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Assessing Diversity of Russian Durum Wheat Cultivars and Breeding Germplasm for Grain Heavy Metal Content Using GWAS

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Abstract

Heavy metal contamination of soil adversely affects wheat growth and productivity, as well as human health when consuming the wheat products. This study aimed to identify the genomic regions associated with grain cadmium (GCdC), lead (GPbC), and chromium (GCrC) contents in a panel of Russian durum wheat varieties by means of genome-wide association study (GWAS). Using the BLINK, FarmCPU, and MLM models, 29, 22, and 14 significant marker–trait associations were identified for grain Cd, Pb, and Cr concentration respectively. GWAS showed that most of the loci were trait-specific, indicating that the genetic architecture underlying the accumulation of individual metals differs significantly. Prominent association was detected on chromosome 5B, where two loci (*GCdC-icg-5B-1* and *GCdC-icg-5B-2*) showed strong relations with grain cadmium accumulation. Locus *GCdC-icg-5B-1* exhibited associations with both grain cadmium and lead content. Ten closely linked SNPs within the *GCdC-icg-5B-2* locus formed two haplotypes—CCAGGCTGAC (Haplotype_1) and ATCAAACAGT (Haplotype_2), among which Haplotype_1 was associated with reduced grain cadmium content. Analysis of the association between allelic variants of the known *TdHMA3* gene and grain Cd content revealed strong linkage between *TdHMA3* alleles and a group of markers within the *GCdC-icg-5B-2* locus. This study provides new insights into the genetic architecture of heavy metal accumulation in durum wheat grains.

Keywords: durum wheat; *Triticum durum*; heavy metal; cadmium; lead; chromium; GWAS

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1. Introduction

Currently, the intensification of crop cultivation, aimed at increasing yields and productivity, has brought the problem of the heavy metal (HM) contamination of agricultural soils to the forefront. The main pollutants include lead, cadmium, copper, zinc, nickel, and mercury, which enter the environment because of large-scale mining operations, ferrous and non-ferrous metallurgy, oil refining vehicle traffic, and other anthropogenic activities [1–3]. In agricultural regions, the concentration and bioavailability of heavy metals are influenced by the physicochemical properties of the soil; these properties can be negatively affected by factors such as the atmospheric deposition of heavy metals, the application of organic and mineral fertilizers, increased soil acidity, and irrigation with wastewater [4–6].

Soil contamination with heavy metals has been reported in most countries around the world. The highest levels of cadmium contamination in agricultural soils have been observed in northern and central regions of India, Pakistan, Bangladesh, Turkey, Ethiopia, South Africa, and Mexico [7]. In coal-related industrial regions in Poland, the concentrations of zinc (Zn), lead (Pb), and cadmium (Cd) in soil are 28, 12, and 33 times higher, respectively, than the EU soil quality standards [2]. Heavy metal contamination is also a serious issue in the United States and China, where Cd and Pb concentrations in industrial regions exceed the national soil quality standards by an order of magnitude [1,8,9]. In the Russian Federation, the total area of agricultural land contaminated with highly toxic HMs exceeds 3 million hectares; a significant portion of this area is located in the European part of Russia (Krasnodarskii Krai and Rostovskaya Oblast) near major industrial centers [10,11].

Unlike micro- and macroelements (magnesium, zinc, iron, copper, selenium), heavy metals such as cadmium, lead, and chromium are not essential for normal physiological processes in plants, humans, or animals. When present in soil, they can inhibit plant growth and development even at low concentrations, affecting seed germination, suppressing root growth, and delaying phenological development [12,13]. Heavy metal-induced oxidative stress leads to cell membrane damage and the degradation of photosynthetic pigments, thereby negatively affecting respiration and photosynthesis and reducing biomass accumulation [14–16].

Heavy metals adversely affect a wide range of biological processes in humans, primarily through the induction of oxidative stress, increased production of reactive oxygen species, DNA damage, and inhibition of detoxification enzymes [17,18]. Chronic exposure to heavy metals, even at low doses, can cause damage to multiple organs and increase cancer risk [19,20]. Cadmium is considered one of the most toxic heavy metals. Chronic exposure to Cd can impair kidney and liver function and increase the risk of malignant tumors [21,22]. Cadmium is eliminated from the body very slowly; prolonged exposure can disrupt calcium metabolism and bone mineralization, increasing the risk of osteoporosis, bone deformities, and spinal curvature. Lead is a highly toxic, potentially carcinogenic metal; exposure to Pb can cause nervous system dysfunction, anemia, hypertension, and neurobehavioral disorders [20,23]. Hexavalent chromium is also classified as a human carcinogen, particularly associated with lung cancer, and can cause kidney and liver damage, as well as various allergic reactions [24,25].

Toxic metals enter the human body primarily through food, with products derived from common and durum wheat being a major source of calories worldwide. Studies conducted in various countries have shown that the concentrations of cadmium, lead, and chromium in wheat grain and flour can exceed the established permissible limits [26–30].

Breeding wheat genotypes with reduced heavy metal accumulation in grain is an important strategy for improving food safety. Screening of common and durum wheat varieties has revealed significant genotypic variation in grain heavy metal concentrations [28,31–33]. To develop genotypes with a low grain HM content, it is necessary to study the genetic control of metal accumulation, identify associated genes and quantitative trait loci (QTLs), and develop molecular markers for marker-assisted selection. The search for genes and QTLs associated with the accumulation of heavy metals in grain has been ongoing since the late 20th century. To date, numerous QTLs associated with the content of micro- and macroelements and heavy metals in common and durum wheat grain have been identified using molecular markers [34–41].

Recently, genome-wide association studies (GWAS) using high-density SNP arrays have enabled the identification of marker–trait associations, facilitating more precise mapping of target loci and the development of markers for plant breeding. The application of

GWAS has led to the identification of novel loci and candidate genes associated with heavy metal tolerance and the accumulation of cadmium, lead, and other HMs in the grain [42–46].

The Russian Federation is a major producer and exporter of both common and durum wheat. With a sown area of approximately 1.2 million hectares and an annual grain production of approximately 2.5 million tonnes, durum wheat ranks as the fourth most important cereal crop in the Russian Federation, following common wheat, barley, and maize [47]. The recent growth in durum wheat production in Russia has been driven by an increase in domestic consumption and growing exports, reaching 1.5 million tonnes. However, despite the importance of this crop, the genetic diversity of Russian durum wheat varieties with respect to grain heavy metal accumulation remains largely unexplored. The objectives of this study were: (i) to assess genotypic variations in grain cadmium, lead, and chromium content in a collection of Russian durum wheat varieties and breeding lines; and (ii) to conduct a genome-wide association study (GWAS) to identify genomic loci underlying the variability of these traits.

2. Results

2.1. Phenotyping

Grain concentrations of all three metals varied substantially across both experimental sites and years (Figure 1, Table S2). The distribution of most traits deviated from normal, indicating pronounced skewness and the presence of outliers. The exception was the cadmium content in the Novosibirsk region in both years, the chromium content in Bezenchuk in 2023, and lead content in Novosibirsk in 2023.

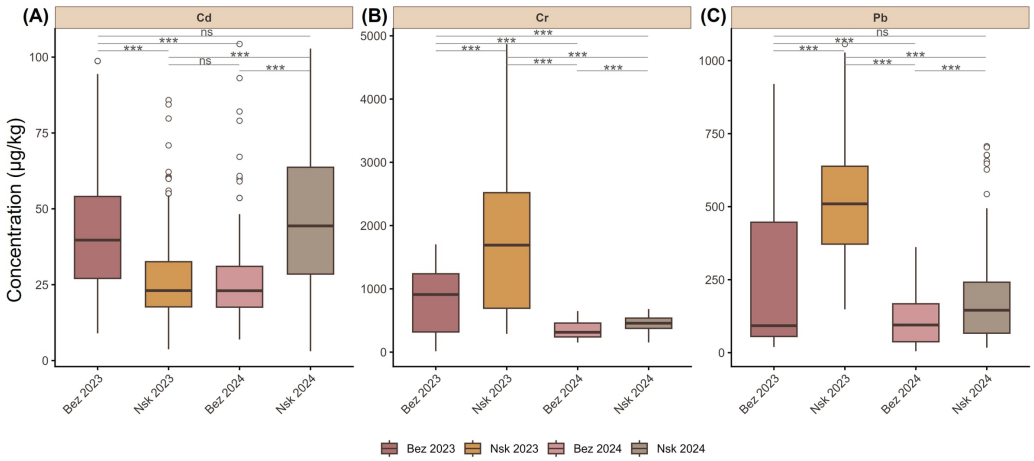

Figure 1. Distribution of Cd, Cr, and Pb concentrations in wheat grain across two experimental sites and years. Boxplots show the variation in (**A**) cadmium (Cd), (**B**) chromium (Cr), and (**C**) lead (Pb) concentrations measured at the Bezenchuk (Bez) and Novosibirsk (Nsk) experimental fields in 2023 and 2024. The horizontal line within each box indicates the median, the box represents the interquartile range, whiskers extend to values within 1.5 interquartile ranges, and individual points denote outliers. *** $p < 0.001$, ns—nonsignificant.

