

## Article

# Association Mapping of Quantitative Trait Loci for Agronomic Traits in a Winter Wheat Collection Grown in Kazakhstan

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**Abstract:** Central Asia is an important region for the growth of winter wheat, with a cultivation area of more than 15 million hectares (ha). However, the average yield is approximately 3 tons per ha, which is significantly lower than that in developed countries. Therefore, the development of new competitive high-yielding cultivars, including those based on the application of modern molecular genetics tools, is a key priority in winter wheat breeding projects. One of these tools is the result of the identification of new genes and quantitative trait loci (QTLs) for agronomic traits using diverse germplasm panels and genome-wide association studies (GWAS). In this work, a panel of winter wheat accessions was assembled using 115 accessions from Central Asia and 162 samples from other regions of the world. The GWAS, based on a two-year field evaluation of the collection in Kazakhstan's southern and southeastern regions and 10,481 polymorphic SNP (single-nucleotide polymorphism) markers, allowed for the detection of 173 stable QTLs in nine studied agronomic traits. A survey of the published scientific literature suggests that 23 of these 173 stable QTLs have locations similar to those of previously reported QTLs, supporting the robustness of the research. In addition, 221 and 162 accessions surpassed local standards for grain yield at Kazakhstan's southern and southeastern stations, respectively. Therefore, this study is an additional contribution to the identification of new QTLs for key agronomic traits and valuable genetic lines in winter wheat breeding projects.

**Keywords:** *Triticum aestivum* L.; genome-wide association studies; quantitative trait loci; agronomic traits; winter wheat



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## 1. Introduction

Bread wheat (*Triticum aestivum* L.) is the third most important cereal after maize and rice, with a predicted global production of 794.6 million tons in 2023 [1]. However, due to predicted population growth, climate change, and political instability, there is a worldwide demand for greater wheat production [2], including in Central Asia. In the Central Asian region, wheat is grown on more than 15 million hectares (ha) of land with an average yield of 1.4 tones/ha [3], which is significantly lower than that in advanced agricultural countries, such as in USA (3.0 tones/ha in 2021–2022) [4]. Consequently, to increase the wheat production potential in this region, there is a necessity to generate new cultivars with higher yield and yield stability and increased resistance/tolerance to biotic and abiotic stresses [5].

Wheat is commonly divided into spring and winter types in relationship to their sensitivity to low temperatures and the photoperiod [6], where the spring type does not

require vernalization and can produce fertile plants when sown in spring [7]. Winter wheat is sown in the autumn and differs from spring wheat in its expression of the vernalization response, which is controlled by *Vrn* genes [8]. In Central Asia, the yield of winter wheat (3.1 t/ha in 2020) is nearly three times higher than that of spring wheat (1.1 t/ha in 2020), which is the type predominantly grown in Kazakhstan [3]. Still, there are potentials to increase the yield of winter wheat up to the level achieved in developed countries. Therefore, it is important to promote winter wheat production, improve agronomy technologies, and enhance regional breeding activities, focusing on the generation of new competitive cultivars with higher grain yields. Central Asia's average winter wheat production is significantly lower than that in developed countries. For instance, grain productivity in the U.K. was 7.8 t/ha per year during 2021–2022 [4]. It is well known that grain yield is a complex trait and is associated with main components such as the number of productive spikes, spike length, number of spikelets per spike, number of kernels per spike, kernel size, and thousand-kernel weight [9]. The performance of these quantitative components is affected not only by environmental factors but also by genotype and is controlled by multiple quantitative trait loci (QTLs) located in different parts of the genome [10,11].

The availability of powerful molecular tools, such as genome-wide association studies (GWAS), has dramatically improved the possibility of dissecting the genetic basis of complex quantitative traits in crop plants, including cereals [12]. GWAS has higher resolving power in comparison to bi-parental linkage mapping and is currently considered the method of choice to better understand the genetics of resistance/tolerance to biotic and abiotic stresses and yield-related traits [13–18]. Recently, GWAS of winter wheat have been actively employed to study grain quality [19,20], drought tolerance [21,22], pre-harvest sprouting resistance [23], disease resistance [24,25], and yield and its components [26–30]. As seen from these studies, the success of each GWAS relies on the availability of diverse germplasm. Therefore, to evaluate untapped local germplasm, the CAWBIN (Central Asian Wheat Breeding Initiative) was recently launched as a collective effort of Central Asian and British scientists in Kazakhstan to establish a bank of genotypes of winter wheat collected from Central Asia [3]. The CAWBIN winter wheat collection consists of 277 accessions, including 115 samples from Central Asia (Kazakhstan, Kyrgyzstan, Tajikistan, and Uzbekistan) [3]. Another issue is the consideration of genotype–environment interaction patterns, as a survey of the published GWAS articles for cereal crops, including winter wheat types [4,31], suggests a strong influence of the growth environment on the detection of QTLs for yield components. As environmental conditions may greatly impact the timing of the heading date and seed maturation, they may also significantly alter yield components [32,33]. The particular purpose of this GWAS was to evaluate the CAWBIN winter wheat collection harvested in the southern and southeastern regions of Kazakhstan to identify QTLs of important agronomic traits in this previously poorly studied region. Detection of efficient QTLs for key agronomic traits will be an essential step in breeding activities based on molecular-assisted selection.

## 2. Materials and Methods

### 2.1. Plant Material and Field Experiments

The collection used in this study consisted of 277 winter wheat genotypes from 21 countries. The places of origin of this collection were Europe (84 accessions), Central Asia (115), Afghanistan (32), Russia (25), Turkey (14), the USA (3), Australia (3), and Iran (1). The Central Asian collection consisted of 115 samples and included those from Kazakhstan (52), Kyrgyzstan (27), Uzbekistan (27), and Tajikistan (9) (Table S1). All genotypes were tested in two regions of Kazakhstan at the Kazakh Research Institute of Agriculture and Plant Growing (KRIAPGG, Almaty region, Southeast Kazakhstan) and Krasnovodopad Breeding Station (KBS, Turkestan region, South Kazakhstan) during the 2020–2021 and 2021–2022 growing seasons. The genotypes were planted in both locations with two replications in randomized plots. The distance between rows was 15 cm, with a 5 cm

distance between plants [34]. The climate conditions recorded during the trials are shown in Table 1.

