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RESEARCH ARTICLE

QTL discovery for grain iron and zinc content in wheat: Pathway to biofortification

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Abstract

A deficiency of micronutrients causes hidden hunger, ultimately leading to malnutrition. Biofortification through marker-assisted breeding can be the most effective way to overcome this problem. Therefore, the identification of genomic regions (QTLs) for grain micronutrients like Fe and Zn can articulate the researchers for quicker and more accurate development of micronutrient-rich wheat varieties through marker-assisted breeding. In this study, QTLs were identified for grain Fe and Zn concentration of 214 mapping lines in $F_{2:3}$ population derived from a biofortified variety, HD 3298 and M-160, a mutant line deficient for Fe and Zn developed from DPW 62150. Person correlation coefficient analysis between GFeC and GZnC content revealed that there is a significant positive correlation between the micronutrients ($r = 0.95$). Again, 700 SSR markers were used for genotyping of which 547 were monomorphic, while 153 were polymorphic in both the parents with a polymorphic rate of 21.11%. Linkage map construction discovered that a total of 17 QTLs were dispersed on nine chromosomes out of which five QTLs correspond to GFeC with an average LOD value of 14.73% which mapped on chromosomes, 1A and 7A. Similarly, 12 QTLs correspond to the trait GZnC with an average LOD of 14.03% and are mapped on chromosomes, 2A, 4A, 5B, 5D, 6A, 7B and 7D. The mean GFeC content of mapping lines was 34.46 mg/kg and for GZnC was 22.59 mg/kg with SE of 0.65 and 0.40, respectively. These identified QTLs can be triggered as valuable tools for advanced molecular breeding practices.

Keywords: Biofortification, micronutrients, QTL mapping, iron, Zznc.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important food crops globally, serving as a primary source of dietary energy for a large portion of the world's population. As a staple food in many countries, wheat provides a substantial share of daily calories through carbohydrates, contributing between 20 and 50% or more of the caloric intake in regions where it is a dietary staple (FAO 2022). Human nutrition depends primarily on plant foods, either directly or indirectly (El-Ramady et al. 2022). However, malnutrition due to a lack of essential mineral nutrients, particularly deficiencies in Zinc (Zn) and Iron (Fe), affects approximately 3 billion people globally (Kurmiet al. 2023; Kumar et al. 2024). This leads to poor health outcomes such as anemia, increased morbidity and mortality, and reduced worker productivity (Lee 2020; Zulfiqar et al. 2024). Such micronutrient deficiencies are often referred to as "hidden hunger" (Lowe 2021).

Addressing hidden hunger through the genetic enhancement of staple crops to increase their micronutrient content is a cost-effective strategy with minimal ongoing expenses (Nayak et al. 2023; Kumar et al. 2024; Pal et al. 2024). For biofortification breeding programs, the

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availability of genetic variability in germplasm and a thorough understanding of its genetic architecture are crucial prerequisites (Tiwari et al. 2009). Recognizing the importance of biofortification, numerous studies have been conducted to evaluate grain Fe and Zn content in advanced wheat breeding lines and germplasm (Roy et al. 2022; Wani et al. 2022; Gaurav et al. 2022; Morgounov et al. 2007; Rawat et al. 2009). While some research has explored the genetic mechanisms influencing mineral accumulation in major cereal crops like wheat and rice (Pradhan et al. 2022; Alomari et al. 2021; Raman et al.2024; Long et al. 2024), yet there remains a need for a deeper understanding of the underlying genetic factors. Biofortification, the process of enhancing the nutritional value of plants through breeding, has been recognized as a sustainable and cost-effective strategy to combat malnutrition. It was identified as one of the primary approaches to addressing micronutrient malnutrition by the Copenhagen Consensus (Horton and Alderman 2008).

Wheat biofortification specifically offers an encouraging approach to address deficiencies in Fe and Zn, which affect billions of people worldwide (Kamble et al. 2022; Gupta et al. 2022). Researchers have made significant progress in developing biofortified wheat cultivars with improved micronutrient content, leveraging natural genetic variation and advanced technologies like genome-wide association studies (GWAS) and precise gene editing (Gupta et al. 2022). Studies suggest that the consumption of biofortified wheat can significantly improve hemoglobin, protein, iron, and zinc levels in humans (Kumari et al. 2023). Despite some challenges, biofortification remains a sustainable strategy to ensure nutritional security, particularly in developing countries where wheat is a dietary cornerstone (Kamble et al. 2022; Sharma et al. 2020).

