472 ~ SYN18069, explained 12.0% of phenotypic variation with increasing effect of 4.04 cm on plant heights. *qKB-PH6-1* and *qKB-PH9-1* were detected in genomic regions of PZE-106089546 ~ PZE-106098680 and PZE-109053554 ~ PZE-109075980, respectively.

Shoot dry weight *qKB-SDW2-1*, *qKB-SDW2-2*, *qKB-SDW1-1*, *qKB-SDW3-1* controlling shoot weight in maize were mapped on chromosome 1, 2 and 3, explaining 8.5% - 10.1% of phenotypic variation. In the -Zn treatment, *qKB-SDW2-1* and *qKB-SDW2-2* were identified on chromosome 2 in the regions of PZE-102009755 ~ SYN37566 and PZE-102017472 ~ PUT-163a-60342470-2456, respectively. Alleles from By815, at these two loci, increased shoot dry weights under Zn deficiency by 0.13 g. *qKB-SDW1-1*, *qKB-SDW3-1* was detected in the intervals of PUT-163a-31558578-1965 ~ PUT-163a-71311320-3113 and PUT-163a-86473168-4518 ~ SYN36395 in the CK and -Zn/CK treatment respectively, each loci explaining 8.5% of phenotypic variation.

Root dry weight Six QTLs (*qKB-RDW1-1*, *qKB-RDW1-2*, *qKB-RDW3-1*, *qKB-RDW1-3*, *qKB-RDW9-1*, *qKB-RDW9-2*) identified at different Zn nutrition status were distributed on chromosome 1, 3 and 9. *qKB-RDW1-1* controlling the root dry weight under Zn deficiency was flanked by PZE-101213588 and PZE-101217291, explaining 5.9% of phenotypic variation. In the CK treatment, *qKB-RDW1-2* and *qKB-RDW3-1* were localized in the genomic regions of PUT-163a-31558578-1965 ~ PZE-101026148 and SYN1579 ~ PZE-103187323, explaining 9.4% and 6.5% of phenotypic variation, respectively. Alleles from By815 increased root dry weights by 0.07 g and 0.05 g at these two loci, respectively. Three QTLs were identified in the -Zn/CK treatment, explaining 8.8% - 11.2% of phenotypic variation. *qKB-RDW1-3* detected on chromosome 1 was flanked by PZE-101225664 and PZE-101233241, with an additive effect of K22 alleles. *qKB-RDW9-1*, the third largest loci, explaining 11.2% of phenotypic variation, was identified in the interval of PZE-109023988 ~ PZE-109025227. *qKB-RDW9-2* localized in the genomic region of PZE-109027610 ~ PZE-109053554 on chromosome 9 explained 9.8% of phenotypic variation. Alleles from By815 increased root dry weights in the -Zn/CK treatment by 0.28 and 0.23 at these two loci localized on chromosome 9, respectively.

R/S ratio Three QTLs for R/S ratio were identified on chromosome 5 and 7, explaining 6.4% - 16.6% of phenotypic variation. *qKB-R/S5-1* detected on chromosome 5 was in the region of SYN20689 ~ PZA00069.4 with an additive effect of 0.02. *qKB-R/S7-1* mapped on chromosome 7 was flanked by PZE-

107011423 and SYN18112, explaining 7.8% of phenotypic variation. *qKB-R/S7-2*, major effect QTL in the K22×By815 RIL population, was localized in the interval of PZE-107132535 - SYN32833, explaining 16.6% of phenotypic variation.

QTL Co-localizations and candidate genes identification

Five co-localizations of loci for different traits were identified on chromosome 1, 2, 6 and 9 with additive effects of By815 alleles, explaining 5.5% - 12.0% of phenotypic variation (Table 4). Furthermore, the first co-localization was detected at 12.5 - 15.7 Mb on chromosome 1, containing *qKB-RDW1-2* and *qKB-SDW1-1* which explained 9.4% and 8.5% of phenotypic variation, respectively. On chromosome 2, the second largest-effect locus *qKB-PH2-1* together with *qKB-SDW2-2* were co-localized in the region of 7.7 - 8.8 Mb, explaining 12.0% and 9.2% of phenotypic variation, respectively. At 150.7 - 152.3 Mb on chromosome 6, *qKB-PH6-1* and *qKB-ZnSc6-1* were co-localized under Zn deficiency, explaining 5.5% - 9.2% of phenotypic variation. Two co-localizations, including *qKB-ZnSc9-1* and *qKB-RDW9-2* as well as *qKB-PH9-1*and *qKB-ZnSc9-2*, mapped on chromosome 9 were detected at 28.0 - 89.4 Mb and 100.9 - 107.3 Mb respectively, explaining 5.2% - 9.8% of phenotypic variation.

Combined with the high-density bin map of K22×By815 RIL population, 12 physical intervals determined by 9 QTLs where there were co-localizations as well as the largest effect QTL *qKB-R/S7-2* and the single locus *qKB-SDW2-1* were narrowed the range to two adjacent bins for each QTL peak, varying from 30.3 kb to 31.1 Mb (Table S1 in the additional file). And 223 genes identified by 11 refined physical intervals were annotated in total (Table S1): 7 genes for *qKB-RDW1-2*, 15 genes for *qKB-SDW1-1*, 9 genes for *qKB-SDW2-1*, 4 genes for *qKB-PH2-1* and *qKB-SDW2-2*, 8 genes for *qKB-PH6-1*, 6 genes for *qKB-ZnSc6-1*, 6 genes for *qKB-R/S7-2*, 21 genes for *qKB-ZnSc9-1*, 106 genes for *qKB-RDW9-2*, 27 genes for *qKB-PH9-1* and 14 genes for *qKB-ZnSc9-2*.

Discussion

Comparison of loci among different populations

Previous results pertaining to the genomic locations, confidence intervals or phenotypic variance explained by QTLs were inconsistent due to the differences in genetic backgrounds, environments, and/or mapping populations (Jin et al. 2015). Quantifying target traits in plants is time consuming, laborious, and expensive. Consequently, comparing QTL for different traits detected by independent experiments is important. Evidences for comparative QTLs detected by different populations leading to identify candidate genes which are probably responsible for target traits have been recorded by multiple studies in maize (Prioul et al. 1999; Pelleschi et al. 1999; Duble et al. 2000; Liu et al. 2012; Osman et al. 2013; Jin et al. 2015).

By comparing our previous results of linkage analysis in different RIL populations and genome-wide association study (unpublished), we found that 7 QTLs in the present study were also detected in Wu312×Ye478 and K22×Dan340 RIL population. On chromosome 1, *qKB-PH1-1* was co-localized by *qKD-PH1-2* which explained 14.2% of phenotypic variation in K22×Dan340 population. Besides, *qKB-RDW1-1* was also detected in the genomic regions covered by *qKD-PH1-1*, *qKD-RDW1-1* and *qKD-ZnSc1-3*, explaining 11.4%-15.5% of phenotypic variation in K22×Dan340 population. On chromosome 2, *qKB-PH2-1* and *qKB-SDW2-2* as well as *qKB-SDW2-1* were all mapped inside the major effect QTL *qWY-ZnSc2-1* explaining 63.5% of phenotypic variation in Wu312×Ye478 RIL population. On chromosome 9, genomic regions mapped by *qKB-ZnSc9-1* and *qKB-RDW9-2* also contained the SNP (chr9.S_59587835) which control shoot and root dry weights under Zn deficiency and explained 10% of phenotypic variation in our GWAS studies (unpublished).

