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VALIDATION OF KASP MARKERS FOR ADAPTATION AND YIELD-RELATED TRAITS IN SPRING BREAD WHEAT BREEDING LINES OF KAZAKHSTAN

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ABSTRACT

The validation of molecular markers under target environmental conditions is essential for the effective implementation of marker-assisted selection in wheat breeding. This study aimed to assess the genetic diversity of spring bread wheat germplasm from Kazakhstan, validate KASP markers associated with adaptation and yield-related traits, and identify favorable alleles for breeding applications. A total of 107 spring bread wheat genotypes originating from five breeding organizations were evaluated in four contrasting environments of Kazakhstan and genotyped using 43 KASP markers developed from previous genome-wide association studies (GWAS) and quantitative trait locus (QTL) mapping studies. Of the analyzed markers, 33 were polymorphic and successfully differentiated the studied germplasm. Genetic diversity analysis revealed moderate variation among breeding collections, with the highest level of polymorphism observed in the lines from Karabalyk Agricultural Experimental Station collection (100%). Principal coordinate analysis demonstrated clear genetic differentiation among breeding programs. Association analysis identified numerous significant marker–trait relationships across environments, with the largest number detected in North Kazakhstan. Five markers (*ipbb_ta_152*, *ipbb_ta_219*, *ipbb_ta_223*, *ipbb_ta_183*, and *ipbb_ta_162*) showed stable associations with adaptation- and yield-related traits across all testing locations. Evaluation of allelic effects revealed that marker *ipbb_ta_162* had the strongest positive influence on grain yield, particularly under North Kazakhstan conditions, whereas *ipbb_ta_183* exhibited the most stable favorable allele across environments. The results confirm the usefulness of KASP markers for marker-assisted selection and provide valuable molecular tools for the development of high-yielding and environmentally adapted wheat cultivars in Kazakhstan.

Key words: *Triticum aestivum* L., KASP markers, genetic diversity, marker-assisted selection, adaptation, grain yield

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important staple crops worldwide, providing approximately 20% of the calories consumed by the global population [1-3]. In Kazakhstan, wheat production plays a central role in ensuring food security and maintaining export potential, particularly in the northern regions, which are characterized by a continental climate and high interannual variability [4-5]. These environmental fluctuations impose strong selective pressure on wheat genotypes, making the development of high-yielding and well-adapted cultivars a major priority for breeding programs. Traditionally, wheat breeding in Kazakhstan has relied on phenotypic selection; however, the efficiency of this approach is often limited by environmental influences and the complex inheritance of quantitative traits such as grain yield, drought tolerance, and adaptation [6]. Cultivars are developed separately for different regions of the country, taking into account specific natural and climatic conditions, as reflected in the State Register of Breeding Achievements of the Republic of Kazakhstan (2021) [7]. Consequently, the development of modern breeding approaches that accelerate genetic gain and improve selection efficiency remains an important objective.

Recent advances in genomic technologies have significantly improved the ability to investigate the genetic basis of economically important traits [8]. High-throughput single nucleotide polymorphisms (SNP) genotyping platforms, including the 90K Illumina iSelect array and Affymetrix array, have enabled large-scale characterization of wheat germplasm collections and facilitated the identification of genomic regions associated with yield components, phenology, adaptation, and stress tolerance [9-11]. Genome-wide associ-

ation studies (GWAS) and quantitative trait locus (QTL) mapping have become powerful tools for identifying loci that control agronomic performance and environmental adaptation in wheat [10-14].