Ameliorating grain micronutrients through conventional breeding is arduous due to the quantitative nature of these traits and their interplay with the environment. With the inception of new molecular techniques, breeders have made progress by using marker-assisted breeding to postmortem genetic architecture of plants, identifying QTLs and assimilating them into practical breeding programs (Kumar et al. 2024). Discovering genomic regions for GFeC and GZnC with high PVE and their interlinked markers could substantially accelerate the molecular breeding program. Multiple QTLs have previously been identified derived from hexaploidy wheat populations (Rathan et al. 2021; Krishnappa et al. 2021) but QTLs identified between cross from a high Fe line and a Fe deficient mutant line are infrequent. Therefore, this study aims to ascertain the novel and stable QTLs in $F_{2:3}$ population derived from iron-deficient mutant lines (M-160) and biofortified wheat cultivar HD 3298.

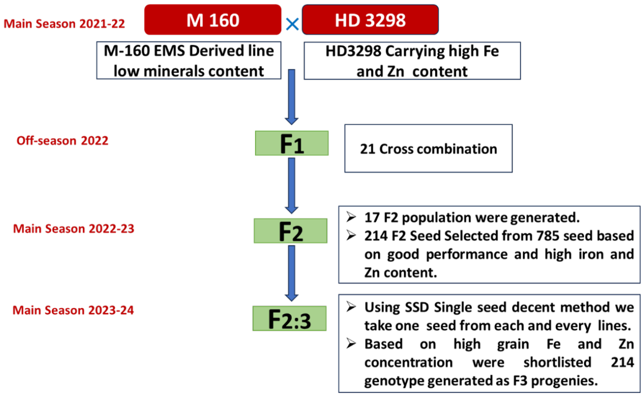
Materials and methods

Plant materials

A panel consisting of 214 mapping lines was implemented to map QTLs that could influence the iron and zinc content in grains. These lines were developed from a cross between the Indian biofortified cultivar HD 3298 and the M 160 line, which was developed through ethyl methane sulfonate (EMS) treatment. Single seed descent (SSD) method was utilized to develop the entire population to maintain genetic purity and integrity. Staggered sowing was performed at 7-day intervals to facilitate the synchronization between female and male parents for effective pollination in 2021-22 at the Norman E. Borlaug Crop Research Centre (CRC), affiliated with G. B. Pant University of Agriculture and Technology in Pantnagar, Uttarakhand with a spacing of 30 X 10 cm. In April 2022, the F_1 seeds were harvested, and an off-season planting initiative was executed at the ICAR Off-Season Research Station in Keylong, located in the Lahaul- Spiti district of Himachal Pradesh, to generate $F_{2:3}$ seeds (Fig. 1). The $F_{2:3}$ seeds were utilized for comprehensive assessments, including both phenotypic and genotypic evaluations, with a specific focus on analyzing the grain micronutrient content of Fe and Zn.

Micronutrient analysis

Ensuring the purity of these seeds was paramount, and to achieve this, an extensive cleansing process was initiated. Initially, the seeds underwent a one-minute wash with N/10 hydrochloric acid, effectively eliminating any dust or impurities adhering to the seed surfaces (Antoine et al. 2004). Subsequently, the seeds were dried in a hot air oven at 80°C until their weight was stabilized. For micronutrient studies, 0.5 g samples underwent digestion with a 5 ml mixture of nitric acid (65%, w/w) and hydrogen peroxide (30%, w/w) at a 3:1 ratio. The digestion process occurred within a microwave digestion system (Multiwave Go; Anton Paar GmbH, Austria). The volume of the digested sample



- 17 F2 population were generated.
- 214 F2 Seed Selected from 785 seed based on good performance and high iron and Zn content.

- Using SSD Single seed decent method we take one seed from each and every lines.
- Based on high grain Fe and Zn concentration were shortlisted 214 genotype generated as F3 progenies.