Exploration of genes associated with Zn deficiency tolerance

Genes associated with Zn nutrition in plants were widely studied, however, were rarely verified in maize. It is the first time that 7 genes (*GRMZM5G855347*, *GRMZM2G025680*, *GRMZM2G118821*, *GRMZM2G009368*, *GRMZM2G000219*, *GRMZM2G151406* and *GRMZM2G404702*) which belong to ZIP (ZRT, IRT-like protein), HMA and NRAMP (The natural resistance associated macrophage protein) families were discovered based on the natural phenotypic variations in maize in the use of single-family linkage analysis at a low level of Zn nutrition.

qKB-PH1-1 which was co-localized by *qKD-PH1-2* in K22×Dan340 population, contained *ZmIRT1* and *ZmNRAMP6* which were also known as *GRMZM2G118821* and *GRMZM2G028036*, respectively. ZIP family were characterized to transport various divalent cations, including Fe^{2+}, Zn^{2+}, Mn^{2+} and Cd^{2+} (Guerinot 2000; Colangelo and Guerinot 2006). Previous results indicated that IRT-like genes encode major Fe transporters at the root surface in plants (Eide et al. 1996; Varotto et al. 2002; Vert et al. 2002). Recently, *AtIRT3* could complement the Zn and Fe uptake double yeast mutants, indicating that *AtIRT3* is involved in both Zn and Fe translocation, which was recorded by Lin et al. (2009). In maize, the expression of *ZmIRT1* was significantly up-regulated in shoots under Zn deficiency, and *ZmIRT1* remarkably reversed the growth defects in the yeast mutants while the effect of the other proteins in ZIP family were relatively inferior, indicating that *ZmIRT1* may be likely to play an essential role in Zn uptake in maize, which was evidenced by Li et al. (2013). Besides, results from Li et al. (2015a) indicated that *ZmIRT1* has a high selectivity for iron transportation and overexpression of *ZmIRT1* enhances Fe and Zn concentration in the roots and seeds of transgenic *Arabidopsis*. Therefore, *ZmIRT1* was predicted to be Zn and Fe homeostasis gene and its physiological function remains unclear in maize.

ZmNRAMP6, a member of NRAMP family. NRAMP genes, in general, are considered to be associated with membrane-spanning proteins (Cellier et al. 1995) and function as transporters for a variety of divalent cations in plants (Gunshin et al. 1997; Thomine et al. 2000; Curie et al. 2000; Bereczky et al. 2003; Nevo

and Nelson 2006). However, specific function of *ZmNRAMP6* still remains largely unknown and needs further functional characterization. *EIN2* corresponding to *GRMZM2G009368* in maize has been discovered in *qKB-RDW1-1* in this study. *EIN2*, a central signal transducer in the ethylene-signaling pathway, contains sequence similarity (21% identity) to the NRAMP family of proteins (Alonso 1999). Physiological studies indicated that ethylene perception requires a transition metal such as Cu or Zn (Rodríguez et al. 1999) and studies in *Arabidopsis thaliana* have provided complementary evidence for the role of Cu in ethylene perception (Hirayama et al. 1999), but there was no further verification for Zn. These studies suggest that metal metabolism may have a critical role not only in ethylene perception but also in ethylene signaling.

The heavy metal ATPases (HMAs) belong to P_{1B} subfamily of P-type ATPase superfamily responsible for metal cations transport. 3 members of HMA family were identified on chromosome 1 and 9 in this study. *GRMZM5G855347* on chromosome 1 covered by *qKB-SDW1-1* whose functional characterization in *Arabidopsis thaliana* indicated that *AtHMA8* (also known as *PAA2*) transports Cu into the thylakoid lumen to supply plastocyanin (Abdel-Ghany et al. 2005). Besides, *qKB-PH9-1* on chromosome 9 contained *GRMZM2G000219*, *GRMZM2G151406* and *GRMZM2G404702* whose homology gene correspond to *AtHMA6* (also known as *PAA1*) and *AtHMA7* (also known as *RAN1*) respectively. And it is reported that both of them contributed to Cu transport (Hirayama et al. 1999; Shikanai et al. 2003). However, few genes in HMA family in maize have been cloned and described. Thus, these genes are still valuable for further functional verifications.

The QTL mapping resolution is mainly limited by population size and marker density (Mackay et al. 2009). Generally, increasing the marker density can increase the resolution of the genetic map and enhance the resolution and precision of QTL mapping. The quality and accuracy of high density bin map for QTL detection has been validated by studies on multiple traits in maize (Pan et al. 2012; Stange et al. 2013; Unterseer et al. 2014; Guimaraes et al. 2014; Li et al. 2015b; Zhou et al. 2016). Therefore, the QTL intervals narrowed down via high density SNP map within the peak bin is more likely to exhibit potential for target genes responsible for Zn deficiency tolerance than those outside of the peak bins. In the present study, 9 genes are selected out of 223 candidate genes on chromosome 1, 2, 6 and 9.

For the largest-effect locus *qKB-R/S7-2*, the leading gene *GRMZM2G149040* was characterized as bZIP-transcription factor 58 (also known as bZIP58) in maize. Transcription factors bZIP19 and bZIP23 in *Arabidopsis thaliana* were identified to regulate the mechanism in tolerance to Zn deficiency by increasing the transcription of ZIPs and other genes (Laurie et al. 2004), implying that maize bZIP-like transcription factors may play essential roles in the regulation of ZmZIP expression under Zn deficient conditions.

GRMZM2G395114, a leading gene detected in a refined interval (217 kb) of *qKB-SDW2-1* which was co-localized by the largest effect locus *qWY-ZnSc2-1* in WY population, is known as an expressed gene *AST91* (Anti-sigma factor antagonist domain of sulfate transporter 91) and *ZmSULTR3;3* in maize, also a member of SULTR family which contribute to mediate the uptake and translocation of sulfate in higher

plants (Huang et al. 2018). *ZmSULTR3;3* is similar to sulfate transporters which is a high-affinity H(+)/sulfate co-transporter in *Arabidopsis thaliana*. Furthermore, Zn was partly absorbed by plants from soil in the way of Zn^{2+} coupled with SO_4^{2-}. Therefore, variations in *ZmSULTR3;3* might be likely to regulate the amount of sulfate transporter thus participating in the control of Zn transport by roots.

GRMZM2G059314 located inside *qKB-ZnSc6-1* together with *GRMZM2G004128* covered by *qKB-RDW9-2* both encode the NAD(P)-binding oxidoreductase family proteins which are considered to participate in the produce and remove of oxygen free radical (OFR), revealing that the plant growth may be influenced by the expression of *GRMZM2G004128* via redox process under Zn deficiency. Under the Zn-deficient conditions, the inhibitions in the removal of OFR and the oxidation of membrane not only lead to plasma membrane leak and leaf chlorosis, but also result in the oxidative degradation of IAA thus causing suppressions in shoot growth (Marschner 1995). It is reported that IAA level of the shoot tips and young leaves in the Zn-deficient plants decreased to about 50% of that in Zn-sufficient plants (Cakmak et al. 1989). In our work, *GRMZM2G344993* which is identified within *qKB-RDW9-2* encodes indole-3-acetate beta-D-glucosyltransferase which is also known as indol-3-ylacetylglucose synthase (Michalczuk and Bandurski 1982), IAA-glucose synthase (Leznicki and Bandurski 1988), IAGlu synthase (Kesy and Bandurski 1990). The enzyme IAGlc synthase catalyzes the reversible reaction: IAA + UDP ↔ 1-O-IA-glucose + UDP, which is the first step in the biosynthesis of IAA-ester conjugates in monocotyledonous plants (Ostrowski et al. 2015), revealing that *GRMZM2G344993* was predicted to participated in the metabolism of IAA then affecting plant growth at a low status of Zn nutrition.