A wide range of studies conducted in different wheat-growing regions worldwide have successfully applied GWAS to identify genes and QTLs associated with productivity, stress tolerance, and adaptation [14-16]. In Kazakhstan, several investigations have focused on the environmental evaluation of domestic and foreign wheat germplasm [17-25], as well as large-scale GWAS analyses of yield, adaptation, and disease resistance traits [17-20]. Studies conducted under Kazakhstan conditions have also demonstrated substantial genetic variation for grain quality and agronomic traits. For example, analyses of the double-haploid mapping population Chinese Spring × SQ1, evaluated in southern Kazakhstan, revealed significant variation in agronomic traits, grain protein concentration, and mineral composition [21]. More recently, QTL mapping studies conducted using two locally adapted mapping populations, Pamyati Azieva × Paragon and Avalon × Cadenza, identified genomic regions associated with agronomic traits, grain protein content, gluten-related characteristics, and starch quality parameters, thereby providing valuable molecular resources for marker-assisted selection (MAS) in Kazakhstan wheat breeding programs [22-25]. These findings have expanded our understanding of the genetic architecture of economically important traits and provided valuable molecular tools for MAS in Kazakhstan wheat breeding programs. However, the practical implementation of GWAS results in breeding programs requires the validation of identified marker–trait associations under target environmental

conditions. Due to the considerable environmental heterogeneity of Kazakhstan and the strong influence of genotype $\times$ environment interactions (GEI), marker effects identified in one population or environment may not always be reproducible in other breeding materials or growing regions. Therefore, additional regional validation studies are essential before molecular markers can be effectively incorporated into breeding programs.

Although SNP arrays provide highly informative genomic data, their routine application in breeding programs is often constrained by cost and technical complexity. To overcome these limitations, informative SNPs can be converted into Kompetitive Allele Specific PCR (KASP) assays, which represent a flexible, cost-effective, and high-throughput genotyping platform suitable for MAS [26-28]. KASP markers have been widely used in wheat breeding programs for QTL validation, genetic diversity studies, and the identification of favorable alleles associated with important agronomic traits [29-30]. Furthermore, the identification of favorable alleles, defined as alleles that significantly improve trait performance, represents a critical step toward the successful implementation of MAS. The accumulation of favorable alleles in breeding germplasm has been shown to enhance yield potential, adaptation, and performance stability across diverse environments. Therefore, the validation of KASP markers and the identification of effective allelic variants are of particular importance for wheat improvement in Kazakhstan.

The objectives of this study were to 1) assess the genetic diversity of spring bread wheat germplasm originating from major breeding centers of Kazakhstan using KASP markers, 2) evaluate marker-trait associations across multiple environments, and 3) identify favorable alleles associated with yield and adaptation-related traits. The results provide a basis for implementing MAS and contribute to developing high-yielding wheat cultivars adapted to Kazakhstan's diverse agroecological conditions.

MATERIALS AND METHODS

Plant materials. A total of 107 spring bread wheat (*Triticum aestivum* L.) genotypes were selected for genotyping using KASP markers associated with adaptation and yield-related traits. The plant material originated from five major breeding stations in Kazakhstan: Karabalyk Agricultural Experimental Station – 36 genotypes, A.F. Khristenko Karaganda Agricultural Experimental Station – 19 genotypes, A.I. Barayev Research and Production Centre for Grain Farming – 23 genotypes, North Kazakhstan Agricultural Experimental Station – 17 genotypes, and Kazakh Research Institute of Livestock and Forage Production, Foraging (name of before Aktobe Agricultural Experimental Station) – 12 genotypes. All accessions represented advanced breeding lines and released cultivars selected for high agronomic performance and adaptation to regional environmental conditions.

Evaluation of the breeding lines for variation in agronomic traits. Field trials were conducted during the 2022-2023 growing seasons at four experimental sites: Karabalyk Agricultural Experimental Station (KB, Kostanay region), A.F. Khristenko Karaganda Agricultural Experimental Station (KA, Karaganda region), A.I. Barayev Research and Production Centre for Grain Farming (SH, Akmola region), and

North Kazakhstan Agricultural Experimental Station (NK, North Kazakhstan region). These environments represent contrasting agro-climatic zones of Kazakhstan. Climatic conditions during the growing seasons were described previously in Anuabek et al. (2022) [31]. The rows were spaced 15 cm apart, and the distance between plants within a row was 5 cm. In total, data on the mean values of eight agronomic traits for the 107 genotypes harvested in the 2022-2023 trials were analyzed [32]. The traits studied are divided into two groups: 1) plant adaptation-related traits and 2) yield components. The first group included the following traits: heading date (HD, days), seed maturation date (SMD, days), vegetation period (VP, days), and plant height (PH, cm). The group of yield components consisted of spike length (SL, cm), number of kernels per spike (NKS, pcs), thousand-kernel weight (TKW, g), and yield (Ych, c/ha).