Cd concentrations ranged from 3.08 to 104.30 µg/kg (Figure 1A). According to the Technical Regulation of the Customs Union (TR CU 015/2011) [48], the maximum permissible concentration (MPC) of cadmium in cereal grain intended for human consumption is 0.1 mg/kg. Only three genotypes slightly exceeded this limit: Line 512 (100.9 µg/kg) and Line 513 (102.8 µg/kg) grown in the Novosibirsk region in 2024 and the variety ISD19 (104.3 µg/kg) grown in Bezenchuk in 2024. Mean Cd concentrations differed between environments, with the lowest mean observed in Novosibirsk in 2023 (27.77 µg/kg) and the highest in Novosibirsk in 2024 (46.61 µg/kg).

Pb concentrations exhibited greater variation across environments than Cd, ranging from 4.93 to 1057.00 µg/kg (Figure 1C). The MPC for lead in cereal grain intended for human consumption is 0.5 mg/kg (TR CU 015/2011). Exceedances of this limit were particularly frequent among genotypes grown on both experimental fields in 2023, with the mean Pb concentration in Novosibirsk reaching 521.94 µg/kg (Table S2). In 2024, only eight accessions (Bezenchukskaya 210, Gord1082, Mech, Real, SI Atlante, 804d_94, Leuk3462, ISD22) grown in Novosibirsk exceeded the regulatory limit, whereas Pb concentrations in Bezenchuk in all analyzed accessions remained below the permissible level.

Unlike Cd and Pb, chromium is currently not regulated under the Technical Regulation (TR CU 015/2011) for cereal grains intended for human consumption. However, in the Russian Federation, the approximate permissible concentration (APC) of chromium is 0.5 mg/kg [49]. Cr concentrations exhibited the widest range of variation among the analyzed metals, ranging from 13.53 to 4870.00 µg/kg (Figure 1B). Particularly high concentrations were observed in Novosibirsk in 2023, where both the mean (1711.68 µg/kg) and maximum concentrations greatly exceeded those recorded in the other environments. In contrast, Cr concentrations were considerably lower in 2024 at both locations, with mean values of 343.85 and 447.73 µg/kg in Bezenchuk and Novosibirsk, respectively.

Based on the two-year mean values, no statistically significant differences in grain Cd content were observed between the Bezenchuk (34.64 µg/kg) and Novosibirsk regions (37.09 µg/kg). In contrast, the mean lead content was significantly higher in Novosibirsk than in Bezenchuk (365.60 vs. 171.89 µg/kg, $p < 0.000001$), representing a 2.1-fold difference. Similarly, the mean grain chromium content was 1.86-fold higher in the Novosibirsk field than in the Bezenchuk field (1081.94 vs. 583.23, $p < 0.000001$).

Analysis of variance (ANOVA) showed a greater contribution of genotype than environment to variation in grain Cd concentration (Table S3). Broad-sense heritability for Cd was high ($H^2 = 0.73$). However, the genotype × environment interaction also contributed substantially to phenotypic variation. ANOVA indicated a lower contribution of genotype to the content of lead and chromium compared to cadmium. Broad-sense heritability for Pb and Cr contents was moderate at 0.58 and 0.57, respectively, which may reflect the substantial contributions of environmental effect and genotype × environment interactions.

Spearman correlation analysis of the mean trace metal content in grain across each region revealed a moderate positive correlation between Cd and Cr ($r = 0.43$, $p < 0.0001$), a weaker positive correlation between Pb and Cr ($r = 0.30$, $p < 0.001$), and a weak correlation between Cd and Pb ($r = 0.22$, $p < 0.01$) under field conditions in the Bezenchuk region. In the Novosibirsk region, a stronger positive correlation was observed between the Pb and Cr concentrations ($r = 0.59$, $p < 0.0001$), while a weak negative correlation was detected between Cd and Cr ($r = -0.23$, $p < 0.01$).

2.2. Population Structure

Investigation of the population structure performed using sNMF revealed two major genetic clusters within the analyzed spring durum wheat panel (Figure 2A,B). Principal component analysis (PCA) supported this population subdivision, with the first two principal components explaining 18.05% and 5.19% of the total genetic variation, respectively (Figure 2C). The clustering pattern observed in PCA was consistent with the ancestry coefficients estimated by sNMF. Most accessions were assigned predominantly to a single ancestral population, whereas some exhibited admixed ancestry. Cluster 1 mainly consisted of genotypes of foreign origin, whereas cluster 2 was primarily represented by accessions developed by Russian breeding centers (Table S1).

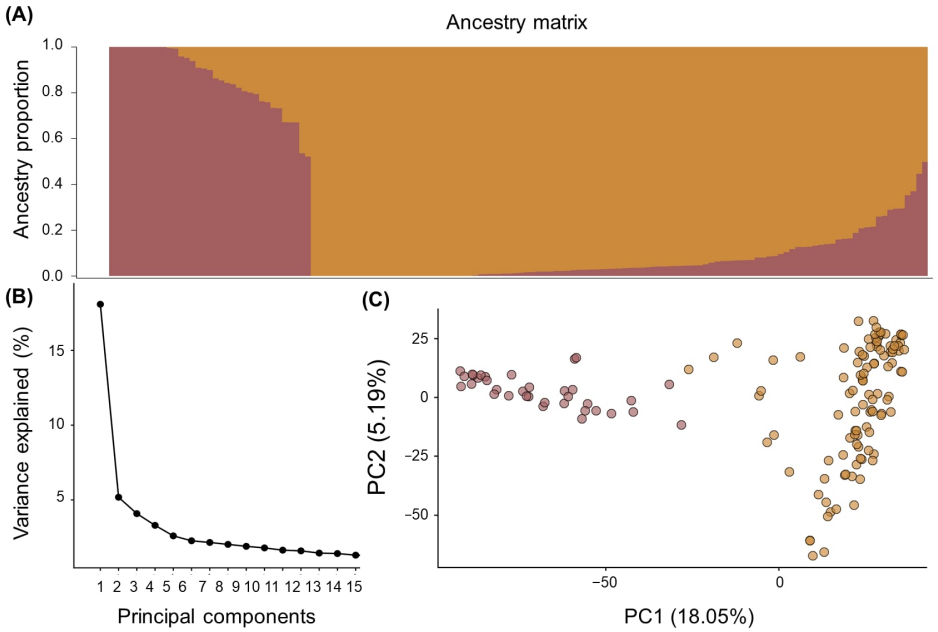

Figure 2. Population structure analysis of the spring durum wheat collection. (**A**) Ancestry coefficients estimated using sNMF for K = 2, with each vertical bar representing a single accession and colors indicating the inferred ancestral populations. Accessions are ordered according to their dominant ancestry component. (**B**) Scree plot showing the percentage of genetic variation explained by the first 15 principal components. (**C**) Principal component analysis (PCA). Each point represents an accession and is colored according to its dominant ancestral population inferred from the sNMF analysis at K = 2.

