

Article

Exploring the Agronomic Performance and Molecular Characterization of Diverse Spring Durum Wheat Germplasm in Kazakhstan

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Abstract: Spring durum wheat occupies over 0.5 M ha in Kazakhstan and represents an important domestic and export commodity. This study aimed to characterize 151 durum wheat cultivars and advanced lines originating from eight breeding programs of the Kazakhstan–Siberia Spring Wheat Improvement Network (KASIB) between 2003 and 2018. The phenotypic characterization was performed in two contracting evaluation sites more than 1000 km apart (Almaty in the Southeast and Shortandy in the North) for two years and a total of 11 agronomic traits were recorded. Field trials at both locations followed regional agronomy practices, including sowing, harvesting, and genotype evaluation using a randomized complete block design (RCBD). The growing season was longer in Almaty, resulting in a higher number of grains per spike. Though grains are smaller in size with an overall higher yield, 243 g/m² versus 170 g/m², there was no correlation between germplasm performance at the two sites. Molecular characterization was performed with 10 iPBS-retrotransposons primers that resulted in a total of 345 bands and showed a mean polymorphism of 91.9%. Mean values of gene diversity (0.251), Shannon’s information index (0.388), and expected heterozygosity (0.233) revealed a relatively high level of genetic diversity in the KASIB set. AMOVA revealed higher genetic variations due to differences within the populations. Marker-based cluster analysis, including STRUCTURE and neighbor-joining algorithms, divided the material into two populations with clear differences in geographic origin. Superiors and diverse germplasm identified in the study are recommended for marker assisted selection and breeding.

Keywords: *Triticum turgidum* ssp. *durum*; germplasm characterization; molecular markers; KASIB

1. Introduction

Wheat is the main crop in Kazakhstan being grown in an area exceeding 12 mln ha in 2021 [1]. The major area of spring wheat production in the country is in the North as part of a cropping belt extending from the southern Ural Mountains to western Siberia. This production region is fairly uniform and characterized by short seasons (100 days), relatively low precipitation (350–450 mm), and extensive cropping technologies. A detailed description of agroecology, the history of wheat production, and breeding is provided by [2]. Spring wheat is grown from May till early September in rotation with oil crops (sunflower, linseed) and legumes (dry peas, lentils). No-till application has been gradually expanding, currently covering almost 3 mln ha [3]. Drought represents the major abiotic stress and takes place two years out of five on average. Among diseases leaf and stem rust, tan spot and *Septoria* spot blotch are dominating biotic stresses, especially in favorable years with higher rainfall [4]. The average grain yield of spring wheat in northern Kazakhstan is in the range of 1.2–1.3 t/ha, almost twice as low compared to similar regions in North America [5]. The yield gap for spring wheat in Kazakhstan is substantial and can be reduced through intensification of production including fertilizers and crop protection. Durum wheat varieties currently grown in Kazakhstan represent tall day-length sensitive material with limited yield potential as compared to European cultivars [6]. Enhancement of wheat breeding and development of cultivars with broad adaptation, response to inputs, and disease resistance is one of the important avenues for raising yield in the country.

Though spring bread wheat dominates in northern Kazakhstan, durum wheat occupies a substantial share (estimated 700,000 ha). Its area has been growing due to both domestic and foreign demand. There are several spring durum wheat breeding programs in the country: Aktobe (Aktobe region) and Karabalyk (Kostanay region) Agricultural Experimental Stations and Scientific Production Center of Grain Farming named after A.I. Barayev (near Astana) in the North and North-West; Kazakh Scientific Research Institute of Agriculture and Plant Growing in the Southeast. These four durum breeding programs and four breeding programs in Russia (Saratov, Samara, Omsk, and Barnaul regions) have been united in the Kazakhstan–Siberia Spring Wheat Improvement Network (KASIB) since 2003. The network operates bi-annual cooperative yield trials with each participant contributing 2–4 new varieties or advanced lines and conducting the trials. More than 200 genotypes of spring durum wheat have been exchanged and tested in multi-locational trials.

Tajibayev et al. [7] analyzed KASIB data from nine trials conducted in 2003–2020 across five sites in Kazakhstan and Russia to evaluate genotype × environment interactions and identify superior germplasm. Analysis of weather variation between years demonstrated that all sites were subject to the same climatic events. Air temperature in May–August was negatively associated with grain yield and had a relatively higher effect compared to rainfall. The performance of germplasm was generally similar between all five sites. Grain yield and 1000 kernel weight were closely associated at all sites, suggesting the importance of breeding for grain size. Superior germplasm was identified by combining high grain yield across the five sites, variable response to environments, variable maturity range, and large grain. Breeding programs at Samara and Omsk contributed the most germplasm to high-yielding performers. Evdokimov et al. [8] analyzed durum wheat KASIB data from 2000 to 2014 and identified drought-tolerant germplasm across multi-environment testing sites. However, there was no systematic effort to evaluate KASIB durum wheat material exchanged during different years in one uniform trial.

Marker-assisted selection can be achieved by characterizing germplasm that allows the breeders to identify new genetic variations and use them to select parent lines for further crossing [9]. To date, a large number of genetic markers have been developed to identify genetic variations in various crop species [10–12]. Among the markers, retrotransposons are mobile elements that have the ability to change their position, duplicate themselves, and play a key role in plant genome size, and can be used as molecular markers [13]. There are two types of retrotransposons, i.e., LTRs (long terminal repeats

found in plant genes) and non-LTR retrotransposons. Researchers have developed various retrotransposon-based markers like Retrotransposon-Based Insertional Polymorphism (RBIP), inter-retrotransposon amplified polymorphism (IRAP), and retrotransposon microsatellite amplified polymorphism (REMAP). Limitations in these markers resulted in the development of a new marker system named “inter primer binding site (iPBS)” [14]. The iPBS-retrotransposon amplification methodology is very practical, and it contains a robust DNA fingerprinting technology that does not need previous sequence information [15]. The versatility of iPBS retrotransposons lies in the presence of a tRNA complement as the binding site for the reverse transcriptase (PBS) primer in LTR retrotransposons [16]. Due to their general applicability, ease of use, and genotype resolution systems, retrotransposon DNA markers have found widespread use in numerous evolutionary and genetic studies [17–23].

A set of 151 spring durum wheat genotypes from KASIB network trials conducted in 2003–2020 was selected for a detailed diversity and adaptation study. The material represented the breeding outcome of eight main spring durum wheat breeding programs in Kazakhstan and Russia. The field trials were conducted at two key sites at the Scientific Production Center of Grain Farming named after A.I. Barayev (near Astana) in the North and Kazakh Scientific Research Institute of Agriculture and Plant Growing in the Southeast (near Almaty). These two institutions are the main crop improvement centers targeting their respective regions but also overlap geographically in the release of their cultivars. The two sites are situated at a distance of over 1100 km and represent very different and contracting production systems.