DNA extraction and KASP Assay. Genomic DNA from 107 genotypes was isolated from a single seedling of each line using the Delaporta protocol with modifications [33]. The 43 KASP markers were selected from significant SNPs and QTLs identified in our previous GWAS and biparental QTL mapping studies conducted using wheat materials evaluated in Kazakhstan [17-25, 34]. Marker selection was based on the significance of the original association, relevance to adaptation- and yield-related traits, evidence of stability across environments, representation of different wheat chromosomes. Priority was given to loci associated with phenological development, plant height, spike productivity, thousand-kernel weight, and grain yield, because these traits are major breeding targets under the contrasting agroclimatic conditions of Kazakhstan (Table 1).

KASP assays were carried out using reagents provided by LGC Genomics. Each reaction was performed in a total volume of 10 μ L, consisting of 5 μ L of genomic DNA (adjusted to approximately 30 ng/ μ L) and 5 μ L of KASP reaction mix containing 2 $\times$ KASP Master Mix and allele-specific primers. PCR amplification was conducted using the following thermal cycling profile: an initial denaturation at 94 °C for 15 minutes, followed by 40 cycles of denaturation at 94 °C for 20 seconds and annealing/extension at 60 °C for 60 seconds. Fluorescence signals were detected using a real-time PCR system, and genotype calling was performed based on allele-specific fluorescence clustering [35].

Statistical data analysis. Descriptive statistics, including means and ranges, were calculated separately for each trait and environment using Microsoft Excel. Marker-trait associations were evaluated independently within each environment by comparing the mean phenotypic values of the two homozygous allelic classes of each polymorphic KASP marker using a two-tailed independent-samples Student's t-test in SPSS version 16.0 [36]. Monomorphic markers were excluded from association testing, and rare heterozygous genotypes were not included in comparisons between homozygous allelic classes. Statistical significance was declared at $P < 0.05$. Because the markers were selected a priori from previously reported GWAS and QTL results, the analysis was considered a targeted validation test; associations reproduced in multiple environments were interpreted as having stronger evidence of stability. Pearson's correlation coefficients were calculated in R/RStudio using the *stats* package, and boxplots were gener-

Table 1. List of 43 KASP markers used for genotyping of spring bread wheat collection