AC210595.3_FG004 detected inside *qKB-ZnSc9-1* encodes plasma-membrane associated cation-binding protein 1, also known as *PCaP1*. Previous studies *Arabidopsis thaliana* verified that PCaP1 binds Ca^{2+}, Cu^{2+} and other cations and is stably associated with the plasma membrane (Ide et al. 2007; Nagasaki-Takeuchi et al. 2008). Similarly, PCaP1 in maize probably functions as a metal cations transport protein. *GRMZM2G034015* identified in *qKB-RDW9-2*, which was also the only candidate gene in the leading SNP (chr9.S_59587835) mapped in the GWAS study in maize (unpublished), was found to encode transmembrane protein in plants. To our knowledge, transmembrane proteins mediate signal transduction between cells and the environments, and perform important functions in lots of cellular biological processes, such as the receptors for signaling molecules and hormones, transmembrane channels for some ions and transport proteins for cations. The function of *GRMZM2G034015* was identified associated with cations transport involving in metal homeostasis.

Conclusions

We detected 21 QTLs associated with Zn deficiency tolerance in maize in K22×By815 RIL population using a high-density linkage map. By comparing the linkage analysis and GWAS in different populations, 14 target genes considered as being associated with the mechanistic metabolism in tolerance to Zn deficiency were mined in combination of candidate genes located in refined QTLs and the gene families already characterized to transport metal cations. It is the first time that ZmIRT1, ZmNRAMP6, ZmHMAs

were identified using linkage analysis under Zn deficiency in maize. Our findings have assisted us untangling the genetic basis of possible mechanisms in response to Zn deficiency in maize.

Methods

RIL population

A recombinant inbred population, consisting of 209 lines, was derived from the cross between inbred lines K22 and By815. All the seeds are provided by Professor Xiaohong Yang from National Maize Improvement Center of China in China Agricultural University. The results of our previous studies indicated that By815 was highly tolerant to Zn deficiency and K22 was sensitive to Zn deficiency. The molecular map consisting of 2263 recombinant bins covers 1670.4 cM throughout the genome and the average interval is 0.74 cM.

Plant culture in hydroponics

Maize seeds were sterilized for 30 minutes in a 10% solution of H_2O_2, and washed with distilled water. After having been soaked in saturated $CaSO_4$ for 10 h, the seeds were then germinated on moist filter paper in the dark at room temperature. Two days later, the germinated seeds were wrapped in moist filter paper roll and grown. At the stage of two visible leaves, the seedlings were selected and transferred into a 40-L black container. The RIL population and the parents were grown in the Zn-deficient (3×10^{-4} mmol L^{-1} Zn-EDTA) and Zn-sufficient (1×10^{-2} mmol L^{-1} Zn-EDTA) conditions. The adjusted Hoagland nutrient solution contained (mmol L^{-1}): 0.5 NH_4NO_3, 0.5 $CaCl_2$, 1.5 $Ca(NO_3)_2$, 0.75 K_2SO_4, 0.65 $MgSO_4$, 0.1 KCl, 0.25 KH_2PO_4, 1.0×10^{-3} H_3BO_3, 0.35 EDTA-Fe(II), 1.0×10^{-3} $CuSO_4$, 5.0×10^{-3} $MnSO_4$, 5.0×10^{-6} $(NH_4)Mo_7O_{24}$. Solution pH was set at 5.5 - 6.0. Nutrient solution was renewed every 3 days and aerated by a pump. And growth chamber condition was set as a 14-h light period from 8:00 to 22:00 with 28 °C and a 10-h dark period with 22 °C. The average light intensity measured at canopy was 350 μmol m^{-2} s^{-1} and relative humidity was 60%. Each treatment contained 3 duplicates. An each duplicate contained 200 lines and their parents.

Phenotyping methods

Zn deficiency symptoms were observed at the 9^{th}~12^{th} day after transplanting, and would be assessed by scoring during this period. Zn scores were scaled from 0 to 5. Score 0 indicates the severest symptoms of Zn deficiency. 0-scored plants growth was heavily depressed, showing shortened internodes and petioles. Besides, small malformed leaves turned pale yellow and tended to be dead. Score 5 indicates great advantages in plant height, greenness, number of expanded leaves, but still being Zn-deficient when comparing with control plants. The differences in phenotypes between 5-scored plants and control plants

are less than the other lines. Score 1 to 4 would be determined by specific symptoms, including internode length, chlorosis, necrotic spots, as well as the difference between the other lines and control plants. The experiment was terminated at the 15th day after transplanting, and the plant heights were measured first, then the shoots and roots were stored in the envelopes separately before drying. All samples were dried at 105 °C, then shoot and root dry weights were measured and the R/S ratios were calculated.

Statistical analysis

Means of different inbred lines were compared using one-way ANOVA at a 0.05 level of probability by SPSS 20.0. The correlation analysis of Pearson and Spearman were used to investigate the relationships among traits by SPSS version 20.0.The linear mixed effect function lmer in the lme4 package of R version 3.1.1 was fitted to each RIL to obtain the BLUP (Best Linear Unbiased Prediction) value for each traits: $y_i = \mu + f_i + e_i + \varepsilon_i$, where y_i is the phenotypic value of individual i, μ is the grand mean for all environments, f_i is the genetic effect, e_i is the effect of different environments and ε_i is the random error. These variance components were considered to calculate the broad-sense heritability as $h^2 = \sigma_g^2/(\sigma_g^2+\sigma_e^2/e)$, where σ_g^2 is the genetic variance, σ_e^2 is the residual error and e is the number of environments (Holland et al. 2003). And the 95% confidence intervals of the h^2 were calculated following the method of Knapp et al. (1985).

QTL mapping

The identification of QTL was performed using composite interval mapping (CIM) in the Windows QTL Cartographer version 2.5. The scanning interval between markers was set at 0.5 cM, and the window size was set at 10 cM. Model 6 was selected for detecting QTLs and estimating their effects. The threshold logarithm of odds (LOD) values in this study were estimated by permutation tests with minimum of 1000 replicates at a significant level of $P < 0.05$. The confidence interval of the QTL position was determined using the 1-LOD interval method.

Annotation of candidate genes

According to the physical distance of peak bins, genes within the refined QTL peak and their functional description were identified using the maizeB73 reference genome assembly v2 available on the MaizeGDB Genome database (http://www.maizeGDB.org). The function of candidate genes were further confirmed by the annotations of orthologs in *Arabidopsis* or rice.

Abbreviation List

Abbreviation	Full name
ZnSc	Zinc score
PH	Plant height
SDW	Shoot dry weight
RDW	Root dry weight
R/S	R/S ratio

Declarations

Availability of supporting data

All supporting data can be found within the manuscript and its additional files.