The study objective was the evaluation of contemporary spring durum wheat diversity, genotypes \times environment interaction between two contrasting breeding sites, the comparative agronomic performance of germplasm originating from different programs and, finally, identification of superior and genetically distinct genotypes for potential use as varieties and parents.

2. Materials and Methods

2.1. Durum Wheat Material

The list of materials comprised 151 entries and is presented in Supplementary Table S1. The germplasm represented the recent varieties and advanced lines from four breeding programs in Kazakhstan and four in Russia (Table 1). An estimated 70–80% of all spring durum wheat grown in Kazakhstan and Russia originates from these eight breeding programs. The distance from the westernmost site Saratov and easternmost Barnaul exceeds 3000 km, while Almaty (South) and Omsk (North) are more than 1500 km apart. All the breeding sites represent different agroecological zones with variable climates and cropping systems. The germplasm included in the study was tested through KASIB trials from 2003 to 2020. The exception was the material from Samara and Saratov, which joined KASIB in 2011 and 2015, respectively. Three local checks were used in the study: Nauryz-6 (Almaty), Altyn Dala (Karabalyk), and Zhemchuzhina Sibiri (Omsk).

2.2. Experimental Sites and Weather

The material was evaluated in 2020 and 2022 at Kazakh Scientific Research Institute of Agriculture and Plant Growing near Almaty. The soil type is light chestnut with organic matter content in a range of 2.5%. The phosphorus and potassium contents are sufficient and nitrogen needs to be provided to meet the crop demand. This site represents a Southeastern cropping system with winter wheat, soybeans, vegetables, and forage crops. This site is at the Tien Shan Mountains foothills at 900 m above sea level. The average annual rainfall is above 500 mm with mild winter and a long growing season from March to October. The 2020 season was characterized by average temperature but almost 50% lower rainfall compared to the long-term average. The year 2022 had a slightly higher temperature and 40% higher rainfall during April–August.

The northern evaluation site was situated at the Scientific Production Center of Grain Farming named after A.I. Barayev (Shortandy, 60 km north of Astana). The soil type is chernozem with organic matter content in a range of 3.5%. This is a typical dryland steppe production zone (spring wheat and barley, pulses, oil crops) with cold winter and a growing season of around 100 days from May to September. The annual rainfall is only 350–400 mm. The winters are characterized by cold temperatures, with temperatures reaching as low as $-40.6\text{ }^{\circ}\text{C}$ ($-41.5\text{ }^{\circ}\text{F}$), while the summers can be quite hot, with temperatures reaching up to $39.4\text{ }^{\circ}\text{C}$ ($103\text{ }^{\circ}\text{F}$) [24]. The average air temperature during the season was close to the long-term average. The rainfall in 2021 was 30% lower and in 2022 it was close to the long-term average.

Overall, the two contracting evaluation sites provided substantial environmental variation for germplasm evaluation. The fluctuation of seasonal weather at each site also contributed to the detailed characterization of the tested material. The variations in climatic conditions between Shortandy and Almaty, characterized by differences in precipitation patterns, temperature regimes, and duration of the vegetative period, play a crucial role in influencing crop yield and the adaptive capacity of plants to their respective environments.

Table 1. The origin of the germplasm included in the study.

Breeding Program	Geographical Site ¹	Coordinates	Precipitation May–August, mm	Number of Entries in the Study
Aktobe Agric. Research Station (KZ)	Aktobe, Aktobe region	50.3519° N, 57.3928° E	131	20
Karabalyk Agric. Research Station (KZ)	Karabalyk, Kostanay region	53.8540° N, 62.1015° E	218	25
Scientific Production Center of Grain Farming named after A.I. Barayev (KZ)	Shortandy, Akmola region	51.4024° N, 71.0049° E	225	14
Kazakh Scientific Research Institute of Agriculture and Plant Growing (KZ)	Almaty, Almaty region	43.2475° N, 76.6959° E	509	25
Altay Agric. Research Institute (RU)	Barnaul, Altay region	53.4125° N, 83.5190° E	230	20
Omsk Agricultural Research Center (RU)	Omsk, Omsk region	55.0404° N, 73.3604° E	238	25
Samara Agric. Research Institute (RU)	Bezenchuk, Samara region ²	52.9644° N, 49.4187° E	180	14
Southeast Agricultural Research Institute (RU)	Saratov, Saratov region ²	51.3420° N, 45.5952° E	175	5

¹ Sites names are used in the following tables and text to identify the respective breeding programs. ² Samara and Saratov germplasm is combined in one group for the analysis and called Sam-Sar.

2.3. Field Experimentation

The field trials at both locations followed common agronomy practices for the respective regions. Due to the unavailability of suitable seeds for the study, yield performance trials in Almaty were not conducted in 2021. The decision not to conduct the trials was made based on the availability of materials, with a focus placed on alternative locations where seeds were accessible and data collection opportunities existed. The experiments were planted in the field after black fallow. Fertilizers were used before planting at a rate of N30P30K30. Planting took place in mid-March in Almaty and in mid-May in Shortandy. The harvesting was conducted in July and September, respectively. Weeds were effectively managed through the application of conventional herbicides, utilizing a carefully calibrated blend of Granstar Pro and Trend 90 at the prescribed dosage. Neither insecticides or fungicides were used for crop protection. The statistical design used for genotype evaluation was a randomized complete block design (RCBD) in both locations. The trial at the Kazakh Scientific Research Institute of Agriculture and Plant Growing in Almaty was conducted in 1 m² plots with two replications. The plot size was 3 m², with two replications at the Scientific Production Center of Grain Farming named after A.I. Barayev. Agronomic traits (days

to heading, plant height, yield components) were recorded following CIMMYT Physiology Manual-2 [25].

2.4. Molecular Analysis

2.4.1. DNA Isolation

Healthy seeds were sown in germination trays at Sivas University of Science and Technology, Sivas, Türkiye. After germination, fresh, young, and healthy leaves were collected for the isolation of genomic DNA. The CTAB protocol described by Doyle and Doyle [26], a specific protocol recommended by Diversity Arrays Technology (Available online: <https://www.diversityarrays.com/orderinstructions/plant-dna-extraction-protocol-for-dart/> accessed on 15 May 2023), was used. The isolated DNA was diluted and stored at $-20\text{ }^{\circ}\text{C}$. After extraction, the concentration and quality of genomic DNA was measured by Spectrophotometry using nanodrop 2000c (Thermo Scientific, Waltham, MA, USA), and the quality of DNA was also analyzed with 0.8% agarose gel. The final concentration of genomic DNA was $5\text{ ng }\mu\text{L}^{-1}$ with double distilled water (ddH_2O) and stored at $-20\text{ }^{\circ}\text{C}$ for Polymerase chain reaction (PCR) amplification.