2.3. GWAS Results

Three GWAS models (MLM, BLINK, and FarmCPU) were used to identify and map loci associated with HM content in durum wheat grain. Compared with MLM, BLINK and FarmCPU identified fewer significant SNPs within the same chromosomal regions because these models iteratively account for the effects of associated loci and may reduce redundant associations arising from linkage disequilibrium (LD) (Figure 3, Table S4). Consequently, they may identify fewer significant markers representing the same association regions. In contrast, MLM identified clusters of correlated SNPs spanning the same LD blocks. Therefore, MLM results were used to characterize the genomic regions surrounding the lead SNPs detected by BLINK and FarmCPU, facilitating candidate gene identification.

2.3.1. Cd Content

For grain cadmium concentration, 29 significant marker–trait associations (p-value < 0.0001) were identified using the BLINK and FarmCPU models on chromosomes 1A, 1B, 2A, 2B, 3A, 4A, 4B, 5A, 5B, 6B, and 7B (Table S4, Figure 3). Most loci were detected only in specific environments or years, whereas several genomic regions showed consistent associations across datasets and GWAS models.

The most prominent associations were detected on chromosome 5B, where two distinct genomic regions demonstrated strong and reproducible associations with grain cadmium accumulation. The first region, located at 583.77 Mb (locus *GCdC-icg-5B-1*), was represented by the marker BS00000137_51, which was identified by both BLINK and FarmCPU using the multi-environment mean values from the Novosibirsk region. The strongest association signal within this locus was detected by BLINK in 2024 ($p = 2.28 \times 10^{-9}$).

An even stronger association was observed within the second 5B locus (*GCdC-icg-5B-2*) spanning 602.54–603.08 Mb. This region included four closely linked markers

(wsnp_Ex_c210_411604, Tdurum_contig32812_325, Kukri_c12910_908, and Ra_c22730_460) that were detected in both environments. The marker Kukri_c12910_908 exhibited the highest statistical significance among all detected associations, being identified by both BLINK and FarmCPU in the Novosibirsk datasets from 2023 and 2024 as well as in the multi-environment mean dataset (p-value down to 1.16×10^{-20}). Likewise, marker Tdurum_contig32812_325 showed highly significant associations in both the 2023 dataset and the multi-environment mean dataset ($p = 1.47 \times 10^{-14}$ and 5.11×10^{-14}, respectively), while wsnp_Ex_c210_411604 represented the strongest association in the Bezenchuk environment ($p = 4.91 \times 10^{-14}$).

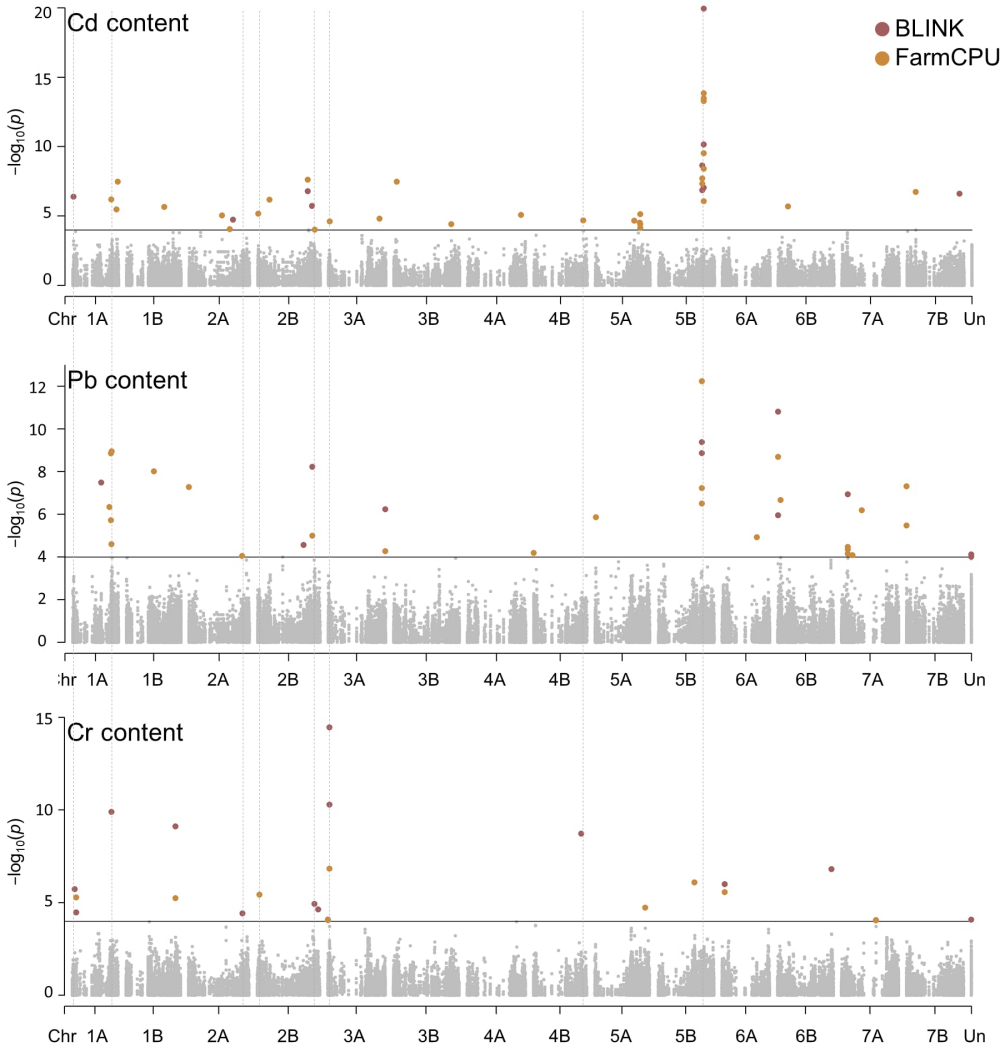

Figure 3. Genome-wide association analysis of grain cadmium (Cd), lead (Pb), and chromium (Cr) contents in spring durum wheat using the BLINK and FarmCPU models. Manhattan plots show the distribution of association signals across all chromosomes. Significant SNPs identified by the BLINK and FarmCPU models are highlighted in burgundy and orange, respectively, whereas non-significant markers are shown in gray. The horizontal line indicates the significance threshold ($p < 1 \times 10^{-4}$). Chromosomes are labeled according to the Svevo v2 durum wheat reference genome [50].

Additional associations detected across multiple datasets or GWAS models were detected on chromosome 2B, where marker AX-94392210 was identified by both BLINK and FarmCPU in the Novosibirsk dataset from 2023, and on chromosome 5A, where adjacent markers Kukri_rep_c103366_421 and wsnp_Ra_c12183_19587379 were associated with grain cadmium concentration in the analysis of the multi-environment mean, with one of these loci

also detected in the 2024 dataset. Marker AX-95631713 on chromosome 7B was associated with grain cadmium concentration in the multi-environment mean dataset, whereas AX-95143224 was identified in the 2024 Novosibirsk dataset, indicating additional association signals on this chromosome.

2.3.2. Pb Content

For grain lead concentration, 22 significant marker–trait associations ($p < 0.0001$) were identified across several chromosomes. Among these, loci on chromosomes 1A, 2A, 2B, 5B, 6B, 7A, and 7B showed recurrent association signals across different years, environments, or GWAS models, suggesting that some of these genomic regions may contribute consistently to variation in grain lead accumulation (Figure 3, Table S4).

The most prominent association was detected on chromosome 5B at 583.77 Mb, where marker BS00000137_51 was identified in both the Bezenchuk and Novosibirsk environments based on the multi-environment mean values. This locus was detected by both BLINK and FarmCPU, as well as in the Bezenchuk dataset in 2023. The strongest association was observed using FarmCPU in the analysis of the multi-environment mean ($p = 5.81 \times 10^{-13}$), while BLINK also identified this marker with high significance ($p = 4.09 \times 10^{-10}$). Interestingly, although BS00000137_51 was associated with grain lead concentration in both environments, the estimated allelic effect was negative in the Bezenchuk dataset but positive in the Novosibirsk dataset, indicating an environment-dependent direction of the marker effect.