**Table 1.** Location, environment, and weather data for the two study regions in Kazakhstan.

Site/Region	KRIAPG (Almaty Region)		KBS (Turkestan Region)	
Latitude/Longitude	43°21′/76°53′		41°46′/69°45′	
Soil type	Light chestnut (humus 2.0–2.5%)		Light serozem (humus 1.1%)	
Conditions	Rainfed		Rainfed	
Year	2020–2021	2021–2022	2020–2021	2021–2022
Annual rainfall, mm	464.7	568.9	279.4	421.0
Mean temperature, °C	10.5	12.2	17.5	11.7
Max temperature, °C	26.9	26.5	31.6	23.3
Min temperature, °C	1.8	1.1	2.7	4.0

## 2.2. Phenotyping of the Collection

The collection was analyzed for two groups of traits: plant adaptation-related traits and yield components. The plant adaptation traits included the heading date (HD, days), seed maturation days (SMD, days), plant height (PH, cm), and peduncle length (PL, cm). The yield components included the spike length (SL, cm), number of productive spikes (NPS, pcs), number of kernels per spike (NKS, pcs), thousand-kernel weight (TKW, g), and yield per square meter (YM2, g/m<sup>2</sup>). A phenological evaluation of these traits was carried out according to the protocol of the “ADAPTAWHEAT” project [35].

## 2.3. Genotyping of the Collection

A panel of 188 winter wheat accessions from Central Asia, Afghanistan, Russia, Turkey, and Ukraine was genotyped using the Axiom Wheat Breeder’s Genotyping Array with 35 K single-nucleotide polymorphism (SNPs) [36]. A panel consisting of 89 accessions from Europe, the USA, and Australia was genotyped previously using the same SNP array [37]. The monomorphic markers with a minor allele frequency (MAF) of <5% and >15% missing data were removed from the analysis. In total, 10,481 polymorphic SNP markers were used in the GWAS analysis.

## 2.4. Analysis of Linkage Disequilibrium and Population Structure

The population structure of the winter wheat panel was assessed using a model-based clustering method (admixture models with correlated allele frequencies) in STRUCTURE v.2.3.4 software (USA, Pritchard Lab, Stanford University) [38]. The population structure was determined by inferring  $K$  ranging from 2 to 10 using 100,000 burn-in lengths and 100,000 Markov chain Monte Carlo (MCMC) iterations, with five replications for each  $K$ . The optimal number of clusters ( $K$ ) was chosen based on  $\Delta K$  as described by using a STRUCTURE harvester v.0.6.94 (USA, University of California) [39]. The obtained results were then converted into a population structure matrix ( $Q$ ). The linkage disequilibrium (LD) in the studied collection was separately calculated for each hexaploid common wheat genome (genome A, genome B, and genome D), in addition to the average LD for the three genomes, using Java-based TASSEL v.5.2.90 software (USA, Cornell University) [40]. The R statistical platform was used to build a plot between the pairwise  $R^2$  and the genetic distance (LD decay plot) [41]. The analysis of variance (ANOVA) and correlation analysis were performed using Rstudio v. 2023.03.1 software (USA, Boston) [41]. The broad-sense heritability index ( $h_b^2$ ), describing the proportion of phenotypic variation due to genetic factors, was calculated based on the ANOVA results as follows:

$$h_b^2 = \frac{SS_g}{SS_t}$$

where  $SS_g$  is the sum of squares for the genotype, and  $SS_t$  is the total sum of squares [42].

### 2.5. Genome-Wide Association Study

The GWAS was performed using the R package Genome Association and Prediction Integrated Tool (GAPIT) v.3 [43]. The Mixed Linear Multiple Loci Model (MLMM) with the application of  $K$  and  $Q$  matrices was applied for the identification of QTLs for the studied traits in the two regions. The evaluations of nine agronomic traits generated in field trials over two years in the two locations were used as phenotypic data. The criterion  $p < 1 \times 10^{-3}$  was used as a significance threshold for the identified QTLs. The Bonferroni correction index was applied to determine the statistical significance threshold in GWAS. The distribution lines in each quantile–quantile (Q–Q) plot were analyzed to confirm the correction due to the  $K$  and  $Q$  matrices. In the case of several significant QTLs positioned close to each other, the SNP with the lowest  $p$ -value was chosen. Manhattan plots and SNP density plots were generated using the rMVP package [44].

### 2.6. Candidate Gene Analysis

To identify the protein-coding genes that overlapped the identified significant QTLs, the sequence for each marker was used in the BLAST tool of Ensembl Plants [45] for comparison against the reference genome of *T. aestivum*.

