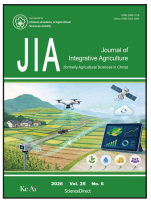
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RESEARCH ARTICLE

Genome-wide association study of novel genetic loci for cadmium accumulation and germplasm screening for low cadmium accumulation in common wheat (*Triticum aestivum* L.)

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Highlights

- Identification of 30 core quantitative trait loci (QTLs) for wheat cadmium (Cd) accumulation.
- Genome-wide analysis reveals four potential candidate genes.
- Development of two kompetitive allele specific PCR (KASP) markers for breeding selection.

Abstract

Cadmium (Cd) contamination in wheat farmland is increasing at an alarming rate, posing threats to food security and public health. Breeding and utilizing wheat varieties characterized by low Cd accumulation levels constitute an effective strategy in the battle against wheat Cd contamination. The adoption of molecular marker-assisted approaches can greatly expedite the selection and enhancement of wheat varieties with low Cd accumulation. Nonetheless, research concerning the genes associated with wheat cadmium accumulation remains scarce. In this study, a high-density 660K SNP array was employed for conducting a genome-wide association study (GWAS) on the grain Cd concentration (GCdC), bioconcentration factor (BCF) and translocation factor (TF) in 175 wheat germplasms. The findings revealed 401 significant SNPs identified across three diverse environments. Linkage disequilibrium analysis revealed 30 core quantitative trait loci (QTLs) capable of reliably modulating wheat Cd accumulation phenotypes. Through gene annotation, transcriptomics, and gene molecular features, four candidate genes (*TraesCS7B02G000200*, *TraesCS4A02G035900*, *TraesCS4A02G040900*, and *TraesCS5D02G564000*) were identified as potential constituents in the biological process of wheat Cd accumulation. Furthermore, six wheat germplasms exhibiting low grain Cd accumulation were isolated, and two kompetitive allele specific PCR (KASP) markers conducive to breeding selection were developed. These findings provide valuable genetic resources for cultivating wheat with low Cd accumulation and establish a foundation for understanding the molecular mechanisms underlying low Cd accumulation in wheat. The candidate genes and KASP markers elucidated in this research have potential for effective use in genetic enhancement and marker-assisted selection in the breeding of wheat with low Cd accumulation.

Keywords: *Triticum aestivum*, cadmium accumulation, genome-wide association analysis, functional prediction, molecular marker

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1. Introduction

As a toxic heavy metal, cadmium (Cd) is extremely harmful to human health (Reyes-Hinojosa *et al.* 2019). Cd is not biodegradable, but it is persistent in the natural environment and highly mobile between soil and plants. Human exposure to Cd from agricultural contamination is a problem that cannot be ignored. Wheat (*Triticum aestivum* L.) is the second largest grain crop in the world, and the second most important grain crop in China after rice. However, findings from the Chinese Academy of Sciences have revealed an increase in heavy metal contamination of agricultural land in China's major grain-producing regions. According to the National Soil Pollution Survey Bulletin, the prevalence of heavy metal contaminated sites has increased from 19.4% in 2014 to 21.49% in 2019, which poses a serious threat in terms of farmland pollution (Ludwig and Slamet-Loedin 2019; Wang *et al.* 2020). Cd initially enters the plant roots and then moves from the stem and leaves to the grains. This process leads to the production of a substantial quantity of reactive oxygen radicals in the plant body, disrupts metabolic balance, impairs membrane functionality and adversely affects the photosynthetic structure, which consequently results in plant cell death and ultimately a decline in crop yield (Ci *et al.* 2010; Suneja *et al.* 2017). Many studies have proposed various methods to reduce Cd contamination in wheat crops. Methods such as chemical washing, *in situ* passivation or high enrichment plant adsorption can remediate Cd contaminated soil and reduce Cd accumulation in wheat (Song *et al.* 2022). Breeding and applying low Cd-enriched wheat can ensure food safety, improve the use of Cd contaminated land, and increase the area and yield of cultivated wheat.

Previous research has demonstrated significant differences in the absorption, transport, and accumulation of heavy metals among different plants and different genotypes within a given species (Adomako *et al.* 2023). Numerous genes that regulate these functions have been extensively studied, particularly in rice (*Oryza sativa* L.). Cd transport from the roots to the shoots plays a crucial role in the accumulation of this metal in rice. Extensive research has elucidated the molecular mechanisms of Cd transport in rice and their implications for phytoremediation. For example, one study revealed that the uptake and transport of Cd in rice are regulated by various transporters, including members of the ZIP and NRAMP families, whose expression is influenced by various abiotic stress factors, including exposure to Cd. Furthermore, these transporters can be manipulated to develop rice varieties with increased resistance to Cd toxicity (Ai *et al.* 2022). Another study revealed that root-specific transporter expression is associated with the ability of rice to withstand Cd exposure, and that study further revealed that the *OsNramp5* mutant greatly reduces Cd uptake by rice roots, thereby reducing Cd accumulation in stalks and seeds (Zhang *et al.* 2024). *OsHMA3* transports Cd to the vesicles, which sequesters it and reduces the transport of Cd to aboveground areas, thus reducing Cd toxicity. These findings suggest that the manipulation of these root-specific transporters can be used to breed rice varieties with greater Cd tolerance. These studies provide comprehensive insights into Cd accumulation and transport in rice crops, particularly highlighting the importance of genes such as *OsNramp5* and *OsHMA3* (Miyadate *et al.* 2011; Ishimaru *et al.* 2012). However, studies focusing on the corresponding QTLs/genes in wheat are rare.

Several Cd-associated marker loci in wheat have been reported, including SNPs associated with Cd uptake (Guttieri *et al.* 2015; Safdar *et al.* 2020), random amplified polymorphic DNA (RAPD) markers for Cd exclusion (Penner *et al.* 1995) and quantitative trait loci for Cd tolerance (Ci *et al.* 2012). On chromosome 5BL, a SNP responsible for low Cd uptake was mapped and it explained approximately 54.3% of the variation in that phenotype (AbuHammad *et al.* 2016). In durum wheat, variations in the *HMA3* gene resulted in a 96% reduction in the variation of Cd uptake in wheat grains, which could be significantly reduced by overexpression of *HMA3* (Maccaferri *et al.* 2019; Zhang L *et al.* 2020). To develop wheat varieties with low Cd accumulation, it will be essential to acquire wheat germplasm resources with low Cd levels and to identify the genes responsible for Cd uptake and transport. The discovery of genetic loci related to Cd accumulation in wheat still lags behind rice, and the number of molecular markers available for screening and identification is very limited, which hinders the selection and identification of low-Cd-accumulating wheat cultivars. With the rapid development of next-generation sequencing (NGS), genome-wide association analysis (GWAS) has emerged as a powerful tool for rapidly analyzing the genetic basis of complex traits controlled by multiple genes. Originally, this approach was applied to the genetic analysis of human diseases and traits (Liu *et al.* 2018), and it has subsequently gained widespread use in the genetic dissection of complex plant traits (Qiu *et al.* 2015; Zhang *et al.* 2015). With the expansion of research on heavy metal stress and its mitigation in crops, this approach has been employed to study the Cd stress responses in wheat (Zaid *et al.* 2022), maize (Zhao X *et al.* 2018), and rice (Zhao J *et al.* 2018). Specifically, it has been instrumental for the development of a high-density single nucleotide polymorphism (SNP) gene analysis matrix for wheat. Among the various resources, the Wheat Axiom 660K SNP array has abundant polymorphisms in the wheat genome with high-density marker coverage and low heterozygosity. These new techniques and methods provide tools for the rapid localization and identification of important quantitative traits such as Cd accumulation (Voss-Fels and Snowdon 2016).

The present study aimed to explore the genetic basis of Cd accumulation in common wheat to develop reliable molecular markers that can facilitate the breeding of low-Cd-accumulating wheat. This study investigated grain Cd concentration (GCdC) and related traits, such as the translocation factor (TF) and bioconcentration factor (BCF), of 175 wheat genotypes in Cd-contaminated environments, and a GWAS of those three traits was performed using three models with wheat 660K arrays. The objectives of this study were to (1) screen wheat germplasms with low Cd accumulation and (2) mine the important genomic regions or loci and related candidate genes affecting the GCdC, TF and BCF.

2. Materials and methods

2.1. Plant materials and field trials

To ensure the wide diversity of common wheat, a total of 175 wheat germplasms originating from 14 Chinese provinces and six from foreign countries were investigated. This panel comprised both modern germplasms and foreign germplasms (Appendix A). All germplasms were sourced from the College of Agronomy, Northwest A&F University, Yangling, Shaanxi Province, China. They were grown during the crop growing seasons (October–June) in 2020–2021 and 2021–2022 on Cd-contaminated farmland in Hanzhong, Shaanxi, China (107.02°E, 33.07°N), following local wheat agronomic practices under natural conditions. The field trial was arranged in a completely randomized block design with three replications, and each genotype was planted on a unit plot size of 3 m^2. The physical and chemical properties of the soil are given in Appendix B and were determined through the following standard procedures. The concentrations of Pb and Cd in soil were determined by inductively coupled plasma mass spectrometry (ICP-MS), whereas the concentrations of Zn and Cu were determined by inductively coupled plasma emission spectrometry (ICP-OES), and the concentrations of Hg and As were estimated using an atomic fluorescence photometer (AFS-930). In addition, Ca, Mg and Na were measured with an atomic absorption spectrophotometer. The samples were quality controlled by the standard substance GBW07403 (GSS-3) throughout the determination.

2.2. Phenotype determination

After harvesting, the wheat material was divided by plot using mesh bags and then threshed by hand after complete drying. For each germplasm, the material was divided separately into grains, stems and roots, which were cleaned with distilled water, soaked in 10 mmol L^{-1} EDTA for 5 min, rinsed 2 or 3 times with distilled water, and dried in an oven at 70°C to constant weight. Three replicates of each germplasm were individually ground using a grinder, sieved through 100 mesh screens and bagged for storage. The material was digested using a microwave digester (Multiwave PRO, Anton Paar GmbH, Austria) and the digested wheat tissue samples were measured for Cd concentration by inductively coupled plasma mass spectrometry (iCAP™ RQ ICP-MS, Thermofisher). The standard substance GBW10010 (GSB-1) was used for quality control throughout the process.