Another highly significant association signal was identified on chromosome 6B at 7.1 Mb, where marker Excalibur_c4789_2748 was detected by both BLINK and FarmCPU in the analysis of the multi-environment mean and was also identified in the Novosibirsk dataset from 2023. The strongest association within this region was observed using BLINK in the analysis of the multi-environment mean ($p = 1.53 \times 10^{-11}$).

Several additional loci demonstrated recurrent associations with GPbC. On chromosome 1A, marker AX-158536815 (505.1 Mb) was associated with grain lead concentration in Bezenchuk in 2023 and was also significantly associated with the multi-environment mean. Another recurrently detected association signal on chromosome 7A was represented by marker AX-158553484, which was detected in the Novosibirsk dataset from 2024 and in the analysis of the multi-environment mean using FarmCPU. Likewise, marker RAC875_c6650_838 on chromosome 7B showed significant associations in both the multi-environment mean analysis and the Novosibirsk dataset from 2023. Additional loci detected by multiple GWAS models included markers wsnp_Ex_rep_c102127_87360034 on chromosome 2B, AVRIG22576 on chromosome 3A, and RAC875_c21906_247 on chromosome 2A, with the latter being identified by both BLINK and FarmCPU in the Bezenchuk dataset from 2024.

2.3.3. Cr Content

For grain chromium concentration, 14 significant marker–trait associations ($p < 0.0001$) were identified on chromosomes 1A, 1B, 2B, 3A, 4B, 5B, 6A, 6B, and 7A (Figure 3, Table S4). Most significant associations were detected in the Bezenchuk environment, whereas associations on only chromosomes 3A and 7A were identified in the Novosibirsk datasets. Loci on chromosomes 1A, 1B, 3A, and 7A were detected by multiple GWAS models and/or in analyses of the multi-environment mean.

The strongest association was identified on chromosome 3A at 26.0 Mb, where marker AX-158524110 was detected in the Bezenchuk dataset from 2023 and in the analysis of the multi-environment mean. This association was identified by both BLINK and FarmCPU, with the highest significance observed in the 2023 dataset ($p = 3.51 \times 10^{-15}$). Another highly

significant association was detected on chromosome 1A at 516.8 Mb, where marker AX-109279209 showed a strong association with grain chromium concentration in the Bezenchuk dataset from 2023 ($p = 1.27 \times 10^{-10}$).

Several additional loci were detected in multiple GWAS analyses. On chromosome 1A, marker Tdurum_contig5008_635 (54.2 Mb) was identified by both BLINK and FarmCPU in the analysis of the multi-environment mean. Likewise, marker BobWhite_c2844_569 on chromosome 1B (653.2 Mb) was detected by both GWAS models in the analysis of the multi-environment mean, providing additional support for its association with grain chromium accumulation. On chromosome 6A, two closely linked markers (IAAV7418 and RAC875_c34617_143) located within a 1-Mb interval were significantly associated with grain chromium concentration in the analysis of the multi-environment mean.

Comparison of GWAS results for grain cadmium, lead, and chromium concentrations revealed only a limited number of overlapping genomic regions associated with multiple heavy metals. Most significant associations were metal-specific, indicating that the genetic architecture underlying the accumulation of individual metals is largely independent. The most prominent shared association signal was identified on chromosome 5B at 583.77 Mb, where marker BS00000137_51 was significantly associated with both cadmium and lead concentrations (Figure 3, Table S4). This marker was repeatedly detected across multiple datasets, representing one of the most robust associations identified in the present study. Apart from this marker, no identical SNPs were significantly associated with different metals.

2.4. Dissection of Highly Significant Locus GCdC-icg-5B-2, Associated with Grain Cd Content

To further characterize the stable locus *GCdC-icg-5B-2* located within the 602–603 Mb region of chromosome 5B, the set of associated markers was expanded to include additional significant SNPs identified by the MLM model. Linkage disequilibrium (LD) analysis of the expanded marker set revealed several LD blocks within the associated interval, with the strongest LD observed among a cluster of closely linked SNPs (Figure 4A). This LD block included ten SNPs (Ra_c22730_460, AX-109830951, wsnp_Ex_c210_411604, wsnp_JD_c37023_27225840, AX-108760164, wsnp_Ex_c29304_38355434, Excalibur_c29304_176, Tdurum_contig32812_325, BS00022000_51, Kukri_c12910_908) and defined two major haplotypes—CCAGGCTGAC (Haplotype_1) and ATCAAACAGT (Haplotype_2).

Among the studied genotypes, Haplotype_1 and Haplotype_2 were identified in 94 and 30 accessions, respectively, while the remaining samples were characterized as heterogeneous. These haplotypes showed a diverse effect on grain cadmium accumulation across both experimental locations and years (Figure 4B). Accessions carrying Haplotype_2 (ATCAAACAGT) had significantly higher Cd concentrations than those carrying Haplotype_1 (CCAGGCTGAC) in Novosibirsk in the 2023 and 2024 growing seasons and in Bezenchuk in 2023 ($p < 0.001$). Haplotype_1 was predominantly found among Russian varieties and breeding lines, whereas Haplotype_2 was more frequently observed among accessions of foreign origin (Table S1).

Physical mapping of the associated interval revealed that the significant SNPs are distributed across a ~0.64-Mb region containing 32 annotated genes (Figure 4C). Expression profiling revealed substantial variation in the expression patterns of genes within this region across tissues and developmental stages (Figure S2, Table S5). Two genes within the *GCdC-icg-5B-2* locus exhibited predominantly root-specific expression: *TrturSVE5B02G01523030*, encoding Myosin family proteins, and *TrturSVE5B02G01523270*, encoding maltase-glucoamylase (Table S6). The functional annotations of these genes do not suggest a possible mechanism for their involvement in the accumulation of cadmium in durum wheat grain. Based on their functional annotations, three other genes were

considered potential candidates: *TrturSVE5B02G01523140*, *TrturSVE5B02G01523150*, and *TrturSVE5B02G01523250*, all encoding protein kinase domain-containing proteins (Table S6). Previous research in rice has demonstrated that mitogen-activated protein kinase signaling is involved in the regulation of root growth under Cd stress through changes in auxin signaling and the expression of cell cycle-related genes [51].