2.4.2. PCR Amplification

Eighty-three iPBS primers taken from the study of [14] were screened on 8 randomly selected genotypes and highly polymorphic primers were selected for further PCR amplification. Details of selected iPBS-retrotransposon primers and their annealing temperature and sequence are given in Table 2. PCR amplification was performed using a $20\text{ }\mu\text{L}$ volume mixture comprising $2\text{ }\mu\text{L}$ $1\times$ PCR assay buffer (Thermo Scientific), $2\text{ }\mu\text{L}$ dNTPs (2 mM) (Thermo Scientific), $5.6\text{ }\mu\text{L}$ distilled water, $3.2\text{ }\mu\text{L}$ primer (5 mM), and $2\text{ }\mu\text{L}$ MgCl_2 (2 mM), $0.2\text{ }\mu\text{L}$ U Taq DNA polymerase (Thermo Scientific) and $5\text{ }\mu\text{L}$ (25 ng) of genomic DNA. The PCR protocol of [14] was followed; initial denaturation at $95\text{ }^{\circ}\text{C}$ for 3 min; 30 cycles at $95\text{ }^{\circ}\text{C}$ for 15 s, 50 to $65\text{ }^{\circ}\text{C}$ annealing temperature (depending on the primer) for 1 min, and $68\text{ }^{\circ}\text{C}$ for 1 min; and final extension at $72\text{ }^{\circ}\text{C}$ for 5 min. PCR products were separated via electrophoresis (Bio-Rad, Hercules, CA, USA) using 2% agarose gel in a $1\times$ Tris Borate ETDA (TBE) buffer with a constant voltage of 120 V for 2.30 h. Gels were stained with ethidium bromide. The gel image was taken using a gel documentation system (Bio-Rad, Hercules, CA, USA). A 100 bp plus gene ruler was used as a molecular weight during the scoring of gel images.

Table 2. Ten iPBS markers used to evaluate genetic diversity among 151 durum wheat genotypes.

Primer Name	Sequence	Annealing Temperature ($^{\circ}\text{C}$)
2228	CATTGGCTCTTGATACCA	53
2074	GCTCTGATACCA	50
2226	CGGTGACCTTTGATACCA	53
2239	ACCTAGGCTCGGATGCCA	55
2245	GAGGTGGCTCTTATACCA	50
2252	TCATGGCTCATGATACCA	52
2256	GACCTAGCTCTAATACCA	51
2270	ACCTGGCGTGCCA	55
2271	GGCTCGGATGCCA	55
2389	ACATCCTTCCCA	50

2.5. Statistical Analysis

2.5.1. Morphological Data Analysis

ANOVA was conducted for agronomic traits in a replicated trial using R Studio software version 2022.12.0+353. Average values, standard errors, and coefficients of correlation were calculated using MS Excel.

2.5.2. Molecular Markers Data Analysis

All clear and distinguishable gel bands were scored and the band's presence and absence were represented as 1 and 0, respectively. PopGen software version 1.32 [27] was used for the determination of genetic parameters such as total gene diversity (Ht), genetic diversity (He), Shannon's information index (I), the effective number of alleles (Ne), and genetic distance (GD). The polymorphism information content (PIC) was determined using the formula, i.e., $PIC = 2f_i(1 - f_i)$ [28]. Here, f_i represents the frequency of molecular marker loci present and $(1 - f_i)$ represents the frequency of absent loci. Analysis of molecular variance (AMOVA) was calculated and analyzed using GenAlExV6.5 software [29]. To evaluate the genetic resemblance among 151 durum wheat accessions, a neighbor-joining analysis was conducted employing arithmetic means in cluster analysis, supported by 1000 bootstrapping iterations, to assess the statistical significance and reliability of the phylogenetic tree. The analysis was performed using R statistical software R 4.2.3 version. STRUCTURE program was run to determine the number of subgroups (K) among the studied germplasm, with K values ranging from 1 to 10, and 10 independent runs for each K value. Thereafter, a Markov Chain Monte Carlo (MCMC) algorithm was adopted to obtain the most suitable K value. The initial burn-in period was set to 50,000 with 300,000 MCMC (Markov chain Monte Carlo) iterations with no prior information on the origin of individuals. For the determination of a suitable number of clusters (number of K; the number of subpopulations) in the STRUCTURE analysis, we followed the criteria suggested by [30]. The best number of K was selected by using STRUCTURE Harvester online <https://taylor0.biology.ucla.edu/structureHarvester> (accessed on 15 May 2023), according to the principle of the highest value of ΔK .

3. Results

3.1. Comparison of Durum Wheat Agronomic Performance at Two Sites

The agronomic performance of durum wheat germplasm at the two sites was substantially different (Table 3). The southern location in Almaty had much earlier planting and the period from emergence to heading exceeded 60 days compared to over 42 days in Shortandy. The plants were 32 cm taller and the spike length was 40% longer in Almaty compared to Shortandy. As a result, the spike productivity in Almaty was 2.07 gr versus 1.15 gr. This advantage was determined by the higher number of spikelets per spike (18.8. versus 12.1) and number of grains per spike (49.9 vs. 24.6). However, 1000 kernel weight was 10.8% lower in Almaty as compared to Shortandy (39.8 vs. 44.1). The grain yield was 243 g/m² in Almaty compared to 170 g/m² in Shortandy. Overall, the environment in the southern location of Almaty was more suitable for spring durum wheat: longer growing seasons, taller plants, and more productive spikes resulted in a higher grain yield.

The correlation between agronomic traits expression at the two sites was highest for 1000 kernel weight (0.699), number of days to heading (0.698), and awn length (0.633) (Table 3). Average values of correlations were observed for plant height (0.534), spike length (0.453), and number of spikes per spikelet (0.450). For other yield components, the correspondence between the two sites was low. For grain yield, there was no correlation between the two sites.

The relationship between grain yield and other agronomic traits for individual sites and years is presented in Table 4. In Almaty, there was a tendency for later germplasm being lower yielding. This tendency was better expressed in 2022, with the negative coefficient of correlation between the number of days to heading and grain yield being -0.412 . In contrast, in Shortandy, the later maturing material tended to be higher yielding. Plant

height, the number of grains per spike and per spikelet, and grain weight per spike had a positive effect on grain yield across all four sites and years. However, the values of the coefficient of correlations varied depending on the environment. In Shortandy under dry conditions of 2021, almost all traits had an average degree of correlation with grain yield. Thousand kernel weight had a weak significant association with grain yield in Almaty in 2022 (0.377). The main difference in grain yield formation at the two sites was a reverse relationship with maturity. While in the Southeast earlier heading material tended to perform better, in the north it was the opposite.

Table 3. Agronomic parameters of the spring durum wheat germplasm tested in Almaty (2020 and 2022) and Shortandy (2021–2022) and coefficients of correlation between two sites.

