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PLANT BREEDING AND SEED PRODUCTION

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Original article

BREEDING AND GENETIC EVALUATION
OF INTERNATIONAL WINTER WHEAT COLLECTION
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A.S. Chursin, A.M. Kovalchuk, T.V. Savin, V.P. Shamanin***Abstract**

Winter wheat is considered one of the sources of increased grain production according to higher yield and better use of bioclimatic resources in Western Siberia.

The objective of this study was to breeding evaluate and identify genetically distinct genotypes among an international collection of winter bread wheat accessions for potential use as parents and for the development of high-yielding varieties resistant to adverse environmental factors.

Materials and methods. The breeding evaluation of 96 accessions of winter wheat generated from different breeding programs was performed in 2022 and 2023, using generally accepted agronomy techniques. All 96 accessions were genotyped using 55 KASP markers developed in Institute of Plant Biology and Biotechnology (Kazakhstan).

Results. The accessions from Turkey had a longer growing period. The accessions from the USA were characterized by short stature (57.5 cm), more spikes on plants (2.42), and increased yield (271 g/m²). The samples from Russia and Bulgaria were distinguished by high levels of winter hardiness (66.7% and 69.9%, respectively). The average number of alleles was 2, and the effective number of alleles was 1.42. The analysis of the genotypic structure of the population determined the division of genotypes into 6 subclusters regardless of their origin. Shannon's information index ranged from 0.058 to 0.693, with an average value of 0.40 and an expected

heterozygosity of 0.25, which indicated a relatively high genetic diversity of the studied accessions.

Conclusion. The results of this research can be successfully used in breeding to develop high-yielding winter wheat varieties, that are resistant to adverse environmental factors and have high grain quality.

Keywords: winter wheat; collection; genotyping; KASP markers; agronomic traits

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Научная статья

СЕЛЕКЦИОННО-ГЕНЕТИЧЕСКАЯ ОЦЕНКА МЕЖДУНАРОДНОЙ КОЛЛЕКЦИИ ОЗИМОЙ ПШЕНИЦЫ В УСЛОВИЯХ ЗАПАДНОЙ СИБИРИ

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Аннотация

В Западной Сибири озимая пшеница рассматривается в качестве одного из источника увеличения производства зерна благодаря повышенной урожайности и лучшему использованию биоклиматических ресурсов региона.

Целью исследования являлась селекционная оценка и выделение генетических источников из международной коллекции образцов озимой мягкой пшеницы в качестве исходного материала для создания высокоурожайных сортов, устойчивых к неблагоприятным факторам среды.

Материалы и методы. Проведена полевая оценка 96 образцов озимой мягкой пшеницы из разных стран в 2022–2023 гг. с применением общепринятых селекционных методик. 96 образцов генотипированы с использованием 55 KASP-маркеров, разработанных в Институте биологии и биотехнологии растений (Казахстан).

Результаты. У селекционного материала из Турции преобладали более позднеспелые формы (313 сут.); образцы американской селекции отличались низкорослостью (57,5 см), высокой продуктивной кустистостью (2,42 шт./

раст.) и повышенной урожайностью (271 г/м²); образцы из России и Болгарии имели высокие показатели зимостойкости растений – 66,7% и 69,9% соответственно. Среднее число аллелей составило 2, число эффективных аллелей – 1,42. Изучение популяционной структуры и генетического сходства образцов коллекции из разных географических групп выявило их деление на шесть субпопуляций, независимо от происхождения образцов. Информационный индекс Шеннона варьировал от 0,058 до 0,693 со средним значением 0,40; ожидаемая гетерозиготность (0,25) свидетельствуют об относительно высоком генетическом разнообразии изученных форм.

Заключение. Результаты проведенных исследований могут быть успешно использованы в селекции для создания высокоурожайных сортов озимой пшеницы, устойчивых к неблагоприятным факторам среды и отличающихся высоким качеством зерна.

Ключевые слова: озимая пшеница; коллекция; генотипирование; KASP-маркеры; хозяйственно-ценные признаки