2.3. Statistical analyses

Analysis of variance (ANOVA), Pearson correlation, and Student's *t*-test were performed using Origin 2022 software and correlation plots were subsequently generated. The BCF, TF, and generalized heritability (H^2) were calculated using the following equations (Maiti and Jaiswal 2008):

TF (grain/root)=GHMC/RHMC

BCF (grain/soil)=GHMC/SHMC

$$H^2=\frac{\sigma_G^2}{\sigma_G^2+\frac{\sigma_\varepsilon^2}{r}}$$

where TF (grain/root, g/r) represents the degree of heavy metal transport from roots to grains; BCF (grain/soil, g/s) represents the bioconcentration factor, which reflects the uptake capacity of plants for heavy metals; GHMC represents the concentration of heavy metals in grains; RHMC represents the concentration of heavy metals in roots; SHMC represents the concentration of heavy metals in soil; σ_G^2 represents the genotype; σ_ε^2 represents the environment interaction and error; and r represents the number of replicates in each environment. For the phenotypic traits of wheat, the best linear unbiased estimate (BLUE) was calculated using Rv4.1.3 for each germplasm in multiple environments *via* a linear model (Naserkheil *et al.* 2020).

2.4. Genome-wide association study

Genomic DNA was extracted from the 175 wheat germplasms using a modified CTAB method (Fu *et al.* 2017), and genotyping was carried out with the Affymetrix wheat 660K (including 660,023 SNPs) array. Markers with heterozygous genotypes, individual deletions, minimal allele frequencies<5% or gene deletions>20% were filtered using Plink software (Pang *et al.* 2020). Population structure was analyzed using admixture with 10-fold cross-validation, with the number of iterations of kinship matrix (K) ranging from 2 to 10, and the cross-validation error was calculated to evaluate the fit for different values. Principal component analysis and visualization of genotypic data were accomplished using Origin 2022 software. The VanRaden kinship matrix for the 175 genotypes was calculated using GAPIT software (K) (VanRaden 2008), and GWAS was subsequently carried out based on the population structure (Q) and a kinship matrix (K) for statistical correction. Genome-wide linkage disequilibrium (LD) analyses were carried out using PopLDdecay software, and LD decay maps for wheat subgenomes A, B, D, and the whole genome were developed to investigate the average decay distances for each genome (Kaewcheenchai *et al.* 2021).

The whole-GWAS of the three traits was performed using three models, namely, the compressed linear mixed model (CMLM), mixed linear model (MLM) (Q+K) (Zhang *et al.* 2010), and the Bayesian-information and linkage-disequilibrium iterative nested keyway (BLINK) (Huang *et al.* 2019), through GAPIT (V.3). To avoid losing important loci due to an overly conservative Bonferroni correction, we set the threshold for marker-trait association (MTA) as $P<1.0\times10^{-3}$. In multiple environments (with the BLUE value as one environment), the SNPs commonly associated with all three models were considered stable associated loci. Based on the LD decay distances in different subgenomes, a LD analysis of significant loci was performed to select candidate QTLs based on the local LD. Manhattan and quantile-quantile (Q-Q) plots were generated using the CMplot package in R v4.1.3.

2.5. Candidate gene and KASP marker development

The candidate genes of the stable QTL regions in this study were identified through gene annotation based on IWGSC RefSeq v1.1 in the EnsemblPlant (http://plants.ensembl.org/index.html) database. The functional genes related to rice Cd accumulation were obtained from the China Rice Research

Center (https://www.ricedata.cn) and their orthologues in wheat were identified using BLASTP (https://blast.ncbi.nlm.nih.gov). Genes within or close to the physical interval of an identified QTL were considered as possible candidate genes when they were involved in either: (1) biological processes related to metal ion homeostasis, transport and binding, such as root uptake, xylem and phloem transport, seed storage and regulatory factors; or (2) molecular functions related to the response to cationic stress. The spatio-temporal transcriptional dynamics of the candidate genes were investigated using the expVIP8 database to obtain the spatiotemporal expression profiles of the candidate genes in plant tissues at different developmental stages.

In accordance with the methods of Alvarez-Fernandez *et al.* (2021), KASP markers were developed for the QTL regions containing the candidate genes, and chromosome-specific primers were designed using Polymarker software (Ramirez-Gonzalez *et al.* 2015). To ensure the reliability of the KASP markers, all the markers were verified in two populations: an associated population with 175 wheat germplasms and a natural population with 89 wheat germplasms. Based on the phenotypic data of the population, *t*-tests were conducted to verify allelic effects.

2.6. Sample processing and RNA-seq library construction

To extract RNA, the samples from different treatments were ground into powder in liquid nitrogen and RNA-seq libraries were then constructed following the procedures given by Sarantopoulou *et al.* (2019). For RNA-seq, total RNA was first extracted from cells using the TIANamp Polysaccharide & Polyphenols DNA Kit (QIAGEN, Germany) and treated with DNase to eliminate any genomic DNA contamination. Then, the mRNA was selectively captured using oligo (dT) magnetic beads and fragmented using a heat mixer (Eppendorf, Hamburg, Germany) with interrupting reagents. The fragmented mRNA was used as a template for cDNA synthesis. Double-stranded cDNA was generated using a double-stranded cDNA synthesis reaction system and purified using a purification kit. The purified cDNA was then subjected to sticky end repair, followed by the addition of an "A" base and adapter ligation at the 3′ end. cDNA fragments of the desired size were selected and amplified by PCR. Following the manufacturer's protocol, the resulting library was sequenced through paired-end sequencing on the Illumina NovaSeq 6000 platform (Illumina, USA).

2.7. Library preparation, sequencing, and read mapping

The original sequence data were analyzed using Fastp software. The following parameters were used for the analysis: fastp -g -q 5 -u 50 -n 15 -| 150. This removed reads with adapter contamination, reads containing N (indicating undetermined nucleotide information), and low-quality reads (where the percentage of bases with Qphred≤20 exceeded 50% of the read length). In addition, the Q20, Q30, and GC contents of the filtered data were calculated. All downstream analyses were carried out using only the high-quality filtered data. The reference genome and gene model annotation files were based on Chinese Spring wheat gene information (http:/plants.ensembl.org/info/data/ftp/index.html). The reference genome index, constructed with HISAT2 (v2.2.1) (Kim *et al.* 2019), was used to align the paired-end filtered reads to the reference genome. The DEGseq2 R package (1.36.0) (Love *et al.* 2014) was used for gene expression analysis. Thresholds of $|\log_2(\text{fold change})|\geq 1$ and P_{adj} value≤0.05, based on quantile normalization, were used to identify differentially expressed genes (DEGs). The data were normalized based on the *Z*-score.

2.8. Differential expression and functional enrichment analysis

Gene Ontology (GO) enrichment analysis of the differentially expressed genes was conducted using the ClusterProfiler (4.4.4) R package (Wu *et al.* 2021). GO terms with a P_{adj} value less than 0.05 were considered significantly enriched with DEGs. The differentially expressed genes were also subjected to statistical enrichment analysis for the KEGG pathways using the ClusterProfiler (4.4.4) R package.

3. Results

3.1. Phenotyping analysis

The present study focused on three wheat traits: GCdC; TFg/r, which was determined by dividing GCdC by root Cd concentration; and BCFg/s, calculated as the ratio of GCdC to rhizosphere soil Cd concentration. The phenotyping analysis revealed that the GCdC, TFg/r, and BCFg/s values in the 175 wheat germplasms presented continuous variation with distributions closely resembling the normal distribution in all environments (Appendix C). Correlation analysis revealed highly significant positive correlations between GCdC and BCFg/s with TFg/r (r=0.839, P<0.001; r=0.445, P<0.001), in the blue environment (Appendix C). Meanwhile, a weak correlation was observed between GCdC and yield (r=0.35), while no significant correlations were found with plant height and grain size (Appendix D). The ANOVA results indicated that the phenotypic variations in GCdC, BCFg/s, and TFg/r in the 175 wheat materials were influenced by both the genotype and environment (Appendix E). Analysis of the broad-sense heritability (H^2) revealed that GCdC and BCFg/s had H^2 values of 0.75 and 0.59, respectively, suggesting that genetic factors primarily influence their phenotypic variation. The coefficients of variation (CVs) for GCdC, BCFg/s, and TFg/r were significantly different among the different genotypes, with CV values of 47, 41, and 57%, respectively, in 2022. These traits significantly differed in both genotype and environment, highlighting the suitability of the natural population constructed from these 175 source materials for genome-wide association studies.

By performing clustering analysis on GCdC, BCFg/s, and TFg/r (Fig. 1), and determining the frequency statistics of the results (Fig. 1), the pool of 175 wheat germplasms was categorized into three distinct groups. Category 1 consists of wheat germplasms with low GCdC, BCFg/s, and TFg/r values (GCdC range, 0.07–0.169; BCFg/s range, 0.015–0.033; TFg/r range, 0.02–0.133). This category comprised 37 wheat germplasms, including notable germplasms such as Luohan 7 and Ningdong 11 (Fig. 1-G). To screen the wheat

germplasms characterized by low Cd accumulation and ensure that they meet the safety standards for consumption (GCdC<0.1 mg kg^{-1}) specified in the National Food Safety Standard (GB 2762-2017 2017), a cluster analysis was conducted in tandem with enrichment and GCdC ranking methods. This screening revealed six wheat germplasms characterized by low Cd accumulation (Appendix F). These germplasms were stable in all environments, with BLUE values of GCdC less than 0.1 mg kg^{-1}, BCF less than 0.015 and TF less than 0.15. Among them, Serim 82 had the lowest GCdC and BCF, while Luohan 7 had the lowest TF.

3.2. Population structure, genotyping, principal component analysis and LD

Through quality control of the 660k genetic data, a total of 280,198 high-quality polymorphic SNPs were identified. The screening criteria included a minor allele frequency (MAF) threshold of less than 0.05 and a missing data threshold of less than 0.2. The population structure analysis revealed that the optimal value of K as 8 (Fig. 2-A). A comprehensive analysis of the genetic background of 175 wheat germplasms was carried out using a phylogenetic tree and principal component analysis, resulting in their successful classification into eight distinct subgroups (Fig. 2-B and C). Furthermore, the LD results indicated that the decay distance of LD varied unevenly among the different subgenomes of wheat (Fig. 2-D). Specifically, the B subgenome exhibited slower decay, potentially due to stronger artificial selection, whereas the D subgenome showed faster decay, possibly a consequence of reduced artificial selection following integration into the wheat genome. The average LD decay distance across the entire genome was estimated to be approximately 2.4 Mb, providing valuable criteria for the subsequent selection of associated genomic regions.