Fig. 1. Breeding scheme followed to transfer high grain Fe and Zn into wheat line M-160

was calibrated to 25 mL in a 50 mL graduated Falcon tube with ionized water. They re-analyzed components utilizing an AAS instrument (Shimadzu AA-7000 from Japan). The instrument was initially calibrated to standards of 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 ppm for Fe at 248.3 nm, as well as 0.1, 0.2, 0.3, 0.4, 0.6, and 0.8 ppm for Zn at 213.92 nm. Each sample was replicated to ensure the precision of grain nutrient concentration measurements, and the results were expressed in milligrams per kilogram (ppm) based on the dry weight of the samples.

DNA extraction and genotyping

Genomic DNA was extracted from the leaves of 21-day-old seedlings in the $F_{2:3}$ mapping population and their parental lines by using the CTAB method (Murray and Thompson 1980).

Purified DNA was used for genotyping in a 20 µL reaction mixture which includes 5.0 µL of template DNA (20 ng/µL), 2.0 µL of 10× Taq buffer with $MgCl_2$, 2.0 µL of dNTPs (25 mM), 2.0 µL each of forward and reverse SSR primers (30 ng/µL), 0.3 µL of Taq DNA polymerase (3 U/µL), and 6.7 µL of double-distilled water. The PCR was done using a ProFlex thermocycler, which includes an initial denaturation at 94°C for 4 minutes, succeeded by 35 cycles of denaturation at 94°C for 1 minute, annealing at 49 to 55°C for 1 minute, and extension at 72°C for 1 minute, finishing in a final extension at 72°C for 10 minutes. The amplified DNA was separated through a 3% agarose gel, stained with ethidium bromide in 1× TAE buffer, and put under an electric current of 80V for a duration of 2.5 to 3 hours. The gel results can be seen with a gel documentation system (Avegene). Seven hundred SSR markers, equally distributed throughout the wheat genome, were selected from a consensus genetic map to determine the genetic variation between parental lines.

Linkage analysis and QTL mapping

Genotyping was executed in 214 $F_{2:3}$ mapping lines with 153 polymorphic markers and the resulting bands were scored manually. In a scoring panel, 'A' is assigned a band similar to HD 3298, 'B' is assigned to M-160 and «H» for heterozygous loci. This data is used for linkage map construction using the QTL IciMapping (Version 4.2) program (Meng et al. 2015). The linkage group was identified using grouped markers having a minimum LOD score threshold of 3. This linkage group was converted into maps using a regression algorithm with specific parameters such as recombination value below 0.49. The critical threshold for LOD scores for QTL discovery is determined by one thousand different combinations of p >0.05 (Churchill and Doerge 1994)

The quantity of observed morphological modifications correlated to particular QTLs was evaluated by determining the coefficient (R_2) utilizing the Maximum likelihood composite interval mapping (CIM) method. Genetic distance was calculated utilizing Kosambi's mapping function (Huehn et al. 2011).

Correlation coefficient analysis

The Pearson correlation coefficient has been calculated to determine the relationship between the accumulation GFe and GZn. The student's t-test was employed to determine the significant differences in parental data.

Results

Grain Fe and Zn concentration

The micronutrient estimation of both parental genotypes demonstrated that HD-3298 exhibits higher amounts of GFeC and GZnC compared to M-160, since HD-3298 is an elite, high-quality variety, whereas M-160 is an EMS-treated line (Table 1). The mean concentrations of GFeC and GZnC in the two samples for HD-3298 were 55.91 and 36.54 mg/kg, respectively. The estimated value M-160 values were 25.31 and 18.595 mg/kg. Significant variations occurred in $F_{2:3}$ populations. The population exhibited a normal distribution (Fig. 2), and transgressive segregants were identified in both Fe and Zn concentrations. (Table 1, Fig. 2).

Correlation studies

The statistical relationship between GFeC and GZnC concentrations in the $F_{2:3}$ Population, as determined by The Pearson correlation coefficient (r) is calculated for all data sets. Studies revealed a positive correlation between the concentrations of Fe and Zn in grains indicating that the rate of accumulation of Fe and Zn could mutually influence both micronutrients (Table 2, Fig. 3).

Studies of Quantitative Trait Regions (QTR) responsible for iron and zinc contents in wheat grain

Scoring of the 153 SSR markers was done manually (Fig. 4). A linkage map framework (Kordenaeej 2008), consisting of 153 SSR polymorphic markers was mapped 214 $F_{2:3}$ lines from the HD-3298 × M-160 cross.