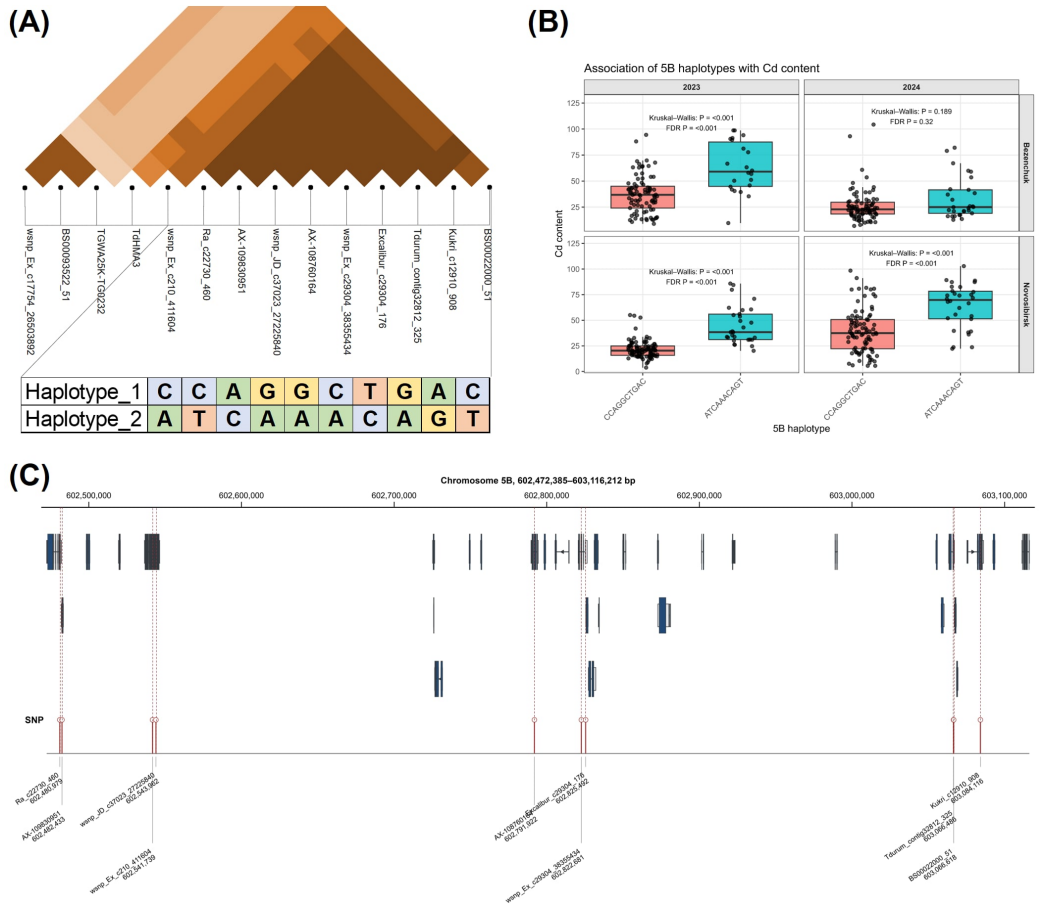

Figure 4. Haplotype structure of the chromosome 5B locus associated with grain cadmium content in durum wheat. (**A**) Linkage disequilibrium (LD) among significant SNPs identified within the chromosome 5BL. Pairwise LD relationships are shown above the markers, whereas the two major haplotypes identified for the block of closely linked SNPs are presented below. Darker shades of orange indicate stronger linkage disequilibrium. (**B**) Comparison of grain cadmium content between the two haplotypes in two environments (Bezenchuk and Novosibirsk) during the 2023 and 2024 growing seasons. Boxplots show the median; individual accessions are shown as dots. Differences between haplotypes were evaluated using the Kruskal–Wallis test. (**C**) Physical map of the associated interval (Chr5B: 602,472,385–603,116,212 bp) showing the positions of annotated genes and significant SNPs used for haplotype construction. Red dotted lines indicate SNP positions relative to genes. Boxes represent exons, and connecting black lines represent introns.

2.5. Association of TdHMA3 Allelic Variation with Grain Cadmium Content

When comparing the GWAS results with known genes and loci associated with grain Cd, Pb, and Cr content, it was found that the *GCdC-icg-5B-1* and *GCdC-icg-5B-2* loci are located near the previously identified *Cdu-B1* locus harboring the *TdHMA3-B1* gene [39]. We genotyped the durum wheat panel using allele-specific primers for *TdHMA3-B1* and evaluated the association of its alleles with grain heavy metal content, as well as linkage disequilibrium (LD) between the *TdHMA3-B1* and nearby Cd-associated SNP markers.

The *TdHMA3-B1a* allele, associated with low grain Cd content, was found in 102 wheat accessions, *TdHMA3b* in 35 accessions, and five genotypes were heterozygous at this locus

(Table S1). LD analysis showed that *TdHMA3-B1* is located close to the *GCdC-icg-5B-2* loci with LD r^2 values ranging from 0.6 to 0.77 depending on the SNP marker within the *GCdC-icg-5B-2* locus (Figure 4A).

ANOVA revealed significant differences in grain Cd content among accessions carrying different *TdHMA3-B1* alleles in all four site-year combinations (Figure 5, Table S7). Significant but weaker associations with grain Cr content were also identified in two environments: Novosibirsk in 2023 and Bezenchuk in 2024 (Table S7).

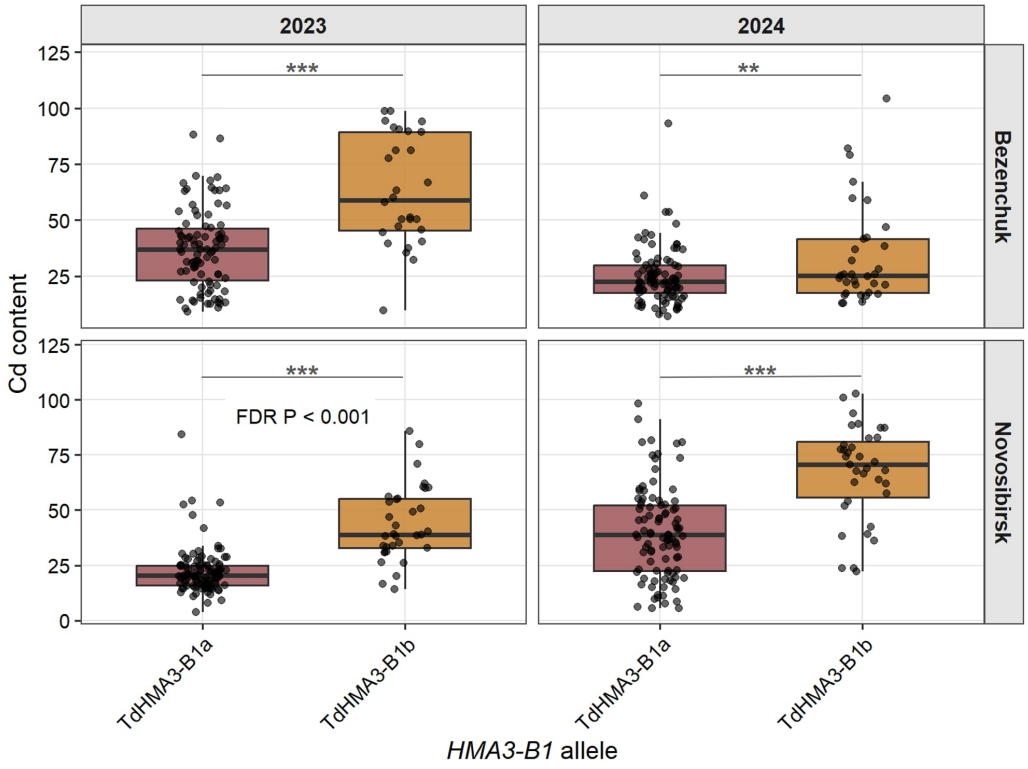

Figure 5. Comparison of grain cadmium content between different alleles of *TdHMA3-B1* in two environments (Bezenchuk and Novosibirsk) during the 2023 and 2024 growing seasons. Boxplots show the median; individual accessions are shown as dots. Pairwise differences between alleles were assessed using ANOVA. *** $p < 0.001$, ** $p < 0.01$.

2.6. Conditional Association Analysis of GCdC-icg 5B-2 and TdHMA3-B1

To evaluate whether the association of *GCdC-icg-5B-2* with grain cadmium content was independent of the known *TdHMA3-B1* gene, we performed conditional GWAS using *TdHMA3-B1* allelic status as a covariate. The analysis was conducted using the MLM, FarmCPU, and BLINK models across individual environments and location-specific mean datasets. After accounting for *TdHMA3-B1*, association signals within the *GCdC-icg-5B-2* region were substantially reduced (Table S8).

We additionally compared linear models including *TdHMA3-B1* alone or *TdHMA3-B1* together with the *GCdC-icg-5B-2* haplotype. The addition of haplotype information significantly improved the model fit for Novosibirsk 2023 ($\Delta R^2 = 0.056$, $p < 0.001$) and the Novosibirsk mean dataset ($\Delta R^2 = 0.044$, $p < 0.01$). However, these associations were strongly influenced by individual accessions carrying discordant *TdHMA3-B1* allele-haplotype combinations (Table S9). Together, these results indicate that the association detected at *GCdC-icg-5B-2* is largely explained by its linkage with *TdHMA3-B1*, although a minor additional genetic contribution cannot be excluded.

3. Discussion

3.1. Variation in Grain Heavy Metal Concentrations in Durum Wheat

In this study, we investigated the grain concentrations of three heavy metals—cadmium, lead, and chromium—in a panel of durum wheat varieties cultivated in two agroecological regions of the Russian Federation. The results revealed substantial variation in grain metal concentrations, depending on the cultivation region and environmental conditions (Figure 1, Table S2). Grain Cd content remained below the maximum permissible concentration (MPC) in nearly all samples, with only three varieties slightly exceeding the regulatory limit. The mean Cd concentrations observed in our study were comparable to those previously reported for durum wheat [28,52,53] and common wheat [54,55].