Trait	Almaty	Shortandy	LSD 0.05	Correlation Almaty-Shortandy ²
Days to heading	60.7	42.7	0.6	0.698 ***
Plant height, cm	103.2	71.9	1.3	0.534 ***
Spikes/plant	1.17 ¹	1.50	-	-0.067
Spike length, cm	8.8	6.2	0.2	0.453 ***
Awn length, cm	11.5 ¹	8.9	-	0.633 ***
Spikelets/spike	18.8	12.1	0.2	0.450 ***
Grains/spike	49.9	24.6	0.7	0.277 ***
Grains/spikelet	2.66	2.03	0.05	0.279 ***
Grain weight/spike, g	2.07	1.15	0.04	0.164 *
1000 KW	39.8	44.1	0.7	0.699 ***
Yield, g/m ²	243	170	9	-0.006

¹—values for year 2020 only. ²—*, ** and ***—correlation coefficients are significant at $p < 0.05$ and $p < 0.001$, respectively.

Table 4. Coefficients of correlation between agronomic traits and grain yield for spring durum wheat germplasm tested in Almaty (2020 and 2022) and Shortandy (2021–2022).

Trait	Coefficients of Correlation with Grain Yield:			
	Almaty		Shortandy	
	2020	2022	2021	2022
Days to heading	-0.140	-0.412 ***	0.209 **	0.290 ***
Plant height, cm	0.241 **	0.202 *	0.570 ***	0.219 **
Spikes/plant	0.025	-	0.197 *	0.082
Spike length, cm	0.173 *	-0.078	0.429 ***	0.221 **
Awn length, cm	0.101	-	0.215 **	0.013
Spikelets/spike	0.151	-0.084	0.423 ***	0.216 **
Grains/spike	0.229 **	0.193 *	0.422 ***	0.213 **
Grains/spikelet	0.164 *	0.275 ***	0.252 **	0.092
Grain weight/spike, g	0.330 ***	0.509 ***	0.422 ***	0.265 ***
1000 KW	0.100	0.377 ***	0.194 *	0.063

Values for year 2020 only. *, ** and ***—correlation coefficients are significant at $p < 0.05$; $p < 0.01$ and $p < 0.001$, respectively.

3.2. Agronomic Performance of Durum Wheat Germplasm Originating from Different Breeding Programs

KASIB spring durum wheat set used in the study comprised material from four breeding programs in Kazakhstan and four breeding programs in Russia (Table 1). It

represented typical material bred by these institutions over the last 20 years. Hence, it was well justified to compare the performance of cultivars and breeding lines originating from environmentally diverse breeding programs using different breeding approaches and methodologies. The mean values for the agronomic traits across two seasons at each site and for each breeding program are presented in Supplementary Table S2 and Figure 1. The difference in the number of days to heading between the materials from different programs was over five days. The material from Shortandy was the latest when tested in Almaty and Shortandy followed by material from Karabalyk. The earliest germplasm originated from the Samara and Saratov (Sam-Sar) program followed by the Almaty germplasm. Karabalyk material was the tallest among all groups, while the Sam-Sar germplasm was the shortest. For the number of grains per spike, Shortandy material was the highest in Almaty even when compared to local Almaty material. In Shortandy, Karabalyk and Omsk material had the highest number of grains per spike compared to grains in the Sam-Sar group. For 1000 kernel weight, the highest values were observed in the Sam-Sar group, followed by the Aktope group and the Barnaul group. The smallest grain size was observed in material originating from Shortandy and Karabalyk.

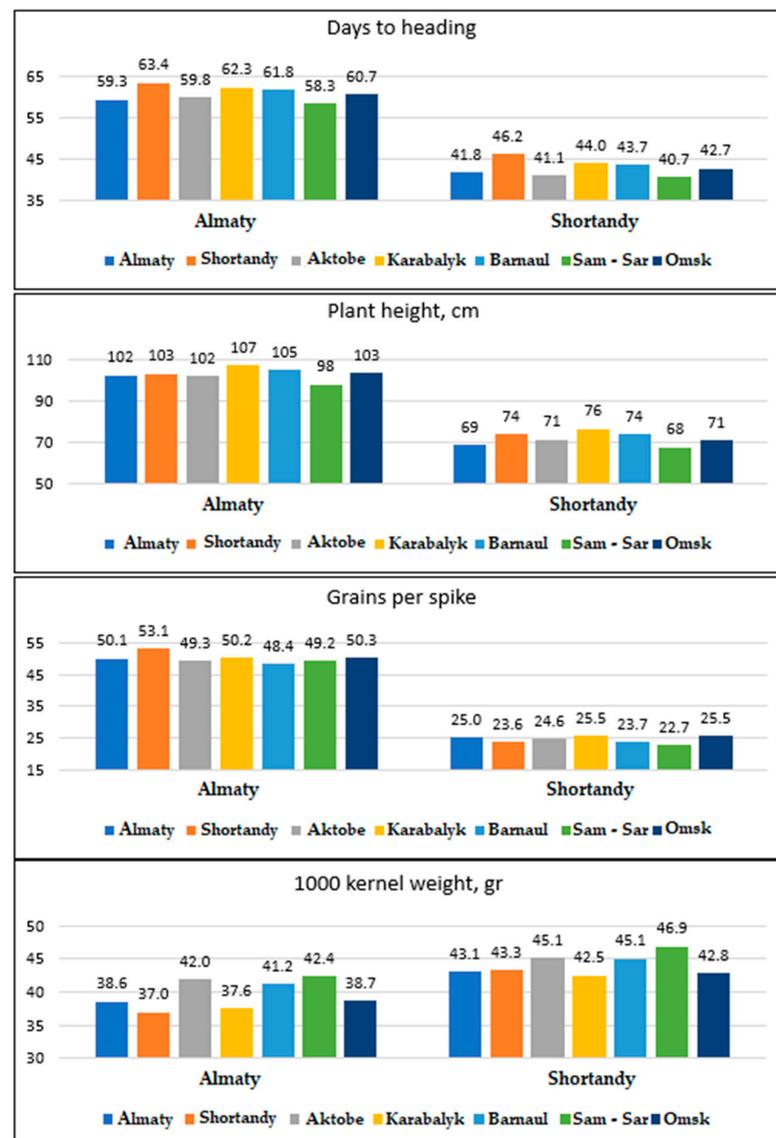


Figure 1. Average values of agronomic traits for eight groups of material originating from different programs tested at Almaty (2020 and 2022) and Shortandy (2021–2022).

The grain yield of spring durum wheat germplasm from eight groups is presented in Supplementary Table S2 and Figure 2. Cultivars and breeding lines originating from Omsk were the highest yielding in Almaty in 2020 (294 g/m²) and across two seasons (263 g/m²). It was also marginally the highest in Shortandy in two years and across them. Spring durum wheat from Omsk demonstrated by far the most superior performance across all sites and years. The Shortandy germplasm was very competitive when tested in Almaty—the second highest across two years with a productivity of 253 g/m². However, when tested in Shortandy its performance was moderate compared to other groups (161 g/m²). The Almaty-originated germplasm was relatively competitive when tested in Almaty—fourth across two years (241 g/m²)—but was among lower-yielding groups when tested in Shortandy (158 g/m²). Material from Karabalyk performed poorly in Almaty (229 g/m²) but was the second highest yielding across two years in Shortandy (178 g/m²). The lowest-yielding group was Sam-Sar material at both sites.