Acknowledgement

The authors would like to thank Professor Xiaohong Yang of China Agricultural University for K22, By815 and their RIL population and the genetic map.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The dataset used and analyzed during the current study are included in this published article and its supplementary information files. And all the data used and analyzed in this study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interest.

Funding

This study is supported by the National Key Research and Development Program of China (2016YFD0200405).

Authors' contributions

Jianqin Xu performed and carried out the experiments, and wrote the manuscript; Futong Yu designed the study; Xiaoyang Zhu modified the manuscript; Xiuyi Fu assisted in analyzing data. All authors have read

and approved the final version of the manuscript.

Acknowledgements

The authors would like to thank Dr Xiaohong Yang of China Agricultural University for K22, By815 and their RIL population and the genetic map.

References

Marschner H. Mineral nutrition of higher plants. 2nd ed. London: Academic Press; 1995.

Broadley MR, White PJ, Hammond JP, Zelko I, Lux A. Zinc in plants. New Phytol. 2007;173:677-702.

Graham RD. Effect of nutrient stress on susceptibility of plants to disease with particular reference to trace elements. Adv in Bot Res. 1983;10:221-276.

Cakmak I. Role of zinc in protecting plant cells from reactive oxygen species. New Phytol. 2000;146:185-205.

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

Kumssa DB, Joy EJM, Ander EL, Watts MJ, Young SD, Walker S, Broadley MR. Dietary calcium and zinc deficiency risks are decreasing but remain prevalent. Sci Rep. 2015;5:10974.

United Nations, Food and Agriculture Organization, Statistics Division (FAOSTAT). “Maize production in 2014, Crops/Regions/Production Quantity from pick lists” 2014.

Sadeghzadeh B. A review of zinc nutrition and plant breeding. J Soil Sci Plant Nutr. 2013;13:905-927.

Alloway BJ. Soil factors associated with zinc deficiency in crops and humans. Environ Geochem Health. 2009;31:537-548.

Hacisalihoglu G, Kochian LV. How do some plants tolerate low levels of soil zinc? Mechanisms of zinc efficiency in crop plants. New Phytol. 2003;159:341-350.

Prasanna BM, Pixley K, Warburton ML, Xie CX. Molecular marker-assisted breeding options for maize improvement in Asia. Mol Breed. 2010;26:339-356.

Zhan J, Wang F, Xing W, Liu J, Fan Z, Tao Y. Fine mapping and candidate gene prediction of a major QTL for kernel number per ear in maize. Mol Breed. 2018;38:27.

Zeng ZB. Precision mapping of quantitative trait loci. Genetics. 1994;136:999-1009.

Alonso-Blanco C, Aarts MGM, Bentsink L, Keurentjes JJB, Reymond M, Vreugdenhil D, Koornneef M. What has natural variation taught us about plant development, physiology, and adaptation? Plant Cell. 2009;21:1877-1896.

Li P, Zhang Y, Yin S, Zhu P, Pan T, Xu Y, Wang J, Hao D, Fang H, Xu C, Yang Z. QTL-by-environment interaction in the response of maize root and shoot traits to different water regimes. Front Plant Sci. 2018;9:229.

Gao ZY, Zhao SC, He WM, Guo LB, Peng YL, Wang JJ, et al. Dissecting yield-associated loci in super hybrid rice by resequencing recombinant inbred lines and improving parental genome sequences. Proc Natl Acad Sci U S A. 2013;110:14492-14497.

Velu G, Tutus Y, Gomez-Becerra HF, Hao Y, Demir L, Kara R, et al. QTL mapping for grain zinc and iron concentrations and zinc efficiency in a tetraploid and hexaploid wheat mapping populations. Plant Soil. 2017;411:81-89.

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

Sadeghzadeh B, Rengel Z, Li C, Yang H. Molecular marker linked to a chromosome region regulating seed Zn accumulation in barley. Mol Breed. 2010;25:167-177.

Ghandilyan A, Kutman UB, Kutman BY, Cakmak I, Aarts MGM. Genetic analysis of the effect of zinc deficiency on Arabidopsis growth and mineral concentrations. Plant Soil. 2012;361:227-239.

Genc Y, Verbyla AP, Torun AA, Carmak I, Willsmore K, Wallwork H, McDonald GK. Quantitative trait loci analysis of zinc efficiency and grain zinc concentration in wheat using whole genome average interval mapping. Plant Soil. 2009;314:49-66.

Wissuwa M, Ismail A M, Yanagihara S. Effects of zinc deficiency on rice growth and genetic factors contributing to tolerance. Plant Physiol. 2006;142:731-741.

Šimić D, Mladenovic Drinic S, Zdunic Z, Jambrović A, Ledenčan T, Brkić J, Brkić A, Brkić I. Quantitative trait loci for biofortification traits in maize grain. J Hered. 2012;103:47-54.

Jin T, Chen J, Zhu L, Zhao Y, Guo J, Huang Y. Comparative mapping combined with homology-based cloning of the rice genome reveals candidate genes for grain zinc and iron concentration in maize. BMC Genetics. 2015;16:17.

Zhang H, Liu J, Jin T, Huang Y, Chen J, Zhu L, Zhao Y, Guo J. Identification of quantitative trait locus and prediction of candidate genes for grain mineral concentration in maize across multiple environments. Euphytica. 2017;213:90.

Li S , Zhou X , Huang Y , Zhu L, Zhang S, Zhao Y, Guo J, Chen J, Chen R. Identification and characterization of the zinc-regulated transporters, iron-regulated transporter-like protein (ZIP) gene family in maize. BMC Plant Biol. 2013;13:114.

Li S, Zhou X, Li H, Liu Y, Zhu L, Guo J, Liu X, Fan Y, Chen J, Chen R. Overexpression of *ZmIRT1* and *ZmZIP3* enhances iron and zinc accumulation in transgenic *Arabidopsis*. PLoS One. 2015a;10:e0136647.

Holland JB, Nyquist WE, Cervantes-Martinez CT. Estimating and interpreting heritability for plant breeding: An update. Plant Breed Rev. 2003;22:9-12.

Knapp S, Stroup W, Ross W. Exact confidence intervals for heritability on a progeny mean basis. Crop Sci. 1985;25:194-195.

Prioul JL, Pelleschi S, Séne M, Thévenot C, Causse M, Vienne D, Leonardi A. From QTLs for enzyme activity to candidate genes in maize. J Exp Bot. 1999;50:1281-1288.

Pelleschi S, Guy S, Kim JY, Pointe C, Mahé A, Barthes L, Leonardi A, Prioul JL. *Ivr2*, a candidate gene for a QTL of vacuolar invertase activity in maize leaves. Gene-specific expression under water stress. Plant Mol Biol. 1999;39:373-380.

Duble CM, Melchinger AE, Kuntze L, Stork A, Lubberstedt T. Molecular mapping and gene action of *Scm1* and *Scm2*, two major QTL contributing to SCMV resistance in maize. Plant Breed 2000;119:299-303.

Liu R, Zhang H, Zhao P, Zhang Z, Liang W, Tian Z, Zheng Y. Mining of candidate maize genes for nitrogen use efficiency by integrating gene expression and QTL data. Plant Mol Biol Rep. 2012;30:297-308.

Osman KA, Tang B, Wang Y, Chen J, Yu F, Li L, Han X, Zhang Z, Yan J, Zheng Y, Yue B, Qiu F. Dynamic QTL analysis and candidate gene mapping for waterlogging tolerance at maize seedling stage. PLoS One. 2013;8:e79305.