## 3. Results

### 3.1. Descriptive Statistics of Phenotypic Traits

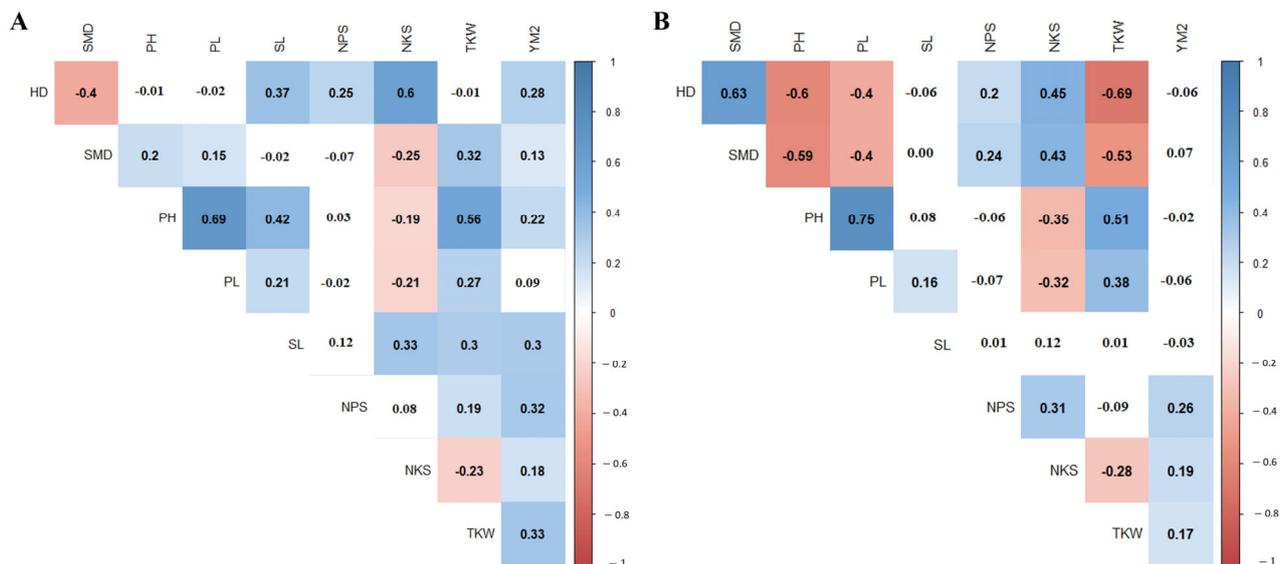
The field performance of the 277 winter wheat accessions was analyzed at KRIAPG and KBS during the 2020–2021 and 2021–2022 growing seasons (Table S2). We recorded the phenotypic variability of nine traits between the two regions, including the average SMD, which ranged from  $35.35 \pm 0.18$  days at KRIAPG to  $39.46 \pm 0.31$  days at KBS. The average PH ranged from  $70.58 \pm 0.81$  cm at KRIAPG to  $41.95 \pm 0.45$  cm at KBS (Table 2), which showed that the mean PH was 1.7 times is taller at the southeastern station. Most of the average yield components (SL, NKS, TKW, and YM2) showed a higher value at KRIAPG; on the contrary, the average NPS was higher at KBS.

The assessment of HD and SMD showed that in the Almaty region, the mean performance of the collection was shorter than that of the local standard “Zhetisu” (Table 2). In contrast, in the Turkestan region, the average HD was ten days longer and the average SMD was three days longer than those of the local standard “Pamyat 47”. The evaluation of the mean YM2 revealed that the yield at the southeastern station at 4.8 times higher than that at the southern station. In total, 162 and 221 accessions demonstrated higher values in comparison to local standards at KRIAPG and KBS, respectively. The ANOVA showed that the region and year of the trials (Table S3) highly significantly affected all nine studied phenotypic traits. In addition, PH and YM2 were affected by three factors (genotype, region, and year). The index of heritability ( $h_b^2$ ) was analyzed for all traits under all conditions (Table S3), and the highest  $h_b^2$  value was recorded for NKS (0.44) and TKW (0.36). Pearson’s correlation of the average phenotypic values in the two regions indicated that YM2 was positively correlated with NPS, NKS, and TKW (Figure 1). The results of correlation analysis at KBS showed a positive correlation between HD and SMD and between SMD and NKS, but at KRIAPG, there was a negative correlation between these traits (Figure 1). Notably, a shorter SMD was favorable for higher YM2 at KRIAPG, but it was not a significant factor of yield at KBS. At KRIAPG, taller PH positively correlated with YM2, and longer PL positively correlated with TKW (Figure 1A). At KBS, HD negatively correlated with PH, PL, and TKW (Figure 1B).

**Table 2.** Phenotypic variability of the studied traits of 277 accessions of winter wheat in the two regions.

Traits	Site	Check Cultivar	Min	Max	Mean $\pm$ SE
HD, days	KRIAPG	102.75 $\pm$ 31.75	44.00	143.00	102.37 $\pm$ 1.29
	KBS	92.00 $\pm$ 12.00	82.50	115.00	102.98 $\pm$ 0.38
SMD, days	KRIAPG	36.50 $\pm$ 5.50	24.50	44.00	35.35 $\pm$ 0.18
	KBS	36.00 $\pm$ 6.50	26.50	52.00	39.46 $\pm$ 0.31
PH, cm	KRIAPG	71.92 $\pm$ 20.75	35.00	132.00	70.58 $\pm$ 0.81
	KBS	42.00 $\pm$ 0.00	26.40	58.25	41.95 $\pm$ 0.45
PL, cm	KRIAPG	30.83 $\pm$ 8.83	13.42	61.33	25.47 $\pm$ 0.41
	KBS	16.75 $\pm$ 0.25	6.90	31.55	17.38 $\pm$ 0.30
SL, cm	KRIAPG	8.52 $\pm$ 0.19	3.17	12.65	9.52 $\pm$ 0.08
	KBS	7.20 $\pm$ 1.00	5.40	28.85	8.29 $\pm$ 0.14
NPS, pcs	KRIAPG	3.17 $\pm$ 0.83	1.33	6.17	3.27 $\pm$ 0.06
	KBS	2.50 $\pm$ 0.50	3.00	8.00	4.57 $\pm$ 0.06
NKS, pcs	KRIAPG	50.49 $\pm$ 4.86	23.17	70.88	47.04 $\pm$ 0.48
	KBS	18.50 $\pm$ 1.50	25.00	70.00	42.60 $\pm$ 0.50
TKW, g	KRIAPG	40.66 $\pm$ 6.61	19.85	58.80	35.25 $\pm$ 0.43
	KBS	30.27 $\pm$ 4.15	13.49	33.42	20.44 $\pm$ 0.29
YM2, g/m <sup>2</sup>	KRIAPG	354.80 $\pm$ 176.09	22.95	997.28	393.48 $\pm$ 7.88
	KBS	36.72 $\pm$ 1.85	15.05	266.98	81.33 $\pm$ 2.16

Note: min—minimum value, max—maximum value, mean—mean value, SE—standard error.