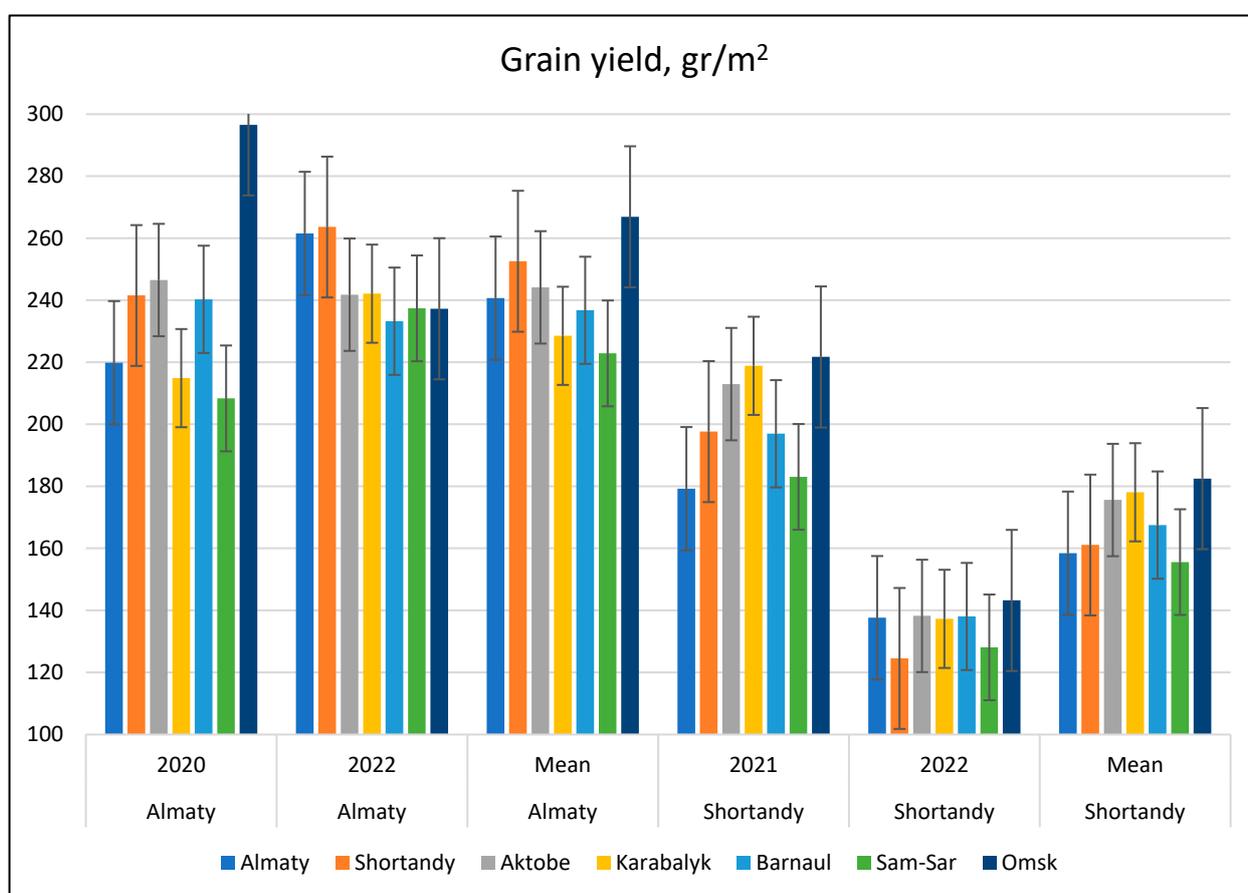


Figure 2. Grain yield of eight groups of material originating from different programs tested at Almaty (2020 and 2022) and Shortandy (2021–2022).

3.3. Superior Spring Durum Wheat Germplasm Identified in the Study

The trial results allowed the identification of high-yielding genotypes at each of the testing sites. Table 5 presents the data for three local checks and ten-top yielding lines at Almaty and Shortandy averaged across two years. At Almaty, Line-250-06-14 from Shortandy demonstrated the highest grain yield—436 g/m²—followed by Omsk breeding line Gordeiforme-91-22-2 (399 g/m²) and cultivar Ertol from Almaty (399 g/m²). In addition, the top ten highest-yielding lines comprised three genotypes from Omsk and one each from Aktobe, Almaty, Barnaul, and Samara. The highest-yielding lines in Almaty had a variable number of days to heading from 52.5 (Gordeiforme-91-102-6) to 66.5 (Line-250-06-14). All lines were tall, exceeding 95 cm. Samara Line-1970d-5 ranked

fifth for yield and had 55.5 grains per spike. The cultivar Ertol from Almaty had the highest 1000 kernel weight—49.4 g.

The three high-yielding lines in Shortandy were Gordeiforme-00-96-8 from Omsk (286 g/m²), Gordeiforme-924 (268 g/m²), and Gordeiforme-910 (246 g/m²), both from Barnaul. The top ten highest-yielding lines in addition comprised three genotypes from Karabalyk and one each from Omsk, Samara, Aktobe, and Almaty. Strangely enough, none of the Shortandy genotypes were among the top ten yielding. The variation in the number of days to heading among top ten yielding lines in Shortandy was within seven days. The plant height varied from 74 to 81 cm. The cultivar Seymour from Almaty had the highest number of grains per spike (32.2) while Leucurum-1469d-21 had the largest grain (51.1 g).

There was no correspondence between germplasm performances at the two sites. The top highest yielding lines in Almaty were ranked from 16 to 127 in Shortandy, while the best ten lines in Shortandy were ranked from 15 to 144 in Almaty. These two groups of lines had a contracting performance at the two sites. The highest yielding lines in Almaty were 3–4 days earlier compared to Shortandy best performers at both sites. They were also 7 cm shorter when tested in Shortandy. Obviously, these two groups had different combinations of traits and different adaptations.

3.4. Molecular Characterization

Appropriate, clear, and scoreable bands were obtained from all primers included in the study. Ten primers amplified a total of 345 bands, 317 of which were polymorphic. The number of bands ranged from 23 (iPBS-2228) to 44 (iPBS-2226) with an average of 34.5 bands per primer (Table 6). The polymorphism (%) ranged from 75 to 100% with an average value of 91.88%. The highest and lowest values of polymorphism information content (PIC) were calculated as 0.308 (2226) and 0.118 (2228), respectively, with an average value of 0.251. The effective number of alleles (Ne) varied from 1.524 (2239) to 1.186 (2389), with a mean value of 1.418. The highest level of gene diversity (0.309) was recorded for 2226, and the lowest (0.118) for 2389, with an average of 0.251. The Shannon's information index range was from 0.201 (2389) to 0.472 (2239) with an average of 0.388. The maximum overall gene diversity was found in 2245 (0.293) and the minimum in 2389 (0.056), with an average value of 0.233.