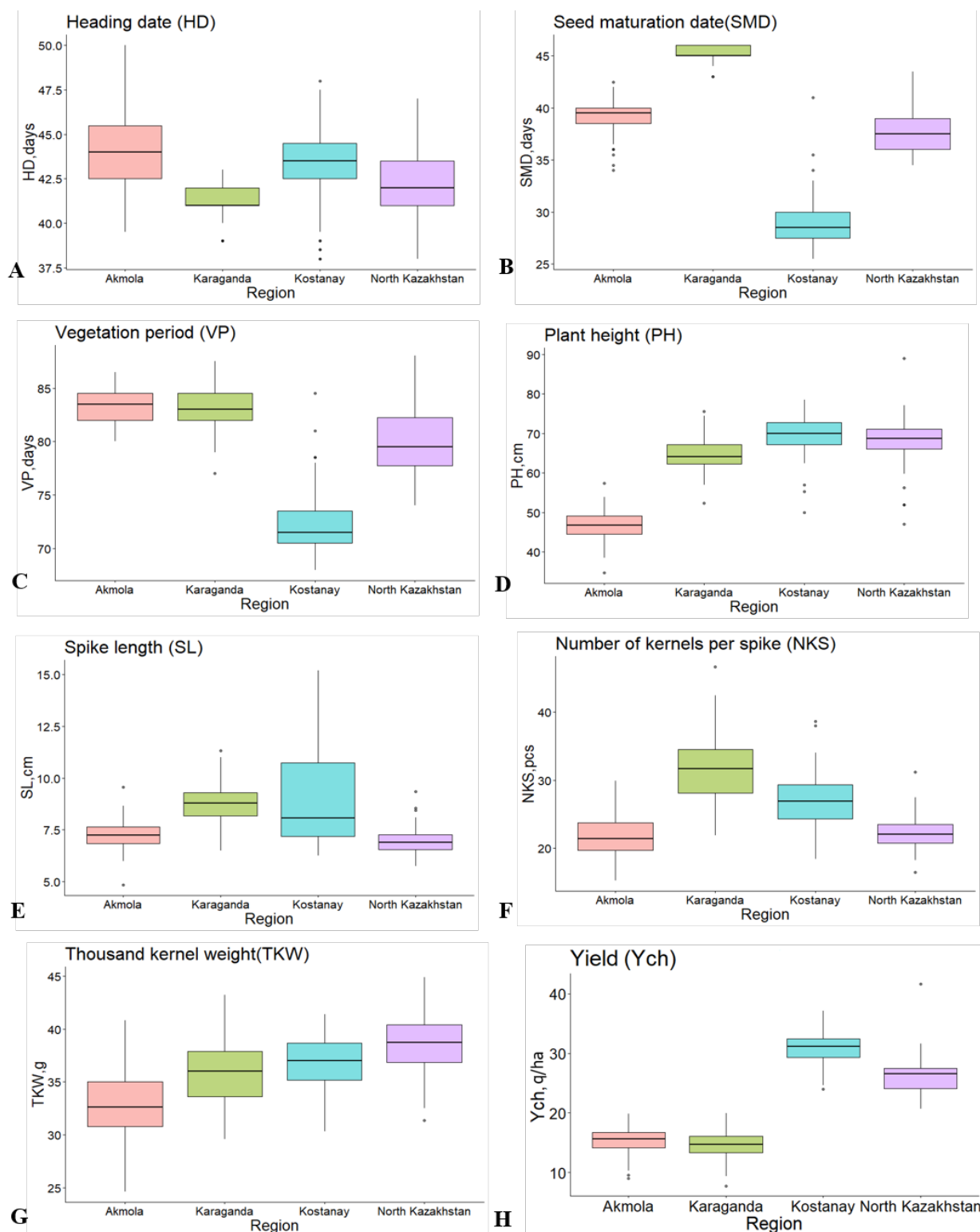
Trait	KASP ID	Chromosome	Position
FT	<i>ipbb_ta_148</i>	2D	50.8
FT	<i>ipbb_ta_223</i>	7D	84
SMD	<i>ipbb_ta_152</i>	2A	101.9
SMD	<i>ipbb_ta_233</i>	2A	101.9
SMD	<i>ipbb_ta_234</i>	3B	56.6
SMD	<i>ipbb_ta_238</i>	4A	120.1
SMD	<i>ipbb_ta_239</i>	5D	167
PH	<i>ipbb_ta_155</i>	6A	123.5
PH	<i>ipbb_ta_157</i>	6A	134.2
PH	<i>ipbb_ta_180</i>	7A	101.4
PL	<i>ipbb_ta_161</i>	6A	99.1
PL	<i>ipbb_ta_190</i>	7A	55.4
PL	<i>ipbb_ta_217</i>	3B	68.8
SL	<i>ipbb_ta_298</i>	2D	30.00
NPS	<i>ipbb_ta_106</i>	5B	51.3
NPS	<i>ipbb_ta_306</i>	7A	208.70
NKS/WKS	<i>ipbb_ta_165</i>	3B	32.9
NKS/YM2	<i>ipbb_ta_185</i>	2D	76.5
NKS	<i>ipbb_ta_222</i>	6D	1.12
NKS	<i>ipbb_ta_297</i>	2B	84.00
NKS	<i>ipbb_ta_305</i>	6A	100.10