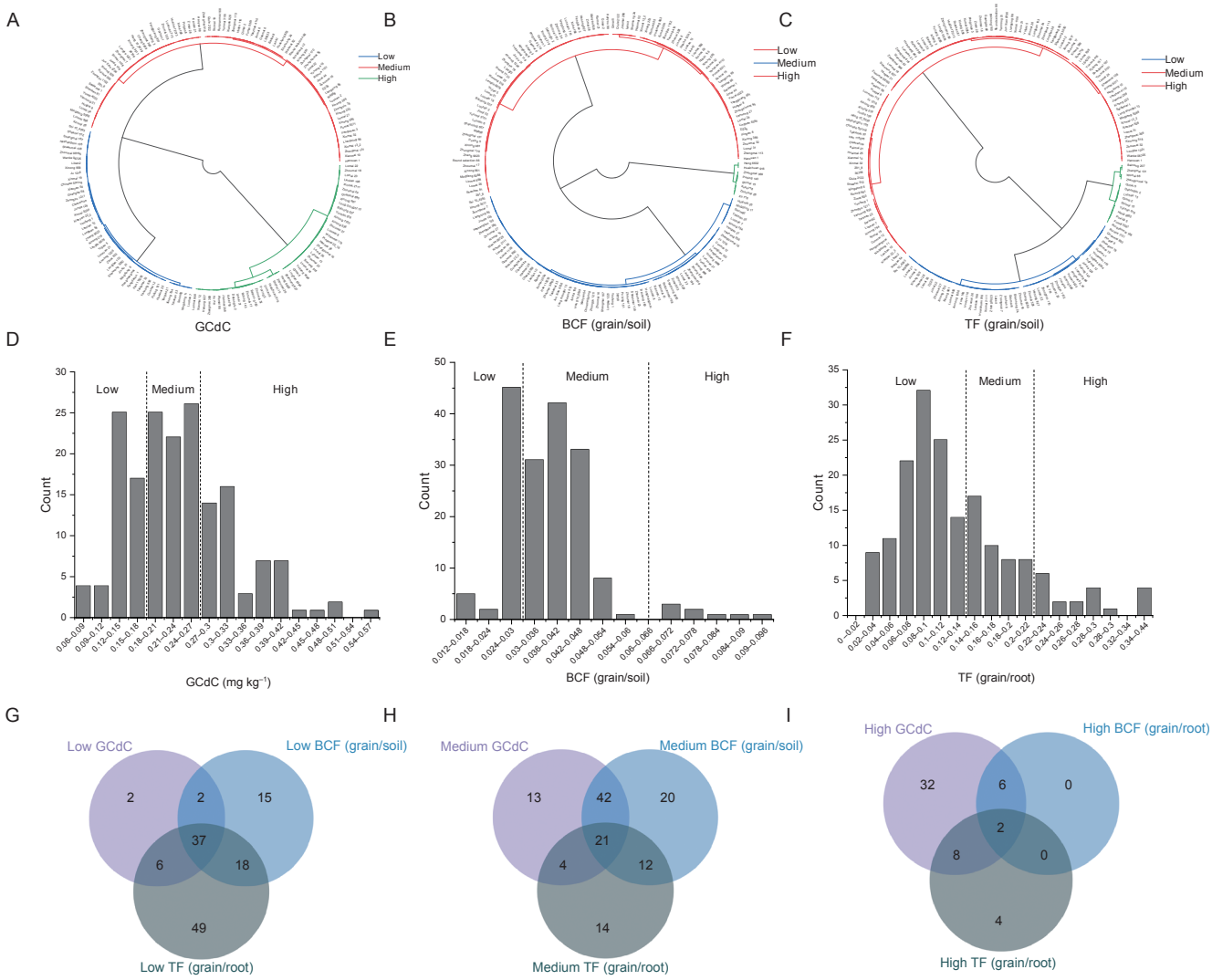


Fig. 1 Cluster analysis, frequency distribution, and Venn diagram analysis of 175 wheat germplasm accessions based on the phenotypic data of grain cadmium concentration (GCdC), bioconcentration factor (BCF), and translocation factor (TF). A–C, cluster analysis plots of three phenotypes. D–F, frequency counts of the three phenotypes categorized as low, medium, and high. G–I, Venn diagram analyses of different categories within the three phenotypes.

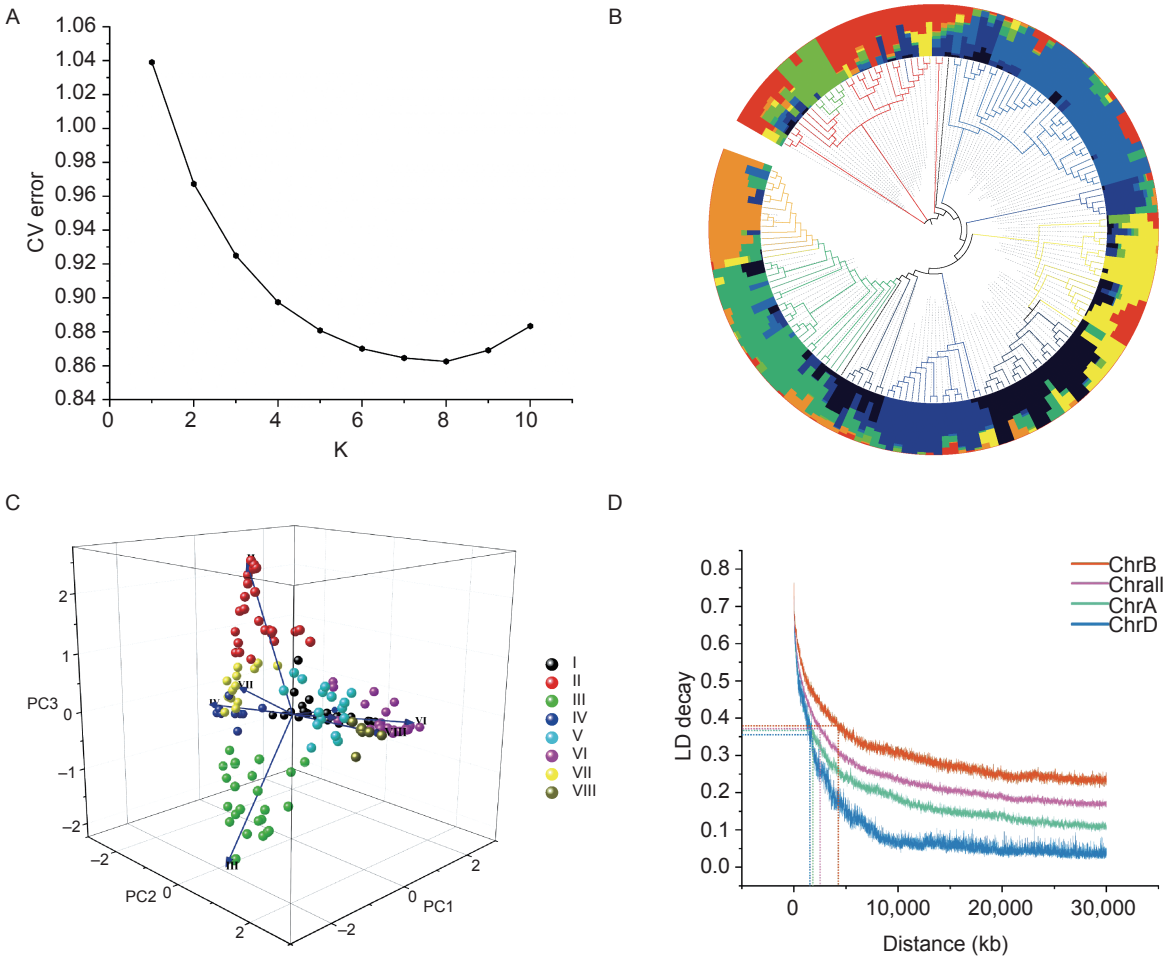


Fig. 2 Genotypic characteristics of 175 wheat germplasm accessions based on SNP variation information. A, line chart of coefficient of variation (CV) error values performed by admixture. B and C, population structure and principal component analysis of 175 wheat germplasm accessions, respectively. D, attenuation distance of the linkage disequilibrium (LD) of wheat.

3.3. Marker-trait associations and QTLs

Association analysis was conducted using the MLM, CMLM, and Blink models, while accounting for the BLUE value and phenotypic traits specific to each environment. Fig. 3 illustrates the association results for GCdC, whereas Appendices G and H summarize the results for BCF and TF. To ensure the reliability of the loci, significant SNPs, which are commonly associated with the three models, were used as the basis for further analysis. For the GCdC trait, 46 significantly associated SNPs were identified in at least two environments, BCF had 155 significant SNPs, and TF had 200 stably associated loci in at least two environments. The results are summarized in Appendix I.

The aggregation of the associated loci revealed that they predominantly reside on chromosomes 2B, 2D, 4A, 5D, 6A, and 7B. For a more comprehensive assessment of SNP linkage, a LD analysis of the significant SNPs resulted in the identification of linkage blocks within a 2.4 Mb range upstream and downstream of the loci. Blocks encompassing two or more significant SNPs were designated as quantitative trait loci (QTLs). A total of 30 QTL regions were identified and consolidated (Appendix J).

3.4. Candidate gene mining

Fifteen candidate genes were identified within the QTL physical intervals, which may be directly or indirectly involved in Cd uptake, transport, storage and regulation (Table 1). Most of these genes represent wheat homologues of known functional genes in rice, along with genes encompassing domains related to the heavy metal gene family. These genes are expected to play vital roles in the processes of Cd absorption, transport, defense, and regulation in wheat, particularly under Cd-induced stress.