To understand the genetic diversity more clearly, genetic distance was calculated among all the studied germplasm. The mean genetic distance was 0.287, the maximum genetic distance (0.708) was found between Seymour-17 and Gordeiforme-430-88, and the lowest genetic distance (0.038) was found between Gordeiforme-98-42-1 and Altyn_Shygys. To understand the genetic relationship among the genotypes, various clustering algorithms were performed. The structure analysis separated all the accessions into two classified populations (K = 2, A and B) and one unclassified population (population C/admixture population) based on $Q \geq 75\%$ individual ancestry/membership coefficients (Figure 3). A total of 82, 54 and 15 accessions clustered in population A, B and C, respectively. Population A clustered the germplasm from all collection regions except Saratov. Population B clustered genotypes from all eight breeding programs. A possible reason for the clustering of accessions from Saratov in population was their common pedigree with accessions from the Samara region. The NJ tree also divided all 151 accessions into two populations, i.e., A and B. Population A contained 86 accessions while population B comprised 65 accessions (Figure 4). Analysis of molecular variance (AMOVA) explored the existence of higher genetic diversity within populations accounting for 59% compared to among populations (Table 7).

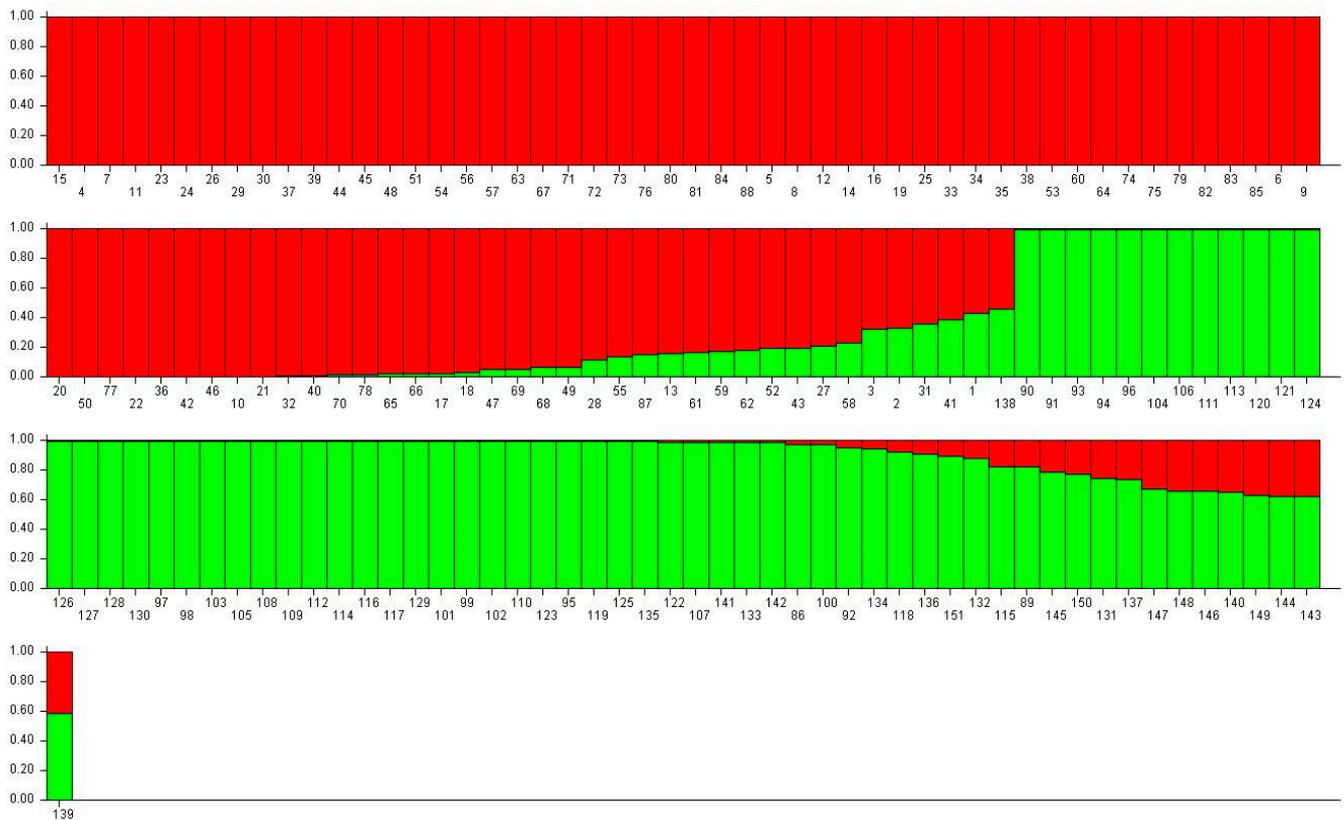
Table 5. High-yielding genotypes of spring durum wheat identified based on evaluation at Almaty (2020 and 2022) and Shortandy (2021–2022).

Entry	Germplasm Name	Originator	Days to Heading		Plant Height, cm		Grains/Main Spike		1000 KW, g		Yield, g/m ² -Rank			
			Almaty	Shortandy	Almaty	Shortandy	Almaty	Shortandy	Almaty	Shortandy	Almaty	Shortandy	Almaty	Shortandy
172	Nauryz-6 (LC-1)	Almaty	60.2	41.8	108	67	43.9	27.2	36.2	40.6	203		200	
47	Altyn-Dala (LC-2)	Karabalyk	61.5	43.5	107	75	46.7	23.3	44.8	48.3	286		190	
121	Zhemch. Sibiri (LC-3)	Omsk	61.0	37.5	110	70	49.7	29.8	41.2	43.1	274		204	
149	Line-250-06-14	Shortandy	66.5	49.8	96	71	54.4	24.4	38.6	44.7	436	1	208	16
27	Gordeiforme-91-22-2	Omsk	53.5	41.5	106	72	49.8	23.1	44.6	42.3	411	2	147	117
68	Ertol	Almaty	60.5	43.0	105	71	47.8	20.4	49.4	44.9	399	3	156	97
26	Gordeiforme-91-102-6	Omsk	52.5	38.8	107	70	45.3	30.0	45.4	42.2	357	4	151	110
160	Line-1970d-5	Samara	60.5	42.0	101	70	55.5	24.4	41.7	46.7	341	5	146	119
69	Altayskiy Yantar	Barnaul	61.0	42.8	98	73	50.1	23.4	40.8	44.1	330	6	142	127
25	Gordeiforme-94-71	Omsk	60.0	41.8	108	74	48.4	26.2	46.0	46.5	322	7	189	34
147	Serke	Almaty	58.5	39.0	105	63	50.6	21.4	40.2	38.5	321	8	167	67
157	Gordeiforme-08-67-1	Omsk	64.0	45.8	108	73	64.2	28.7	36.4	41.5	315	9	169	64
89	Kargala-1408	Aktobe	56.0	41.5	103	68	47.4	23.6	44.8	48.2	315	10	155	100
	Average for top ten Almaty performers		59.3	42.6	104	70	51.3	24.6	42.8	44.0	355	-	163	-
86	Gordeiforme-00-96-8	Omsk	68.5	46.2	103	74	59.4	31.6	28.2	40.7	214	99	286	1
155	Gordeiforme-924	Barnaul	65.0	46.5	102	81	57.5	23.7	43.9	49.1	310	15	268	2
154	Gordeiforme-910	Barnaul	63.0	45.0	107	74	53.1	29.1	42.0	48.4	174	132	246	3
59	Gordeiforme-95-139-4	Omsk	61.0	43.8	101	77	53.1	25.8	44.3	50.6	258	62	242	4
23	Gordeiforme-242-93	Karabalyk	61.5	44.7	103	77	43.8	24.1	37.2	46.1	224	94	241	5
118	Leucurum-1469d-21	Samara	58.0	40.8	107	78	47.5	28.8	49.0	51.1	266	47	240	6
123	Kargala-238	Aktobe	60.5	48.3	106	77	51.7	22.3	40.6	45.7	194	117	239	7
45	Seymur	Almaty	65.0	48.8	96	71	60.8	32.2	29.2	37.9	144	144	235	8
151	Gordeiforme-1790	Karabalyk	64.0	47.0	110	80	56.9	27.8	37.4	43.0	292	29	225	9
150	Kostanayskaya-15	Karabalyk	63.5	47.8	108	81	41.9	25.9	40.0	44.4	263	53	222	10
	Average for top ten Shrotandy performers		63.0	45.9	104	77	52.6	27.1	39.2	45.7	234	-	244	-