WKP	<i>ipbb_ta_111</i>	2A	76
WKS	<i>ipbb_ta_162</i>	2A	106.3
TKW	<i>ipbb_ta_241</i>	7B	99.2
TKW	<i>ipbb_ta_290</i>	2B	94.34
TKW	<i>ipbb_ta_294</i>	6A	69.13
TKW	<i>ipbb_ta_302</i>	5A	17.00
TKW	<i>ipbb_ta_304</i>	6A	57.50
YM2	<i>ipbb_ta_242</i>	3D	149.8
YM2	<i>ipbb_ta_243</i>	7B	77.2
YM2	<i>ipbb_ta_291</i>	5A	20.04
YM2	<i>ipbb_ta_301</i>	4D	69.60
NPS/YM2	<i>ipbb_ta_166</i>	2D	70.0
WKP/YM2	<i>ipbb_ta_169</i>	4D	70.2
PL/NPS/NKS/TKW	<i>ipbb_ta_171</i>	6A	31.6
SMD/YM2	<i>ipbb_ta_179</i>	7B	120.8
PH/SL/NKS	<i>ipbb_ta_183</i>	5A	41.05
HD/FT/SMD	<i>ipbb_ta_184</i>	7B	23.1
PL/YM2	<i>ipbb_ta_188</i>	6B	65.1
NPS	<i>ipbb_ta_193</i>	2D	43.4
HD/SMD/PL/WKP	<i>ipbb_ta_197</i>	7A	82.9
WKP/YM2	<i>ipbb_ta_219</i>	1D	81.4
WKS	<i>ipbb_ta_220</i>	6A	72

