

Identification of Novel Major Quantitative Trait Loci Associated with Selenium and Zinc Accumulation in *Aegilops tauschii*

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Research Article

Keywords: *Ae. tauschii*, Selenium, Zinc, RIL population, QTL, Candidate genes

Posted Date: March 23rd, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1468596/v1>

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Abstract

Background and aims Wheat is the major nutrient source for numerous people. *Aegilops tauschii* Coss. (DD), the D genome donor of common wheat (BBAADD), exhibits abundant genetic variations in selenium and zinc concentrations. *Ae. tauschii* accession AS2407 exhibited prominent Se and Zn accumulation ability. The objectives of this study were to map Se and Zn accumulation-related quantitative trait loci (QTLs) and to identify candidate genes controlling Se and Zn accumulation in *Ae. tauschii*.

Methods In this study, quantitative trait locus (QTL) mapping was carried out for selenium and zinc accumulation in the aerial parts of recombinant inbred lines (RILs) derived from two subspecies, AS2407 (*Ae. tauschii* ssp. *strangulate*) and AS65 (*Ae. tauschii* ssp. *tauschii*), and candidate genes were screened. Kompetitive allele-specific polymerase chain reaction (KASP) markers, which are closely linked to QTLs, were developed, and their distributions were detected in a natural population of *Ae. tauschii*.

Results QTLs controlling selenium and zinc content were identified on chromosomes 1D and 2D, named *QSe-1D* and *QZn-2D*, respectively, which could explain 11.94% and 13.49% of the phenotypic variation, respectively. Five selenium-related candidate genes and three zinc-related candidate genes were screened. KASP markers (KASP_Chr1_22217614 and KASP_Chr2_57200260) were developed. *QSe-1D* and *QZn-2D* are the first mapped QTLs controlling selenium and zinc accumulation in the aerial part of *Ae. tauschii*.

Conclusions These QTLs and KASP markers will be useful for quality improvement of wheat high nutrition and valuable for understanding the biological basis of Se and Zn intake in *Ae. tauschii*.

Introduction

Microelement malnutrition is a worldwide human health problem. Approximately 3 billion people are affected by deficiencies in iron (Fe), zinc (Zn), selenium (Se), vitamin A, and iodine (I) (Brundtland 2002; Welch and Graham 2004; White and Broadley 2009; Wu et al. 2018). Breeding programs in recent decades have focused mainly on improving grain production and rarely on enhancing the nutrient content of crops. Consequently, the edible parts of many major staple crops are not optimized with micronutrients, which leads to global micronutrient deficiency, particularly in developing countries (Brundtland 2002; Pu et al. 2014; Welch and Graham 2004; White and Broadley 2009). Breeding staple crops with high micronutrient content is an effective way to overcome nutritional deficiency. Selenium (Se), recognized as an essential trace element for humans and animals, consists of the key component of more than 25 mammalian selenoenzymes or selenoproteins with important biological functions (Rayman 2002). The active role of Se as an antioxidant, thyroid hormone and general immune function regulator has been highlighted since the essentiality of Se as a nutrient for humans and animals was first established in the 1950s by Schwarz and Foltz (Rayman 2000; Schwarz and Foltz 1957).

Zinc is an essential micronutrient for plants, humans and animals. Zn deficiency is a well-documented world health problem. Zn plays a role in more than 300 enzymes and is a constituent in thousands of proteins, including transcription factors (Hansch and Mendel 2009). Zn status in plants is directly

correlated with plant growth, crop yield, and product nutritional quality (Cakmak 2008; Sadeghzadeh and Rengel 2011).

Common wheat (*Triticum aestivum* L., genome AABBDD), one of the main staple crops worldwide that supplies a major energy source and nutrients for a large number of people in the world, is an allohexaploid that originated from hybridization between cultivated tetraploid wheat (*T. turgidum*, BBAA) and the diploid *Ae. tauschii* (DD). Since only limited tetraploid and diploid genotypes were involved in its origination, bread wheat has reduced genetic diversity compared with its donor species. The genetic variation of bread wheat has been further narrowed by subsequent domestication and modern breeding (Khan and Shewry 2009). Identifying genotypes with higher selenium and zinc accumulation is important for wheat biofortification improvement. Previous studies showed that *Ae. tauschii* exhibited higher and more abundant genetic variations in selenium and zinc concentrations than bread wheat (Cakmak 2008; Lyons et al. 2005; Wu et al. 2018; Zhao et al. 2017). The higher selenium and zinc concentrations of *Ae. tauschii* could not be fully exhibited in their corresponding allohexaploid synthetic hexaploid wheat (SHW) lines (Zhao et al. 2017). Obtaining a better understanding of the genetic control of Se and Zn accumulation in *Ae. tauschii* is therefore very necessary for breeding wheat cultivars with the capacity of simultaneous accumulation of high concentrations of Se and Zn by biotechnological (transgenic and gene editing) as well as synthetic hexaploid wheat approaches.

Ae. tauschii accession AS2407 exhibited a high Se and Zn accumulation ability, while *Ae. tauschii* accession AS65 exhibited low Se and Zn accumulation ability. The objectives of this study were to map Se and Zn accumulation-related quantitative trait loci (QTLs) and to identify candidate genes controlling Se and Zn accumulation in *Ae. tauschii*, with the ultimate goal of providing markers that can be used in marker-assisted breeding as well as the target genes for transgenic and gene-edited wheat lines with high Se and Zn content.

Materials And Methods

Plant materials

AS2407 (*Ae. tauschii*. ssp. *strangulate*) has been proven to accumulate much higher selenium and zinc than AS65 (*Ae. tauschii*. ssp. *tauschii*) (Wu et al. 2019; Zhao et al. 2017). A mapping population consisting of 95 F₇ recombinant inbred lines (RILs) derived from a cross between AS2407 and AS65 was used in the current study for genetic mapping of mineral QTLs.