The concentration of Fe was linked with five significant quantitative trait loci (LOD 4.32), whereas the concentration of Zn was linked to twelve significant quantitative trait loci (Table 3; Fig. 5). Out of 5 QTLs, one is located on chromosome no 1A and four QTLs are linked to chromosome 7A.

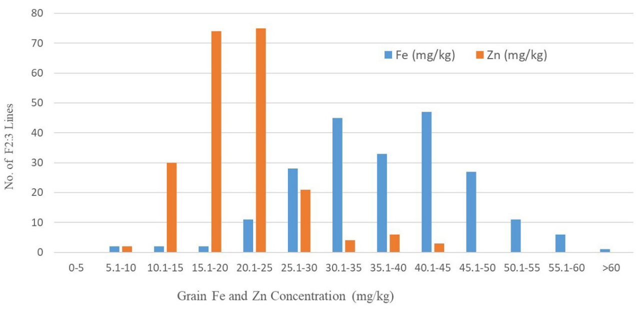


Fig. 2. Distribution of HD-3298/M-160 $F_{2:3}$ mapping population for GFe and GZn. The average iron content in grains (milligrams per kilogram) for HD3298 and M-160 were 55.91 and 25.315, respectively

Table 1. Grain Fe and Zn concentrations (mg/kg) of the parental varieties and the $F_{2:3}$ population

	Parents			
Element	HD3298	M160	$F_{2:3}$ Mapping population Mean	Standard Error
Fe (mg/kg)	55.91	25.315	34.46	0.651
Zn (mg/kg)	36.54	18.595	22.59	0.401

Table 2. The Pearson correlation coefficient between grain iron and grain zinc concentration in the $F_{2:3}$ population

	Fe	Zn
Fe	1	0.9547
Zn	0.9547	1

**Significant at *p* <0.0001."

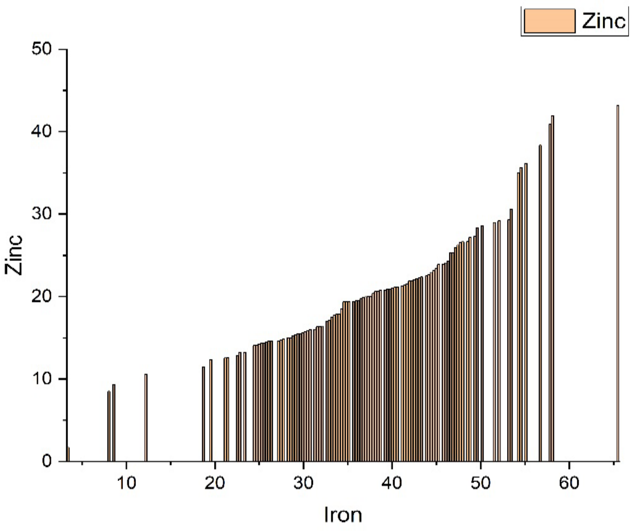


Fig. 3. Distribution and Pearson correlation of GFeC and GZnc

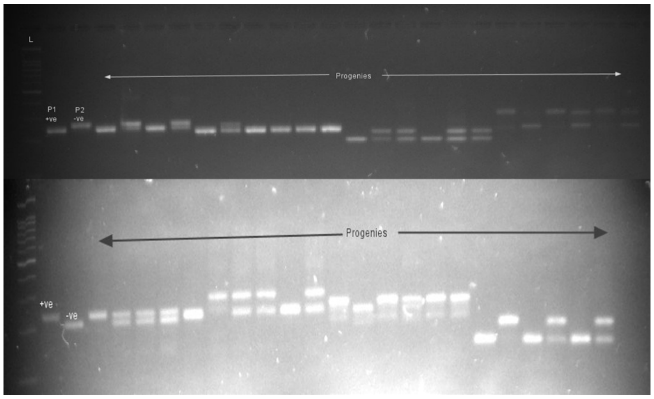


Fig. 4. Polymorphism study of SSR marker *Xwmc475* resolved on 3% Agarose gel electrophoresis in the $F_{2:3}$ population derived from M 160 X HD 3298

qGFeC-1A-1 is flanked by GWM413 and Bglu7BLt with PVE of 4.32%.Similarly, *qGFeC-7A-1* flanked by wmc479and cfa2049 having PVE 12.83%; *qGFeC-7A-2*and *qGFeC-7A-3*flanked by *Xgwm282* and *Xwmc475* with PVE of 13.97%, 13.34%, respectively at last *qGFeC-7A-4* flanked by *Xwmc475* and *Xwmc662* with PVE of 29.2%. The LOD values of the QTLs on 7A were 12.8,13.9,13.3 and 29.1 respectively. These QTLs, *qGFeC-7A-1*, *qGFeC-7A-2* and *qGFeC-7A-4* possess desirable alleles that enhance the content of grain iron from HD3298, in contrast, those QTLs *qGFeC-1A-1* and *qGFeC-7A-3* possessed negative alleles from M106 (Table 3; Fig. 5)