These findings are also consistent with data reported by Pompa et al., who found that Cd concentrations in Russian wheat grain imported into Italy were generally within the EU regulatory limits and lower than those reported for wheat originating from Kazakhstan, Turkey, and the United States [53].

In contrast to Cd, grain Pb and Cr content exhibited greater variability, particularly among varieties cultivated in the Novosibirsk region, potentially reflecting differences in soil metal availability and other environmental factors between the experimental fields. To date, relatively few studies have investigated lead and chromium concentrations in durum wheat grain. Nevertheless, existing research indicates that despite genotypic differences, environmental conditions, particularly the soil concentrations of these elements, are the primary factors influencing their accumulation in grain [56–59].

On average, the concentrations of toxic metals in soils across the Russian Federation remain below the MPC, although higher levels may be observed in certain regions. Long-term monitoring of mobile forms of heavy metals (Cu, Zn, Cd, Pb, Ni, Cr, Hg, As) in Russian agroecosystems (2000–2024) has revealed no consistent temporal trends, suggesting the absence of widespread heavy metal contamination in agricultural soils [10,11,60]. Long-term soil monitoring in the Samara and Novosibirsk regions has shown that the content of mobile forms of cadmium, lead, nickel, chromium, copper, and zinc do not exceed the corresponding MPCs across soil types differing in composition and humus content [61–63]. However, elevated concentrations of chromium, cobalt, lead, nickel, copper, and zinc in agricultural soils have been associated with proximity to highways and industrial facilities [4,64].

Long-term fertilizer application in the Russian Federation can also contribute to the accumulation of various heavy metals in agricultural soils. The extent of this effect depends on the types and formulations of fertilizers applied. For certain crops, fertilizer application can increase the concentrations of Ni, Cr, Cd, and Pb to levels exceeding their MPCs, even in soils with initially low to moderate concentrations of the mobile forms of these elements [65]. In addition, sowing date and nitrogen fertilizer application can influence cadmium concentration, although the magnitude of these effects depends on genotype and environmental conditions [66]. However, in this study, the concentrations of toxic metals in the soils of the experimental fields were not determined, highlighting the need for further investigation.

It should also be noted that in this study, we analyzed the total chromium content, without regard to its valence state—Cr(III) or Cr(VI). It is known that plant uptake of Cr(III) is a passive process compared to Cr(VI), which requires active transport using carrier ions such as sulfate or iron [46]. The toxicity of Cr(VI) arises from the formation of free radicals inside the cell during the reduction of Cr(VI) to Cr(III). Chromium(III), in addition to generating reactive oxygen species, can also cause toxic effects inhibiting some metalloenzyme systems [14]. Since soil immobilizes both trivalent and hexavalent chromium, it is difficult to separately evaluate the consequence of the two types of Cr

on plants. Therefore, the contributions of the more toxic form Cr(VI) to the quality of Cr-contaminated durum wheat grain and its ecological risk are now unclear.

3.2. Comparison of Mapped QTLs with Known T. durum Loci

Based on the results obtained, chromosomes 5B, 2B, and 5A can be highlighted, as significant associations with GCdC were identified using multiple GWAS models and/or across different environments. Two independent loci detected in the present study coincided with previously reported GIC (grain ionome content) intervals. The first, *GCdC-icg-5B-1* located at 583.8 Mb, harbored the marker BS00000137_51, which showed reproducible associations with both cadmium and lead concentrations. The second, *GCdC-icg-5B-2* located at 602.5–603.1 Mb, encompassed a cluster of highly significant cadmium-associated markers and closely linked with the *TdHMA3* locus [39]. According to the most recent durum wheat genome assembly and annotation [50], neither locus overlaps with the previously mapped *Cdu1* and *Cdu2* loci on chromosome 5B.

It has previously been shown that grain Cd content in durum wheat is controlled by the dominant gene *Cdu1* (*Cdu-B1*), located on the long arm of chromosome 5B [67,68]. The RAPD marker OPC20, the SCAR marker ScOPC20, and the CAPS marker usw47, developed for Cdu1, have proven effective at identifying low-Cd genotypes [38,67,69,70]. Allelic variation at *Cdu1* has been shown to account for more than 80% of the phenotypic variation in grain Cd concentration in a doubled-haploid population of durum wheat [71]. The application of these markers in durum wheat breeding programs has facilitated the development of cultivars ('Strongfield', 'CDC Alloy', 'CDC Dinamic', among others) combining low cadmium content with high productivity [72–74]. Another locus, *Cdu2*, distinct from *Cdu1*, was subsequently mapped to chromosome 5B within the interval flanked by the SNP markers wsnp_Ex_c1343_2570756 and Kukri_c66357_357 (566.68–566.97 Mb) [75,76].

The *TdHMA3* gene has been proposed as a candidate for the *Cdu-B1* locus, with its allelic variants *TdHMA3-B1a* and *TdHMA3-B1b* associated with low and high grain Cd content, respectively. Exome sequencing and comparative genomic analysis of the durum wheat accessions Zavitan and Svevo, which differ in GCdC, revealed a 17-bp duplication in the first exon of *TdHMA3* [39]. This duplication results in a nonfunctional *TdHMA3-B1b* allele associated with increased grain cadmium concentration. We assessed LD between *GCdC-icg-5B-2* and *TdHMA3* and revealed from moderate to strong linkage between *TdHMA3* and a group of SNP markers located 4–5 Mb distal to the *GCdC-icg-5B-2 locus* ($r2 = 0.6–0.77$) (Figure 4).

According to currently available durum wheat genome information, the *TdHMA3-B1* gene is located at 598.88–598.89 Mb on chromosome 5B [50], whereas *GCdC-icg-5B-2* spans the 602.47–603.11 Mb interval, indicating that the two loci are physically separated. Conditional GWAS revealed that the associations within *GCdC-icg-5B-2* were no longer significant after accounting for *TdHMA3-B1* allelic variation, suggesting that the observed association largely reflects linkage with the known gene. Although joint haplotype analysis indicated an additional statistical effect in certain environments, this effect was sensitive to individual accessions with discordant allele *TdHMA3-B1*–haplotype *GCdC-icg-5B-2* combinations. Therefore, the limited number of informative accessions (only five individuals in our panel exhibited recombination between markers at *TdHMA3-B1* and *GCdC-icg-5B-2*) and the strong linkage disequilibrium between the two regions prevented us from reliably distinguishing their independent genetic contributions.

In addition to the *GCdC-icg-5B-1* and *GCdC-icg-5B-2* loci, we identified QTLs on chromosome 2BL (at the 662.49 Mb) and 5AL (at 592.53 Mb). A previous study reported a QTL associated with GCdC on chromosome 2BS at 11.72 Mb in durum wheat [75]; however, this QTL is located in a different genomic region from the locus identified in our study.

Previous research on the genetic control of GCdC in durum wheat has primarily focused on the *Cdu-B1* locus. In contrast, the contribution of loci on other chromosomes, including 5AL, remains poorly understood.

Data on the chromosomal localization of QTLs associated with grain lead and chromium content remain limited for both durum and common wheat. In the present study, significant associations with grain Pb content were identified on chromosomes 1A, 2A, 2B, 3A, 5B, 6B, 7A, and 7B using different GWAS models (BLINK, FarmCPU) (Table S4). Loci in chromosomes 2B, 5B, and 7A were identified based on phenotyping data from both regions, whereas other QTLs were mapped only under the environmental conditions of either Bezenchuk (chromosomes 2A and 5B) or Novosibirsk (chromosomes 3A and 7B). It should be noted that the expression of the loci on chromosomes 2B, 5B, and 7A is region-specific and exhibits opposite effects. Such differences may be attributed to the environmental conditions and variations in the soil content of the element. In a previous study, we identified the locus *QPb.1102-icg-2B* associated with grain Pb content in the synthetic line 1102, on chromosome 2B between the markers Ex_c9729_243 and BS00063365_51 (~721.9 Mb) [77]. This locus is located in close proximity to the QTL identified in durum wheat in the present study at 723.31 Mb.

Mourad et al., who studied 103 bread wheat genotypes differing in their tolerance to lead exposure, hypothesized that chromosome 7B may harbor genes involved in the tolerance of wheat plants to Pb at the seedling stage [36]. Similarly, Wang et al. reported the involvement of chromosome 7B in the genetic regulation of grain Pb accumulation in Chinese winter wheat varieties [52].