Experimental design and implementation

The seeds of AS2407, AS65 and the 95 RILs were soaked in double distilled water (ddH₂O), placed into a refrigerator at 4 °C for 24 h for dormancy breaking, and then germinated in sterilized Petri dishes with moist filter paper at a constant temperature (25 °C) in darkness. At the three-leaf stage, the seedlings were transplanted into 72-well seedling hole trays (530 mm×270 mm×40 mm) containing a quarter of Hoagland's nutrient solution under daily conditions in a greenhouse (Day 25°C, 16 h; dark 20°C, 8 h) for

growth. During the seedling growth period, the humidity was adjusted to approximately 60%. Zinc is supplied by zinc sulfate (ZnSO_4), which is one of the components of Hoagland's nutrient solution. After two weeks, half of the seedlings were treated with sodium selenate (Na_2SeO_4) to a final concentration of 10 μM . Two weeks later, the aerial parts of all samples were collected for element measurement and genomic DNA extraction. All lines contain three biological replicates. The solution was replaced by a fresh solution every 2 days during plant growth to ensure a constant concentration and pH value.

Element measurement

The aerial part of each seedling was harvested and dried at 80°C to constant weight. Dried samples were ground into a fine powder, and a 0.2 g sample for each genotype was digested by hydrochloric acid. Selenium and zinc concentrations were measured by inductively coupled plasma–mass spectrometry (ICP-MS) (Akune et al. 2010).

RAD-seq analysis

The plant genomic DNA Kit (TIANGEN Biotech, Beijing, China) was used to extract DNA from the parents and all RILs. A total of 1 μL of DNA solution was used to identify the DNA quality in 1% agarose gels by electrophoresis, and the DNA concentration was measured with a Nano Drop 2000C spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Qualified genomic DNA was used to construct a RAD-seq library (NEB Next [®]MLtra™ DNA Library Prep Kit, NEB, USA) and sequenced on an Illumina NovaSeq 6000 (Illumina, San Diego, CA, USA) using 150 bp paired-end reads.

Construction of a high-density genetic map

FASTP 18.0 (Chen, et al. 2018) was used to filter the raw data, and the filtering criteria were as follows: (1) reads containing 10% unknown nucleotides (N) were removed; (2) reads with a Phred quality score ≤ 20 and $\geq 50\%$ were rejected; and (3) reads containing joints were eliminated. To identify SNPs, Burrows-Wheeler Aligner (BWA) was used to BLAST the clean data against the reference genome (Aet_T093_v1.0). Variant calling was performed for all samples using GATK's unified Genotyper.

Markers with homozygous polymorphisms and sequencing depths greater than 4 were selected. Among these markers, markers with deletion rates greater than 10%, heterozygosity ratios greater than 75% and severe segregation ($P < 0.001$) were removed. Then, the filtered markers were used to construct the genetic map by the OrderMarkers2 module of LEP-MAP3 (identical Limit = 0.01, min error = 0.001, sex Averaged = 0, informative Mask = 123) (Pasi 2017).

QTL analysis and candidate gene screening

QTL IciMapping 4.1 was used for QTL analysis. The QTLs related to selenium and zinc concentrations were detected in the biparental populations (BIPs), in which an inclusive composite interval mapping (ICIM) module was used. The mapping parameters were set as follows: QTL walking speed of 1.00 cM, stepwise regression probability threshold value of 0.001, and the threshold LOD was determined by

permutation tests (Times = 1000 and Type I Error = 0.05). The genetic linkage map was drawn by MapChart 2.2.

The rice annotation project (RAP) database (<http://rapdb.dna.affrc.go.jp/>) was used for gene annotation analysis and assembly (<https://db.cngb.org/search/assembly/CNA0019289/>). Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis screened genes corresponding to biological functions. Genes related to zinc metabolism and selenoprotein metabolism were selected as candidate genes for further study.

Validation of the QTLs in the natural population

Closely linked KASP markers were developed on the online platform (<http://www.polymarker.info/>) to validate these QTLs in a natural population of *Ae. tauschii*. The 96-well plate amplification reaction system (10 µL) was as follows: 2×KASP Master mix (5 µL), Forward-1 (0.5 µL), Forward-2 (0.5 µL), Reverse (1 µL), DNA (2 µL), and ddH₂O (1 µL). Thermal cycle conditions were set according to the KASP genotyping instructions. After the PCR was completed, the QuantStudio®3 Real-Time PCR instrument read the data and performed genotyping. If the genotyping was not obvious, three cycles were added, and the total number of cycles did not exceed 48.

Statistical analysis

Excel 2019 was used to statistically analyze the zinc and selenium concentration data in the RIL population and two parents. A normal distribution test and the correlation between selenium and zinc concentrations in the RIL population were computed using Origin 2018. Analysis of single factor variance was performed by SPSS 18.0.

Results

Statistical analysis of the concentrations of zinc and selenium

The concentrations of selenium and zinc in the two parents and 95 RILs were measured by ICP-MS. The results showed that: (1) AS2407 exhibited 6- and 2-fold higher concentrations of Se and Zn than AS65 in hydroponically cultivated seedlings, respectively (Table 1; Fig. 1). Significant differences in the concentrations of zinc and selenium between the two parents were detected ($p < 0.05$). (2) The average content of selenium and zinc in the RIL population was 10.89 mg/kg and 39.03 mg/kg, and the coefficients of variation were 28% and 25%, respectively. Selenium content in 70% of the RILs ranged from 8 mg/kg to 14 mg/kg (Fig. 1a). Up to 68% of the RILs showed higher zinc concentrations than AS2407 (Fig. 1b). (3) The degree and kurtosis of the content of two trace elements in the RIL population were close to 1, which conformed approximately to the normal distribution and was suitable for QTL analysis.