Guerinot ML. The ZIP family of metal transporters. Biochim Biophys Acta. 2000;1465:190-198.

Colangelo EP, Guerinot ML. Put the metal to the petal: metal uptake and transport throughout plants. Curr Opin Plant Biol. 2006;9:322-330.

Eide D, Broderius M, Fett J, Guerinot ML. A novel iron-regulated metal transporter from plants identified by functional expression in yeast. Proc Natl Acad Sci U S A. 1996;93:5624-5628.

Varotto C, Maiwald D, Pesaresi P, Jahns P, Salamini F, Leister D. The metal ion transporter IRT1 is necessary for iron homeostasis and efficient photosynthesis in *Arabidopsis thaliana*. Plant J. 2002;31:589-599.

Vert G, Grotz N, Dedaldechamp F, Gaymard F, Guerinot ML, Briat JF, Curie C. IRT1, an Arabidopsis transporter essential for iron uptake from the soil and for plant growth. Plant Cell. 2002;14:1223-1233.

Lin YF, Liang HM, Yang SY, Boch A, Clemens S, Chen CC, Wu JF, Huang JL, Yeh KC. Arabidopsis IRT3 is a zinc-regulated and plasma membrane localized zinc/iron transporter. New Phytol. 2009;182:392-404.

Cellier M, Prive G, Belouchi A, Kwan T, Rodrigues V, Chia W, Gros P. Nramp defines a family of membrane proteins. Proc Natl Acad Sci U S A. 1995;92:10089-10093.

Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, Nussberger S, Gollan JL, Hediger MA. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature. 1997;388:482-488.

Thomine S, Wang R, Ward JM, Crawford NM, Schroeder JI. Cadmium and iron transport by members of a plant metal transporter family in *Arabidopsis* with homology to Nramp Genes. Proc Natl Acad Sci U S A. 2000;97:4991-4996.

Curie C, Alonso JM, Jean ML, Ecker JR, Briat JF. Involvement of NRAMP1 from *Arabidopsis thaliana* in iron transport. Biochem J. 2000;347:749-755.

Bereczky Z, Wang H Y, Schubert V, Ganal M, Bauer P. Differential regulation of *nramp* and *irt* metal transporter genes in wild type and iron uptake mutants of tomato. J Biol Chem. 2003;278:24697.

Nevo Y, Nelson N. The NRAMP family of metal-ion transporters. Biochim Biophys Acta. 2006;1763:609-620.

Alonso JM. EIN2, a bifunctional transducer of ethylene and stress responses in *Arabidopsis*. Science, 1999;284:2148-2152.

Rodríguez FI, Esch JJ, Hall AE, Binder BM, Schaller GE, Bleecker AB. A copper cofactor for the ethylene receptor ETR1 from Arabidopsis. Science. 1999;283:996-998.

Hirayama T, Kieber JJ, Hirayama N, Kogan M, Guzman P, Nourizadeh S, Alonso JM, Dailey WP, Dancis A, Ecker JR. RESPONSIVE-TO-ANTAGONIST1, a Menkes/Wilson disease-related copper transport, is required for ethylene signaling in *Arabidopsis*. Cell. 1999;97:383-393.

Abdel-Ghany SE, Muller-Moule P, Niyogi KK, Pilon M, Shikanai T. Two P-type ATPases are required for copper delivery in *Arabidopsis thaliana* chloroplasts. Plant Cell. 2005;17:1233-1251.

Shikanai T, Muller-Moule P, Munekage Y, Niyogi KK, Pilon M. PAA1, a P-type ATPase of Arabidopsis, functions in copper transport in chloroplasts. Plant Cell. 2003;15:1333-1346.

Mackay TF, Stone EA, Ayroles JF. The genetics of quantitative traits: challenges and prospects. Nat Rev Genet. 2009;10:565-577.

Pan Q, Farhan A, Yang X, Li J, Yan J. Exploring the genetic characteristics of two recombinant inbred line populations via high-density SNP markers in maize. PLoS One. 2012;7:e52777.

Stange M, Utz HF, Schrag TA, Melchinger AE, Würschum T. High-density genotyping: an overkill for QTL mapping? Lessons learned from a case study in maize and simulations. Theor Appl Genet. 2013;126:2563-2574.

Unterseer S, Bauer E, Haberer G, Seidel M, Knaak C, Ouzunova M, et al. A powerful tool for genome analysis in maize: development and evaluation of the high density 600 k SNP genotyping array. BMC Genomics. 2014;15:823.

Guimaraes CT, Simoes CC, Pastina MM, Maron LG, Magalhaes JV, Vasconcellos RCC, et al. Genetic dissection of Al tolerance QTLs in the maize genome by high density SNP scan. BMC Genomics. 2014;15:153.

Li C, Li Y, Shi Y, Song Y, Zhang D, Buckler ES, Zhang Z, Wang T, Li Y. Genetic control of the leaf angle and leaf orientation value as revealed by ultra-high density maps in three connected maize populations. PLoS One. 2015b;10:e0121624.

Zhou Z, Zhang C, Yu Z, Hao Z, Wang Z, Zeng X, Di H, Li M, Zhang D, Yong H, Zhang S, Weng J, Li X. Genetic dissection of maize plant architecture with an ultra-high density bin map based on recombinant inbred lines. BMC Genomics. 2016;17:178.

Laurie CC, Chasalow SD, LeDeaux JR, McCarroll R, Bush D, Hauge B, Lai C, Clark D, Rocheford TR, Dudley JW. The genetic architecture of response to long-term artificial selection for oil concentration in the maize kernel. Genetics. 2004;168:2141-2155.

Huang Q, Wang M, Xia Z. The SULTR gene family in maize (*Zea mays* L.): Gene cloning and expression analyses under sulfate starvation and abiotic stress. J Plant Physiol. 2018;220:24.

Cakmak I, Marschner H, Bangerth F. Effect of zinc nutritional status on growth, protein metabolism and levels of indole-3-acetic acid and other phytohormones in bean (*Phaseolus vulgaris* L.). J Exp Bot. 1989;40:405-412.

Michalczuk L, Bandurski RS. Enzymic synthesis of 1-O-indol-3-ylacetyl-beta-D-glucose and indol-3-ylacetyl-myo-inositol. Biochem J. 1982;207:273-281.

Leznicki AJ, Bandurski RS. Enzymic synthesis of indole-3-acetyl-1-O-beta-d-glucose. II. Metabolic characteristics of the enzyme. Plant Physiol. 1988. 88:1481-1485.

Kesy JM, Bandurski RS. Partial purification and characterization of indol-3-ylacetylglucosemyo-inositol indol-3-ylacetyltransferase (indoleacetic acid-inositol synthase). Plant Physiol. 1990;94:1598-1604.

Ostrowski M, Hetmann A, Jakubowska A. Indole-3-acetic acid UDP-glucosyltransferase from immature seeds of pea is involved in modification of glycoproteins. Phytochemistry. 2015;117:25-33.

Ide Y, Nagasaki N, Tomioka R, Suito M, Kamiya T, Maeshima M. Molecular properties of a novel, hydrophilic cation-binding protein associated with the plasma membrane. J Exp Bot. 2007;58:1173-1183.