Regarding the loci associated with grain Cr content, two highly significant QTLs were identified on chromosomes 1BL (653.15 Mb) and 3AS (26.01 Mb). These loci were detected using different GWAS models, and the associated SNPs exhibited a significant effect in reducing the Cr concentration (Table S4). Almas et al. investigated the effect of chromium stress on several economically important traits in bread wheat and identified associated loci on chromosomes 2A, 3B, 4A, and 6B [44]. In a previous study, we identified two QTLs associated with grain Cr accumulation (*QCr.1102-icg-3B* and *QCr.1102-icg-4B*) on chromosomes 3B and 4B, respectively, in the synthetic line 1102 [77]. In the present study, the chromium-associated marker AVRIG18688 was found to overlap with the previously reported Cd-associated interval GCdC-Svevo2.M_1120-2B [42,50]. This finding suggests that the same genomic region may be involved in the accumulation of both Cd and Cr in wheat grain.

3.3. Putative Gene Candidates

Compared with bread wheat, the molecular mechanisms underlying cadmium uptake, transport, and accumulation in durum wheat remain poorly characterized. Due to the limited availability of genetic mapping data for QTLs associated with grain Cd accumulation in durum wheat, the identification of candidate genes remains challenging. A greater number of QTLs associated with grain Cd accumulation have been reported in bread than in durum wheat. In addition to chromosome 5BL, QTLs explaining varying proportions of phenotypic variation have been identified on chromosomes 1A, 1D, 1B, 4B, 6A, 6B, 7A, 7B, and 7D in bread wheat [37,78–80]. A homolog of *Cdu1* has also been reported in the 579.84 Mb region of chromosome 5AL of winter bread wheat [81].

Based on findings in bread wheat and its wild tetraploid relatives, Cd uptake and its subsequent transport from the roots to aboveground tissues and grain are likely mediated by members of several transporter families, including ZIP, NRAMP, YSL, and others [82]. Several genes involved in cadmium transport and accumulation have been characterized in wheat. The gene *TaHMA2b-7A* has been identified as an important Cd transporter involved in

its translocation in hexaploid wheat [83]. In durum wheat, *TdHMA3-B1*, located within the *Cdu1* locus, has been proposed as a candidate gene [71]. Subsequent studies demonstrated that the rice *OsHMA3* gene, which is an ortholog of the functional *TdHMA3-B1a* allele, encodes a P-type ATPase ion transporter that promotes cadmium accumulation in root cells, thereby limiting its translocation to the grain [84]. The *Cdu2* locus, located separately from *Cdu-B1*, has been associated with a candidate gene encoding a protein homologous to an aluminum-induced protein (AIP), which may be involved in heavy metal transport [76].

Members of the NRAMP transporter family are involved in Cd uptake and translocation in wheat. The role of the *TaNRAMP5* in regulating grain Cd accumulation in common wheat (*Triticum aestivum*) was demonstrated using CRISPR/Cas9-mediated gene-knockout [41]. Knockout of *TaNRAMP5* resulted in reduced Cd accumulation in wheat grain.

The *TtNRAMP6* gene, located on chromosome 3B of *Triticum turgidum* ssp. *turgidum*, encodes a plasma membrane-localized transporter and is predominantly expressed in the roots. Heterologous overexpression of *TtNRAMP6* in *Arabidopsis thaliana* increased Cd accumulation in the roots, stems, and leaves [85].

Previous studies have indicated that the grain-filling stage is a critical period for cadmium accumulation in durum wheat grain, with leaves playing a significant role in this process [86]. In addition, analysis of the expression patterns of key genes involved in cadmium accumulation in common wheat grain revealed that the *TdHMA3* homoeologs (*TraesCS5B02G388000*, *TraesCS5A02G383400*, and *TraesCS5D02G392700*) are predominantly expressed in the roots. The highest expression levels of all three homoeologs were observed at the flag-leaf stage and later developmental stages. Similarly, *NRAMP5* (*TraesCS4A02G004400*) and its homoeologs showed predominantly root-specific expression patterns in common wheat.

Transcriptomic data encompassing different plant parts and developmental stages of durum wheat have recently been published [50]. Using these data, we analyzed the expression patterns of key genes involved in grain cadmium accumulation to assess the potential of transcriptomic information for prioritizing candidate genes located within the genomic loci significantly associated with grain cadmium content in this study. *TdHMA3* (*TrturSVE5B02G01522140*) showed no detectable expression in most tissues, with low expression levels (maximum 0.34 TPM) observed only in the peduncle at 30 days after anthesis. *TdNRAMP5* (*TrturSVE4A02G00973950*) was expressed in the roots at the seedling stage, as well as in the pistils and the grain 3 days after anthesis. However, compared with bread wheat, the currently available transcriptomic data for durum wheat are insufficient to fully assess and compare gene expression patterns across different developmental stages or to effectively use such data as a basis for prioritizing genes within the target genomic regions.

4. Materials and Methods

4.1. Plant Material and Phenotyping

The plant material consisted of 142 durum wheat accessions, including 52 Russian cultivars, 60 Russian advanced breeding lines, 29 foreign varieties, and accession of tetraploid wheat *Triticum turanicum* (Table S1). The genotypes were grown under field trial conditions in the Novosibirsk and Samara regions in 2023 and 2024. In the Novosibirsk region, sowing was carried out at the experimental field of the Institute of Cytology and Genetics SB RAS (54°55′14″ N, 82°59′31″ E). The soils of the experimental site are mainly leached chernozem of medium depth. The humus content in the topsoil is 5.7–6.9%. Sowing was conducted manually in rows at a depth of 5–7 cm. The plot size was 0.4 m^2, with two replicates. The experimental fields of the Samara Scientific Research Agriculture Institute are located in the urban-type settlement of Bezenchuk (52°58′55″ N, 49°25′59″ E). The main soil type is common chernozem with a medium- to heavy-loam texture. The average humus content

is 4.8%. The variety was sown in a randomized layout on plots of 7.4 m^2 in two replicates. The samples were sown at the end of April in the Samara region and in mid-May in the Novosibirsk region.

A comparison of climatic conditions between the regions during the growing season and with long-term averages showed that in 2023, the Novosibirsk region experienced elevated air temperatures throughout the growing season, with the exception of the second ten-day periods of May and August, when temperatures were 1.3 and 2.0 °C below the long-term averages, respectively. The most significant increase (+8.4 °C) above the long-term average temperature was recorded in the first ten days of June, while precipitation during this period was only 27.8% of the long-term average. In the Samara region, there was a general precipitation deficit throughout the 2023 growing season, accompanied by moderate temperatures. From the seedling stage to wax ripeness, the total precipitation was 89.3 mm. The 2024 growing season in the Novosibirsk region was generally characterized by excessive precipitation. Precipitation in May, July, and August was 185%, 205%, and 229% of the long-term averages, respectively. Temperatures were also 1.5 to 2.1 °C above the long-term average in June, July, and August. Conversely, the growing season in the Samara region was characterized by arid conditions. Precipitation was no more than 20% of normal; average monthly temperatures in May and June were below normal, while in July, they were within the long-term average range.

For the analysis of cadmium, lead, and chromium content, 300 mg of wheat grain was ground and treated with 1 mL of hydrogen peroxide (30%) and 5 mL of concentrated nitric acid. Sample mineralization was carried out in a microwave oven for 40 min. The sample volume was then adjusted to 50 mL with deionized water, and the resulting solution was used for elemental quantification. Elemental concentrations were determined by atomic absorption spectrometry with electrothermal atomization in a graphite cell using a ContrAA 800 D (Analytik Jena, Jena, Germany) spectrometer. Aliquots of the samples were added into the graphite tube using an AS-GF autosampler (Analytic Jena, Jena, Germany). Calibration curves for each element were prepared according to the manufacturer's instructions. Samples from each field replicate were analyzed in duplicate.