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Introduction

It is necessary to double wheat grain production and develop high-yielding wheat varieties adapted to negative environmental factors to meet the demand for food in recent decades due to the growth of the world's population. The Russian Federation's grain production may increase if winter wheat is replaced with spring wheat due to its relatively high yield [2; 3]. More than 200 varieties of winter wheat were developed at the National Grain Center P.P. Lukyanenko, including the famous wheat variety Bezostaya 1. This variety is widely involved in the genealogy of many wheat varieties. Modern varieties of winter wheat are capable of producing approximately 130 c/ha on basic intensive technologies [1]. Krasnodar Krai, Stavropol Krai, and Rostov Oblast are the main regions, in which winter wheat is cultivated in the Russian Federation [6]. However, due to the increase in heat stress and drought conditions in the southern regions of Russia, it is possible to predict yield reductions in winter wheat [19]. Compared with those in the southern regions of Russia, the cultivation of winter wheat in Western Siberia was limited by less favorable conditions, such as early summer drought, a lack of snow cover at the beginning of winter and severe winters. Recently, conditions for

planting winter wheat in Western Siberia have become more favorable due to increases in average temperatures in the autumn and winter periods, and the sum of effective temperatures during the growing season. This might contribute to the growth of the winter wheat planting area in this region [7; 35]. The involvement of diverse genetic resources in modern wheat breeding, such as the genotyping of germoplasm via functional markers associated with genes that control key agronomic traits, has played an important role in increasing winter wheat yield and broadening their genetic variety [38]. Among SNP genotyping platforms, the Competitive Allele-Specific PCR (KASP, <http://www.lgcgenomics.com>) assay is a promising technology for high throughput SNP genotyping based on the FRET method. In this system, each allele-specific primer has a proprietary tail sequence that is labeled with one corresponding FRET cassette, and each of the two primers is labeled with a dye. KASP assays can accommodate SNPs, insertions and deletions (InDels) [32; 24].

KASP markers tested on different genetic backgrounds can be used to improve yield components and resistance to biotic and abiotic stresses in winter wheat [14; 22; 26; 27]. KASP technology provides more efficient and less expensive genotyping than genotyping by sequencing (GBS). It is efficient at identifying polymorphic loci and conducting haplotype analysis [29]. Low frost and winter hardiness, and the biotic and abiotic stress resistance of winter wheat varieties are the most significant factors limiting yield under conditions in Western Siberia. This is a set of quantitative traits. The assessment of their phenology is quite complex, considering their morphological, physiological, and biochemical characteristics. In this regard, the identification of genes that control such traits would benefit for the adaptation and breeding of winter wheat varieties [11; 16; 25].

A collection of 96 winter bread wheat genotypes from different countries of the world was genotyped via 55 KASP markers associated with genes of the main agronomic traits, including the number of days to heading and maturity, yield, grain quality, and resistance to fungal diseases. The objective of this study was to breeding evaluate and identify genetically distinct genotypes among an international collection of winter bread wheat accessions for potential use as parents and for the development of high-yielding varieties resistant to adverse environmental factors.

Materials and methods

Wheat material and field experimentation. A set of 96 accessions of winter bread wheat from different geographical locations. The germoplasm represented the released varieties and advanced lines from Russian breeding institutions:

Rostov Agricultural Research Center (7 accessions), Donskoy Agricultural Research Center (8 accessions), Rostov Oblast; North Caucasus Federal Scientific Center (1 accession), Stavropol Krai; and National Grain Center P.P. Lukyanenko, Krasnodar Krai (12 accessions). The remaining accessions were from different countries of the world: Germany (1 accession); Finland (1 accession); Bulgaria (7 accessions); Turkey (7 accessions); Kazakhstan (1 accession); the USA (17 accessions); and the International Winter Wheat Improvement Program (Türkiye-CIMMYT-ICARDA, TCI) (33 accessions). The collection of winter bread wheat accessions was studied on the experimental field of Omsk SAU (Omsk, Omsk region, 55.0404°N, 73.3604°E) in 2022 and 2023. The experiments were conducted in the field after black fallow. The standard for this plot size was 3 m², and two replications were used with the local winter wheat variety Omskaya 4. The agronomic traits were recorded following methodical instructions for exploring the wheat collection [2]. ANOVA of agronomic traits in a replicated trial was performed using MS Excel.

Experimental sites and weather. The soil type of the experimental field is thin meadow chernozem, with an organic matter content in the range of 3.5–4%. The climate of the region is sharply continental, the average annual rainfall is approximately 300–380 mm, the sum of effective temperatures is 2000–2100 °C. Factors limiting of the yield and quality of wheat grain include insufficient rainfall, the threat of wind erosion of soils and the low phosphorus content. The average duration of the winter period is 164 days. The soil freezes to 165–220 cm, and the snow cover varies between 20–25 cm. The growing seasons of 2022 and 2023 were characterized by average temperatures; an uneven distribution of rainfall in May 2022, a large lack of rainfall in May, and excess rainfall in August–early September in 2023.