Similarly for Zn, a total of 12 QTLs were mapped across 2A (*qGZnC-2A-1*), 4A (*qGZnC-4A-1*), 5B(*qGZnC-5B-1*), 5D(*qGZnC-5D-1*), 6A(*qGZnC-6A-1*), 7B(*qGZnC-7B-1, qGZnC-7B-2, qGZnC-7B-3,qGZnC-7B-4,qGZnC-7B-5*) and 7D (*qGZnC-7D-1, qGZnC-7D-2*) chromosomes. Out of them chromosomes 2A, 4A, 5B, 5D and 6A harbor one QTL While chromosome 7B is linked with five QTLs and 7D possesses two QTLs. The details of the flanked markers and LOD value PVE score are mentioned in Table 3. Out of all these QTLs *qGZnC-7B-1* has a higher PVE of 6.31 which means it has more impact on Zn concentration.

QTLs such as*qGZnC-4A-1*, *qGZnC-5D-1*, *qGZnC-7B-1*, and *qGZnC-7B-2* possessed a favourable allele from HD-3298, in contrast to the QTLs*qGZnC-2A-1*, *qGZnC-5B-1*, *qGZnC-6A-1*, *qGZnC-7B-3*, *qGZnC-7B-4*,*qGZnC-7B-5A* and *qGZnC-7D-1*, *qGZnC-7D-2* had a negative allele with marker region *Xwmc181-Xgwm445*, *Xwmc149-Xgwm213*, *Xcfd190-Xwmc672*,*Xwmc276-Xbarc176*,*Xgwm146-Xgwm577*,*Xgwm577-Xgwm611*,*Xbarc184-wmc221* and *Xwmc221-Xwmc606* had a negative allele from HD3298 (Table 3). From the results, it is also deduced that chromosome no 2A showed the highest no of polymorphism (36.11%) followed by 2B (35.0%) and 7B (34.04%). On the contrary, chromosome no 3A showed the lowest polymorphism (5.71%), followed by 4B (6.67%) and 6B (6.90%) (Table 4).

Discussion

Amalgamation of minerals in cereal grains can be performed through various mechanisms like soil mobilization, root translocation, rhizosphere integration and facilitated bio-available form of the ion in grain (Afzal et al. 2020). These mechanisms are articulated through various genomic regions (Kamaral et al. 2022). So far, few studies have reported genetic factors affecting the accumulation of Zn and Fe in wheat (Aiqing et al. 2022; Wang et al. 2022; Chandu et al. 2024). Therefore, QTL mapping is a pressing necessity to study the inheritance pattern of grain iron and zinc.

In the present study, a strong positive correlation (r = 0.95) between Fe and Zn was recorded in 214 lines. This result is similar to the earlier reports by Chatrath et al. (2018).