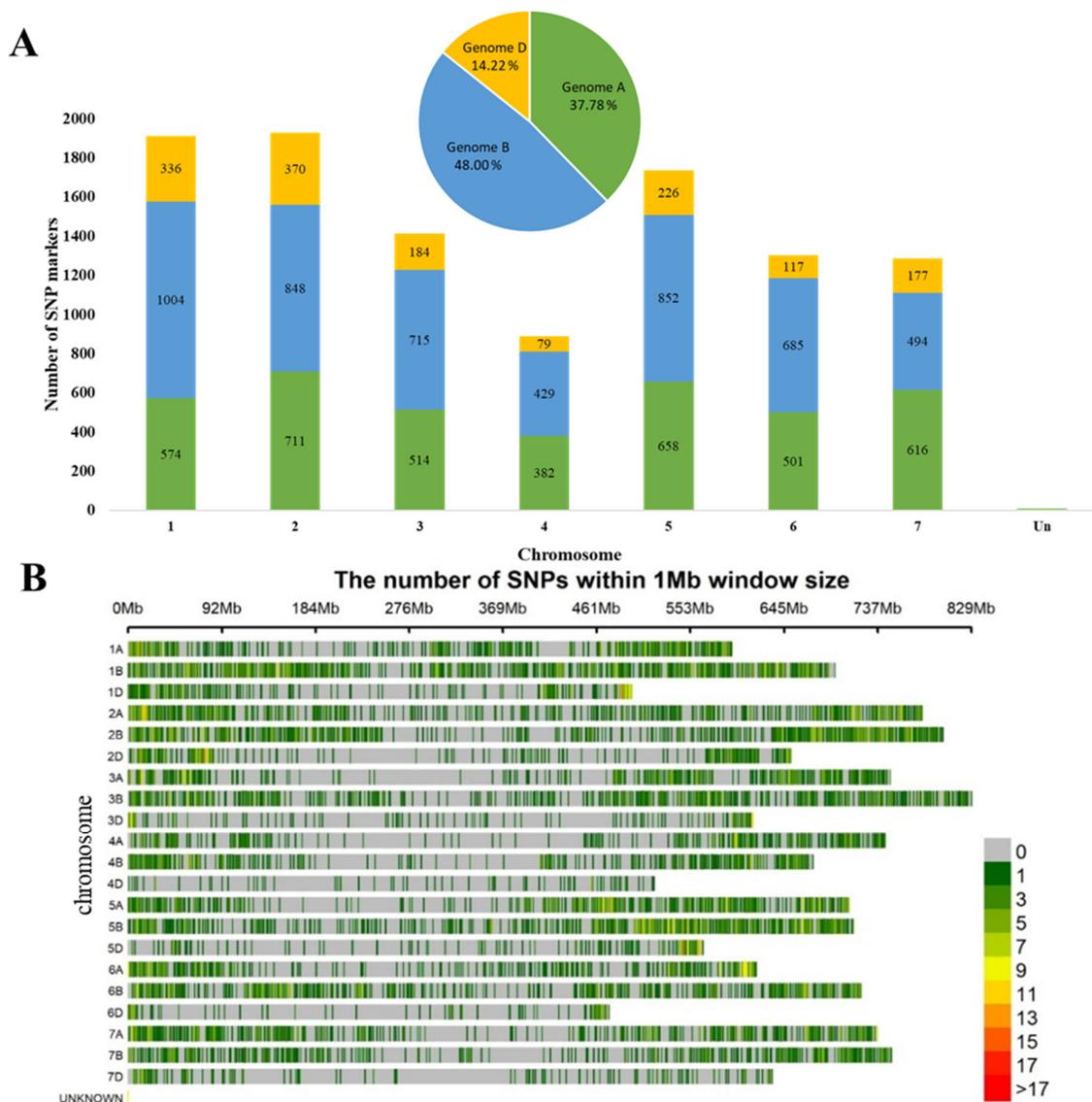


**Figure 1.** Pearson's correlation index based on average data for two years at KRIAPG (A) and KBS (B). Note: Correlations with  $p < 0.05$  are highlighted in color. The color indicates either positive (blue) or negative (red) correlation.

### 3.2. SNP Genotyping and Population Structure in the Studied Winter Wheat Collection

The GWAS relied on the use of 10,481 informative SNP markers, of which 37.78% mapped to the A genome, 48% mapped to the B genome, and 14.22% mapped to the D genome (Figure 2). The minimum number of SNPs (79) was assigned to chromosome 4D, while the maximum number of SNPs was assigned to chromosome 1B (1004) (Figure 2).