DNA isolation. The protocol with modifications described by Delaporta et al. was used to isolate genomic DNA from the leaves of 96 winter wheat accessions at a speed of 3 fold (1983). The purification of dedicated DNA was conducted using the commercial kits (Qiagen, Germany). The concentration and quality of genomic DNA were measured by spectrophotometry (BioRad, USA). The composition of the isolated DNA was determined according to the optical density OD260/OD280. The DNA concentration for each sample was adjusted to 50 ng/μL. All 96 accessions were genotyped using 55 KASP markers developed at the Institute of Plant Biology and Biotechnology (Kazakhstan) [5; 18; 36]. A set of used KASP markers is presented in Table 2.

Molecular marker data analysis. Statistical methods using software packages were used to determine genetic parameters: Na – the effective number of

alleles; N_e – the effective number of alleles; I – Shannon's information index; H_o – the observed heterozygosity; H_e – the expected heterozygosity; uH_e – the unbiased expected heterozygosity. The analysis of molecular variance (AMOVA) was performed using GenAlEx software [30]. The STRUCTURE HARVEST Web v. program. 0.6.94 [12; 31] was used to conduct a cluster analysis of the genotypic population structure of the winter wheat accessions. The number of subgroups (K) was calculated based on the highest value of ΔK , with K values ranging from 1 to 10, and 10 independent runs for each K value [15] A dendrogram of the genetic similarity of the winter wheat accessions was constructed using the nearest neighbor method in the PAST v. program. 4.03 [20].

Results

The main agronomic traits of a set of winter bread wheat accessions from different breeding programs were evaluated under conditions in Western Siberia (Table 1).

Table 1.

Agronomic parameters of germplasms from different geographical sites tested in Omsk (2022 and 2023)

Trait	Russia	Bulgaria	Türkiye	USA	TCI	LSD _{0.05}
No. of accessions	29	7	7	17	33	
Days to maturity	312	312	313	311	311	0.39
Winter hardness, %	66.7	69.9	65.1	65.6	58.2	3.77
Plant height, cm	60.3	58.4	59.4	57.5	60.9	1.93
Spikes / plant	2.17	2.23	2.31	2.42	2.26	0.17
Grains / spike	30.9	32.4	33.6	32.4	29.5	1.49
Grain weight / spike, g	1.14	1.14	1.13	1.07	1.12	0.07
TKW, g	36.7	34.6	33.2	32.8	37.9	2.15
Yield, g/m ²	256	199	235	271	196	21.3

Note: Single accessions from Kazakhstan, Finland, and Germany are not presented in the Table

Across two seasons, the difference in the number of days to maturity between breeding material from Turkey and genotypes from the USA and TCI programs was 2 days. The accessions from the USA were the shortest (57.5 cm), had more number of spikes on the plant (2.42), and had the highest yield among the studied genotypes (271 g/m²). The accessions from Russia and Bulgaria were characterized by similar means of winter hardness (66.7% and 69.9%, respectively), number of spikes on the plant (2.17 and 2.23), and grain weight on

spike (1.14 g). The Russian accessions had a greater yield (256 g/m²) than the Bulgarian accessions (199 g/m²). The highest values of thousand kernel weight (37.9 g), and the lowest values of winter hardness (58.2%) and yield (196 g/m²) were observed in the TCI group.

Genotyping the germoplasm using 55 KASP markers localized in the three wheat genomes revealed that 36 of these markers were polymorphic, 5 were monomorphic, and 14 had a minor allele frequency (MAF <0.05). Among the 96 accessions, 21 were heterozygous for 17 KASP markers associated with agronomic traits. Table 2 presents the polymorphism information for 50 polymorphic KASP markers, including the number of effective alleles and Shannon's index.

Table 2.

Genetic diversity indices for 50 polymorphic KASP markers used for genotyping of the winter wheat accessions