Among the identified candidate genes, *TraesCS6A02G005700* is thought to be involved in controlling arsenic (As) accumulation and regulating wheat tolerance to As (V). *TraesCS6A02G194700* is an orthologue of rice *OsSULTR2*, which functions in sulfate transport and Zn/Fe homeostasis. The genes *TraesCS4A02G035900*, *TraesCS2B02G569300*, *TraesCS3B02G288600*, and *TraesCS6A02G240400* belong to the HMA, ZIP, and ABC transporter families, providing additional support for their roles in Cd uptake and transport in wheat. The rice orthologue of the gene *TraesCS4A02G040900*, known as *OsCLT1*, can impact the detoxification of As and Cd in rice. The remaining genes are associated with abiotic stress tolerance, zinc finger

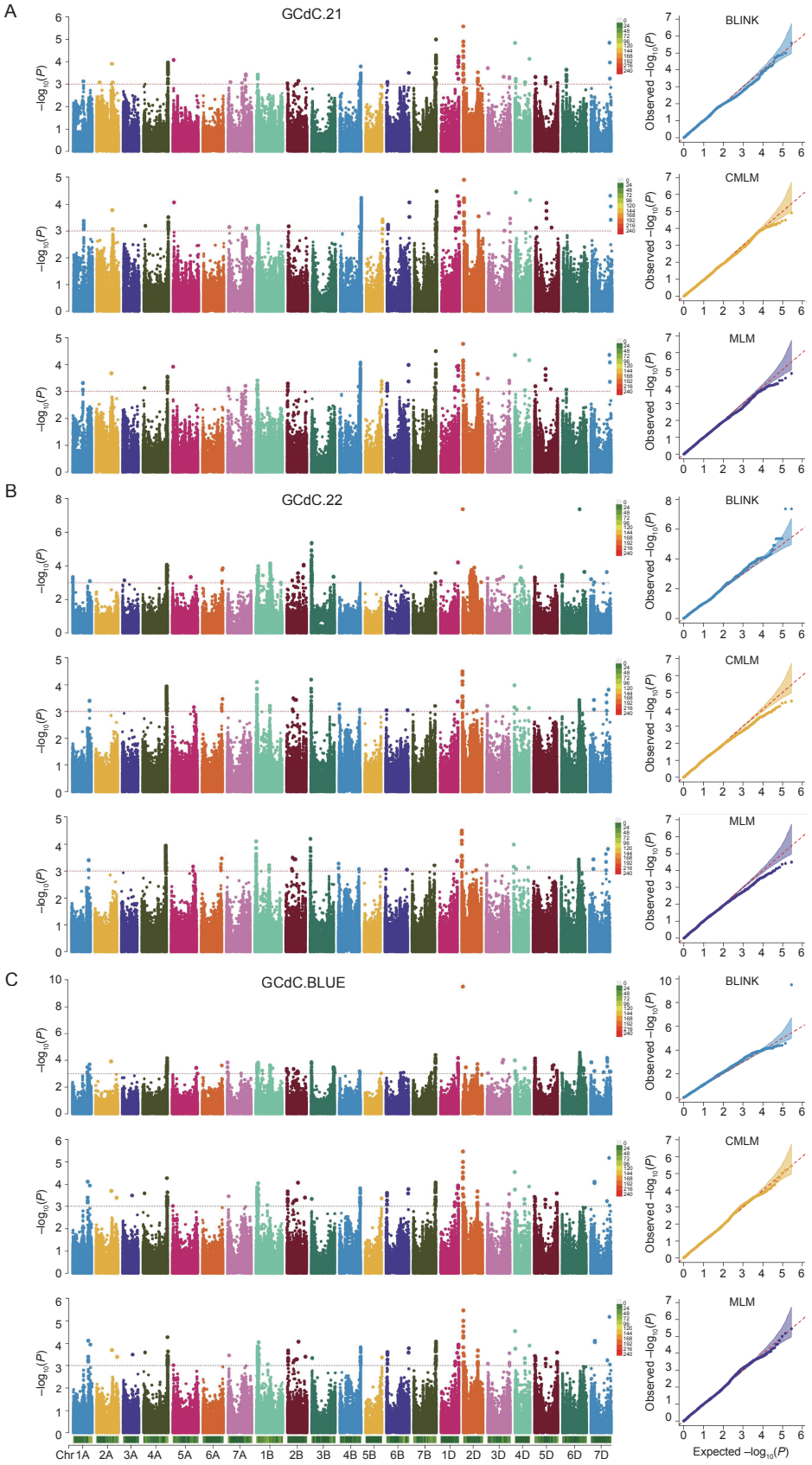


Fig. 3 Manhattan plots for grain cadmium concentration (GCdC) analyzed by the mixed linear model (MLM), the Compressed Linear Mixed Model (CMLM), and the Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway (BLINK) (red line). The threshold of P=1.0×10^{-3} (–log$_{10}$(P)=3.0) was used for calling significant SNPs. A and B, GCdC.21 and GCdC.22 indicate grain Cd concentrations in the two environments. C, best linear unbiased estimation (BLUE) indicates the best linear unbiased estimations across environments.

Table 1 Putative candidate genes associated within the QTL intervals[1)]

QTL region	Chr	Candidate gene	Physical position (Mb)		Gene annotation or coding protein	Orthologs[2)]
Nwafu.7B.1	7B	*TraesCS7B02G000200*	0.043	0.051	In the ABC transporter superfamily. PDR subfamily	*OsPDR12* (Zhou *et al.* 2013)
Nwafu.6A.1	6A	*TraesCS6A02G000700*	0.617	0.621	HSP40 abiotic stress tolerance	*HSP40* (Cui *et al.* 2016)
Nwafu.6A.2	6A	*TraesCS6A02G005700*	2.221	2.222	As (V) tolerance, control of arsenic (As) accumulation	NA
Nwafu.4A.2	4A	*TraesCS4A02G035900*	28.600	28.602	HMA-heavy metal transport	NA
Nwafu.4A.4	4A	*TraesCS4A02G040900*	34.047	34.052	CRT-like transporter 1	*OsCLT1* (Yang *et al.* 2016)
Nwafu.4A.5	4A	*TraesCS4A02G134800*	183.970	183.977	In the cation transport ATPase (P-type) family	NA
Nwafu.6A.6	6A	*TraesCS6A02G194700*	272.559	272.561	Low affinity sulphate transporter	*OsSULTR2* (Yang *et al.* 2022)
Nwafu.6A.8	6A	*TraesCS6A02G214500*	392.937	392.943	Vesicle transport protein	*GOT1B* (Wang *et al.* 2016)
Nwafu.6A.10	6A	*TraesCS6A02G240400*	451.660	451.662	ZIP I protein, transcription activator	NA
Nwafu.3B.3	3B	*TraesCS3B02G288600*	463.115	463.122	ABC transporter-ATP binding protein	NA
Nwafu.5D.4	5D	*TraesCS5D02G562000*	562.062	562.064	Heavy-metal-associated domain	NA
Nwafu.5D.4	5D	*TraesCS5D02G564000*	562.720	562.727	In the ABC transporter superfamily PDR subfamily	NA
Nwafu.2B.1	2B	*TraesCS2B02G569300*	759.818	759.819	Heavy-metal-associated domain	*OsHIPP24* (Chen and Xiong 2021)
Nwafu.2B.2	2B	*TraesCS2B02G569500*	760.118	760.120	Heavy metal transport/detoxification superfamily protein	*OsHIPP24* (Chen and Xiong 2021)
Nwafu.2B.2	2B	*TraesCS2B02G569600*	760.153	760.154	Heavy metal transport/detoxification superfamily protein	*OsHIPP24* (Chen and Xiong 2021)

[1)] Chr, chromosome. The physical positions of annotated genes were based on IWGSC RefSeq v.1.1; gene ID and functional annotations were based on IWGSC RefSeq v.1.1 and China Rice Recearch Center (https://www.ricedata.cn/).
[2)] NA, no known rice ortholog has been identified for this gene.

proteins, and antioxidation, but have not been extensively studied in model plants.

3.5. Biological basis and transcriptome analysis of differential Cd accumulation in wheat

Considering the notable differences in morphology and physiology among wheat genotypes, various samples of the plants were analyzed for Cd accumulation-related phenotypic traits using ICP-MS technology. The data presented in Fig. 4 depict the variations in Cd-related traits among different genotypes, where HS and LS represent genotypes with extremely high and low Cd accumulation, respectively. The whole-plant weight, weight of shoots and weight of roots of the LS genotype were significantly greater than those of the HS genotype (Fig. 4-A–C). Compared with LS, HS resulted in significantly greater whole-plant Cd concentrations (Fig. 4-D), with 24% greater Cd accumulation in HS than in LS (Fig. 4-E). Furthermore, the concentrations of Cd in the stems and roots of HS were significantly greater than those in LS (Fig. 4-F). The plant biomass and Cd concentration can be used to determine the Cd contents in both the aboveground and underground parts of plants. The results indicated significant increases in Cd accumulation in both the aboveground and underground parts of HS compared with those in LS (Fig. 4-H and I). A comparison of the transfer coefficient of Cd from the roots to the aboveground parts revealed that the value of HS was significantly greater than that of LS (Fig. 4-J). Moreover, the Cd contents in the grains of HS were significantly greater than those in the grains of LS (Fig. 4-K).

Based on the genomic and phenotypic differences, to further determine the differences in genotype-related Cd accumulation in wheat regulated by DEGs, high-throughput whole-genome transcriptome sequencing was performed on LS and HS. Principal component analysis revealed that in response to Cd toxicity, the two genotypes and their respective organs exhibited distinct transcriptional characteristics that were significantly different from those of the control (Fig. 5-A), indicating specific transcriptional responses to Cd toxicity based on genotype and organ specificity. In addition, the Pearson correlation coefficients among the RNA-seq groups revealed that irrespective of LS or HS designation, the correlation within a given organ (shoot/root) was significantly greater than that within a given treatment (control/Cd) (Fig. 5-B). LS plants exhibited a greater number of Cd-responsive DEGs in shoots and roots than the HS plants (Fig. 5-C). Furthermore, DEGs between HS and LS presented elevated expression levels in both shoots and roots, with LS demonstrating higher expression levels than HS under both the control and Cd conditions (Fig. 5-D). Venn diagram analysis revealed that 231 genes in the shoots and roots of LS and 47 genes in the shoots and roots of HS responded differentially to Cd toxicity (Fig. 5-E and F), suggesting that these 47 DEGs may serve as key regulatory factors for Cd transport from the roots to the aboveground parts. GO enrichment analysis revealed that the DEGs associated with the Cd response were associated primarily with influences on phenylpropane metabolism, zinc ion transmembrane transport, and divalent metal ion transmembrane transport, as were the responsive processes related to Cd and sulfate transmembrane transport activity (Appendix K-a). KEGG analysis revealed that under Cd toxicity, the DEGs were enriched primarily in ABC transporters, flavonoid biosynthesis, glutathione metabolism, and cysteine and