Table 1
The content of zinc and selenium in aerial parts of the RIL population

Trait	Parents	RILs population					
	AS2407	AS65	Mean	SD	CV (%)	SK.	Ku.
Zinc content (mg/kg)	33.25	16.68	39.03	9.65	25	1.17	1.01
Selenium content (mg/kg)	31.05	5.36	10.89	3.07	28	1.12	0.93
SD, standard deviation; CV, coefficient of variation; SK., skewness; Ku., kurtosis							

Construction of high-density genetic maps and QTL analysis

A total of 12944233 high-quality single nucleotide polymorphisms (SNPs) and indels were obtained by RAD-seq analysis in the parents and the RIL population. After filtering redundant markers, 930,562 markers were used to construct a genetic map with a genetic length of 884.313 cM. These markers were distributed on 7 chromosomes, and the length of linkage groups ranged from 87.385 cM to 174.752 cM. Chromosome 3D has the largest number of polymorphic markers (196,827), while 4D has the least number of polymorphic markers (10,838). The mean interval length was only 0.001 cM. The 930,562 markers were divided into 1308 bins, with an average distance of 0.680 cM between contiguous bins. The bin numbers in the maps ranged from 115 (Chr4D) to 276 (Chr7D) (Table 2).

Table 2
Genetic map statistics

Chrome	Length (cM)	Marker (num)	Average (cM)	Number of Bin markers	cM per Bin marker
Chr1D	104.221	142868	0.001	154	0.68
Chr2D	120.537	196717	0.001	191	0.63
Chr3D	114.748	196827	0.001	173	0.66
Chr4D	87.385	10838	0.008	115	0.76
Chr5D	172.130	171493	0.001	238	0.72
Chr6D	110.540	151106	0.001	161	0.69
Chr7D	174.752	60713	0.003	276	0.63
Total	884.313	930562	0.001	1308	0.68

The BIP function of IciMapping with the ICIM-ADD module was used to analyze the loci related to selenium and zinc concentrations of the RIL population. After 1000 permutation tests, the logarithm of the odds (LOD) thresholds were determined to be 2.58 and 2.45, respectively. QTLs for Se and Zn concentrations were mapped on chromosomes 1D and 2D and named *QSe-1D* and *QZn-2D*, respectively. The LOD values of the two QTLs were 2.68 and 3.05. *QSe-1D* was located on chromosome 1D in the

interval of Chr1_22149326 and Chr1_22217614 at 70 kb, within a 0.5 cM genetic distance from 17.9 cM to 18.4 cM. The phenotypic variance explained (PVE) by *QSe-1D* was 11.94% (Fig. 2a; Table 3). *QZn-2D* was located at 10 kb with a flanking region between Chr2_57200260 and Chr2_57210419 on chromosome 2D, and the genetic distance was 1.1 cM from 24.2 cM to 25.3 cM. The PVE by the *QZn-2D* was 13.49% (Fig. 2b; Table 3). The order of almost all SNP markers in the genetic map was consistent with the reference genome in *Aegilops tauschii*. In the RIL population, the lines carrying the *QSe-1D* and *QZn-2D* alleles had significantly higher ($p < 0.05$, $p < 0.01$) Se (27.56%) and Zn (17.11%) concentrations than those lines without *QSe-1D* and *QZn-2D*, respectively (Fig. 2).

Table 3
QTLs for selenium and zinc in RILs

Trait	QTL	Chr	Interval (cM)	Flanking markers	LOD	Add	PVE%	Physical position (Mb)
Selenium	<i>QSe-1D</i>	1D	17.9–18.4	Chr1_22149326– Chr1_22217614	2.68	1.28	11.94	22.15– 22.22
Zinc	<i>QZn-2D</i>	2D	24.2–25.3	Chr2_57200260– Chr2_57210419	3.05	3.61	13.49	57.20– 57.21

Validation of the QTLs in a natural population of *Ae. tauschii*

Closely linked KASP markers (KASP_Chr1_22217614 and KASP_Chr2_57200260) were developed based on the flanking sequence of SNPs in *QSe-1D* (Chr1_22217614) and *QZn-2D* (Chr2_57200260). A natural population containing 119 accessions of *Ae. tauschii* was used to validate *QSe-1D* and *QZn-2D*. The closely linked KASP marker KASP_Chr1_22217614 was used to validate *QSe-1D*. In 119 accessions of *Ae. tauschii*, 33 was the same as AS2407, and 55 lines were identical to AS65. The results showed that 100 accessions were homozygous for *QZn-2D* by using KASP_Chr2_57200260. Among these accessions, 45 accessions belonged to *Ae. tauschii* ssp. *stragulata*, and 55 accessions were *Ae. tauschii* ssp. *tauschii*. Another 6 accessions contained the nonZn allele, and they were all *Ae. tauschii* ssp. *tauschii* (Fig. 3).

Potential candidate genes of *QSe-1D* and *QZn-2D*

Eight high-confidence genes were annotated from the interval of *QSe-1D* on the Aet_T093 genome, of which five genes were related to selenocompound metabolism (Table 4; Fig. 4). Five genes (*AetT093_1Dv1G085200*, *AetT093_1Dv1G085300*, *AetT093_1Dv1G085400*, *AetT093_1Dv1G085700*, *AetT093_1Dv1G085800*) encode phenylalanine ammonia-lyase (PAL) in the amino acid metabolism metabolic pathway. Further analysis of these amino acid metabolism pathways revealed a pathway related to selenocompound metabolism (PATH: ats00450) downstream.