Locus	Maf	N	Na	Ne	I	Ho	He	uHe
<i>ipbb_ta_106</i>	0.208	96	2	1.492	0.512	0.000	0.330	0.332
<i>ipbb_ta_107</i>	0.021	96	2	1.043	0.101	0.000	0.041	0.041
<i>ipbb_ta_114</i>	0.116	96	2	1.267	0.367	0.010	0.211	0.212
<i>ipbb_ta_116</i>	0.365	96	2	1.863	0.656	0.000	0.463	0.466
<i>ipbb_ta_123</i>	0.202	96	2	1.492	0.512	0.021	0.330	0.332
<i>ipbb_ta_124</i>	0.448	96	2	1.979	0.688	0.000	0.495	0.497
<i>ipbb_ta_126</i>	0.312	96	2	1.765	0.625	0.031	0.434	0.436
<i>ipbb_ta_130</i>	0.167	96	2	1.385	0.451	0.000	0.278	0.279
<i>ipbb_ta_131</i>	0.115	96	2	1.255	0.356	0.000	0.203	0.204
<i>ipbb_ta_132</i>	0.063	96	2	1.133	0.234	0.000	0.117	0.118
<i>ipbb_ta_133</i>	0.156	96	2	1.358	0.433	0.000	0.264	0.265
<i>ipbb_ta_141</i>	0.115	96	2	1.255	0.356	0.000	0.203	0.204
<i>ipbb_ta_149</i>	0.063	96	2	1.133	0.234	0.000	0.117	0.118
<i>ipbb_ta_151</i>	0.394	96	2	1.917	0.671	0.021	0.478	0.481
<i>ipbb_ta_165</i>	0.458	96	2	1.986	0.690	0.000	0.497	0.499
<i>ipbb_ta_171</i>	0.394	96	2	1.917	0.671	0.021	0.478	0.481
<i>ipbb_ta_188</i>	0.031	96	2	1.064	0.139	0.000	0.061	0.061
<i>ipbb_ta_196</i>	0.010	96	2	1.021	0.058	0.000	0.021	0.021
<i>ipbb_ta_197</i>	0.021	96	2	1.043	0.101	0.000	0.041	0.041
<i>ipbb_ta_219</i>	0.167	96	2	1.385	0.451	0.000	0.278	0.279
<i>ipbb_ta_220</i>	0.010	96	2	1.021	0.058	0.000	0.021	0.021
<i>ipbb_ta_225</i>	0.083	96	2	1.180	0.287	0.000	0.153	0.154
<i>ipbb_ta_226</i>	0.105	96	2	1.242	0.345	0.010	0.195	0.196

<i>ipbb ta 227</i>	0.011	96	2	1.064	0.139	0.042	0.061	0.061
<i>ipbb ta 229</i>	0.010	96	2	1.021	0.058	0.000	0.021	0.021
<i>ipbb ta 230</i>	0.375	96	2	1.882	0.662	0.000	0.469	0.471
<i>ipbb ta 231</i>	0.010	96	2	1.021	0.058	0.000	0.021	0.021
<i>ipbb ta 236</i>	0.097	96	2	1.242	0.345	0.031	0.195	0.196
<i>ipbb ta 239</i>	0.106	96	2	1.255	0.356	0.021	0.203	0.204
<i>ipbb ta 241</i>	0.022	96	2	1.087	0.173	0.042	0.080	0.080
<i>ipbb ta 246</i>	0.011	96	2	1.053	0.121	0.031	0.051	0.051
<i>ipbb ta 247</i>	0.365	96	2	1.863	0.656	0.000	0.463	0.466
<i>ipbb ta 253</i>	0.323	96	2	1.777	0.629	0.000	0.437	0.440
<i>ipbb ta 256</i>	0.022	96	2	1.076	0.157	0.031	0.070	0.071
<i>ipbb ta 257</i>	0.157	96	2	1.306	0.397	0.000	0.234	0.235
<i>ipbb ta 258</i>	0.398	96	2	1.925	0.673	0.031	0.480	0.483
<i>ipbb ta 259</i>	0.340	96	2	1.822	0.643	0.021	0.451	0.454
<i>ipbb ta 260</i>	0.348	96	2	1.853	0.653	0.073	0.460	0.463
<i>ipbb ta 261</i>	0.063	96	2	1.144	0.248	0.010	0.126	0.127
<i>ipbb ta 263</i>	0.042	96	2	1.087	0.173	0.000	0.080	0.080
<i>ipbb ta 272</i>	0.264	96	2	1.666	0.589	0.052	0.400	0.402
<i>ipbb ta 274</i>	0.031	96	2	1.064	0.139	0.000	0.061	0.061
<i>ipbb ta 275</i>	0.490	96	2	1.999	0.693	0.000	0.500	0.502
<i>ipbb ta 278</i>	0.110	96	2	1.293	0.387	0.052	0.227	0.228
<i>ipbb ta 283</i>	0.281	96	2	1.679	0.594	0.000	0.404	0.406
<i>ipbb ta 289</i>	0.389	96	2	1.909	0.669	0.010	0.476	0.479
<i>ipbb ta 291</i>	0.326	96	2	1.800	0.637	0.042	0.444	0.447
<i>ipbb ta 298</i>	0.011	96	2	1.032	0.080	0.010	0.031	0.031
<i>ipbb ta 304</i>	0.302	96	2	1.729	0.613	0.000	0.422	0.424
<i>ipbb ta 305</i>	0.211	96	2	1.506	0.519	0.010	0.336	0.338
Mean +SE		96	2	1.42+0.05	0.40+0.03	0.01+0.00	0.25+0.02	0.25+0.02

Note: N – number of accessions; Na – the effective number of alleles; Ne – the effective number of alleles; I – Shannon’s information index; Ho – the observed heterozygosity; He – the expected heterozygosity; uHe – the unbiased expected heterozygosity