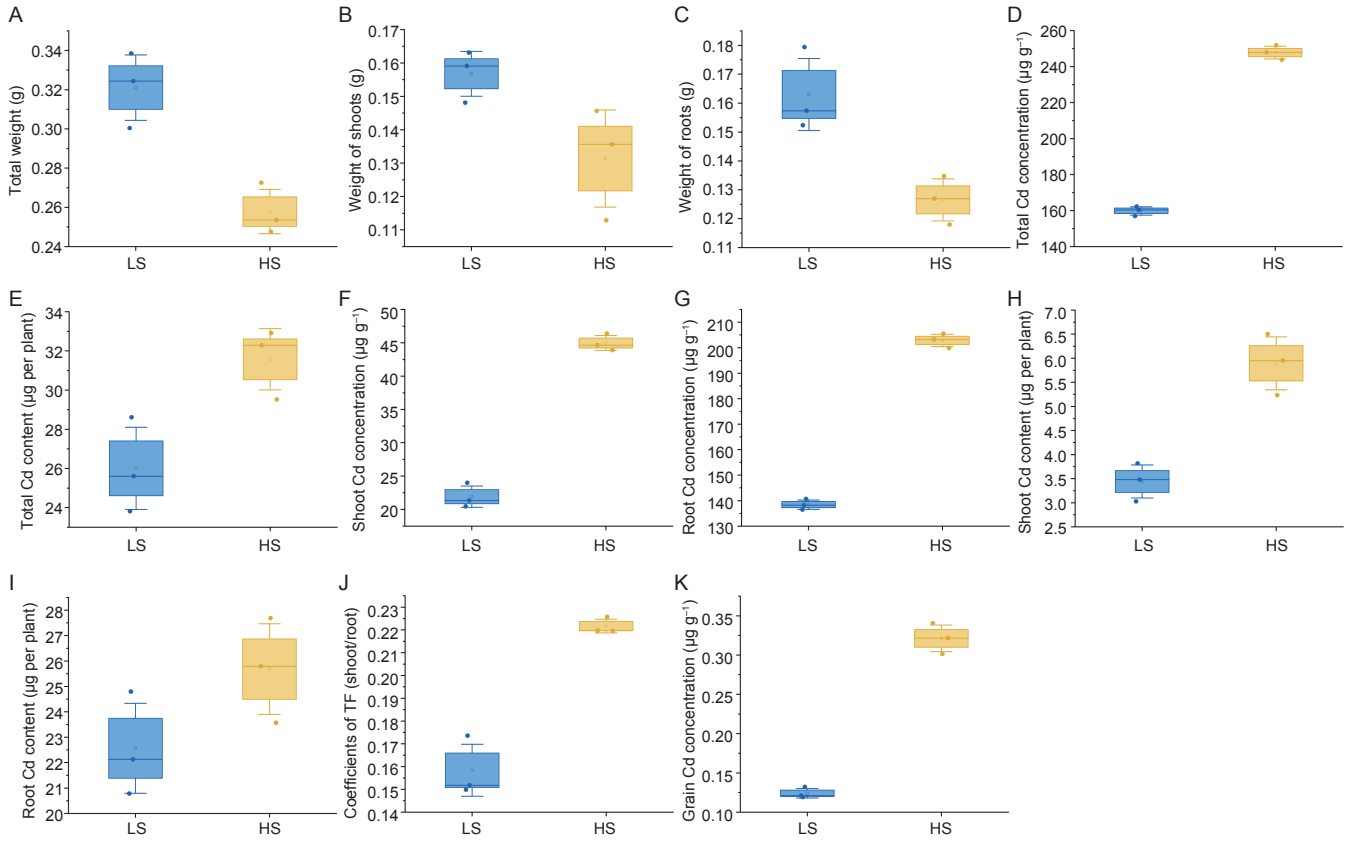


Fig. 4 Comparative analysis of cadmium (Cd) concentrations between low-accumulating variety (LS) and high-accumulating variety (HS). A, total plant weight. B, whole shoot weight. C, root weight. D, total plant Cd concentration. E, total plant Cd content. F, whole shoot Cd concentration. G, root Cd concentration. H, shoot Cd content. I, root Cd content. J, coefficient of Cd translocation from roots to shoots. K, wheat grain Cd concentration.

methionine metabolism; and, notably, the genes in the HS treatment were significantly upregulated, especially those in the ABC transporter pathway. This upregulation may be linked to the high accumulation characteristics as shown in Appendix K-b.

3.6. Analysis of candidate genes responsive to Cd stress

After analyzing the transcript levels of the 15 genes screened by GWAS, eight genes were expressed to varying degrees in both the roots and shoots of the extreme material. The remaining genes were not expressed. Therefore, the expression patterns of these eight genes and their orthologues were analyzed. The expression data for rice genes under Cd stress conditions were obtained from the RED database (http://expression.ic4r.org/). The genes *TraesCS2B02G569300* and *TraesCS5D02G564000* were significantly upregulated in the roots of the low-accumulating variety (LS), whereas their expression levels remained unchanged in the roots of the high-accumulating variety (HS). In addition, the expression of their orthologues was upregulated in rice roots. After 12 h of Cd stress, the gene *TraesCS4A02G035900* was upregulated in the roots of both HS and LS, with higher expression levels in HS than in LS (Appendix L-a). This expression pattern is similar to those of the rice orthologues. Furthermore, the genes *TraesCS2B02G569500* and *TraesCS2B02G569600* were upregulated in the shoots of LS, which differed from the expression patterns of their orthologues. In LS, the *TraesCS7B02G000200* gene was downregulated in both roots and shoots as the duration of Cd stress increased, following the same expression trend as its orthologue *OsPDR12* (Appendix L-b). Notably, the gene *TraesCS4A02G040900* was upregulated in both LS and HS shoots, with no observed changes in roots, and its orthologue gene *OsCLT1* was also significantly upregulated in rice shoots (Appendix L-b). Fewer genes were differentially expressed in HS than in LS. The upregulation of genes in LS may be due to the specific low-accumulation characteristics under these conditions, which confirms the reliability of these candidate genes.

3.7. Molecular characterization of the candidate genes

Phylogenetic analysis revealed that the six candidate genes could be effectively classified into two branches aligned with the related gene families (the HMAs and ABC gene families) in rice and *Arabidopsis* (Appendix M-a). Molecular characteristic predictions indicated that three genes do not contain any related motifs, whereas the remaining genes shared identical conserved motifs with the genes within their corresponding branches. For example, *TraesCS7B02G000200* and *TraesCS5D02G564000* display 12 identical conservative motifs and two conservative domains (ABC2_membrane and

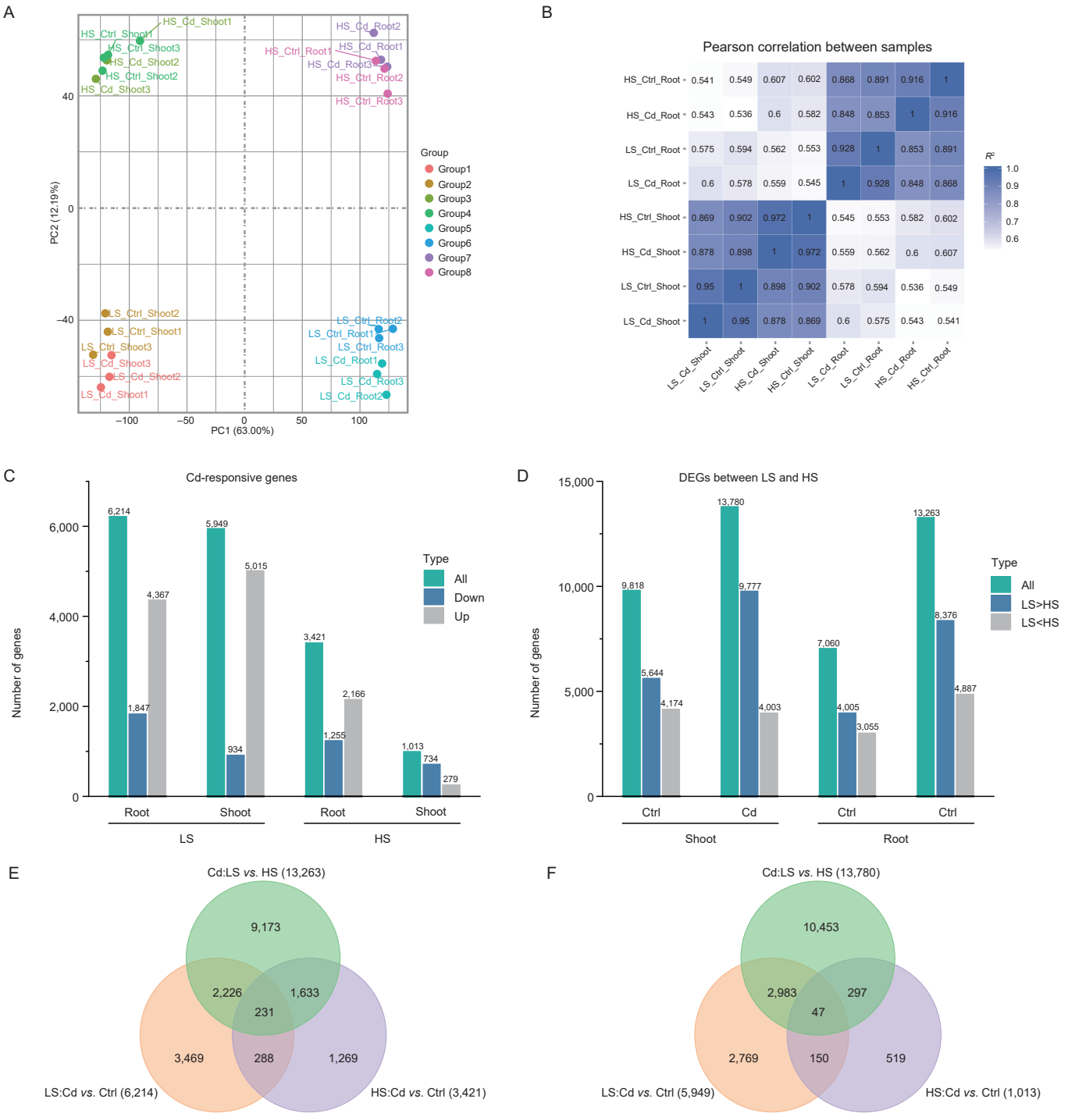


Fig. 5 Overview of transcriptome sequencing data for low-accumulating variety (LS) and high-accumulating variety (HS). A, principal component (PC) analysis of the RNA-seq samples. B, Circos diagram (http://circos.ca/) showing Pearson correlations between the RNA-seq samples. C and D, numbers of genome-wide Cd-responsive differential expressed genes (DEGs) in the shoots and roots between LS and HS. Up, upregulation; down, downregulation. E and F, Venn diagrams for DEGs in the shoots (E) and roots (F).

ABC_tran), both of which are part of the ABC transporter family (Appendix M-b and c). The gene *TraesCS4A02G040900* and its ortholog gene *OsCLT1* both share the same EamA superfamily structural domain. The gene *TraesCS4A02G035900* and its orthologue *Os03g0152000* possess two conserved HMA domains, which share the same structure as the HMA gene family in *Arabidopsis* and rice, thereby reinforcing their associations with the biological processes of Cd stress in wheat. Moreover, after 12 h of Cd stress, this gene (*TraesCS4A02G035900*) was significantly upregulated in the HS wheat samples, suggesting its potential role as a key gene mediating resistance to Cd stress in HS (Appendix L-a). Sequence characteristic predictions revealed that the candidate genes share the same conserved motifs as the functionally validated genes, further supporting their functional conservation.