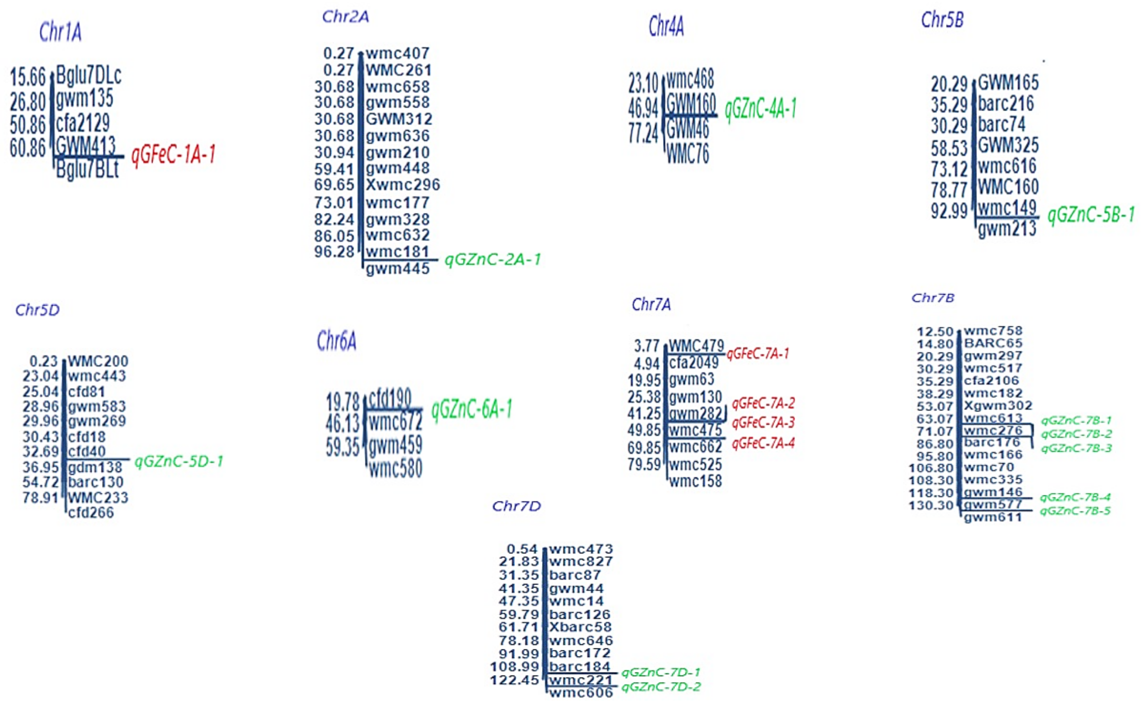


Fig. 5. Chromosomal regions of the significant QTLs for GFeC and GZnC contents. The significant QTLs for GFeC and GZnC contents are situated in the chromosomal regions. The value on the left of the chromosomal map depicts the cM distance for the SSR marker locus

Table 3. Using ICIM detect the QTLs for grain Fe and Zn content in $F_{2:3}$ populations

Chr	Marker interval	Position(cm)	LOD	PVE (%)	Additive	Favourable allele
QTL for Grain Fe						
1A	*Xgwm413-XBglu7BLt*	116	4.32	4.5702	-0.0305	M-160
7A	*Xgwm479-Xcfa2049*	0	12.8	4.4999	0.102	HD 3298
7A	*Xgwm282-Xwmc475*	58	13.9	7.6637	0.0056	HD 3298
7A	*Xgwm282-Xwmc475*	90	13.3	7.982	-0.0036	M-160
7A	*Xwmc475-Xwmc662*	96	29.1	13.1333	0.0001	HD 3298
QTL for Grain Zn						
2A	*Xwmc181-Xgwm445*	575	4.43	1.4865	-0.054	M-160
4A	*Xgwm160-Xgwm46*	50	2.82	0.9988	0.0026	HD 3298
5B	*Xwmc149-Xgwm213*	363	2.66	1.3091	-0.0495	M-160
5D	*Xcfd40-Xgdm138*	143	23.8	3.1052	0.094	HD 3298
6A	*Xcfd190-Xwmc672*	0	39.2	5.5306	-0.1245	M-160
7B	*Xwmc163-Xwmc276*	208	35.7	6.3141	0.0068	HD 3298
7B	*Xwmc276-Xbarc176*	267	39.7	5.858	0.0038	HD 3298
7B	*Xwmc276-Xbarc176*	329	5.12	1.152	-0.0095	M-160
7B	*Xgwm146-Xgwm577*	773	3.28	1.4603	-0.0534	M-160
7B	*Xgwm577-Xgwm611*	951	3.19	1.4476	-0.0534	M-160
7D	*Xbarc184-wmc221*	472	4.16	1.464	-0.0544	M-160
7D	*Xwmc221-Xwmc606*	633	4.1	1.4767	-0.0551	M-160

Table 4. Chromosome-wise distribution of SSR markers and level of polymorphism observed between two parental genotypes