Shannon’s information index ranged from 0.058 to 0.693 with an average mean 0.40. The average value of the effective number of alleles was calculated as 1.42 ranging from 1.021 (*ipbb ta 196*) to 1.999 (*ipbb ta 275*). The comparison of the mean values for Shannon’s index allowed us to determine the loci with the most polymorphism information, which were located on chromosomes 4B, 3B and 1B: *ipbb ta 124*, *ipbb ta 165*, *ipbb ta 275*. The allele frequency for 50 polymorphic KASP markers ranged from 0.010 to 0.990. For 50 of the 100 alleles, an allele frequency greater than 50% was detected. The genotypic

composition of the population was examined by analyzing the genotyping data of 96 winter bread wheat accessions.

The structure analysis separated a set of 96 accessions into six clusters (Figure 1). The nearest neighbor analysis based on the genotyping of the collection of winter wheat using KASP markers allowed us to construct a dendrogram of genetic similarity between the studied accessions. There was no clear division of genotypes into 6 clusters according to their origin from different breeding programs. A total of 5, 11, 32, 9, 8 and 31 accessions clustered, respectively. Cluster III was the largest, and consisted of 32 accessions. Identical alleles of SNP loci were detected in six accessions in this cluster: Don 107 (Russia); Esperia (Bulgaria); Kopru (Finland); WBLL1*2 / Tukuru // Billings; (Attila*2 / ESDA // Mason) / (HBK 0935-7-4 / Betty'S'); AGRI / NAC // Attila /4/ C80.1 / 3*BATAVIA // 2*WBLL1 /3 /2*Kronstad F2004 (TCI). A total of 11 winter wheat varieties from Rostov Oblast and Krasnodar Krai clustered in subpopulation III: Gubernator Dona, Donna, Donera, Donskaya Lira, Donskoy Syurpriz, Donskaya Yubileinyaya, Donskoy Mayak, Tanais, Rostovchanka 7, Graf, and Etnos.

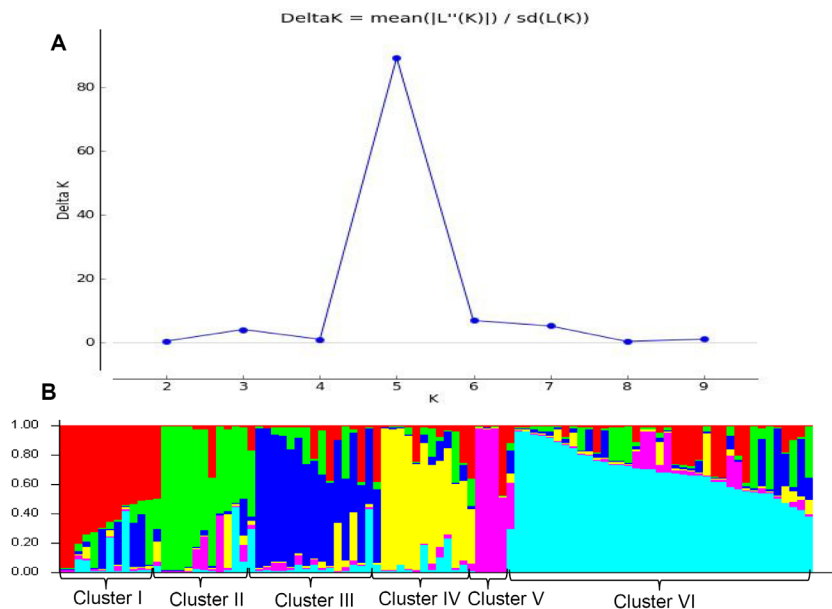


Figure 1. A – Structure analysis of the population based on the best number of clusters (K), B – Population structure of winter wheat collection using the KASP marker system confirmed the genetic similarity

Subpopulation VI also clustered 31 accessions in all five breeding programs, Bulgarian variety Kisla and two lines WBLL1*2 / Tukurku // Billings and Sonmez 01 / Ahmetaga // Sonmez 01 from the TCI group carried identical alleles of SNP loci. Cluster II consisted of 11 accessions, including five from the USA (KS13DH0020-59, KS13DH0030-29, KS13DH0024-48, Whetstone, KS100610K-7) and variety Zhiva from Russia.

Previous GWAS results were used to identify favorable alleles that affect important economic traits. For each group of traits, markers with favorable alleles that had a positive effect on the number of days to heading and maturity, yield components, grain quality indicators, and resistance to fungal diseases were identified. The data obtained divided the studied accessions into four groups based on the number of favorable alleles (Figure 2).