Table 2 Candidate genes corresponding to the 660K marker locus

Chr	Candidate gene	SNP	Physical position (Mb)
7B	*TraesCS7B02G000200*	AX-111475888	0.046
4A	*TraesCS4A02G035900*	AX-108741888	28.603
4A	*TraesCS4A02G040900*	AX-94388233	34.050
5D	*TraesCS5D02G564000*	AX-108993493	562.726

3.8. KASP marker development and quantitative trait loci validation

Four candidate genes were identified based on RNA-seq analysis and direct homologue sequence feature comparison (Table 2). The 660K microarray was subsequently screened for differential SNPs located within or between the genes to develop high-throughput KASP markers. To assess the feasibility of these markers, the associated population was genotyped, resulting in the successful typing of two markers (4A: *XNK0900* and 4A: *XNK5900*) (Fig. 6-A; Appendix N). To ensure that these two markers could be used to screen wheat germplasms with low Cd accumulation and translocation in seeds, they were tested on a new population of 89 wheat germplasms. The results revealed significant differences in GCdC and TF among the plants with different alleles in the population (Fig. 6-B).

4. Discussion

4.1. Wheat cultivars of practical importance in farmland Cd pollution control

Due to increasing urbanization and industrialization, soil Cd pollution is becoming more pronounced. Cd is a nonessential heavy metal for plants that can have detrimental effects on plant growth (Ahmad *et al.* 2011). It can easily enter the food chain, jeopardizing both human health and agricultural ecology. To reduce Cd accumulation in wheat, soil remediation with heavy metal hyperaccumulating plants and intercropping with low-Cd wheat varieties is a common approach (Yaashikaa *et al.* 2022). The selection and breeding of low-Cd, high-yielding and disease-resistant wheat varieties is an effective measure for addressing soil Cd pollution and ensuring national food security (Shen *et al.* 2021). Current studies involving the intercropping of wheat with different plant species have shown that, compared with monoculture wheat, the accumulation of Cd in the shoots of wheat intercropped with *Solanum nigrum* L. increased by 1.13 times. In contrast, Cd accumulation in the shoots and roots of wheat significantly declined when it was intercropped with *Amaranthus mangostanus* L. and *Lolium perence* L. (Wang *et al.* 2015). In the present study, Ningdong 11 was identified as the variety with a low Cd transport coefficient and low grain Cd concentration, which is consistent with the results of previous studies (Appendix F). By studying the characteristics of 119 wheat genotypes under Cd stress, researchers have reported that the enrichment capacity of wheat plants for Cd decreases as the soil Cd concentration increases. The negative correlation between shoot and soil Cd concentrations was significantly stronger than that between roots, leaves and grains, indicating that the accumulation and transport of Cd in the shoots play a key role in the enrichment of Cd in the aboveground parts of wheat plants (Zhang L G *et al.* 2020). Luohan 7 was also identified

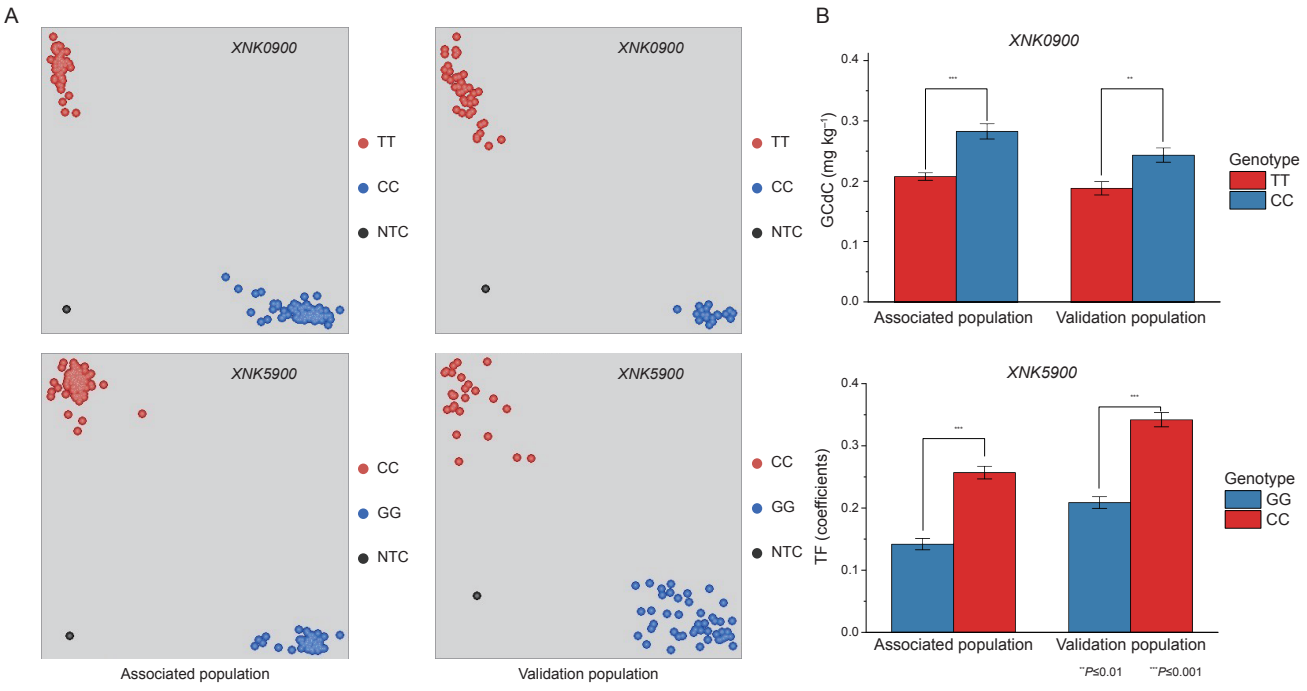


Fig. 6 KASP marker typing maps of the associated and validation populations and phenotypic validation of different genotypes. A, genotyping results of the *XNK0900* and *XNK5900* (KASP) markers in two populations. B, allelic effects of the two markers on grain Cd concentration (GCdC) and translocation factor (TF) in the best linear unbiased estimate (BLUE) environment. T and C indicate two pure genotypes of the marker, and NTC denotes the no-template control. Bars mean SD. ** and ***, $P<0.01$ and $P<0.001$.

as a preferred wheat variety with low accumulation at low to medium-high soil Cd concentrations, which aligns with the findings of this study (Appendix F) (Ren *et al.* 2022). In addition, this study identified six wheat germplasms with low cadmium accumulation. Serim 82 showed the lowest GCdC (0.069 mg kg^{-1}) and a yield of 4,035 kg ha^{-1}. Taishan 23 had the lowest TF, with a GCdC of 0.088 mg kg^{-1} and a yield of 3,624.45 kg ha^{-1}. Previous studies have demonstrated that intercropping wheat with *Sedum plumbizincicola* and rotating with eggplant had no significant impact on the biomass of wheat grains, eggplant plants, or aboveground rock cress. However, intercropping wheat with *S. plumbizincicola* during the wheat growing season significantly reduced the heavy metal concentration in wheat grains, with a reduction of 52.4% compared to wheat monoculture. This biodiversity strategy effectively reduced cadmium accumulation in wheat (Ju *et al.* 2015). In the existing low-Cd wheat germplasm bank, only a few low-Cd-accumulating wheat germplasms have been identified; therefore, there is an urgent need to screen additional low-Cd-accumulating wheat materials and to identify Cd-related genes to accelerate the breeding process. In this study, we evaluated the physiological significance of Cd toxicity in hexaploid wheat and assessed the phenotypic traits and Cd accumulation characteristics of 175 wheat materials with diverse genetic backgrounds when planted in heavy metal-contaminated fields.

4.2. Genetic structure profiling by quantitative trait loci

In recent years, significant progress has been made in our understanding of the genetic mechanisms of heavy metal accumulation in wheat (Safdar *et al.* 2020; Qiao *et al.* 2021; Yan *et al.* 2023). Through GWAS and biparental linkage mapping, several QTLs related to Cd, Pb, Fe, and Zn have been found and located on the wheat chromosomes. This study employed a strategy that combined population structure (Q) and a kinship matrix (K) for association analysis based on the three models, thus minimizing the rate of false positives (Yu *et al.* 2006). The GWAS identified 30 significantly associated QTLs, primarily on chromosomes 3B, 4A, 5D, and 6A. Compared with previous studies, this study identified several loci with consistent or similar positions. For example, *Nwafu.4A.1* and *Nwafu.5B.2* located on 4A and 5B were found to be at the same position as the QTL associated with the Cd translocator in wheat (Zaid *et al.* 2022). Three QTLs (*Nwafu.2A.1*, *Nwafu.5B.1*, and *Nwafu.6D.1*) were found to be the same as the QTL loci for Cd, Ca, and Zn uptake and translocation in wheat reported by Alexey *et al.* (2022). In addition, the QTL Nwafu.2A.1 on 2A was associated with the dynamic equilibrium of Zn in synthetic hexaploid wheat grains (Bhatta *et al.* 2018). In a study reported in 2022, Nwafu.7A.2 was colocalized with a QTL for iron uptake and transport in common wheat grains (Tone *et al.* 2022). The reliability of these QTLs is further supported by the fact that Cd ions can be transported into wheat cells *via* Zn and Fe transporters (Rizwan *et al.* 2016). This study did not identify the SNPs or QTLs reported in earlier studies, possibly because of the complexity of this trait and the use of different genotypes. Overall, colocalization with reported genetic loci confirms the reliability of our results, and the new QTLs are also important for regulating Cd accumulation in wheat. This study identified several potential loci for Cd uptake, translocation, accumulation and regulation in wheat under Cd stress, which are randomly distributed in natural populations. Germplasms with favorable alleles can be used as breeding resources, and the results provide a genetic basis for genomic selection methods.