Table 4
Annotated genes associated with selenium and zinc metabolism in the QTL positioning interval

Trait	Gene ID	Gene Annotation information
selenium	<i>AetT093_1Dv1G085200</i>	phenylalanine ammonia-lyase, PAL
	<i>AetT093_1Dv1G085300</i>	phenylalanine ammonia-lyase, PAL
	<i>AetT093_1Dv1G085400</i>	phenylalanine ammonia-lyase, PAL
	<i>AetT093_1Dv1G085700</i>	phenylalanine ammonia-lyase, PAL
	<i>AetT093_1Dv1G085800</i>	phenylalanine ammonia-lyase, PAL
zinc	<i>AetT093_2Dv1G206900</i>	zinc finger CCCH domain-containing protein
	<i>AetT093_2Dv1G205500</i>	NAC transcription factor NAM-B1-like
	<i>AetT093_2Dv1G205700</i>	NAM-B1 protein ATAF2-like

Candidate genes were screened by blasting the physical regions of QTLs with the reference genome *Aet_T093_v1.0*. In the interval of *QZn-2D*, a total of ten high-confidence genes were detected. Among these genes, three genes were related to zinc content (Table 4; Fig. 4). *AetT093_2Dv1G206900* encodes a series of CCCH-type zinc finger proteins. *AetT093_2Dv1G205500* was similar to the wheat gene *NAM-B1*, and the encoding protein belongs to the NAC-type transcription factor family. *AetT093_2Dv1G205700* encodes the ATAF2 protein, which is also a NAC-type transcription factor.

Discussion

Wheat is the second largest food crop in the world, providing approximately 20% of calorie intake in humans (Viteri and Torún 1975). The Se and Zn content of wheat is an important consideration in human health. Breeding Se- and Zn-enriched wheat varieties may be an ideal strategy for improving nutrition in Se- and Zn-deficient regions worldwide. Common wheat is a nonaccumulator of Se and exhibits narrow genotypic variation in Se and Zn concentrations compared with its diploid D genome donor *Ae. tauschii*, which exhibited a great advantage of accumulating selenium (Lyons et al. 2005; Zhao et al. 2017). Thus, Se- or Zn-enriched *Ae. tauschii* genetic resources would be highly valuable for identifying the genetic basis of Se and Zn accumulation and applying this genetic basis to wheat nutritional quality improvement.

In our previous studies, different *Aegilops tauschii* resources were screened for their Se and Zn concentrations; among these resources, *Aegilops tauschii* AS2407 exhibited the highest Se accumulation capacity, while AS65 exhibited the lowest Se accumulation capacity (Zhao et al. 2017). At the same time, AS2407 exhibited a better Zn accumulation capacity than AS65. To elucidate the genetic basis of Se and Zn accumulation in *Aegilops tauschii*, we created a RIL population derived from a cross between AS2407 and AS65. We determined the chromosomal quantitative trait (QTL) for Se and Zn concentrations using

this RIL population. *Aegilops tauschii* has been proven to have potential selenium-rich properties (Lyons et al. 2005; Wu et al. 2019; Wu et al. 2018; Zhao et al. 2017). To our knowledge, this is the first report of Se and Zn QTLs in a common wheat D genome donor, *Aegilops tauschii*.

Reports of QTLs for Se concentration are limited for wheat. Several studies have been conducted (Pu et al. 2014; Pu et al. 2018; Wang et al. 2017; Yan et al. 2018). Wang et al. (2017) detected QSsece-7D.2, located between marker loci D-3033829 and D-1668160 on chromosome 7D, using a winter wheat RIL population. Pu et al. (2014) identified five QTLs for GSeC on five chromosomes, 3D, 4A, 4D, 5B and 7D, indicating that 3 of 5 QTLs were distributed on the D chromosome. Another work by Pu et al. (2018) reported that a *Qse.sau-3D* located on chromosome 3D enhanced the selenium concentration of wheat grain. These results indicated that the D genome controlled Se intake in wheat. Our work focused on Se-enriched *Aegilops tauschii* accession AS2407 and revealed the genetic basis of Se intake. *QSe-1D* is the first mapped QTL related to selenium concentration in *Aegilops tauschii* and is the only QTL detected in the present study. *QSe-1D* is located on chromosome 1D in the interval of Chr1_22149326 and Chr1_22217614 at 68.288 kb, within a 0.5 cM genetic distance from 17.9 cM to 18.4 cM, explaining 11.94% of the phenotypic variation, indicating a major QTL. The additive effect of the loci was positive, indicating that the allele from AS2407 increased Se accumulation. In addition, Yan et al. (2018) identified seven new QTLs (on chromosomes 1A, 1B, 3A, 5A, 7A, 2×7B) for GSeC and eight QTLs (on chromosomes 1A, 1B, 2×2B, 4B, 5A, 6A, 7B) for GSeY using an RIL population derived from Durum Wheat and Wild Emmer.

QTL mapping related to zinc content has been studied extensively in different mapping populations constructed by diploid, tetraploid or hexaploid wheat (Alomari et al. 2018; Clemens 2016; Crespo-Herrera et al. 2017; Devi et al. 2019; Genc et al. 2008; Krishnappa et al. 2017; Peleg et al. 2009; Pinar et al. 2021; Rehman et al. 2017; Shariatipour et al. 2021; Shi et al. 2007; Tiwari et al. 2009; Velu et al. 2016; Zhou et al. 2020). *QZn.pau-7A* (linkage markers Xcfd31- Xcfa2049) was identified by Tiwari et al. (2009) in the diploid A genome of wheat. Peleg et al. (2009) reported stable QTLs located on chromosomes 5A, 6B, and 7A using a RIL population from durum wheat and wild emmer wheat crosses. Velu et al. (2016) detected two major QTLs for increasing GZn on chromosomes 1B and 6B of the tetraploid and hexaploid mapping RIL populations.