4.2. Genotyping

DNA was extracted following the modified sodium bisulfite protocol described in [87]. DNA purification for SNP genotyping was performed using a Bio-Silica Kit for DNA Purification from Reaction Mixtures (BioSilica Ltd., Novosibirsk, Russia) according to the manufacturer's protocol. DNA was quantified using the Qubit dsDNA BR Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) with a Qubit 4 Fluorometer (Thermo Fisher Scientific).

High-throughput SNP genotyping was performed using the Illumina Infinium 26K Wheat platform by the TraitGenetics-Section of SGS Institute Fresenius GmbH (Gatersleben, Germany, https://www.traitgenetics.com, accessed on 28 October 2026). The SNP chip consists of 26,165 markers mapped to the common wheat genome [88]. To map SNPs onto the durum wheat genome assembly (*Triticum turgidum* Durum Wheat Svevo Rel. 2.0) [50], we used the SNPfix pipeline [89]. Genotyping data were filtered based on minor allele frequency (MAF > 0.1) and a minimum call rate of 80% across the population for further analysis. After filtering, 8380 SNP markers were retained for subsequent analyses.

4.3. Identification of TdHMA3 Alleles

Alleles of the *TdHMA3* gene were identified using the allele-specific marker *Xusw59* described by Maccaferri et al. [39]. PCR amplification was performed according to the published protocol using Hi-Fi BioMaster (Biolabmix, Novosibirsk, Russia). PCR products were analyzed by electrophoresis on a 2% agarose gel in TAE buffer.

4.4. Population Structure and Genome Wide Association Study

Population structure was assessed using the LEA package (version 3.2.0) in R [90]. Ancestry coefficients were estimated using the sparse non-negative matrix factorization algorithm implemented in the snmf function for K values from 1 to 10, with 100 independent runs per K and 5% of genotypes masked for cross-entropy estimation. The run with the lowest cross-entropy was selected for each K. The number of ancestral populations was evaluated using principal component analysis (PCA) based on the filtered genotype matrix and by inspection of the proportion of variance explained by successive principal components. Based on the combined PCA and sNMF results, K = 2 was selected as the most biologically interpretable population structure.

Genome-wide association analysis was conducted using the GAPIT3 R package (version 4.5.1) [91]. To identify robust association signals, GWAS was performed using the BLINK and FarmCPU models, which reduce redundant associations caused by linkage disequilibrium by selecting representative SNPs at each locus. Results obtained using the MLM model were subsequently used to characterize the extent of associated genomic regions by identifying neighboring SNPs in linkage disequilibrium with the lead SNPs detected by BLINK and FarmCPU. In the MLM analysis, the first two principal components were included as covariates to account for population structure, and the kinship matrix was calculated using the VanRaden algorithm to account for genetic relatedness among accessions. A significance threshold of $p < 1 \times 10^{-4}$ was applied to identify potential marker–trait associations, including those exhibiting varying levels of significance across environments, years, and GWAS models. To account for multiple testing, p-values were adjusted using the Benjamini–Hochberg false discovery rate procedure. The corresponding adjusted p-values are provided in Supplementary Table S4. Manhattan and Q–Q plots were generated using the R package CMplot v. 4.5.1 [92]. To assess whether the association signals within the *GCdC-icg-5B-2* region were independent of the known *TdHMA3-B1* gene, conditional GWAS was performed by including the *TdHMA3-B1* allelic state as an additional covariate. Accessions with ambiguous allelic status were excluded. Conditional GWAS was conducted using the MLM, FarmCPU, and BLINK models in GAPIT, applying the same genotyping data, model parameters, and significance threshold as in the original GWAS.

4.5. Gene Annotation and Prioritization

We conducted gene prioritization within quantitative trait loci (QTLs) identified based on markers showing significant associations with the analyzed traits. The boundaries of these loci were determined based on linkage disequilibrium (LD) between markers, as illustrated in Figure S1. LD estimates were obtained using the Genetics R package (version 1.3.8.1.3) [93], and LD decay plots were generated using the R package LDheatmap (version 1.0-6(1).tar.gz) [94].

Gene expression patterns for selected genes were obtained from the Svevo developmental transcriptome dataset [50]. Average transcript abundance values, expressed as transcripts per million (TPM), were extracted for the selected genes across 30 tissues and developmental stages. Expression values were transformed as log2(TPM + 1). To compare relative expression patterns among tissues, row-wise Z-scores were calculated separately for each gene. Heatmaps were generated in R using the pheatmap package (version 1.0.13).

4.6. Statistical Analysis

Statistical analyses were performed in R (version 4.5.1). Descriptive statistics, including the mean, median, standard deviation (SD), standard error (SE), minimum, and maximum values, were calculated separately for each metal, location, and year. The normality of trait distributions was assessed using the Shapiro–Wilk test. Two-way analysis of variance

(ANOVA) was performed to determine the significance of differences among genotypes and environments, with genotype treated as a fixed effect, and environment and replications as random effects. Broad-sense heritability (H^2) across all environments was calculated as:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \left(\frac{\sigma_{g\times e}^2}{Env}\right) + \left(\frac{\sigma_e^2}{Env \times Rep}\right)}$$

where $\sigma^2{}_g$, $\sigma^2{}_{g\times e}$, and $\sigma^2{}_e$ are the genotypic variance, $g \times e$ interaction, and residual (error) variance, respectively, while *Env* and *Rep* are the number of environments and the number of replications, respectively. The effects of *TdHMA3* alleles on Cd, Cr, and Pb concentrations were evaluated separately for each location (Bezenchuk and Novosibirsk) and year (2023 and 2024) using one-way ANOVA, with allele as a factor. Differences in grain Cd content among haplotype groups were assessed using the Kruskal–Wallis test. To account for multiple testing, the resulting *p*-values were adjusted using the Benjamini–Hochberg false discovery rate (FDR) procedure.

5. Conclusions

Thus, the results of the evaluation of a panel of durum wheat varieties for grain cadmium, lead, and chromium content indicate considerable variation in these traits, depending on the cultivation region and environmental conditions. The genotypic contribution was high for cadmium content, whereas for the other metals, the contributions of the environment and the genotype-by-environment interaction were substantially greater.

Despite recent advances, the molecular mechanisms underlying the uptake and transport of heavy metals in durum wheat remain insufficiently understood, and many regulatory pathways have been identified primarily through research on bread wheat and related wheat species. We identified significant loci associated with cadmium Cd content on chromosomes 5B, 2B, and 5A, among them, locus *GCdC-icg-5B-2* on chromosome 5B showed strong associations with phenotypic variation of the trait. Analysis of the composition of this locus revealed that the majority of Russian durum wheat varieties and breeding lines contain a haplotype associated with lower cadmium content in the grains. Analysis of the association between allelic variants of the known *TdHMA3* gene and grain Cd content revealed a strong linkage between *TdHMA3* alleles and a group of markers within the *GCdC-icg-5B-2* locus. Based on our findings and published data, we hypothesize that chromosome 5B may harbor a cluster of genes involved in cadmium uptake by the roots and its subsequent transport and sequestration in different plant tissues. Comparison of GWAS results for grain cadmium, lead, and chromium contents revealed only a limited number of overlapping genomic regions associated with multiple heavy metals. The most prominent association was found on 5B chromosome for grain Pb content, suggesting the existence of a common accumulation mechanism for cadmium and lead.

The transcriptomic data currently available for durum wheat are insufficient to fully evaluate and compare gene expression patterns across different developmental stages or to serve as a basis for prioritizing candidate genes within target genomic regions. Despite the significant influence of environmental factors, the analysis of grain chromium and lead contents made it possible to identify a set of genotypes characterized by low heavy metal accumulation in grain, as well as to identify genomic regions associated with these traits. The obtained data are valuable for further research into the physiological processes underlying heavy metal uptake, transport, and accumulation in wheat, the investigation of HM transport and sequestration pathways, and the development of wheat varieties with low grain HM concentrations.

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Abbreviations

The following abbreviations are used in this manuscript:

GWAS	Genome-wide association studies
QTL	Quantitative trait loci
MTAs	Marker–trait associations
SNP	Single-nucleotide polymorphism
HM	Heavy metal
GCdC	Grain cadmium content
GPbC	Grain lead content
GCrC	Grain chromium content
GIC	Grain ionome content

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