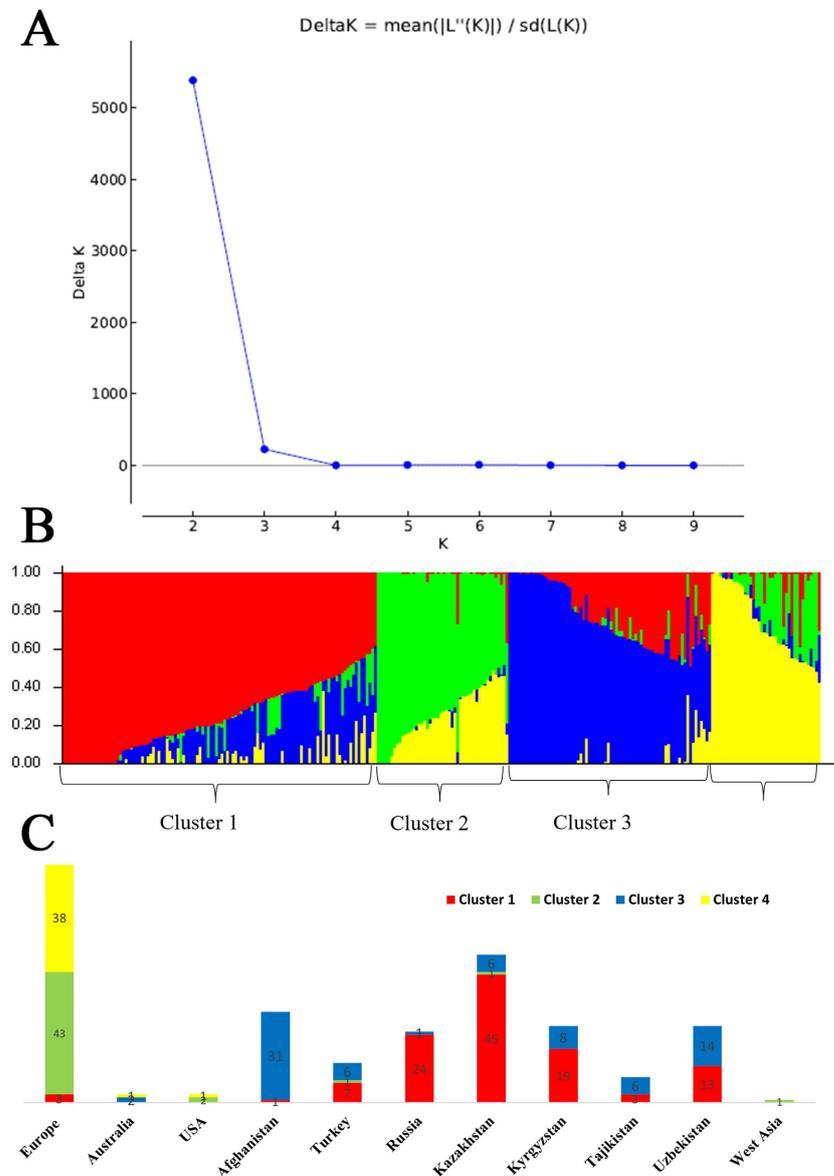
Chromosome 2D contained the largest number of markers, at 1929 SNP markers, having a subgenome A of 711 SNPs, subgenome B of 848 SNPs, and subgenome D of 370 SNPs. Chromosome 4 had the smallest number, with only 890 markers, with a subgenome A of 382 SNPs, subgenome B of 429 SNPs, and subgenome D of 79 SNPs (Figure 2A). The smallest size was found in chromosome 6D (461 Mb), and the longest was found in chromosome 3B (829 Mb). The marker density ranged from 263 SNP/Mb to 450 SNP/Mb, with an average of 365 SNP/Mb. The highest average marker density (450 SNP/Mb) was recorded for chromosome 5B, followed by 442 SNP/Mb for chromosomes 3A and 4A, and the lowest (263 SNP/Mb) was found in chromosome 1D, followed by 281 SNP/Mb and 283 SNP/Mb for chromosomes 7D and 6D (Figure 2B).



**Figure 2.** Distribution of 10,481 single-nucleotide polymorphism (SNP) markers mapped to the wheat genome: (A) distribution of SNPs on seven chromosomal groups and the numbers of mapped SNPs on subgenomes A, B, and D; (B) density plot of SNP markers in wheat chromosomes.

The results of the STRUCTURE and STRUCTURE Harvester analyses suggested that  $K = 4$  was the optimal number of clusters for studying 277 accessions (Figure 3A,B). It can be seen from the STRUCTURE results that most samples from Central Asia were grouped into cluster 1 and cluster 3 (Figure 3B,C). The samples from Kazakhstan (45) and Uzbekistan (14) were predominantly in cluster 1 and cluster 3. Furthermore, all samples from Afghanistan

(31) were grouped into cluster 3, while most of the samples from cluster 1 were from the Russian Federation (24) (Figure 3C). The samples from Europe, Australia, and the USA were grouped into cluster 2 and cluster 4.



**Figure 3.** Population structure of the winter wheat collection based on 10,481 SNP markers: (A) STRUCTURE Harvester output for delta K; (B) separation of samples into clusters based on the STRUCTURE package at K = 4; (C) frequency of populations in clusters of the winter wheat population at K = 4. The colors in the boxes represent the clusters identified in (B).

### 3.3. Identification of Marker–Trait Associations for Agronomic Traits

The GWAS in the two studied regions for the nine agronomic traits allowed the identification of 951 QTLs (Table S4), including 173 stable QTLs that were significant for two or more conditions (Table 3 and Table S5). In all, 59 and 95 stable QTLs were identified at KRIAPG and KBS, respectively. The comparative assessment of the GWAS suggested that 19 QTLs were significant in both regions (Table 3 and Table S5). The highest number of stable QTLs was localized on chromosomes of genome B (83), followed by genomes A (60) and D (30). Among the nine traits, the number of identified QTLs ranged from 1 QTL for TKW to 68 QTLs for PH (Table 3). The total number of stable QTLs identified for the group

of plant-adaptation-related traits was 121 QTLs (Table 3), and for the yield components, it was 52 QTLs (Table 3).

**Table 3.** Summary of identified marker–trait associations in the winter wheat collection based on field performance in the two locations.

Traits	Total QTLs	Stable QTLs	KRIAPG (Almaty Region)	KBS (Shymkent Region)	Both Regions
HD, days	187	37	22	8	7
SMD, days	96	8	3	5	NA
PH, cm	171	68	11	52	5
PL, cm	105	8	3	4	1
SL, cm	76	9	3	6	NA
NPS, pcs	52	4	1	3	NA
NKS, pcs	123	25	10	12	3
TKW, g	55	1	1	NA	NA
YM2, g/m <sup>2</sup>	86	13	5	4	4
Total	951	173	59	94	19

Note: NA—not available.

The GWAS for four traits related to plant adaption allowed the detection of 37 significant QTLs for HD, 8 for SMD and PL, and 68 for PH. However, the application of the Bonferroni threshold suggested that only 18 QTLs can be confidently accepted, comprising 11 QTLs for HD, 1 QTL for SMD, 4 QTLs for PH, and 2 QTLs for PL (Table 4).