Note: FT – flowering time, HD – heading date, SMD – seed maturation date, PH – plant height, PL – peduncle length, NPS – number of productive spikes, SL – spike length, NKS – number of kernel per spikes, WKS – weight of kernel per spike, WKP – weight of kernel per plant, TKW – thousand weight kernel, YM2 – yield per 1 m².

ated using *ggplot2* [37]. Genetic diversity was characterized using the number of alleles, number of effective alleles, Shannon's information index, percentage of polymorphic loci, and principal coordinate analysis (PCOA) implemented in GenALEx version 6.5 [38].

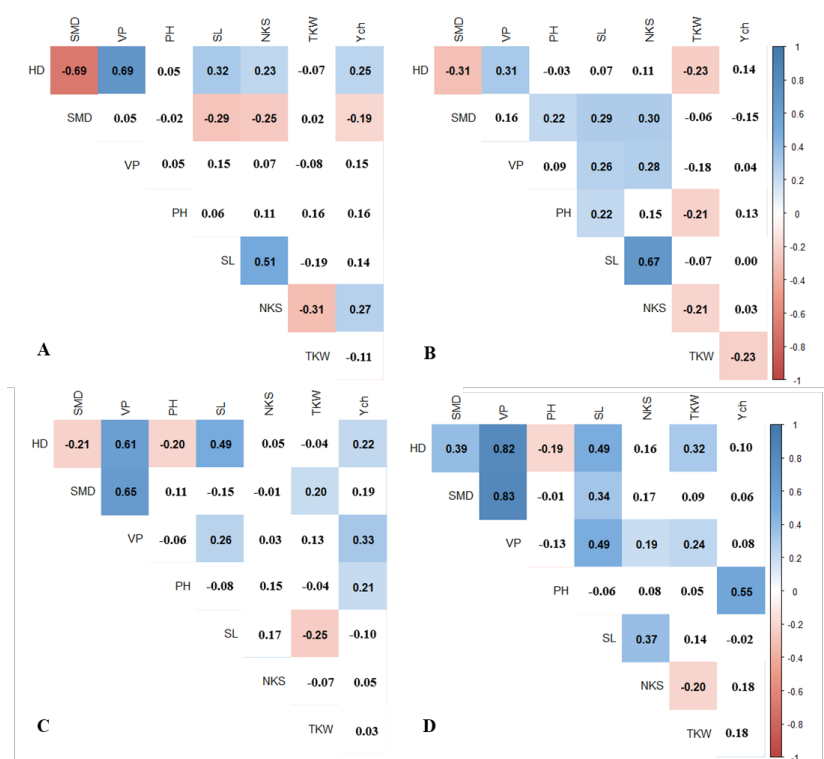
RESULTS

Variation of agronomic traits across regions. Significant variation in key agronomic traits was observed across the four experimental sites located in Akmola, Karaganda, Kostanay, and North Kazakhstan regions (Figure 1).



Note: A – heading date (days), B – seed maturation date (days), C – vegetation period (days), D – plant height (cm), E – spikes length (cm), F – number of kernels per spike (pcs), G – thousand kernels weight (g), H – yield (q/ha)

Figure 1. Distribution of eight agronomic traits among 107 spring bread wheat genotypes evaluated in the Akmola, Karaganda, Kostanay, and North Kazakhstan regions during 2022–2023. Boxes represent the interquartile range, horizontal lines indicate medians, whiskers represent the data range excluding outliers, and individual points indicate outlying observations.



Note: HD – heading date (days), SMD – seed maturation date (days), VP – vegetation period (days), PH – plant height (cm), SL – spike length (cm), NKS – number of kernels per spike (pcs), TKW – thousand kernel weight (g), Ych – yield (c/ha). Correlations with $P < 0.05$ are highlighted in color. The color indicates a positive (blue) or negative (red) correlation.

Figure 2. Pearson's correlation index among the average data of eight agronomic traits of 107 spring wheat genotypes across four regions of Kazakhstan (A – Akmola, B – Karaganda, C – Kostanay, and D – North Kazakhstan).

HD showed moderate variation, with earlier heading in the Karaganda and North Kazakhstan regions and slightly later heading in the Akmola and Kostanay regions. In contrast, SMD varied considerably, with the longest maturation period recorded in Karaganda and the shortest in Kostanay. A similar trend was observed for the VP, which was longest in Akmola and Karaganda (approximately 82-85 days) and shortest in Kostanay (around 70-73 days), while North Kazakhstan displayed intermediate values. PH also differed markedly, with the shortest plants in Akmola and taller genotypes in Kostanay and North Kazakhstan (Figure 1).

Yield-related traits demonstrated clear regional differentiation: SL and NKS were highest in Karaganda and Kostanay, whereas lower values were observed in Akmola and North Kazakhstan. TKW increased progressively from Akmola to North Kazakhstan, indicating improved grain filling conditions in northern environments. Yield (Ych) exhibited the greatest variation, with the highest productivity observed in Kostanay (exceeding 30 c/ha), followed by North Kazakhstan, while Akmola and Karaganda showed significantly lower yields. Overall, these results highlight a strong environmental influence on phenological development and yield formation, with Kostanay conditions favoring early maturation and higher productivity, whereas Karaganda promoted extended growth duration and higher kernel number per spike (Figure 1).