Nagasaki-Takeuchi N, Miyano M, Maeshima M. A plasma membrane-associated protein of *Arabidopsis thaliana* AtPCaP1 binds copper ions and changes its higher order structure. J Biochem. 2008;144:487-497.

Tables

Table 1 Statistical analysis of phenotypic variations of the parents and K22×By815 RIL population

Trait	Treatment	Parents			RIL Population						
		K22	By815	*P*	Mean	Range	CV (%)	Skewness	Kurtosis	H^2 (%)	90% Confidence Interval (%)
ZnSc	-Zn	1	3	0.000	2.43	0-5	55.2	-0.88	-0.42	95.2	92.3 - 97.8
PH (cm)	-Zn	29.5	67.2	0.000	43.3	19.1 - 79.5	26.0	0.44	-0.13	89.5	86.9 - 91.7
SDW (g)	-Zn	0.29	1.88	0.000	0.78	0.18 - 2.63	48.8	0.87	0.64	91.7	89.5 - 93.5
	-Zn/CK	0.23	1.16	0.000	0.51	0.12 - 1.17	47.2	0.67	-0.12	94.1	92.4 - 95.4
RDW (g)	-Zn	0.12	0.64	0.000	0.26	0.07 - 0.66	40.8	0.95	0.97	89.0	86.2 - 91.4
	-Zn/CK	0.29	1.11	0.009	0.64	0.19 - 1.35	40.9	0.71	-0.09	91.5	89.2 - 93.4
R/S	-Zn	0.41	0.29	0.006	0.35	0.21 - 0.55	19.5	0.63	0.00	78.6	73.2 - 83.1
	-Zn/CK	1.31	1.12	0.047	1.23	0.53 - 1.50	14.0	0.04	0.25	85.7	81.9 - 88.7

Note: $P < 0.05$ indicates significant difference between K22 and By815.

Table 2 Analysis of variance for the traits associated with the tolerance to Zn deficiency

Variance	Genotype	Environment	Residuals
ZnSc (-Zn)	1.804**	0.000	0.000
PH (-Zn)	108.678**	3.315**	38.143
SDW (-Zn)	0.112**	0.003**	0.030
SDW (-Zn/CK)	0.105**	0.002**	0.020
RDW (-Zn)	0.009**	0.000**	0.003
RDW (-Zn/CK)	0.082**	0.001**	0.023
R/S (-Zn)	0.004**	0.000**	0.003
R/S (-Zn/CK)	0.084**	0.003**	0.042

Note: ** represents significant difference at $P = 0.01$.

Table 3 Quantitative trait loci (QTL) for traits associated with Zn deficiency tolerance in K22×By815 RIL population

Trait	Treatment	Chr	Name	Marker Interval	Genetic Interval (cM)	Physical Interval (Mb)	LOD	Additive effect	R^2 (%)
ZnSc	–Zn	6	*qKB-ZnSc6-1*	SYN11817 - PZE-106098680	73.1 - 75.8	150.7 - 152.3	5.3	0.19	9.2
		9	*qKB-ZnSc9-1*	PZE-109025227 - PZE-109051633	43.1 - 50.2	25.3 - 89.4	3.6	0.16	6.3
		9	*qKB-ZnSc9-2*	PZE-109059409 - PZE-109064132	53.9 - 58.0	100.9 - 107.3	4.5	0.17	7.7
		10	*qKB-ZnSc10-1*	PZE-110104601 - SYN19780	114.6 - 121.9	146.7 - 148.1	3.1	0.15	5.2
PH	–Zn	1	*qKB-PH1-1*	PZE-101205031 - PZE-101209438	180.0 - 182.9	253.7 - 258.7	5.7	-3.72	10.3
		2	*qKB-PH2-1*	PZE-102017472 - SYN18069	26.6 - 28.7	7.7 - 8.8	6.6	4.04	12.0
		6	*qKB-PH6-1*	PZE-106089546 - PZE-106098680	62.4 - 75.8	146.9 - 152.3	3.3	2.65	5.5
		9	*qKB-PH9-1*	PZE-109053554 - PZE-109075980	50.9 - 64.5	92.6 - 122.9	3.1	2.57	5.2
SDW	–Zn	2	*qKB-SDW2-1*	PZE-102009755 - SYN37566	17.3 - 20.0	4.4 - 4.9	5.0	0.13	10.1
		2	*qKB-SDW2-2*	PZE-102017472 - PUT-163a-60342470-2456	26.6 - 29.0	7.7 - 8.9	4.3	0.13	9.2
	CK	1	*qKB-SDW1-1*	PUT-163a-31558578-1965 - PUT-163a-71311320-3113	40.1 - 49.5	12.5 - 16.8	3.8	0.22	8.5
	–Zn/CK	3	*qKB-SDW3-1*	PUT-163a-86473168-4518 - SYN36395	145.3 - 149.0	212.2 - 213.8	3.8	-0.15	8.5
RDW	–Zn	1	*qKB-RDW1-1*	PZE-101213588 - PZE-101217291	186.2 - 191.3	263.8 - 268.6	3.0	-0.03	5.9
	CK	1	*qKB-RDW1-2*	PUT-163a-31558578-1965 - PZE-101026148	40.1 - 46.3	12.5 - 15.7	4.3	0.07	9.4
		3	*qKB-RDW3-1*	SYN1579 - PZE-103187323	188.7 - 192.8	230.0 - 232.0	3.1	0.05	6.5
	–Zn/CK	1	*qKB-RDW1-3*	PZE-101225664 - PZE-101233241	196.8 - 203.8	276.0 - 281.3	4.1	-0.19	8.8
		9	*qKB-RDW9-1*	PZE-109023988 - PZE-109025227	40.3 - 43.1	24.1 - 25.3	4.6	0.28	11.2
		9	*qKB-RDW9-2*	PZE-109027610 - PZE-109053554	46.4 - 50.9	28.0 - 92.6	4.0	0.23	9.8
R/S	–Zn	5	*qKB-R/S5-1*	SYN20689 - PZA00069.4	159.5 - 166.6	214.1 - 215.3	3.4	0.02	6.4
		7	*qKB-R/S7-1*	PZE-107011423 - SYN18112	41.9 - 45.4	8.0 - 9.3	4.0	-0.03	7.8
		7	*qKB-R/S7-2*	PZE-107132535 - SYN32833	136.9 - 138.9	172.7 - 173.1	8.0	0.04	16.6

Note: Positive values of additive effect indicate By815 alleles are in the direction of increase; negative values indicate K22 alleles are in the direction of increase. The position refers to the B73 reference sequence Version 2.

Table 4 QTLs co-localized by different traits in K22×By815 RIL population

Chr	Trait	Treatment	Name	Physical Interval (Mb)	LOD	Additive effect	R^2 (%)
1	RDW	CK	*qKB-RDW1-2*	12.5 - 15.7	4.3	0.07	9.4
	SDW	CK	*qKB-SDW1-1*	12.5 - 16.8	3.8	0.22	8.5
2	PH	–Zn	*qKB-PH2-1*	7.7 - 8.8	6.6	4.04	12.0
	SDW	–Zn	*qKB-SDW2-2*	7.7 - 8.9	4.3	0.13	9.2
6	PH	–Zn	*qKB-PH6-1*	146.9 - 152.3	3.3	2.65	5.5
	ZnSc	–Zn	*qKB-ZnSc6-1*	150.7 - 152.3	5.3	0.19	9.2
9	ZnSc	–Zn	*qKB-ZnSc9-1*	25.3 - 89.4	3.64	0.16	6.28
	RDW	–Zn/CK	*qKB-RDW9-2*	28.0 - 92.6	3.96	0.23	9.81
9	PH	–Zn	*qKB-PH9-1*	92.6 - 122.9	3.07	2.57	5.17
	ZnSc	–Zn	*qKB-ZnSc9-2*	100.9 - 107.3	4.54	0.17	7.73

Note: Positive values of additive effect indicate By815 alleles are in the direction of increase; negative values indicate K22 alleles are in the direction of increase.