Chr. No.	Markers tested (No.)	Monomorphic markers	Polymorphic markers	Polymorphism (%)	Name of the polymorphic SSR markers used for parental polymorphism survey and genotyping 214 F2:3 populations
1A	34	29	5	14.71	Bglu7DLc, gwm135, cfa2129, gwm413, BgLu7BLt
1B	45	39	6	13.33	wmc134, gwm259.gwm264, gwm494, gdm136, wmc206
1D	22	17	5	22.73	gwm191, cfd63, wmc432, wmc407, wmc261, wmc261
2A	36	23	13	36.11	wmc407, wmc658, gwm558, gwm312, gwm636, gwm210, gwm448, wmc296, wmc177, gwm328, wmc632, wmc181, gwm445
2B	40	26	14	35.00	wmc661, barc91, cfa1101, barc128, gwm429, wmc429, gwm55, wm257, gwm501, barc200, gwm271, bf145935, gpw4043, barc55
2D	33	24	9	27.27	Xbarc59, wmc817, gwm358, barc159,cfd17,wmc41,gwm249,gwm301,gwm382
3A	35	33	2	5.71	wmc11, wmc505
3B	30	21	9	30.00	wmc500, barc133, gwm340, gwm131, gwm566, wmc754, Bglu7ALc, gwm181, wmc54
3D	26	23	3	11.54	wmc630, cfd223, barc71
4A	28	24	4	14.29	wmc468, gwm160, gwm46, wmc76
4B	30	28	2	6.67	wmc16, wmc48
4D	35	24	11	31.43	cfd39, barc98, wmc331, gwm133, wmc89, wmc617, cfd106, wmc74, gwm194, wmc285, gwm624
5A	29	22	7	24.14	cfa,2149, wmc752, wmc489, gwm154, wmc47, barc117, wmc492
5B	31	23	8	25.81	gwm165, barc216, barc74, gwm325, wmc616, wmc160, wmc149, gwm213
5D	40	29	11	27.50	wmc200, wmc443, cfd81, gwm583, gwm269, cfd18, cfd40, gdm138, barc130, wmc233, cfd266
6A	30	26	4	13.33	cfd190, wmc672, gwm459, wmc580
6B	29	27	2	6.90	gwm626, wmc494
6D	27	25	2	7.41	cfd13, wmc749
7A	39	30	9	23.08	wmc479, cfa2049, gwm63, gwm130, gwm282, wmc475, wmc662, wmc525, wmc158
7B	47	31	16	34.04	wmc758, barc65, gwm297, wmc517, cfa2106, wmc182, gwm302, wmc613, wmc276, barc176, wmc166, wmc70, wmc335, gwm146, gwm577, gwm611
7D	34	23	11	32.35	wmc473, wmc827, barc87, gwm44, wmc14, barc126, barc58, wmc646, barc172, barc184, wmc221
Total	700	547	153	21.11	

This positive correlation is mainly due to the common uptake strategy of Fe and Zn. Plants generally follow the reduction strategies or chelation strategy for the uptake of micronutrients. Reduction strategy is generally followed by the non-gramineous dicotyledon plants in which, the micronutrients are reduced first before its absorption. In the chelation strategy, phytosiderophores (PS) are used to chelate Fe^{3+} ions (Prasad et al. 2022; Dey et al. 2020). These PS are chelating agents like mutagenic acid, nicotinamide, and avenic acid and help in the formation of Fe-PS, and Zn-PS (Swarnalatha et al. 2022; Kosakivska et al. 2021). Various transporter proteins like zinc transporter proteins (ZRT), yellow stripe-like transporters (YSL), and zinc/iron-regulated transporter proteins (ZIP) facilitate the uptake mechanism of Fe and Zn (Mahendrakar et al. 2020; Pacheco et al. 2023; Neeraja et al. 2023).

There are studies showing Zn content varies from 34.4 to 25.9 mg. The DH mapping population shows transgressive segregation, with some double haploid populations reaching a concentration of 0.5 grams of Zn in grains (Morgounov et al. 2007). The grain iron concentration in 25 spring wheat cultivars ranged from 39 to 48 mg/kg, whereas in 41 winter wheat cultivars, it ranged from 34 to 43 mg/kg. The zinc concentrations measured for winter and spring wheat were 23 to 33 mg/kg and 20 to 39 mg/kg, respectively (Tiwari et al. 2009) The concentrations of grain iron and Zinc in HD3298 grain were higher than mutant line M160. The disparity between HD3298 and M160 may be due to the mutation of various transporter genes iron-regulated transporter 1 (IRT1), and yellow stripe-like (YSL) transporters (Song et al., 2024; Chowdhury et al. 2022). Likewise, lower Zn concentration in the mutant may be due to the reduced rhizospheres acidification and limited uptake of PS-Zn complex (Grotz et al. 1998; Guerinot 2000; Eide 2006). Due to this EMS mutation, we observed a wide range of variations in 214 $F_{2:3}$ mapping lines.