**Table 4.** List of QTLs after application of the Bonferroni test.

Traits	SNP	Chromosome	Physical Position (Mb)	<i>p</i> -Value	Effect	PVE (%)	Conditions
HD	AX-95186349	1A	102,166,440	$4.19 \times 10^{-6}$	−3.59	6.00	AL2021
HD	AX-94958010	1B	548,536,119	$9.61 \times 10^{-8}$	10.98	12.88	AL2021, 2022, average; SH 2021, average
HD	AX-94687276	2D	5,326,043	$7.24 \times 10^{-11}$	−19.50	39.08	AL2022, average;
HD	AX-94440472	3B	507,287,720	$5.08 \times 10^{-7}$	5.12	64.32	AL2021, SH2022
HD	AX-94567204	3D	496,732,990	$4.15 \times 10^{-9}$	−30.26	95.13	SH2022
HD	AX-94720837	5A	416,225,444	$2.27 \times 10^{-7}$	−3.67	29.92	AL2021
HD	AX-94999352	5A	563,498,900	$7.60 \times 10^{-8}$	−8.64	3.24	AL2022
HD	AX-94464997	5A	591,156,115	$1.08 \times 10^{-8}$	9.18	9.47	AL2021,2022, average
HD	AX-94675648	6B	704,187,628	$5.18 \times 10^{-7}$	−10.74	5.39	AL2022
HD	AX-94994788	7A	446,323,817	$5.18 \times 10^{-7}$	−5.80	40.95	AL2021, average
HD	AX-95074391	7A	468,461,397	$5.39 \times 10^{-9}$	17.34	18.86	AL2022, average
PH	AX-94442698	1B	457,863,915	$4.61 \times 10^{-6}$	2.88	15.74	AL2021, SH2021
PH	AX-94384624	2D	10,323,263	$3.72 \times 10^{-8}$	−13.42	35.21	AL2021, 2022, average

Table 4. Cont.

Traits	SNP	Chromosome	Physical Position (Mb)	<i>p</i> -Value	Effect	PVE (%)	Conditions
PH	AX-94711247	3A	687,675,656	$2.43 \times 10^{-6}$	5.56	48.06	SH2022, average
PH	AX-94517571	5A	585,434,191	$2.44 \times 10^{-6}$	−4.61	10.65	AL2021, average
PL	AX-94599879	2D	32,190,116	$2.72 \times 10^{-7}$	4.13	43.37	AL2022, average
PL	AX-95633357	5D	46,980,690	$3.77 \times 10^{-12}$	−31.38	46.21	SH2021
NKS	AX-94594842	2A	36,131,037	$4.67 \times 10^{-7}$	−3.34	18.44	SH2022, average

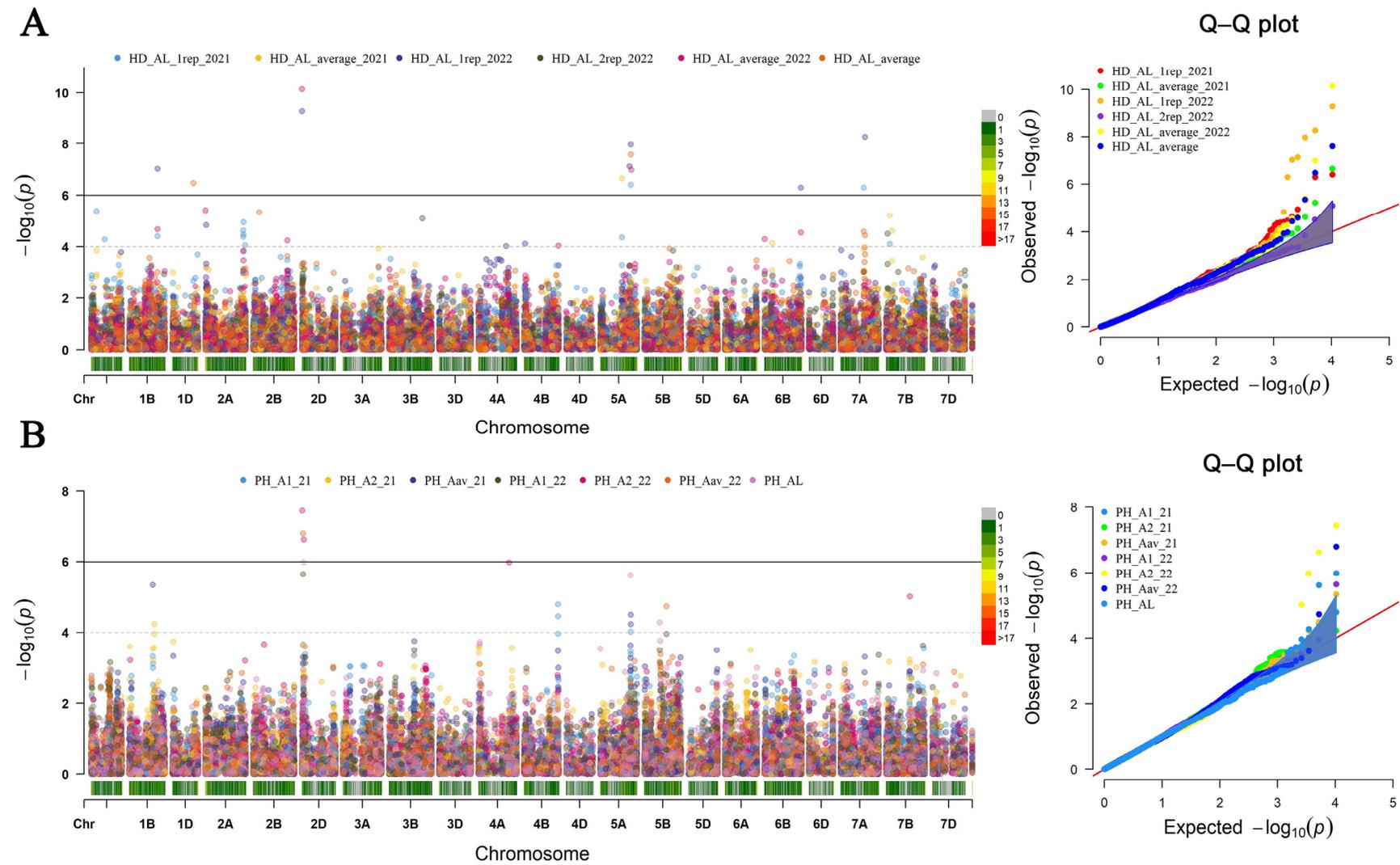
Note: PVE—phenotypic variation, AL2021,2022, average—KRIAPG (Almaty region), SH2021, 2022, average—KBS (Turkestan region).