Table 6. Genetic diversity indices for 151 durum wheat accessions using 10 iPBS-retrotransposons primers.

Primer	TNB ¹	PB	Polymorphism (%)	Ne	h	I	ht	PIC
2228	23	20	86.96	1.248	0.165	0.277	0.165	0.165
2074	36	35	97.22	1.435	0.265	0.412	0.246	0.262
2226	44	44	100.00	1.514	0.309	0.472	0.293	0.308
2239	36	33	91.67	1.524	0.306	0.462	0.270	0.307
2245	32	32	100.00	1.498	0.293	0.446	0.293	0.294
2252	33	32	96.97	1.403	0.251	0.395	0.233	0.251
2256	31	29	93.55	1.407	0.260	0.406	0.260	0.260
2270	36	27	75.00	1.487	0.266	0.385	0.257	0.267
2271	38	33	86.84	1.480	0.280	0.420	0.261	0.281
2389	36	32	88.88	1.186	0.118	0.201	0.056	0.118
Mean	345	317	91.88	1.418	0.251	0.388	0.233	0.251

¹ TNB: total number of bands, Ne: effective number of alleles, PB: polymorphic bands, h: gene diversity, I: Shannon's information index, ht: overall gene diversity, PIC: polymorphism information content.

**Figure 3.** Population structure of KASIB durum wheat germplasm using iPBS-retrotransposons marker system.

The NJ clustering was found mostly in line with structure clustering, with few exceptions, and confirmed the genetic similarity among the studied germplasm due to common parents. Population A was further clustered into sub-population A and B, having 39 and 47 accessions, respectively. Similarly, population B was subdivided into B1 and B2, having 38 and seven accessions, respectively. There was a clear unbalanced distribution of germplasm from different breeding programs among these four clusters. Cluster A was dominated by the Kazakhstan germplasm, which included 62% of all material in the cluster, especially from Karabalyk and Aktobe. Overall, the Russian germplasm was evenly

In the structure algorithm, 20 genotypes did not cluster in any specific population based on their membership coefficient. In NJ analysis, most of these accessions made their separate sub-cluster (Green color) in population B. These “unclustered” germplasms were evenly distributed between Kazakhstan and Russian material, though 35% was represented by genotypes originating from Almaty.

4. Discussion

4.1. Phenotypic Characterization

Despite the importance of durum wheat for Kazakhstan, there is limited information on the agronomic, genetic, and physiological aspects of breeding this crop in the country. Ref. [31] studied the international collection of tetraploid wheat consisting of 85 accessions in the Almaty region during 2018 and 2019. The highest yield performance was demonstrated by local Kazakh material. The collection was analyzed using seven SSR (simple sequence repeats) markers. From 3 to 6 alleles per locus were revealed, with an average of 4.6, while the effective number of alleles was 2.8. Nei's genetic diversity was in the range of 0.45–0.69. Phylogenetic analysis separated the germplasm into six clusters. The local accessions were presented in all six clusters, with the majority of them grouped together with North American material. [32] studied a world tetraploid wheat collection consisting of 184 primitive and domesticated accessions in Northern (Kokshetau) and Southeastern (Almaty) Kazakhstan. The objective was GWAS analysis and identification of potential molecular markers for agronomic traits. Similar to the current study, the authors found a lack of correlation between germplasm yield performances in two distant sites. More than 60 SNPs contributing to agronomic traits were identified. However, the material used was a collection of primarily European and Italian material without representation of local Kazakhstan or Russian germplasm. From this perspective, this study is the first systematic evaluation of modern spring durum wheat germplasm from key breeding programs from Kazakhstan and Russia under Kazakhstan conditions.

The characterization of KASIB material in this study demonstrated its relative uniformity for the key adaptation traits like plant height and development rate (days to heading). There were no semi-dwarf genotypes with *Rht* genes and all material studied was moderately tall or tall. Likewise, despite the variation in development rate, the material lacked the day-length insensitivity genes. In a way, the local material remains pre-Green Revolution type, the same as the bread wheat cultivars in the region [33]. CIMMYT nurseries were introduced and tested in the region but lacked adaptation, especially under moisture stress conditions [34]. Currently, European cultivars are being directly introduced by private companies, officially tested, released, and being adopted by the producers. Large grain production companies apply high-input technologies and local tall cultivars lack the lodging tolerance, input response, as well as quality traits needed by the processing industry. It is well justified for the breeding programs in Kazakhstan and Russia to convert to the development of competitive shorter stature varieties.

Kazakhstan spring durum wheat production is partly based on Russian cultivars. The catalogue of registered varieties in Kazakhstan in 2022 [35] includes 44 spring durum cultivars, including 11 from Russia. However, their share in production is estimated to be 30–40%. The current study demonstrated a high grain yield of material from Omsk Agrarian Research Center both in the South and in the North. The breeding programs in Barnaul and Samara of Russia also contributed best-performing lines at both sites. Kazakh germplasm was also competitive and among the top-ten high yielding lines, but it was outnumbered by the Russian germplasm in a ratio of 6–4. From a breeding strategy and methodology perspective, it makes sense to establish closer cooperative programs between Kazakh and Russian institutions to utilize and share access to modern genomic and phenotyping tools. This will strengthen the spring durum wheat improvement program in the region and, perhaps, will improve competitiveness against European cultivars.