In this study, the molecular mechanism of Cd accumulation in wheat was analyzed by RNA-seq. The results were similar to those of studies on Cd transport and accumulation in wheat (Zhang L *et al.* 2020; Hua *et al.* 2022), including many DEGs associated with ABC transporters, glutathione metabolism, divalent metal ion transmembrane transport, and phenylalanine metabolic pathways detected under Cd stress. Differential ion spectra demonstrated that reduced Cd transport is associated with lower stem Cd concentrations, which play a pivotal role in conferring resistance to Cd in LS compared with HS (Fig. 4). Several transport proteins have been reported to participate in Cd resistance and detoxification. The HMA and ABC gene families are crucial for Cd transportation from roots to stems in plants, and serve as a defense mechanism against Cd toxicity (Lv *et al.* 2020; Zhang L *et al.* 2020). A recent genome-wide comparative analysis confirmed the existence of large-scale convergent selection between the rice and maize genomes (Chen *et al.* 2022), suggesting that orthologues may be functionally conserved among gramineous crop species. Consequently, we collected the verified gene families in rice (*Oryza sativa*) and *Arabidopsis thaliana* and conducted systematic analyses of the phylogenetic trees and conserved motifs with the candidate genes. In addition, through RNA-seq analyses and comparisons of the sequence features of orthologues, we identified four genes that are predicted to be associated with Cd accumulation and transport in wheat: *TraesCS7B02G000200*, *TraesCS4A02G040900*, *TraesCS5D02G564000*, and *TraesCS4A02G035900*. *TraesCS4A02G035900* is located within the 4A QTL region, which contains proteins in the heavy metal transport/detoxification protein domain. In rice, *OSHMA2* is localized primarily in the midcolumn cells of the roots, and its functional absence leads to significant inhibition of Cd transport from the roots to the aboveground parts (Takahashi *et al.* 2012). The *TraesCS7B02G000200* and *TraesCS5D02G564000* genes, located on chromosomes 7B and 5D, respectively, belong to the ABC transporter-like domain-containing protein family. In *Arabidopsis*, the three ABCC-type transporters *ATABCC1*, *ATABCC2*, and *ATABCC3* increase plant tolerance to Cd by transferring phytochelatin (PC) and Cd chelates from the cytoplasm to vesicles (Park *et al.* 2012). Furthermore, *OsCLT1*, an orthologue of *TraesCS4A02G040900*, maintains glutathione homeostasis by exporting γ-glutamylcysteine and glutathione from the plastid to the cytoplasm, thereby affecting the detoxification of arsenic and Cd in rice. This evidence further supports the potential role of *TraesCS4A02G040900* in regulating Cd in wheat (Yang *et al.* 2016).

4.3. Applications for molecular markers

To date, few molecular markers are available for screening low-Cd-accumulating wheat genotypes. Researchers have developed an EST-derived marker (*XBF474090*) that co-segregates with genetic variation for Cd uptake traits, which has been successfully transformed into a co-dominant CAPS marker and designated as *usw47* (Knox *et al*. 2009; Wiebe *et al*. 2010). Researchers have also developed random amplified polymorphic DNA (RAPD) markers for Cd exclusion in wheat (Penner *et al*. 1995). *ScOpc20* is an SCAR marker that has been successfully used in the breeding of new wheat germplasms with reduced Cd uptake, although its application in backcross breeding is limited because it is a dominant marker associated with high Cd levels (Zimmerl *et al*. 2014). Researchers developed the molecular marker *K_AX_94741862* associated with Zn/Fe in wheat grains, but did not verify its utility for screening Cd levels in wheat grains (Tong *et al*. 2022). In contrast, we successfully developed GCdC- and TF-related KASP markers (*XNK0900* and *XNK5900*) (Fig. 6-A) and grouped the associated population of 175 wheat germplasms as well as the validation population of 89 wheat germplasms, revealing significant phenotypic differences between the two alleles (Fig. 6-B). The discovery of new QTL loci and candidate genes in this study has greatly enhanced our comprehension of the complex genetic basis involved in Cd uptake, translocation, accumulation and regulation in wheat. These findings can further facilitate the screening and application of low-Cd-accumulation wheat varieties.

5. Conclusion

This study successfully identified six wheat varieties with low Cd accumulation, which can serve as donor parents for the genetic improvement of wheat. A genome-wide association study (GWAS) revealed 30 quantitative trait loci (QTLs) associated with Cd, including 24 newly identified loci (11 in genome A, nine in genome B, and four in genome D). Four candidate genes that may directly affect the uptake, translocation, accumulation, and regulation of Cd in wheat were identified, suggesting new avenues for exploring the genetic complexity of Cd in wheat. In addition, KASP markers *(XNK0900* and *XNK5900)* were developed and validated for two significant QTLs on chromosome 4A, demonstrating the potential application of these markers in the genetic improvement of Cd in wheat. Overall, this study provides valuable germplasm resources and molecular marker tools that can facilitate the effective screening and breeding of wheat varieties with low Cd accumulation characteristics.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

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References

AbuHammad W A, Mamidi S, Kumar A, Pirseyedi S, Manthey F A, Kianian S F, Alamri M S, Mergoum M, Elias E M. 2016. Identification and validation of a major cadmium accumulation locus and closely associated SNP markers in North Dakota durum wheat cultivars. *Molecular Breeding*, **36**, 112.

Adomako, Opoku M, Yu F. 2023. Effects of resource availability on the growth, Cd accumulation, and photosynthetic efficiency of three hyperaccumulator plant species. *Journal of Environmental Management*, **345**, 118762.

Ahmad M S, Ashraf M, Tabassam Q, Hussain M, Firdous H. 2011. Lead (Pb)-induced regulation of growth, photosynthesis, and mineral nutrition in maize (*Zea mays* L.) plants at early growth stages. *Biological Trace Element Research*, **144**, 1229–1239.

Ai H, Wu D X, Li C L, Hou M M. 2022. Advances in molecular mechanisms underlying cadmium uptake and translocation in rice. *Frontiers in Plant Science*, **13**, 1003953.

Alexey M, Timur S, Paulina F, Adylkhan B, Vladimir C, Anastasiya K, Hamit K, Ivan L, Ram S, Tatyana S, Sergey S, Ekaterina S, Vladimir S. 2022. Effects of environments and cultivars on grain ionome of spring wheat grown in Kazakhstan and Russia. *Crop and Pasture Science*, **73**, 515–527.

Alvarez-Fernandez A, Bernal M J, Fradejas I, Martin Ramirez A, Md Yusuf N A, Lanza M, Hisam S, Perez de Ayala A, Rubio J M. 2021. KASP: A genotyping method to rapid identification of resistance in Plasmodium falciparum. *Malaria Journal*, **20**, 16.

Bhatta M, Baenziger P S, Waters B M, Poudel R, Belamkar V, Poland J, Morgounov A. 2018. Genome-wide association study reveals novel genomic regions associated with 10 grain minerals in synthetic hexaploid wheat. *International Journal of Molecular Sciences*, **19**, 3237.

Chen W K, Chen L, Zhang X, Yang N, Guo J H, Wang M, Ji S H, Zhao X Y, Yin P F, Cai L C, Xu J, Zhang L L, Han Y J, Xiao Y N, Xu G, Wang Y B, Wang S H, Wu S, Yang F, Jackson D, *et al.* 2022. Convergent selection of a WD40 protein that enhances grain yield in maize and rice. *Science*, **375**, 1372.

Ci D W, Jiang D, Li S S, Wollenweber B, Dai T B, Cao W X. 2012. Identification of quantitative trait loci for cadmium tolerance and accumulation in wheat. *Acta Physiologiae Plantarum*, **34**, 191–202.

Ci D W, Jiang D, Wollenweber B, Dai T B, Jing Q, Cao W X. 2010. Cadmium stress in wheat seedlings: Growth, cadmium accumulation and photosynthesis. *Acta Physiologiae Plantarum*, **32**, 365–373.

Fu Z Y, Song J C, Jameson P E. 2017. A rapid and cost effective protocol for plant genomic DNA isolation using regenerated silica columns in combination with CTAB extraction. *Journal of Integrative Agriculture*, **16**, 1682–1688.

GB 2762-2017. 2017. National food safety standard: Maximum levels for contaminants in foods. China Food and Drug Administration. Standards Press of China. pp. 4–5. (in Chinese)

Guttieri M J, Baenziger P S, Frels K, Carver B, Arnall B, Wang S C, Akhunov E, Waters B M. 2015. Prospects for selecting wheat with increased zinc and decreased cadmium concentration in grain. *Crop Science*, **55**, 1712–1728.

Hua Y P, Chen J F, Zhou T, Zhang T Y, Shen D D, Feng Y N, Guan P D, Huang S M, Zhou Z F, Huang J Y, Yue C P. 2022. Multiomics reveals an essential role of long-distance cadmium translocation in regulating plant cadmium resistance and grain cadmium accumulation in allohexaploid wheat (*Triticum aestivum* L.). *Journal of Experimental Botany*, **73**, 7516–7537.

Huang M, Liu X L, Zhou Y, Summers R M, Zhang Z W. 2019. BLINK: A package for the next level of genome-wide association studies with both individuals and markers in the millions. *Gigascience*, **8**, giy154.