Transgressive segregation of the population signifies the presence of a unique gene group in parental genotypes for the desired trait. Five important QTLs were found for grain Fe. Three of them had positive alleles from HD3298 on chromosome 7A, and another two QTLs had dominant negative alleles from M-160 lie on chromosome no 1A and 7A (Table 3). A total of 12 significant QTLs were discovered for Zn, four inherited as favorable alleles from HD-3298 situated on chromosomes 4A, 5D, and 7B. The remaining eight alleles are inherited as a negative allele through the M-160 genotype which is situated on chromosomes 2A, 5B, 6A, 7B, and 7D (Table 3). The qualitative trait loci QTLs for grain iron and Zinc reported in this study are responsible for 13.1% and 6.31% of the total variation in traits, correspondingly. These results are consistent with the previous findings reported by Tiwari et al. (2009). According to their findings, a panel of 93 RILs used of QTL study. Two QTLs were mapped on chromosome 2A and one QTL was mapped in 7A for grain Fe content. In another study, Shi et al. (2008) found one QTL in chromosome 7A with a PVE of 25-30% for GZnC content. In our study, we also found three QTLs for Fe on chromosome 7A, five QTLs for Zn in 7B, and two QTLs for Zn in 7D chromosomes.

The current study highlights that 9 out of 12 genetic loci for GZnC coincide with previous studies, 2A (Wang et al. 2021; Krishnappa et al. 2017; Roshanzamir et al. 2013); 4A (Krishnappa et al. 2017; Roshanzamir et al. 2013); 6A and 7B (Wang et al. 2021); 7B (Krishnappa et al. 2017). The present study also mapped three genomic regions for GZnC in chromosomes 5B, 5D and 7D. Furthermore, five genomic regions (QTLs) for grain Fe were also identified, which coincides with previously reported findings i.e., of 1A (Roshanzamir et al. 2013; Peleg et al. 2009) and 7A (Krishnappa et al. 2017; Tiwari et al. 2009).

Investigations on diploid wheat (AA), tetraploid wheat (AABB), and hexaploid wheat (AABBDD) indicated that zinc absorption increased throughout wheat development. The zinc utilization in tetraploid wheat was 35%, diploid wheat was 60%, and hexaploid wheat was 64% (Cakmak et al. 1999b). The scientists explain the variability of the unique features of the ABD genomes. The researchers demonstrated that the A and D genomes enhance the nutritional value of Zn (Cakmak et al. 1999a).QTL linked to higher mineral content in grain could be originated at different stages of plant development, for example, root absorption, translocation of root-to-shoot, leaves or grain storage, remobilization, and those that make regulatory proteins. Finding the correlation between QTLs that control different mineral nutrients suggests a physiological connection between some mechanisms that control mineral storage in wheat grain (Peleg et al. 2009).

However, we should not ignore chromosomal regions associated with a restricted array of minerals, as they may provide distinct, mineral-specific routes. By mapping QTL for multiple minerals at the same time and looking at their inter- and intra-relationships, we can learn more about how plants work, how genomes are put together, and how mineral accumulation has changed over time in several cereals crops like wheat and others (Peleg et al. 2009). Advancements in QTL mapping will improve understanding of the genetic determinants influencing the mineral concentrations in a wheat crop. Also, finding and tagging the important QTLs which is related to micronutrient traits will allow the selection of extra QTLs in beginning generations using the MAS method. This will significantly accelerate the evolution of varieties of wheat developed to enhance the metal content in the grains (Gautam et al. 2020; Song et al. 2023). To get a more accurate detection of relevant QTL regions on the genome, it is suggested to conduct fine mapping within a subpopulation generated from the current mapping population.

Authors' contribution

Conceptualization of research (SK, RRY); Designing of the experiments (RRY); Contribution of experimental materials (SK); Execution of field/lab experiments and data collection (ST, P, Anvesha); Analysis of data and interpretation (RRY, JK, ST); Preparation of the manuscript (RRY, JK, SKV, NP, IS).

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