Scientific Production Center of Grain Farming, named after A.I. Barayev, in the North (Shortandy) and Kazakh Scientific Research Institute of Agriculture and Plant Growing

in the Southeast (Almaty) are the two main breeding institutions of the country for the field crops. The study clearly demonstrated different wheat adaptation patterns at two sites, resulting in substantial genotype by environment interaction. While Kazakh Grain Research Institute targets only Northern production areas, Kazakh Scientific Research Institute of Agriculture and Plant Growing used to have a nationwide breeding mandate, made crosses, and sent breeding material to the programs in the North of the country. Some of this germplasm was developed into successful varieties. The outcome of this study shows that the adaptation of material developed in the South is different from the North, though some genotypes may be competitive there. Considering the environment, capacity, and needs of the wheat breeding programs across the country, perhaps it is worthwhile to use the institute in Almaty to make crosses for other programs and establish speed breeding and phenotyping facility for specific traits. A mild winter, a long growing season, and access to the Gene Bank would be very beneficial for the nationwide cooperative wheat improvement program.

4.2. Molecular Characterization

Determining the genetic diversity levels of germplasm of a plant species is essential for designing and structuring plant breeding programs [36]. Several studies characterized wheat germplasms and their wild relatives using various types of molecular markers [19,37]. In wheat, different retrotransposons-based markers [19,23,38–42] have been applied to evaluate the level of diversity. A total of 10 polymorphic iPBS retrotransposons primers were used in this study to reveal the genetic diversity and population structure of KASIB durum wheat germplasm. The mean polymorphism obtained in this study was higher compared to results obtained by Marzang et al., [43] and Carvalho et al. [44] using IRAP and REMAP markers in durum wheat germplasm. The current results reflected the high efficiency of iPBS-retrotransposons markers which could be used to investigate the novel variations.

The PIC values indicate the discriminating power of the marker. The mean PIC value obtained in this study was 0.251 and was higher than the study of Alemu et al. [45], who used SNPs markers for the assessment of genetic diversity and population structure in Ethiopian durum wheat (*Triticum turgidum* ssp. *durum*). The iPBS 2226 with the greatest PIC value (0.308) was identified as the best marker for differentiation of the KASIB durum wheat set. Previous studies have proven iPBS-retrotransposon as a highly reproducible, robust, and trustable marker system [14,19–22]. The iPBS-retrotransposons have become a marker of choice for the scientific community due to their low cost and higher efficiency [13].

The mean effective number of alleles (1.42) found in the present study was higher than the one reported by Alemu et al. [45] and Marzang et al. [43]. The mean gene diversity (0.25) resulted in our study was found to be higher than reported by Marzang et al. [43]. Shannon's information index is an important criterion for recognizing the variation, as it differentiates the genetic diversity in a population by combining abundance and evenness [46]. The mean Shannon's information index (0.39) was found to be greater than reported by Marzang et al. [43] using IRAP and REMAP.

The likely reasons for the presence of higher values for various diversity indices might be due to the high efficiency of the iPBS-retrotransposon marker system in assessing the genetic diversity or the higher diversity might come from the nature of germplasm itself. Analysis of molecular variance (AMOVA) revealed the existence of higher variations within the KASIB germplasms. Nadeem [19] used the iPBS-retrotransposons marker system and ref. [47] used the RAPD marker system for the investigation of genetic diversity in bread wheat germplasm and AMOVA results of both studies revealed the existence of higher genetic variations within the populations. These findings were in line with our AMOVA results. Pour et al. [48] stated that higher variations within genotypes could be due to selection, adaptation, gene flow, genetic drift, variation in ecotypes, and the pollination method. Furthermore, human activities and environmental fluctuations over time might also be responsible for higher variations [49].

To clarify the relationship among the studied durum wheat germplasms, structure and NJ were used as clustering algorithms. The model-based structure application proved more robust and informative in the previous investigations [50,51]. The main reason behind clustering of germplasm from various breeding programs together was their similar genetic makeup and common pedigree. The use of iPBS-retrotransposons marker system has proven very useful in this study for the initial evaluation of the germplasm genetic diversity and clustering material in similar groups. Obviously, spring durum wheat from Samara and Saratov was different from the rest of the material, phenotypically as well as based on genomic data. This is logical, as it originates from the European part of Russia in the Volga region with specific adaptation and parentage. No attempt has been made to link iPBS-retrotransposons markers with agronomic traits in the current study. Even if some association was identified, it would be more likely coincidental considering the nature of these markers.

The next logical step for genomic characterization of KASIB spring durum set is genotyping for SNP or similar markers and attempting genome-wide association mapping to identify marker–trait associations for potential use in breeding. However, the results of the current study distributed to KASIB cooperators will contribute to the exploration of phenotypic, agronomic, and genetic diversity. The best genotypes identified in the study may serve as highly valuable parental lines to enhance the genetic gains for grain yield and associated traits.

5. Conclusions

The study conducted in two different regions of Kazakhstan revealed significant differences in agronomic indicators among all genotypes. The genotypes from the Russian and Kazakh breeding programs exhibited substantial variations in yield and other agronomic traits. Promising spring durum wheat germplasm suitable for cultivation in Almaty and Shortandy regions was identified.

Furthermore, the study observed a high level of genetic diversity among the 151 spring durum wheat accessions. The genetic relationships among the genotypes were assessed using iPBS markers, and their origin was determined. Based on the analysis, two major genetic populations were identified within the KASIB set of spring durum wheat genotypes.

Continued collaboration between Kazakh and Russian institutions is crucial to strengthen the spring durum wheat improvement program. Joint efforts can enhance resource sharing, the exchange of germplasm, and the utilization of modern genomic and phenotyping tools, leading to accelerated progress in breeding competitive varieties.

Given the advantages of shorter-stature varieties, there is a need to focus on developing durum wheat cultivars with reduced height. Breeding programs should prioritize the incorporation of genes associated with dwarfism and day-length insensitivity to improve lodging tolerance, input response, and quality traits.

Climate change poses challenges to agricultural systems, including durum wheat production. Future research should consider climate adaptation strategies, such as identifying traits and genetic resources that confer tolerance to drought, heat, or other environmental stresses. This will contribute to the development of climate-resilient durum wheat cultivars.

Efforts should be made to expand the exchange of germplasm, not only within Kazakhstan and Russia but also with international breeding programs. Accessing diverse genetic resources and incorporating them into local breeding programs can broaden the genetic base and introduce novel traits for improved durum wheat varieties.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy13071955/s1>, Table S1: The list of spring durum wheat material included in the study. Table S2: Agronomic performance of spring durum wheat material originating from different breeding programs and tested at Almaty (2020 and 2022) and Shortandy (2021–2022).

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Data Availability Statement: The data are available from the authors upon request. Phenotypic and genomic data generated during this study will be deposited to the database of the project “Rational use of agricultural crops genetic resources of Kazakhstan—adaptation and implementation of the international documentation platform GRIN-Global”.

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