In total, 37 stable QTLs were identified for HD, with 22 and 8 of these QTLs detected at KRIAPG and KBS, respectively. Seven of the thirty-seven QTLs for HD were detected in both regions. The most significant *p*-value was  $7.24 \times 10^{-11}$ , which was for chromosome 2D at KRIAPG (Table 4). The effect of each individual QTL varied significantly, with the highest value detected for *QHD.ta.w.ipbb-3D* (−30 days), explained by the highest phenotypic variation (PVE) of 95.3%. *QHD.ta.w.ipbb-1B.4* (AX-94958010) was also detected in the two regions and had a *p*-value from  $9.73 \times 10^{-4}$  to  $9.61 \times 10^{-8}$ , with phenotypic variation of 12.8%. The second *QHD.ta.w.ipbb-3B.3* (AX-94440472) was detected in the two regions with a PVE of 64.32% (Table 4).

The highest QTL for PH was detected on chromosome 2D (Figure 4), and it was significant in the two-year trials at KRIAPG, with a PVE of 35.2% (Table 4). The QTL AX-94442698 was detected in the two regions and was mapped to chromosome 1B with a PVE of 12.7%. The highest PVE identified for PH, equal to 48.1%, was on chromosome 1B and was detected in both regions.

The details of the identified QTLs are presented in Table S5 and illustrated in Manhattan plots and Q–Q plots in Figure 4A,B.

In the GWAS of the yield components, the highest number of QTLs was detected for NKS (25). However, only one QTL on chromosome 2A (AX-94594842) passed the Bonferroni threshold. In the assessment of the QTLs for NKS, three QTLs (*QNKS.ta.w.ipbb-2A.3*, *QNKS.ta.w.ipbb-5A.5*, *QNKS.ta.w.ipbb-5B*) were detected in the two regions. In total, 13 stable QTLs were identified for YM2, including four QTLs on chromosomes 1B (3) and 3B (1) that were significant at both KRIAPG and KBS. The highest QTL effect value was detected in *QYM2.ta.w.ipbb-7B* (104.8 g/m<sup>2</sup>). Despite the fact that none of those four associations for YM2 passed the Bonferroni threshold, these signals could still be important for consideration in breeding projects. The significant SNPs associated with nine traits were used to identify the putative candidate genes using the annotated wheat reference sequence Chinese Spring and are presented in Table S5. The results showed that out of the 173 identified stable QTLs, the significant SNPs in 107 QTLs were in genic positions (Table S5). The most significant SNPs were associated with controlling plant growth, plant development, and abiotic/biotic stress tolerance.



**Figure 4.** Manhattan and Q-Q plots for the heading date (**A**) and plant height (**B**) in the GWAS panel phenotyped at KRIAPG (Almaty region) during 2020–2022. Note: A grey line represents the threshold from the Bonferroni correction method.

#### 4. Discussion

This evaluation of field data from winter wheat collections indicated a wide range of phenotypic variations in the southern and southeastern regions of the country. A comparative analysis of the meteorological data and soil parameter conditions (Table 1) suggested that higher precipitation and soil quality significantly contributed to the increased productivity at KRIAPG (Table 2). The assessment of the studied traits revealed that the largest differences between the two regions were in PH and YM2 (Table 2). Pearson's correlation analysis showed a sharp difference between the two regions that affected TKW. For instance, at the KRIAPG site, the TKW did not correlate with HD and positively correlated with SMD, while at the KBS site, the TKW negatively correlated with HD and SMD (Figure 1). Similarly, NKS negatively correlated with SMD at KRIAPG and positively correlated with SMD at the KBS site (Figure 1). Expectedly, as detailed in many published reports [46–48], NKS negatively correlated with TKW at both locations. TKW was more strongly correlated with YM2 at KRIAPG (0.33) in comparison to KBS (0.17). The ANOVA among yield components identified highly significant effect of the genotype at  $p < 0.0001$ . The genetic variation among genotypes is very important for breeders to select the high-yielding accessions for local breeding programs. The genotype  $\times$  environment interactions (GEI) were significant at  $p < 0.0001$  for PH and YM2. The significant GEI suggests that genotypes react differently on changes of the environment [5]. The analysis of the average YM2 revealed 162 and 221 accessions that exceeded the YM2 of the local comparison cultivars at KRIAPG and KBS, respectively. Four accessions, CAWBIN-070, CAWBIN-074, CAWBIN-135, and CAWBIN-137, demonstrated adaptability to both regions and showed higher yield than the local comparison cultivars under all studied conditions (Table S2); these can be used for further winter wheat breeding projects in Kazakhstan.

The GWAS analyses of the winter wheat collection in the two regions led to the identification of 173 stable QTLs in nine agronomic traits in two or more environments (Table S5). However, the Bonferroni test suggested that only 19 of these QTLs can be confidently accepted for HD, PH, PL, and NKS. Still, minor QTL hypothetically may play an important role in the expression of complex traits and should be considered in regional breeding projects [19,49], including in Kazakhstan. Notably, the largest number of associations was identified for HD, PH, and NKS, which shows a wide range of variation of these traits in the two regions. The literature survey suggested that 23 of the 173 QTLs identified in our study had also been detected in previous QTL mapping studies and GWAS for HD, SMD, PH, SL, NKS, and YM2 [18,28,50–57] (Table S6). The majority of these matches were found for PH (nine QTLs), followed by HD (six QTLs) and NKS (four QTLs) (Table S6). Four associations matched the results from studies of the UK reference mapping population Avalon  $\times$  Cadenza, where QTL associations with HD, PH, and YM2 were identified in the northern, central, and southern regions of Kazakhstan [55]. Another four associations were identical to the genetic positions of QTLs identified in analyses of eight traits using 94 recombinant inbred lines of the mapping population of Pamyati Azieva  $\times$  Paragon, which were tested in the northern and southern regions of Kazakhstan [56]. Three QTLs (*QHD.ta.w.ipbb-2A.2*, *QHD.ta.w.ipbb-5B.1*, and *QSMD.ta.w.ipbb-3B*) had identical genetic positions to QTLs identified in the GWAS of yield components in a spring wheat collection harvested under two water regimes in northern Kazakhstan [18] (Table S6). Further, *QPH.ta.w.ipbb-5B.2* had an identical to QTLs identified in the analyses of seven traits using a winter wheat doubled haploid population under different soil moisture conditions [28]. Two associations matched the results from the GWAS studies of 290 lines of the Wheat Association Mapping Initiative population in terms of PH and SL, which were identified under drought and terminal heat stress tolerance [57].