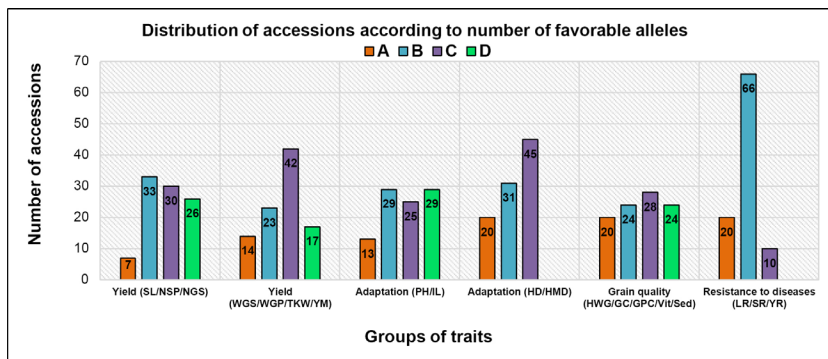


Figure 2. Distribution of the to number of favorable alleles in the winter bread wheat accessions affecting important economic traits

Note: SL – spike length, NSP – number of spikes per plant, NGS – number of grains per spikes, WGS – weight grains per spike, WGP – weight grains per plant, TKW – thousand kernel weight, YM – yield on 1 m², PH – plant height, IL – upper internode length, HD – number of days to heading, HMD – number of days from heading to maturity, HWG – hectoliter weight of grain, GC – gluten content, GPC – grain protein content, Vit – vitreous, Sed – Zeleny sedimentation, LR – resistance to leaf rust, SR – resistance to stem rust, YR – resistance to yellow rust.

For example, group A contains 7 and 14 genotypes with the greatest number of favorable alleles (7–8) affecting the yield components; 13 and 20 accessions have 3–6 alleles with a positive effect on key adaptation traits (plant height, days to heading and maturity); 20 of the 96 accessions possess 10–11 alleles with a positive effect on the grain quality, and 20 accessions possess 6–8 alleles affecting the resistance to rust diseases. The accessions were selected based

on the identification of genes that affect important economic traits. This germplasm can serve as a genetic source for breeding programs for winter wheat: for reducing the duration of the growing period – Konkutent, Line K 18918 (Russia), Pcelina (Bulgaria), Whetstone, KS13DH0030-32 (USA), Kupava /7/ AU /3/ MINN // HK/38MA ... Demir (TCI); for increasing yield components – Alekseich (Russia), CTR Rada (Bulgaria), Bayraktar 2000 (Türkiye), Sy Wolf, CO13D1299 (USA), BEZ / F10S-1 // 233/87-15-37 /6/..., AGRI / NAC // Attila /4/ C80.1... (TCI); for improving grain quality – Nakhodka, Donskaya Lira, Duplet, Postovchanka 7, Donna, Zolushka (Russia), Esperia (Bulgaria), Judee, KS020638~5 / Gallagher (USA), AGRI / NAC // Attila /4/ C80.1... (TCI); for increasing of resistance to diseases – Alekseich, Antonina, Gurt, Duplet, Line K 18918 (Russia), SY Wolf, KS13DH0020-59 (USA), OCW00S106S / Garrison (TCI).

Table 3 presents the highest-yielding accessions from different ecological groups based on field evaluation across the two seasons. These accessions possessed combinations of favorable alleles of genes with a positive effect on agronomic traits.

Table 3.

High-yielding genotypes of winter bread wheat identified via trial results in Omsk (2022 and 2023)

Variety, line	Days to ripeness	Winter hard-ness, %	Spi-kes / plant	Grai-ns / spike	Grain weight/ spike, g	TKW, g	Yield, g/m ²	No. of favorable alleles
<i>Russia</i>								
Donna	311	68.5*	2.16*	30.7*	1.17*	37.8*	379*	34
Zhiva	317	74.5*	1.97	34.5*	1.41*	40.8*	375*	29
Doneko	311	67.0*	2.12*	29.7*	1.19*	49.8*	373*	33
Donskaya Lira	309	68.5*	2.32*	27.0*	1.02*	37.9*	368*	34
Zolushka	314	56.0	1.99	34.6*	1.26*	36.4*	362*	30
Don 107	309	53.0	3.03*	36.3*	1.32*	36.2*	333	31
Donskoy Mayak	311	74.0*	1.57	36.9*	1.87*	37.3*	329	30
K 18918	313	63.5	2.83*	33.6*	1.04*	31.0*	318	32
Л 2293 K 2-4	313	63.0	1.48	32.7*	1.20*	36.9*	309	34
Alekseich	314	57.0	3.09*	29.1	1.08*	37.6*	309	35
<i>Bulgaria</i>								
Pcelina	309	90.5*	2.75*	37.2*	1.29*	34.7*	269	32
Esperia	311	75.5*	2.14*	31.4*	1.06*	33.1*	190	32