Comparatively, many more QTLs related to zinc content were detected in common wheat and synthetic wheat (Alomari et al. 2018; Krishnappa et al. 2017; Pu et al. 2014; Shi et al. 2007; Velu et al. 2016; Zhou et al. 2020). Velu et al. (2016) identified three major QTLs for GZn in a RIL hexaploid population. Shi et al. (2007) detected four QTLs related to the Zn concentration of grain in wheat (on chromosomes 4A, 4D, 5A and 7A) and seven QTLs for Zn content (on chromosomes 1A, 2D, 3A, 4A, 4D, 5A and 7A). Alomari et al. (2018) identified 40 marker-trait associations for grain Zn concentrations in 369 European wheat lines by genome-wide association analysis. These marker trait associations were located on chromosomes 2A, 3A, 3B, 4A, 4D, 5A, 5B, 5D, 6D, 7A, 7B and 7D. Zhou et al. (2020) also detected seven QTLs for grain Zn accumulation in 207 bread wheat varieties using the same method. In the two recombinant inbred synthetic wheat lines, four and three QTLs (chromosomes 2D, 3D, 4D, 5B) were identified in the SC

population (SHW-L1/Chuanmai 32) and CC population (Chuanmai 32/Chuannong 16), respectively (Pu, et al. 2014). Krishnappa et al. (2017) mapped five QTLs using an RIL population (Indian wheat variety “WH542”/synthetic derivative).

QTLs related to Zn were detected on all chromosomes of wheat. However, QTL mapping for zinc content in the diploid D genome is very rare. *QZn-2D* is the only QTL associated with Zn accumulation detected in the present study. *QZn-2D* (linkage markers Chr2_57200260 and Chr2_57210419) is located on chromosome 2D. The genetic distance was a 1.1 cM region from 24.2 cM to 25.3 cM, explaining 13.49% of the phenotypic variation, indicating it is a major QTL. As a closely related species of wheat, the genetic variation of selenium and zinc content in *Aegilops tauschii* is large (Zhao, et al. 2017). QTL analysis was the foundation for exploring the genetic mechanism of selenium and zinc intake, which is helpful for marker-assisted and biotechnological breeding.

Five candidate genes associated with selenium encode phenylalanine ammonia-lyase (PAL) in the amino acid metabolism metabolic pathway. PAL is the first starting enzyme to regulate phenylpropane metabolic pathways (Akbarian 2021; Fock-Bastide 2014). Based on genomic GO and KEGG enrichment analyses, the annotated information of PAL involved sulfur metabolism and selenium compound metabolism pathways. Selenium absorption in plants is closely related to sulfur (Sors et al. 2009). In our previous study of transcriptome analysis, phenylalanine metabolism was an especially highly enriched metabolic pathway in Se-treated AS2407 compared with Se-treated AS65 (Wu et al. 2019). Phenylalanine metabolism and sulfur metabolism were especially highly enriched metabolic pathways in Se-enriched green and purple wheat varieties compared with white wheat varieties under Se treatment (Pu et al. 2021). The PAL gene may indirectly affect selenium metabolism by regulating the sulfur metabolism pathway or be directly involved in the regulation of selenium compound metabolism. The metabolism of trace elements in plants may be strictly regulated by multiple genes (Waters and Grusak 2008). Nevertheless, there are few studies on the genetic mechanism of selenium enrichment in wheat crops, so the annotated genes may be limited.

Three candidate genes related to zinc were screened in the mapping interval. *AetT093_2Dv1G206900* encodes a cascade CCCH zinc finger protein family that has been widely studied in *Arabidopsis* and rice. CCCH zinc finger proteins bind to zinc ions and regulate gene expression by mediating RNA metabolism to affect plant growth and development under biotic and abiotic stress (Bogamuwa et al. 2014; Wang et al. 2008). According to the important role of zinc finger proteins in plant growth, the candidate gene *AetT093_2Dv1G206900* may play a crucial role in regulating the growth and development and zinc tolerance of *Aegilops tauschii*. *AetT093_2Dv1G205500* and *AetT093_2Dv1G205700* are NAC transcription factors. More than 110 and 140 NAC members have been found in *Arabidopsis* and rice, respectively (Wang et al. 2008). The mechanism of stress resistance of NAC genes has been studied in wheat (Shi et al. 2011). Uauy et al. (2006) obtained an NAC gene related to the zinc content of grain in wheat. Therefore, we speculate that *AetT093_2Dv1G205500* and *AetT093_2Dv1G205700* may be related to the zinc accumulation in *Aegilops tauschii*.

Accurate assessment of selenium accumulation under field conditions seems to be difficult because of spatial differences in soil Se availability (Lyons et al. 2005; Zhao et al. 2017). To provide uniform nutritional conditions for genotypes, a hydroponic solution was used in this study. The higher micronutrient content of wild species has often been attributed to their small sizes and lower harvest indices (Calderini and Ortiz-Monasterio 2015; Tan et al. 2006). *Aegilops tauschii* is one of the typical species with small seed sizes and lower harvest indices, this is the reason why we chose the aerial part, not the seed, to investigate Se accumulation in the *Aegilops tauschii* RIL population in the present study.