The GWAS analysis of the winter wheat collection allowed the identification of 150 putative novel, stable QTLs. The most significant numbers of presumably novel QTLs were identified in PH (57) and HD (31), including three QTLs that were revealed in both regions (Table S5). In addition, two of these SNPs (*AX-94450249* and *AX-94711247*) significantly af-

ected both HD and PH (Table 4). In the southern region, it was revealed that *QPH.ta.w.ipbb-7B.6* and *QPL.ta.w.ipbb-7B* shared locations with QTLs relating to PH and PL, respectively (Table S5). Forty-six presumably novel QTLs were identified for yield-related traits, which included eight for SL, four for NPS, twenty-one for NKS, one for TKW, and twelve for YM2 (Table S5).

The significant SNPs in the detected QTLs were analyzed to identify putative candidate genes using the annotated Chinese Spring reference genome [58] in the Wheat Ensembl database [45]. The results showed that out of the 173 identified stable QTLs, 107 were located in genic positions (Table S5). An analysis of these 107 genes suggested that most of them were associated with controlling plant growth, plant development, and abiotic/biotic stress tolerance. For instance, the evaluation suggests that the genes for carboxypeptidase located on chromosome 2A (*TraesCS2A02G133700*) and on chromosome 4A (*TraesCS4A02G035200*) are involved in the regulation of plant growth, development, and pathogen defense [59]. It was determined that AX-94614447 associated with PH and AX-94594842 associated with NKS both encode *Domain of unknown function (DUF)* domain-containing proteins, which play a role in plant development and fitness in rice [60]. *TraesCS3B02G041700* also appears to encode the  $\alpha$ -glucosidase protein, which controls the plant polysaccharide composition at the stage of plant maturation in rice and potato [61]. *TraesCS7A02G322800* (TOM1-like protein 5) genes were shown to be important factors in the uptake of soil iron and its translocation and were significantly associated with HD [62]. TOM1 was first identified in rice and barley. The list of genes related to stress resistance/tolerance genes includes *TraesCS6B02G411900* (protein kinase superfamily protein) [63]; *TraesCS1A02G105700* (YTH domain-containing family protein) [64]; *TraesCS7A02G403900* (basic helix–loop–helix transcription factors) [65]; *TraesCS5B02G057800* (glutathione S-transferase zeta class) for drought tolerance, *TraesCS1B02G323500* (zinc finger protein) [66]; *TraesCS3B02G563200* (protein detoxification) for heavy metal stress tolerance [67]; and *TraesCS1D02G439800* (trimethylguanosine synthase) for chilling tolerance [68]. Finally, *TraesCS2A02G253900* on chromosome 2A is a transcription factor that regulates the photoperiodic control of the flowering time and is associated with PH [69]. The matching of the associations identified in this study with previously published reports confirms the robustness of the results in this work. Although the identified QTLs should be further validated in subsequent experiments, there is a promising assumption that most presumably novel associations have true significance for plant adaptation-related traits and yield components. Therefore, the identified SNPs in the detected QTLs may have great value for successful application in the marker-assisted breeding of winter wheat.

## 5. Conclusions

A collection of winter wheat from Central Asia was compiled, including accessions from Kazakhstan (52), Kyrgyzstan (27), Tajikistan (9), and Uzbekistan (27). The samples from Central Asia, along with accessions from other parts of the world, were united to form the CAWBIN winter wheat collection, consisting of 277 genotypes. The CAWBIN winter wheat collection was tested at stations in Kazakhstan's southern and southeastern regions during the 2021–2022 seasons and comparatively analyzed by examining nine agronomic traits. It was revealed that 162 and 221 accessions surpassed the local standards for YM2 at KRIAPG (Southeast Kazakhstan) and KBS (South Kazakhstan), respectively. Therefore, the collection has tremendous potential for the improvement of winter wheat productivity in Kazakhstan. The CAWBIN collection was genotyped using the Axiom Wheat Breeder's Genotyping Array with 35 K SNPs, and 10,481 polymorphic SNPs were further utilized for a GWAS of the agronomic traits in winter wheat. The results of the GWAS based on the GAPIT package and MLM method allowed the detection of 173 stable QTLs in the nine studied agronomic traits. A survey of the published scientific literature suggested that 23 of the 173 stable QTLs have locations similar to those of previously reported QTLs. Therefore, the remaining revealed QTLs are presumably novel loci for the studied nine

traits and can provide a wealth of genetic information for marker-assisted breeding in winter wheat projects.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy13082054/s1>, Table S1: List of common winter wheat cultivars used in the current study. Table S2. The raw field data at Kazakh Research Institute of Agriculture and Plant Industry (KRIAPG, Almaty region, Southeast Kazakhstan) and Krasnovodopad Breeding Station (KBS, Turkestan region, South Kazakhstan). Table S3. Three-way analysis of variance (ANOVA) for phenotypic traits of the winter wheat. Table S4. GWAS results for nine studied traits identified using 277 winter wheat accessions in condition Kazakh Research Institute of Agriculture and Plant Industry (KRIAPG, Almaty region, Southeast Kazakhstan) and Krasnovodopad Breeding Station (KBS, Turkestan region, South Kazakhstan) in 2020–2022. Table S5. The list of stable QTLs and genes for nine studied traits identified using 277 winter wheat accessions in condition KRIAPG and KBS (2020–2022). Table S6. List of identified QTL based on GWAS analysis of winter wheat collection in comparison to the associations revealed in previously published reports.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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