Table 5 The information of target genes within the loci detected in the K22×By815 RIL population

QTL	Chr	Gene ID	Gene position (bp)	Description
qKB-SDW1-1	1	GRMZM5G855347	16332686 - 16338873	*ZmHMA* - Heavy metal translocating P-type ATPase transports
qKB-PH1-1	1	GRMZM2G025680	255995136 - 255998013	*ZmNRAMP6* - Metal transporter Nramp6 .
qKB-PH1-1	1	GRMZM2G118821	258353073 - 258355277	*ZmIRT1* - A member of ZIP family in maize
qKB-RDW1-1	1	GRMZM2G009368	264267252- 264269646	*ZmEIN2* - Involved in ethylene signal transduction.
qKB-SDW2-1	2	GRMZM2G395114	4890135 - 4895146	*AST91* - Anti-sigma factor antagonist domain of sulfate transporter 91
qKB-ZnSc6-1	6	GRMZM2G059314	151454748 - 151457344	NAD(P)-linked oxidoreductase superfamily protein
qKB-R/S7-2	7	GRMZM2G149040	173148589 - 173154260	bZIP58 - bZIP-transcription factor 58
qKB-ZnSc9-1	9	AC210595.3_FG004	42521681 - 42524448	Plasma-membrane associated cation-binding protein 1
qKB-RDW9-2	9	GRMZM2G034015	59587805 - 59594040	Transmembrane protein
qKB-RDW9-2	9	GRMZM2G004128	86529826 - 86531722	FAD/NAD(P)-binding oxidoreductase family protein.
qKB-RDW9-2	9	GRMZM2G344993	87564495 - 87566420	Indole-3-acetate beta-D-glucosyltransferase
qKB-PH9-1	9	GRMZM2G000219	120249240 - 120251017	*ZmPAA1* - Encodes a putative metal-transporting P-type ATPase.
qKB-PH9-1	9	GRMZM2G151406, GRMZM2G404702	109886385 - 109888147, 109888623 - 109894466	*ZmHMA5* - Encodes a cation-transporting ATPase HMA5

Note: The position refers to the B73 reference sequence Version 2.

Additional File

Table S1 Information of candidate genes

Figures

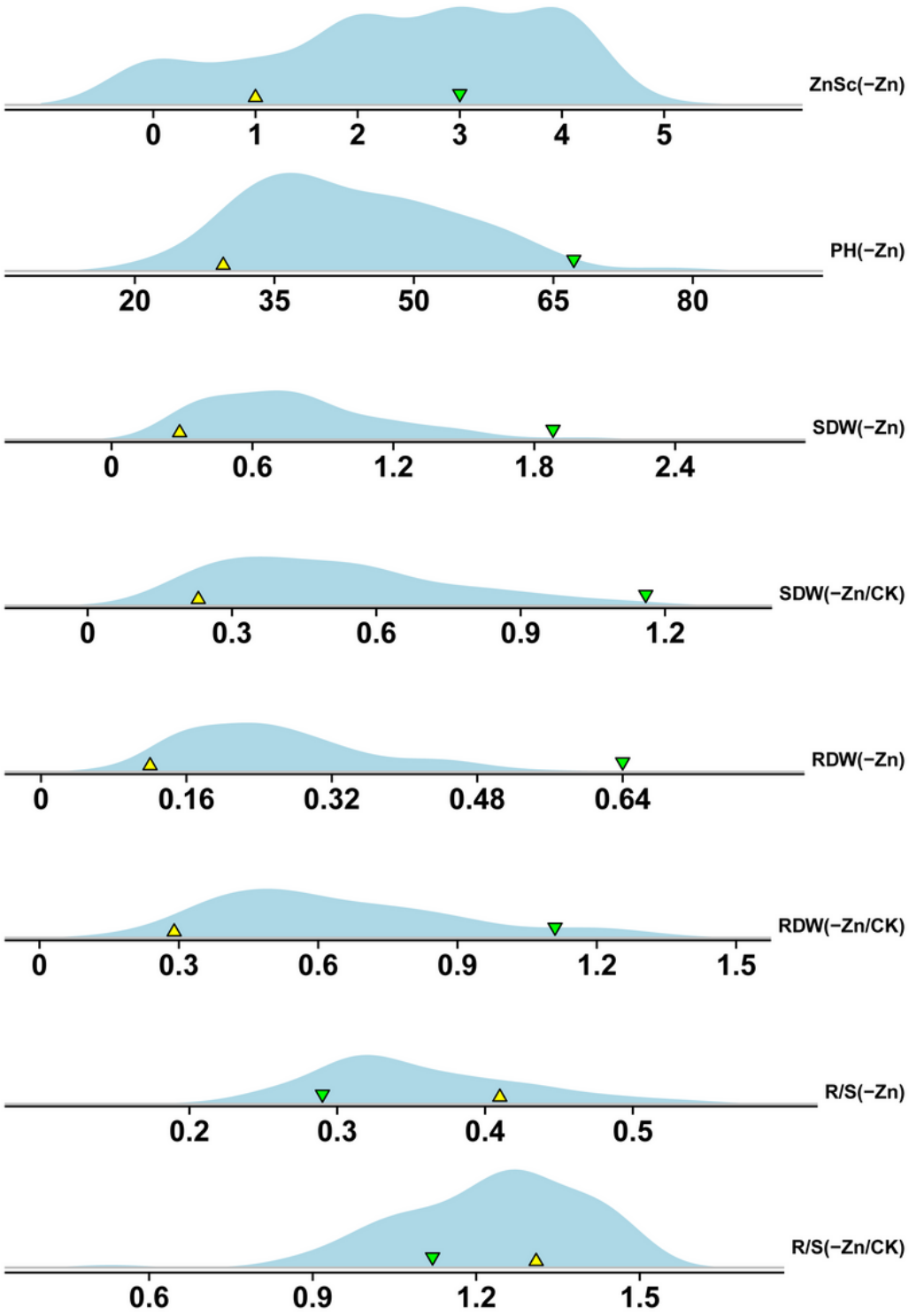

Figure 1

Phenotypic distribution of the traits associated with the tolerance to Zn deficiency in the population. Yellow indicates K22 and blue indicates By815.