Allopolyploidization is an important evolutionary process that merges two distinct genomes into the nucleus to form an allopolyploid species. The merger of two distinct genomes presents a “genomic shock”, to which plants respond with a variety of genomic changes (Mcclintock 1984). Although the high Se and Zn concentrations of *Ae. tauschii* (DD) could not be fully expressed in their corresponding synthetic hexaploid wheat lines (AABBDD) (Zhao et al. 2017). An indirect approach using synthetic hexaploid wheat as a breeding bridge seems to have uncertainty in enhancing Se and Zn. Crossing Se- and Zn-enriched *Aegilops tauschii* accessions directly with common wheat might facilitate the transfer of QTL loci in wheat breeding with marker (KASP_Chr1_22217614 and KASP_Chr2_57200260)-assisted selection (MAS). Fine mapping of these regions harboring *QSe-1D* and *QZn-2D* and finally cloning the genes controlling Se and Zn intake will be valuable for understanding the biological basis of Se and Zn intake in *Ae. tauschii*.

Declarations

Acknowledgements We would like to express our thanks to Dengcai Liu from Triticeae Research Institute, Sichuan Agricultural University for kindly gifted the AS2407 and AS65 *Aegilops tauschii* accessions and provided natural populations of *Ae. tauschii*.

Funding This work was supported by National Natural Science Foundation of China (31101140), CAS "Light of West China" Program (xbzg-zdsys-202109), the Project from Qinghai Province (2020-HZ-807), Strategic Priority Research Program of the Chinese Academy of Sciences (No. XDA26030202), and Joint Research Project of Three-River- Resource National Park funded by Chinese Academy of Sciences and Qinghai Provincial People's Government (LHZX-2020-08).

Author contributions Hong Chen and Tao Liu carried out the experiment and conducted data analysis and drafted the manuscript. Rangrang Zhou helped data analysis. Lianquan Zhang and Shunzong Ning helped to create the RIL population. Lianquan Zhang helped to revise the manuscript. Bo Zhang designed and coordinated this study and revised the manuscript. All authors read and approved the final manuscript.

Competing Interests The authors have no relevant financial or non-financial interests to disclose.

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Figures

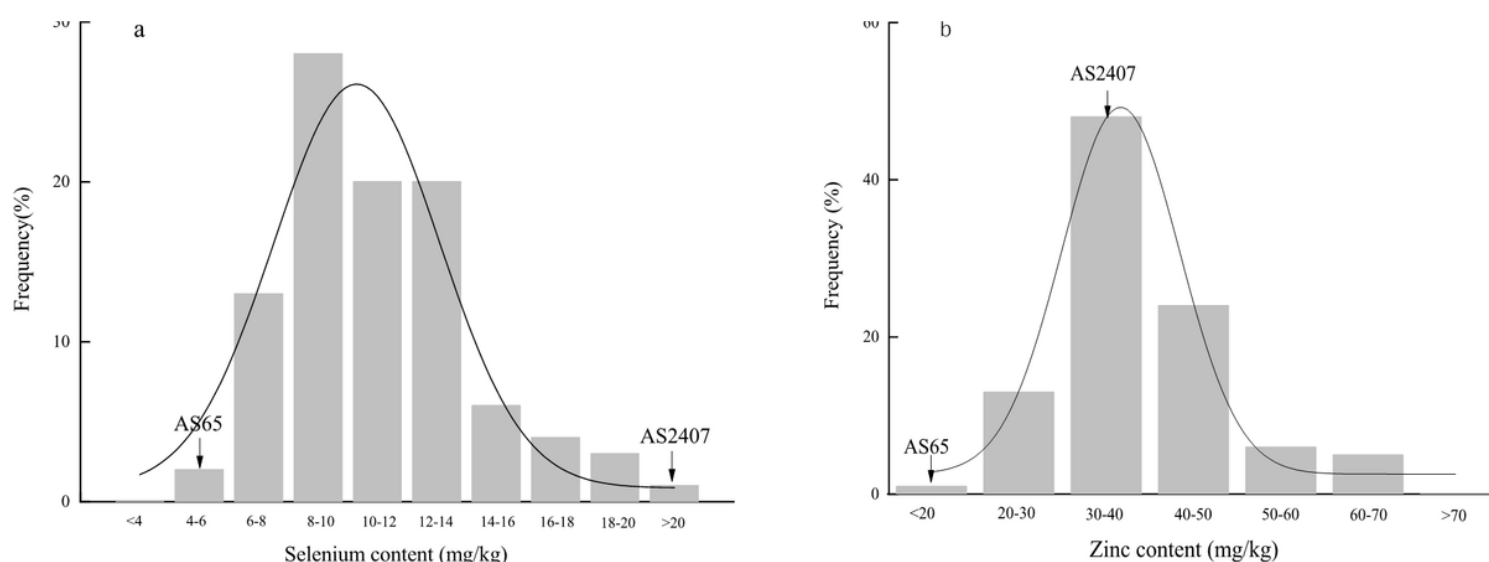


Figure 1

Frequency distribution of selenium (a) and zinc (b) content in aerial parts of the RIL population