Correlation analysis revealed distinct relationships among agronomic traits across the four regions studied (Figure 2). In the Akmola region (Figure 2A), a strong negative correlation was observed between HD and SMD ($r = -0.69$), while HD

showed a positive association with the VP ($r = 0.69$). Among yield components, SL was positively correlated with NKS ($r = 0.51$), whereas NKS was negatively correlated with TKW ($r = -0.31$) but positively correlated with Ych ($r = 0.27$). In the Karaganda region (Figure 2B), correlations were generally weaker, although a moderate positive relationship was observed between SL and NKS ($r = 0.67$). Phenological traits showed limited association with yield, whereas TKW was negatively correlated with Ych ($r = -0.23$), suggesting a trade-off between grain size and productivity under these conditions. In Kostanay (Figure 2C), stronger relationships among phenological traits were identified, particularly between SMD and VP ($r = 0.65$) and between HD and VP ($r = 0.61$). Ych was positively associated with VP ($r = 0.33$), indicating that longer vegetation periods contributed to higher productivity. However, SL showed a negative correlation with TKW ($r = -0.25$), suggesting a compensatory relationship between spike structure and grain weight. In North Kazakhstan (Figure 2D), the strongest correlations were observed among phenological traits, especially between SMD and VP ($r = 0.83$) and between HD and VP ($r = 0.82$). Ych showed a strong positive correlation with PH ($r = 0.55$), indicating the importance of biomass accumulation for productivity in this region. Additionally, moderate positive associations were observed between SL and NKS ($r = 0.37$), while NKS was negatively correlated with TKW ($r = -0.20$).

Overall, the results demonstrate region-specific correlation patterns, highlighting the influence of environmental conditions on the relationships between phenological traits, yield components, and final productivity. The variability in correla-

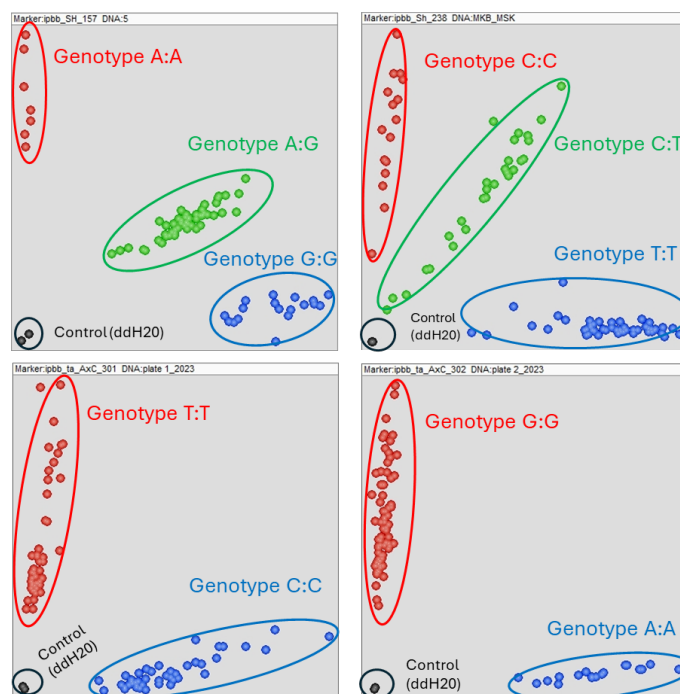


Figure 3. Determination of the allelic status of spring bread wheat collection samples using 4 KASP markers (*ipbb_ta_157*, *ipbb_ta_238*, *ipbb_ta_301*, *ipbb_ta_302*)

tion structure suggests different mechanisms of yield formation across environments

Genotyping and Genetic Diversity Analysis. A total of 107 spring bread wheat breeding genotypes from five breeding organizations in Kazakhstan were genotyped using 43 KASP markers previously developed from significant SNPs identified through GWAS and QTL mapping. Among the analyzed markers, 33 were polymorphic and successfully discriminated the studied wheat genotypes according to their allelic composition, whereas 10 markers were monomorphic across the collection. In addition, heterozygous genotypes were identified for markers *ipbb_ta_SH_157* and *ipbb_ta_SH_238*, which are associated with seed maturation time and plant height, respectively (Figure 3).

Genetic diversity analysis using 33 polymorphic markers revealed moderate variation among breeding collections (Table 2). The highest level of polymorphism was observed

in the Karabalyk collection (100%), followed by North Kazakhstan (94.12%), whereas the Karaganda collection exhibited the lowest (85.29%). Across all accessions, the average polymorphism level reached 91.2%, indicating moderate genetic diversity within the studied germplasm.