<i>Türkiye</i>								
Gelibolu	312	77.0*	1.86	37.8*	1.22*	32.7*	309	30
Saban	309	81.5*	1.39	23.6*	0.52	22.1	212	31
<i>USA</i>								
SY Wolf	313	55.5	2.38*	28.4*	0.98*	34.2*	371*	36
KS13DH0030-29	310	67.5*	2.31*	32.6*	0.99*	30.1*	368*	30
KS13DH0030-32	310	91.0*	2.94*	39.9*	1.39*	34.8*	354	29
KS13DH 002722	310	71.0*	2.67*	27.7*	0.92*	33.1*	326	29
KS- 13DH0020-59	308	65.0	2.75*	27.9*	0.79	28.5*	318	30
KS13DH0039-99	311	86.5*	1.85	27.2*	0.85	31.2*	294	29
<i>Türkiye-CIMMYT-ICARDA</i>								
WBLL1*2 / Kuruku /5 / Chuen-Mai 18...	310	76.0*	3.39*	29.8*	0.86	28.8*	325	34
Gondvana // HBK0935- 29-15 / KS90W077-2-2 / VBF0589-1...	312	37.0	2.87*	34.5*	1.37*	39.1*	309	29
Kupava /7/ AU /3 /MINN // HK / 38MA /4/...	312	83.0*	1.75	29.1*	0.89*	30.0*	253	29
Omskaya 4, standard	319	66.5	1.87	21.4	0.82	25.7	335	29
LSD _{0.05}	3.77	0.39	0.17	1.49	0.07	2.15	21.3	

Note: * – values exceeding the standard are significant at $p < 0.05$

The nine accessions from Russia, which are more adaptive genotypes to local conditions, demonstrated yields of 309–379 g/m². These accessions were characterized by a high number of grains per spike (27.0–34.6), weight of grains per spike (1.02–1.41), and TKW (36.4–49.8). The accessions Pcelina, Esperia (Bulgaria); Saban, Gelibolu (Türkiye); KS13DH0039-99, KS13DH0030-32 (USA); Kupava /7/ AU /3 /MINN // HK / 38MA /4/..., WBLL1*2 / Kuruku /5/ Chuen-Mai 18... (TCI) had the greatest winter hardness (75.5–91.0%), hence, their yield varied from 190 to 354 g/m². Obviously, these accessions had different numbers of productive plants before harvest. Five genotypes from the

USA (KS13DH0030-29, SY Wolf, KS13DH 002722, KS13DH0030-32, KS-13DH0020-59) had the greatest number of spikes per plant (2.31–2.94) and TKW (28.5–34.8). In contrast, the lowest yield was observed for the TCI material (253–335 g/m²). Nevertheless, this material is interesting for breeding because of its high number of grains per spike and TKW (29.1–34.5 and 28.8–39.1, respectively). A total of 29–36 favorable alleles of genes with positives effects on traits of adaptation, yield components, grain yield, and resistance to diseases were identified in the highest-yielding (362–379 g/m²) accessions: Doneko, Donskaya Lira, Zolushka, Donna, Zhiva (Russia), KS13DH0030-29, and SY Wolf (USA).

Discussion

Currently, winter wheat is considered one of the sources for increasing grain production due to its higher yield and better use of the region's bioclimatic resources in Western Siberia. Despite improvements in the methods of growing technologies and the evolution of winter wheat, modern varieties have lost their advantages resistance to negative environmental factors and grain quality [17, 34]. Winter wheat accessions from different geographical groups were studied in an international collection during 2022 and 2023. The studied germplasms differed above all in terms of in frost and winter hardness under conditions in Western Siberia. The accessions from Russia and Bulgaria had higher levels of winter hardness (66.7–69.9%) than did the accessions from other countries. In this study, accessions from the USA demonstrated good winter hardness (65.6%), dwarfism (57.5 cm), greater number of spikes on the plant (2.42), and the highest yield among the studied genotypes (271 g/m²). Genotyping of winter wheat varieties and lines using SNP loci helps to study the genetic structure of populations, detect polymorphism levels, determine varietal composition and determine effective markers associated with valuable agronomic traits [13; 24]. The winter wheat accessions were examined using 55 KASP markers, with 36 polymorphic, 5 monomorphic, and 14 minor-allele-prevalent markers (MAF <0.05). The average number of alleles was 2, and the effective number of alleles was 1.42. The analysis of the genotypic structure of the population revealed that the division of genotypes into 6 subclusters did not depend on their origin. Principally, the germplasms from Rostov Oblast and Krasnodar Krai were distributed between the third and the sixth clusters, together with accessions from Bulgaria, Turkey, the USA, and the TCI program. In contrast, in most of the conducted studies, according to cluster analysis results, the groups of winter wheat accessions formed were closely related to their geographical location [21;

37]. The genetic similarity the collected material can be explained by the limited set of used markers, as well as the supposed common parents in its pedigree.