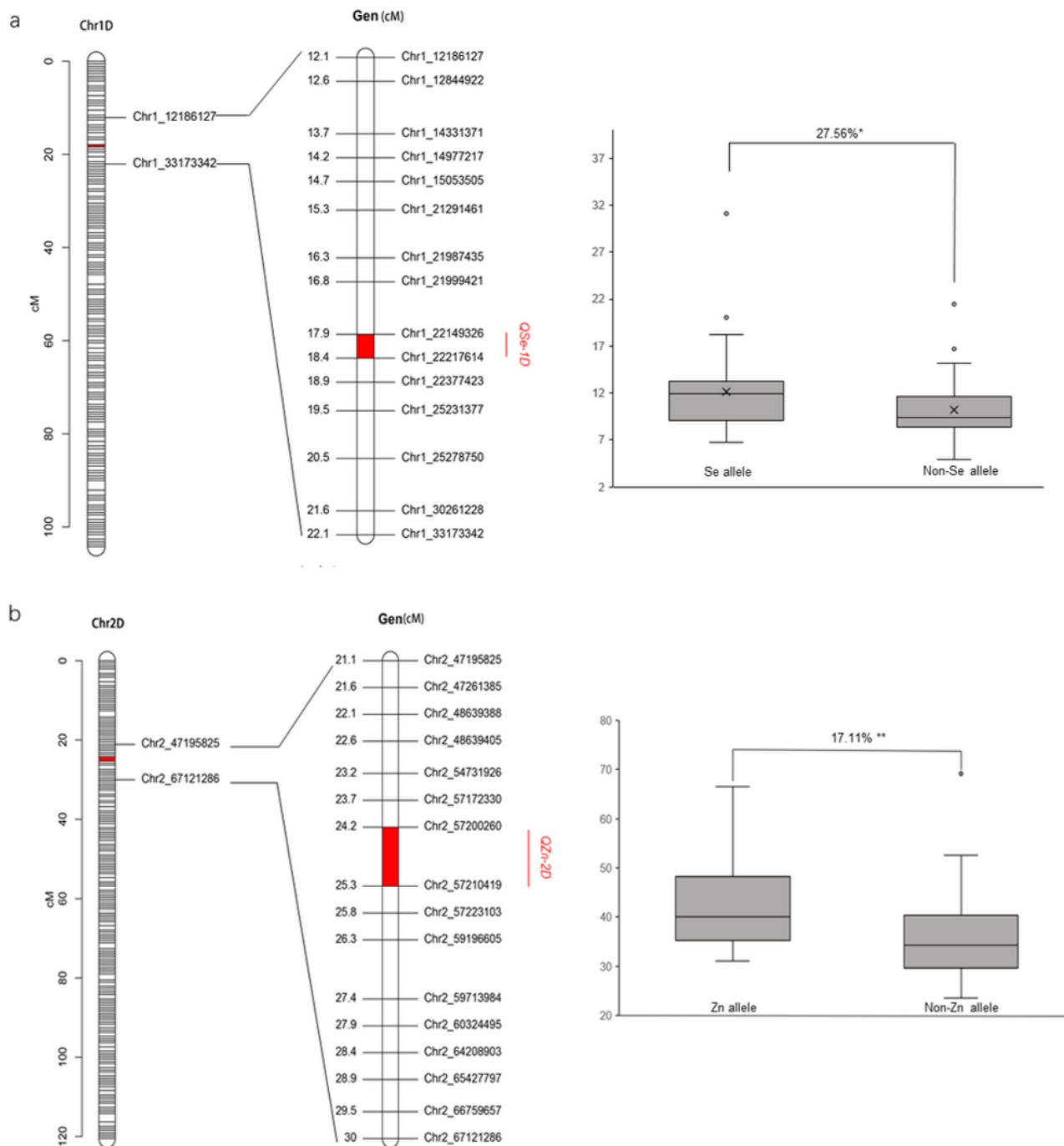


Figure 2

QTL analysis of selenium (a) and zinc (b) concentrations in RILs

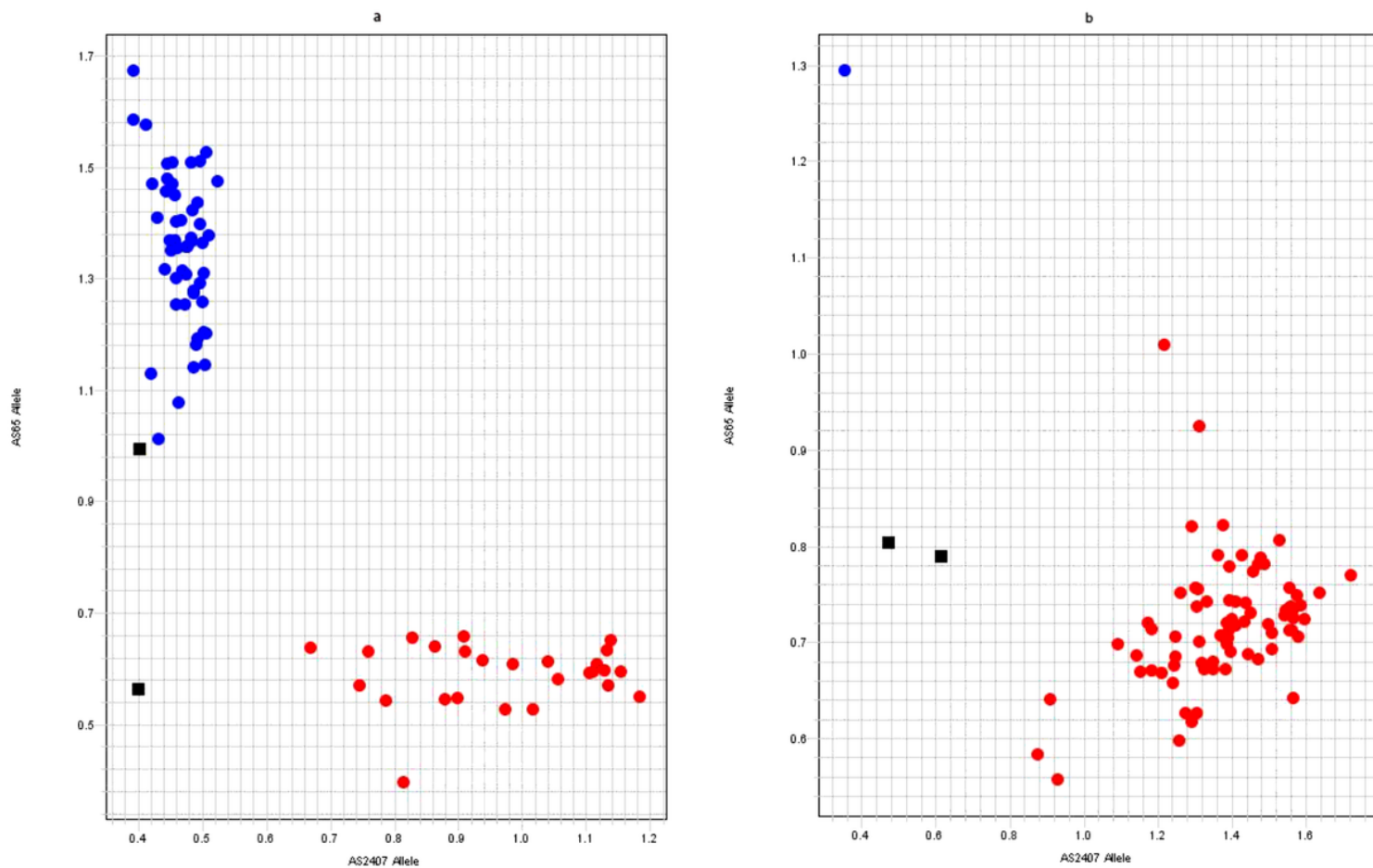


Figure 3

KASP marker genotyping of *Ae. tauschii* natural population.

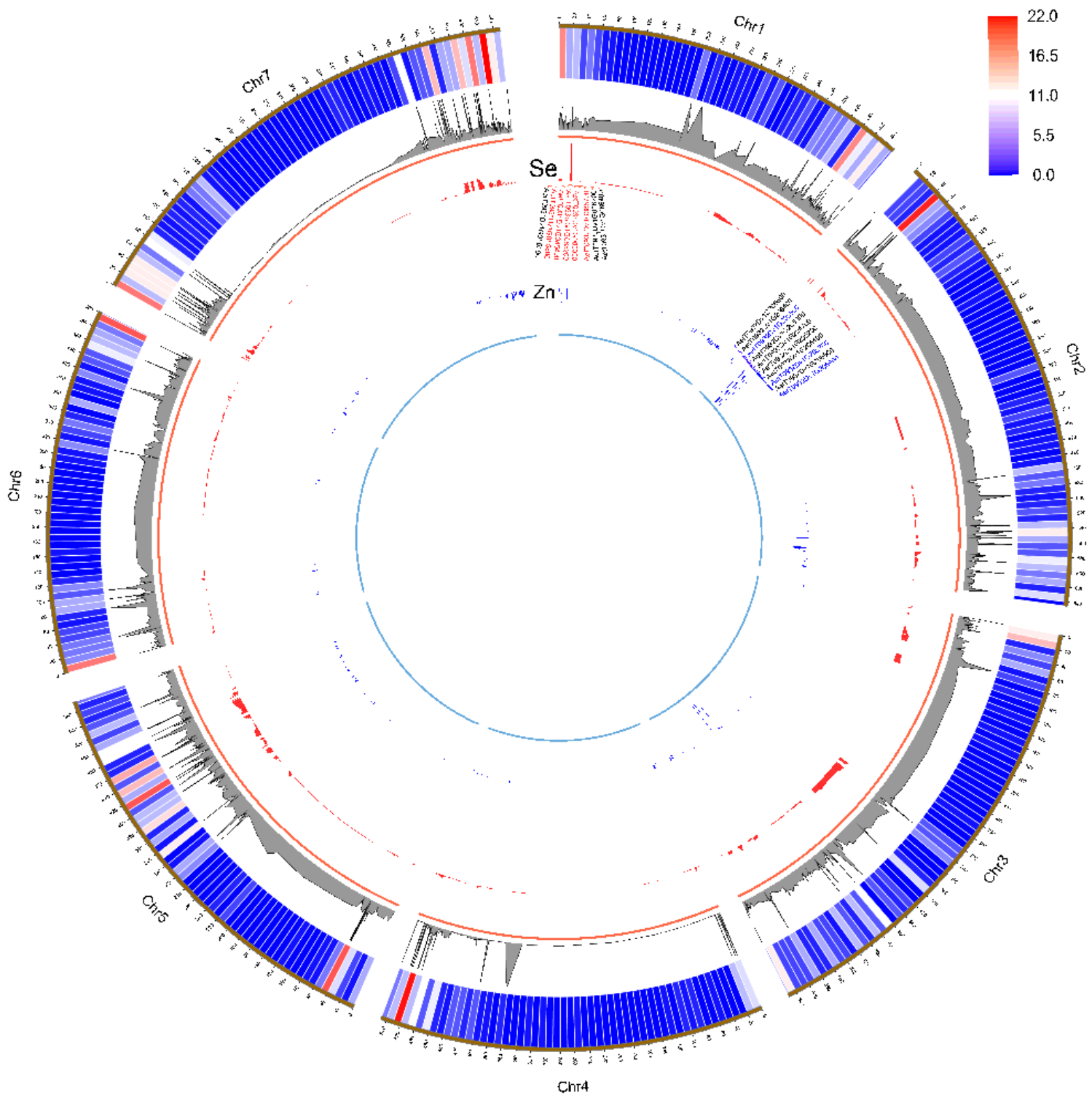


Figure 4

Genome circle map. In the outermost circle it is showed that the markers of all chromosomes used to build the map and the heat map of SNP density for each locus. From blue to red, the density is getting larger and larger. The density of markers was shown in gray peak line. Logarithm of the ods (LOD) of locus in selenium and zinc was displayed in two inner circles (a red and blue peak map). Among them, the solid red and blue circle represent the maximum LOD value of Se and Zinc, respectively. Candidate genes was marked in QTL peak positions, which estimated by maximum LOD values using QTL

IciMapping 4.1. Se-candidate genes and Zn-candidate genes were labeled in red and blue font, respectively.