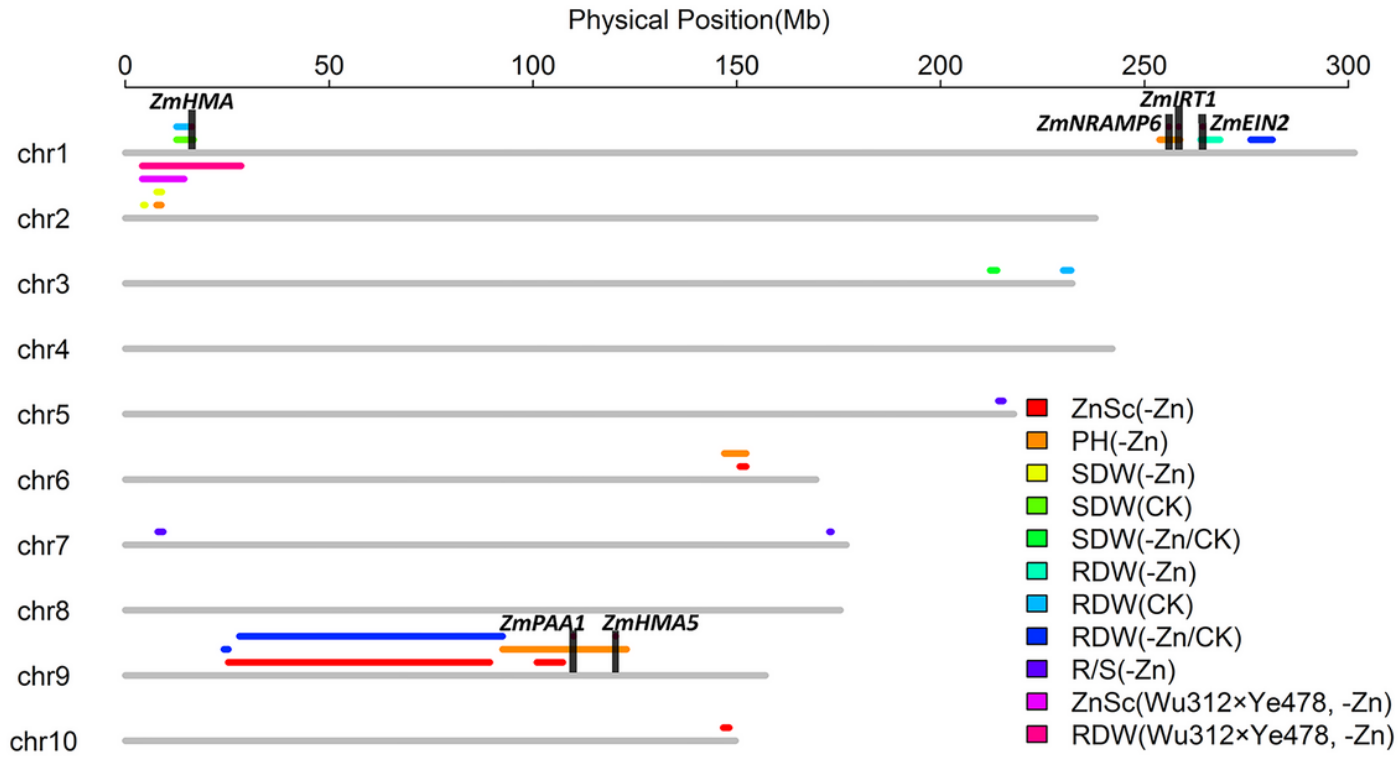

Figure 2

QTLs detected in this study and the co-localization of QTLs identified in Wu312×Ye478 RIL population. Six target genes belonging to ZIP, HMA, NRAMP gene families are also depicted in black columns.

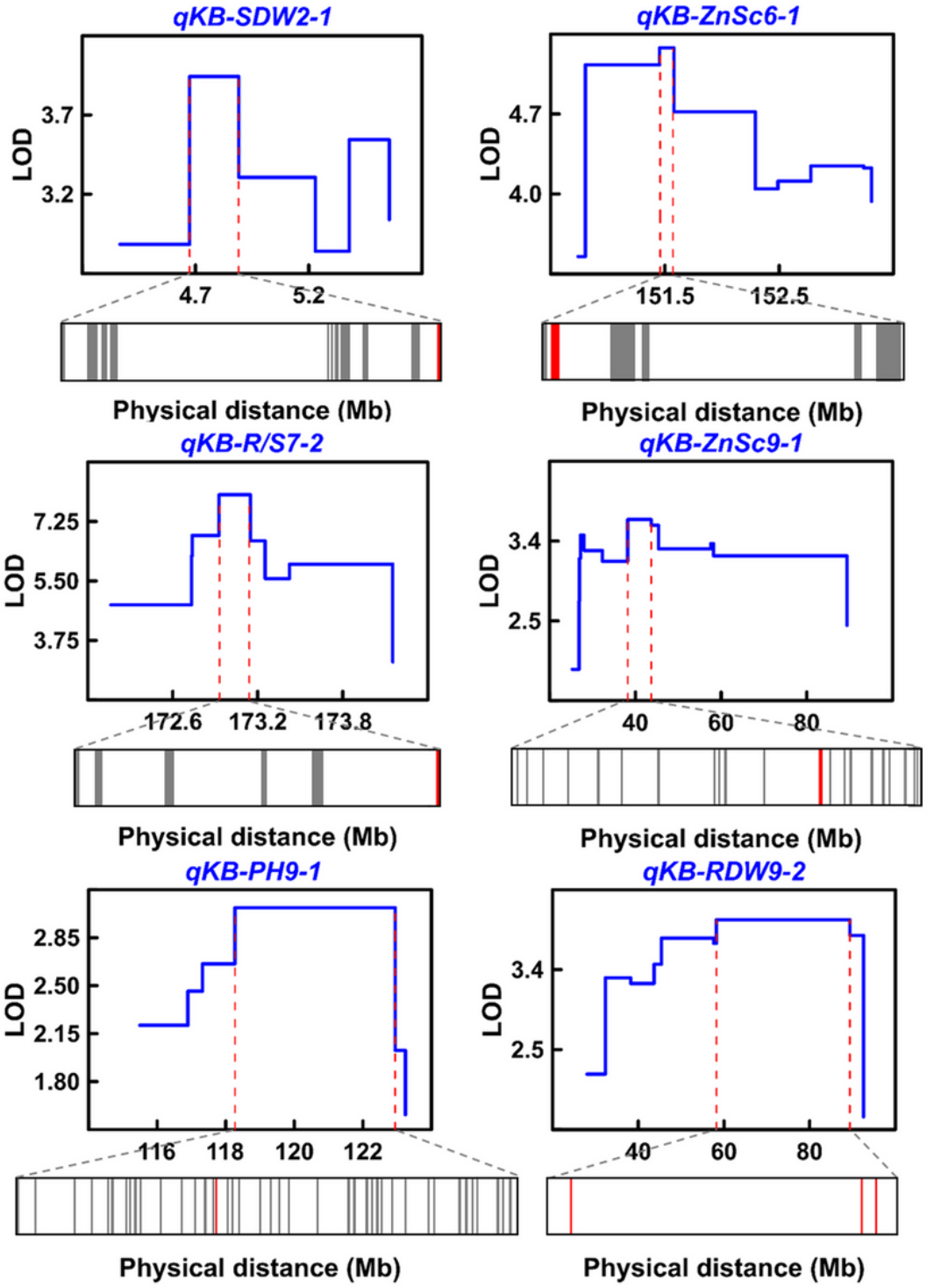

Figure 3

LOD values for QTL bins and candidate genes inside the peak bins. The blue lines represent the LOD profiles of the bins within a QTL interval. Red bands indicate the genes predicted to have putative functions associated with Zn deficiency tolerance; and gray bands indicate the other genes in each peak bin. For qKB-RDW9-2, only 3 target genes are shown.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterial.xlsx