Ishimaru Y, Bashir K, Nakanishi H, Nishizawa N K. 2012. OsNRAMP5,

a major player for constitutive iron and manganese uptake in rice. *Plant Signaling & Behavior*, **7**, 763–766.
Ju S Y, Wang J, Sehn L B, Li Z, Chen Y H, Wu L H, Luo Y M. 2015. Phytoremediation of heavy metal contaminated soils by intercropping with *Sedum plumbi* zincicola and *Triticum aestivum* and rotation with *Solanum melongena*. *Chinese Journal of Ecology*, **8**, 2181–2186. (in Chinese)
Kaewcheenchai R, Vejchasarn P, Hanada K, Shirai K, Jantasuriyarat C, Juntawong P. 2021. Genome-wide association study of local Thai *indica* rice seedlings exposed to excessive iron. *Plants-Basel*, **10**, 798.
Kim D, Paggi J M, Park C, Bennett C, Salzberg S L. 2019. Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. *Nature Biotechnology*, **37**, 907–915.
Knox R E, Pozniak C J, Clarke F R, Clarke J M, Houshmand S, Singh A K. 2009. Chromosomal location of the cadmium uptake gene (*Cdu1*) in durum wheat. *Genome*, **52**, 741–747.
Liu L, Zuo X, Zhu Z, Wen L, Yang C, Zhu C, Tang L, Cheng Y, Chen M, Zhou F, Zheng X, Wang W, Yin X, Tang H, Sun L, Yang S, Sheng Y, Cui Y, Zhang X. 2018. Genome-wide association study identifies three novel susceptibility loci for systemic lupus erythematosus in Han Chinese. *British Journal of Dermatology*, **179**, 506–508.
Love M I, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, **15**, 550.
Ludwig Y, Slamet-Loedin I H. 2019. Genetic biofortification to enrich rice and wheat grain iron: From genes to product. *Frontiers in Plant Science*, **10**, 833.
Lv Q Y, Han M L, Gao Y Q, Zhang C Y, Wang Y L, Chao Z F, Zhong L L, Chao D Y. 2020. Sec24C mediates a Golgi-independent trafficking pathway that is required for tonoplast localization of ABCC1 and ABCC2. *The New Phytologist*, **235**, 1486–1500.
Maccaferri M, Harris N S, Twardziok S O, Pasam R K, Gundlach H, Spannagl M, Ormanbekova D, Lux T, Prade V M, Milner S G, Himmelbach A, Mascher M, Bagnaresi P, Faccioli P, Cozzi P, Lauria M, Lazzari B, Stella A, Manconi A, Gnocchi M, *et al.* 2019. Durum wheat genome highlights past domestication signatures and future improvement targets. *Nature Genetics*, **51**, 885–895.
Maiti S K, Jaiswal S. 2008. Bioaccumulation and translocation of metals in the natural vegetation growing on fly ash lagoons: A field study from Santaldih thermal power plant, West Bengal, India. *Environmental Monitoring and Assessment*, **136**, 355–370.
Miyadate H, Adachi S, Hiraizumi A, Tezuka K, Nakazawa N, Kawamoto T, Katou K, Kodama I, Sakurai K, Takahashi H, Satoh-Nagasawa N, Watanabe A, Fujimura T, Akagi H. 2011. OsHMA3, a P_{1B}-type of ATPase affects root-to-shoot cadmium translocation in rice by mediating efflux into vacuoles. *New Phytologist*, **189**, 190–199.
Naserkheil M, Lee D H, Mehrban H. 2020. Improving the accuracy of genomic evaluation for linear body measurement traits using single-step genomic best linear unbiased prediction in Hanwoo beef cattle. *BMC Genetics*, **21**, 144.
Pang Y, Liu C, Wang D, St Amand P, Bernardo A, Li W, He F, Li L, Wang L, Yuan X, Dong L, Su Y, Zhang H, Zhao M, Liang Y, Jia H, Shen X, Lu Y, Jiang H, Wu Y, *et al.* 2020. High-resolution genome-wide association study identifies genomic regions and candidate genes for important agronomic traits in wheat. *Molecular Plant*, **13**, 1311–1327.
Park W H, Han K H, Ahn S J. 2012. Differences in root-to-shoot Cd and Zn translocation and by HMA3 and 4 could influence chlorophyll and anthocyanin content in *Arabidopsis* Ws and Col-0 ecotypes under excess metals. *Soil Science and Plant Nutrition*, **58**, 334–348.
Penner G A, Bezte L J, Leisle D, Clarke J. 1995. Identification of RAPD markers linked to a gene governing cadmium uptake in durum wheat. *Genome*, **38**, 543–547.
Qiao L, Wheeler J, Wang R, Isham K, Klassen N, Zhao W, Su M, Zhang J, Zheng J, Chen J. 2021. Novel quantitative trait loci for grain cadmium content identified in hard white spring wheat. *Frontiers in Plant Science*, **12**, 756741.
Qiu X, Pang Y, Yuan Z, Xing D, Xu J, Dingkuhn M, Li Z, Ye G. 2015. Genome-wide association study of grain appearance and milling quality in a worldwide collection of *indica* rice germplasm. *PLoS ONE*, **10**, e0145577.
Ramirez-Gonzalez R H, Uauy C, Caccamo M. 2015. PolyMarker: A fast polyploid primer design pipeline. *Bioinformatics*, **31**, 2038–2039.
Ren C, Ren Y Z, Wang H, Zhu L W, Li J T, Du Q Q, Li P. 2022. Cadmium accumulation characteristics of different heat varieties under cadmium stress. *Environmental Science*, **43**, 1606–1619. (in Chinese)
Reyes-Hinojosa D, Lozada-Perez C A, Zamudio Cuevas Y, Lopez-Reyes A, Martinez-Nava G, Fernandez-Torres J, Olivos-Meza A, Landa-Solis C, Gutierrez-Ruiz M C, Rojas Del Castillo E, Martinez-Flores K. 2019. Toxicity of cadmium in musculoskeletal diseases. *Environmental Toxicology and Pharmacology*, **72**, 103219.
Rizwan M, Ali S, Abbas T, Zia-ur-Rehman M, Hannan F, Keller C, Al-Wabel M I, Ok Y S. 2016. Cadmium minimization in wheat: A critical review. *Ecotoxicology and Environmental Safety*, **130**, 43–53.
Safdar L B, Almas F, Sarfraz S, Ejaz M, Ali Z, Mahmood Z, Yang L, Tehseen M M, Ikram M, Liu S, Quraishi U M. 2020. Genome-wide association study identifies five new cadmium uptake loci in wheat. *Plant Genome*, **13**, e20030.
Sarantopoulou D, Tang S Y, Ricciotti E, Lahens N F, Lekkas D, Schug J, Guo X F S, Paschos G K, FitzGerald G A, Pack A, Grant G R. 2019. Comparative evaluation of RNA-Seq library preparation methods for strand-specificity and low input. *Scientific Reports*, **9**, 13477.
Shen X, Dai M, Yang J, Sun L, Tan X, Peng C, Ali I, Naz I J C. 2021. A critical review on the phytoremediation of heavy metals from environment: Performance and challenges. *Chemosphere*, **291**, 132979.
Song P P, Xu D, Yue J Y, Ma Y C, Dong S J, Feng J. 2022. Recent advances in soil remediation technology for heavy metal contaminated sites: A critical review. *The Science of the Total Environment*, **838**, 156417.
Suneja Y, Gupta A K, Bains N S. 2017. Bread wheat progenitors: *Aegilops tauschii* (DD genome) and *Triticum dicoccoides* (AABB genome) reveal differential antioxidative response under water stress. *Physiology and Molecular Biology of Plants*, **23**, 99–114.
Takahashi R, Ishimaru Y, Shimo H, Ogo Y, Senoura T, Nishizawa N K, Nakanishi H. 2012. The OsHMA2 transporter is involved in root-to-shoot translocation of Zn and Cd in rice. *Plant, Cell & Environment*, **35**, 1948–1957.
Tong J, Zhao C, Sun M, Fu L, Song J, Liu D, Zhang Y, Zheng J, Pu Z, Liu L, Rasheed A, Li M, Xia X, He Z, Hao Y. 2022. High resolution genome wide association studies reveal rich genetic architectures of grain zinc and iron in common wheat (*Triticum aestivum* L.). *Frontiers in Plant Science*, **13**, 840614.
VanRaden P M. 2008. Efficient methods to compute genomic predictions. *Journal of Dairy Science*, **91**, 4414–4423.
Voss-Fels K, Snowdon R J. 2016. Understanding and utilizing crop genome diversity *via* high-resolution genotyping. *Plant Biotechnology Journal*, **14**, 1086–1094.
Wang S, Wei S, Ji D, Bai J. 2015. Co-planting Cd contaminated field using hyperaccumulator *Solanum nigrum* L. through interplant with low accumulation welsh onion. *International Journal of Phytoremediation*, **17**, 879–884.
Wang Y F, Su Y, Lu S G. 2020. Predicting accumulation of Cd in rice (*Oryza sativa* L.) and soil threshold concentration of Cd for rice safe production. *Science of the Total Environment*, **738**, 139805.
Wiebe K, Harris N S, Faris J D, Clarke J M, Knox R E, Taylor G J, Pozniak C J. 2010. Targeted mapping of *Cdu1*, a major locus regulating grain cadmium concentration in durum wheat (*Triticum turgidum* L. var durum). *Theoretical and Applied Genetics*, **121**, 1047–1058.
Wu T, Hu E, Xu S, Chen M, Guo P, Dai Z, Feng T, Zhou L, Tang W, Zhan L, Fu X, Liu S, Bo X, Yu G. 2021. clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. *Innovation* (Camb), **2**, 100141.
Yaashikaa P R, Kumar P S, Jeevanantham S, Saravanan R. 2022. A review on bioremediation approach for heavy metal detoxification and accumulation in plants. *Environmental Pollution*, **301**, 119035.
Yan H, Guo H, Xu W, Dai C, Kimani W, Xie J, Zhang H, Li T, Wang F, Yu Y, Ma M, Hao Z F, He Z. 2023. GWAS-assisted genomic prediction of cadmium accumulation in maize kernel with machine learning and linear statistical methods. *Journal of Hazardous Materials*, **441**, 129929.
Yang J, Gao M X, Hu H, Ding X M, Lin H W, Wang L, Xu J M, Mao C Z, Zhao F J, Wu Z C. 2016. OsCLT1, a CRT-like transporter 1, is required for glutathione homeostasis and arsenic tolerance in rice. *New Phytologist*, **211**, 658–670.

Yu J, Pressoir G, Briggs W H, Bi I V, Yamasaki M, Doebley J F, McMullen M D, Gaut B S, Nielsen D M, Holland J B, Kresovich S, Buckler E S. 2006. A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nature Genetics*, **38**, 203–208.

Zaid I U, Muhammad S H, Zhang N, Zheng X, Wang L K, Li X F. 2022. Phenotypic variations of wheat cultivars from the North China Plain in response to cadmium stress and associated single nucleotide polymorphisms identified by a genome-wide association study. *Pedosphere*, **32**, 555–564.

Zhang J, Zhao J, Liang Y, Zou Z. 2015. Genome-wide association-mapping for fruit quality traits in tomato. *Euphytica*, **207**, 439–451.

Zhang L, Gao C, Chen C, Zhang W, Huang X Y, Zhao F J. 2020. Overexpression of rice *OsHMA3* in wheat greatly decreases cadmium accumulation in wheat grains. *Environmental Science & Technology*, **54**, 10100–10108.

Zhang L G, Zhang C, Du B Y, Lu B X, Zhou D M, Zhou J, Zhou J. 2020. Effects of node restriction on cadmium accumulation in eight Chinese wheat (*Triticum turgidum*) cultivars. *Science of the Total Environment*, **725**, 138358.

Zhang Z W, Ersoz E, Lai C Q, Todhunter R J, Tiwari H K, Gore M A, Bradbury P J, Yu J M, Arnett D K, Ordovas J M, Buckler E S. 2010. Mixed linear model approach adapted for genome-wide association studies. *Nature Genetics*, **42**, 355–360.

Zhang W Y, Guan M Y, Chen M X, Lin X Y, Xu, P & Cao, Z Z. 2024. Mutation of reduces cadmium xylem and phloem transport in rice plants and its physiological mechanism. *Environmental Pollution,* **341**, 122928.

Zhao J, Yang W, Zhang S, Yang T, Liu Q, Dong J, Fu H, Mao X, Liu B. 2018. Genome-wide association study and candidate gene analysis of rice cadmium accumulation in grain in a diverse rice collection. *Rice*, **11**, 61.

Zhao X, Luo L, Cao Y, Liu Y, Li Y, Wu W, Lan Y, Jiang Y, Gao S, Zhang Z, Shen Y, Pan G, Lin H. 2018. Genome-wide association analysis and QTL mapping reveal the genetic control of cadmium accumulation in maize leaf. *BMC Genomics*, **19**, 91.

Zimmerl S, Lafferty J, Buerstmayr H, Hartl L. 2014. Assessing diversity in *Triticum durum* cultivars and breeding lines for high versus low cadmium content in seeds using the CAPS marker *usw47*. *Plant Breeding*, **133**, 712–717.

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