The genetic information indices indicated a relatively high genetic diversity of the 96 winter wheat accessions based on genotyping using KASP markers (Table 2). Shannon's information index ranged from 0.058 to 0.693, with an average of 0.40. The observed heterozygosity was 0.25. The genotyping of the world collection of bread wheat using 47 KASP markers revealed that the genetic diversity index ranged from 0.26 for the CIMMYT varieties to 0.32 for the modern Chinese varieties [38]. The highest Shannon's index (0.688–0.693) was detected for loci located in the chromosomes of the B genome: *ipbb_ta_124*, *ipbb_ta_165*, *ipbb_ta_275*. Chen et al. [9] used 269 SSR markers to analyze the 90 accessions of Chinese winter wheat genotypes, and reported that the average genetic diversity index was 0.60. In this study the B genome had the greatest genetic diversity (0.63), while the D genome had the lowest (0.56), which was a significant difference between these genomes. As reported by Sthapit et al. [33] an evaluation of polymorphisms in American winter wheat varieties over the past 120 years revealed that there was the lowest diversity in 2000–2019 compared to that in the period before the 1930s. In contrast, the genetic diversity analysis of 187 lines of winter wheat grown in the eastern United States and released from 1808 to 2005 using SNP and DArT markers showed that the allele composition of the winter bread wheat genome became more diverse; approximately 11% of the genome exhibited significant changes in allele frequencies [8]. Undoubtedly, the disparities observed in the evaluations of the genetic diversity of winter wheat collections can be attributed to diverse breeding methodologies and approaches.

The use of various DNA markers in practical breeding to study the genetic diversity of winter wheat collections is a key task for the improvement of winter wheat and the identification of the effective genetic sources as parental forms for hybridization programs [23; 28]. The highest allele frequency (0.833–0.990) in the studied population of winter wheat had favorable alleles affecting the number of days to heading (*ipbb_ta_229*), number of days from heading to maturity (*ipbb_ta_197*), spike length (*ipbb_ta_196*, *ipbb_ta_114*), number of spikes per plant (*ipbb_ta_107*), TKW (*ipbb_ta_241*), yield (*ipbb_ta_188*), grain protein content (*ipbb_ta_220*, *ipbb_ta_261*), grain gluten content (*ipbb_ta_219*, *ipbb_ta_220*), grain vitreous (*ipbb_ta_274*), and resistance to yellow rust (*ipbb_ta_141*).

Genotypes with the lowest frequency of favorable alleles should be specifically involved in breeding programs to improve winter wheat varieties. The fol-

lowing alleles with positive effects on important agronomic traits were identified: number of days to heading (*ipbb_ta_225*, *ipbb_ta_227*, *ipbb_ta_246*), number of days from heading to maturity (*ipbb_ta_149*), upper internode length (*ipbb_ta_188*, *ipbb_ta_197*), spike length (*ipbb_ta_263*), grain content protein (*ipbb_ta_278*), and resistance to stem rust (*ipbb_ta_131*, *ipbb_ta_132*, *ipbb_ta_133*). A set of the highest-yielding (362–379 g/m²) accessions that possessed 29–36 favorable alleles were recommended for the development of new germplasms and extension of the genetic diversity of winter wheat: Doneko, Donskaya Lira, Zolushka, Donna, Zhiva (Russia), KS13DH0030-29, and SY Wolf (USA).

Conclusion

The study of the international collection of winter wheat accessions from different geographical groups under conditions in Western Siberia revealed significant differences in yield and agronomic traits. The accessions from Russia and Bulgaria were characterized by the highest winter hardiness, while accessions from the USA had the highest yield and early maturity. The identification of genes related to valuable traits of collection accessions is a key task for the improvement of winter wheat and identification of the effective genetic sources as parental forms for hybridization programs. Genotyping using 55 KASP markers was used to determine the genetic diversity of 96 winter wheat accessions. The population structure was studied, and the level of genetic similarity between international accessions was assessed. The promising varieties and lines that were identified were characterized by favorable alleles associated with significant agronomic traits. The results of the research can be successfully used in breeding to develop high-yielding winter wheat varieties that are resistant to adverse environmental factors and have high grain quality.

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