PCoA based on 33 polymorphic KASP markers revealed a clear genetic differentiation among the studied accessions (Figure 4). The first coordinate separated the collection into two major groups: genotypes from the A.I. Barayev Research and Production Centre for Grain Farming in Akmola region and the Aktobe breeding programs clustered on one side, while most Karabalyk accessions formed a distinct group on the opposite side. Furthermore, genotypes from the Aktobe breeding centers and Karabalyk breeding centers clustered closely together, suggesting a shared genetic background and/or the use of common parental germplasm in their breeding programs. The North Kazakhstan accessions occupied an intermediate position between the major clusters, whereas the

Table 2. Indicators of the genetic information content of KASP markers used in genotyping bread wheat samples from five breeding organizations

Breeding organizations	N	Na	Ne	I	% P
Aktobe Agricultural Experimental Station	12	1.88+0.06	1.51+0.06	0.46+0.04	88.24
A.F. Khristenko Karaganda Agricultural Experimental Station	19	1.85+0.06	1.42+0.06	0.39+0.04	85.29
A.I. Barayev Research and Production Centre for Grain Farming	23	1.88+0.06	1.53+0.06	0.45+0.04	88.24
North Kazakhstan Agricultural Experimental Station	17	1.94+0.04	1.55+0.05	0.50+0.03	94.12
Karabalyk Agricultural Experimental Station	36	2.00+0.00	1.53+0.06	0.48+0.03	100
Mean +SE	107	1.91+0.02	1.51+0.03	0.46+0.02	91.2+2.63

Notes: n is the number of samples; Na is the number of alleles; Ne is the number of effective alleles; I is the Shannon informative index; % P is the percentage of polymorphic loci

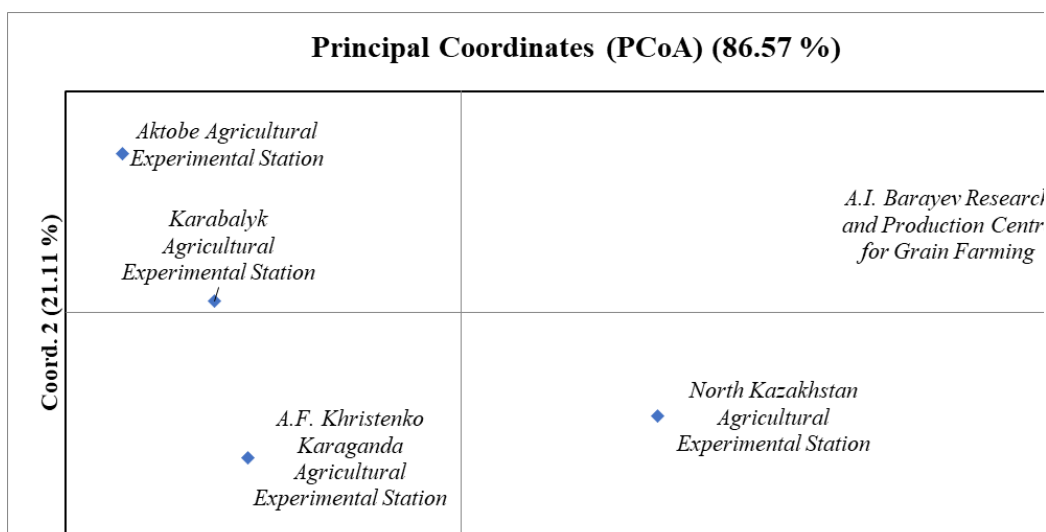


Figure 4. Principal coordinate analysis of 107 spring bread wheat genotypes from five Kazakhstan breeding organizations based on the allelic profiles of 33 polymorphic KASP markers. Colors indicate the breeding organization of origin

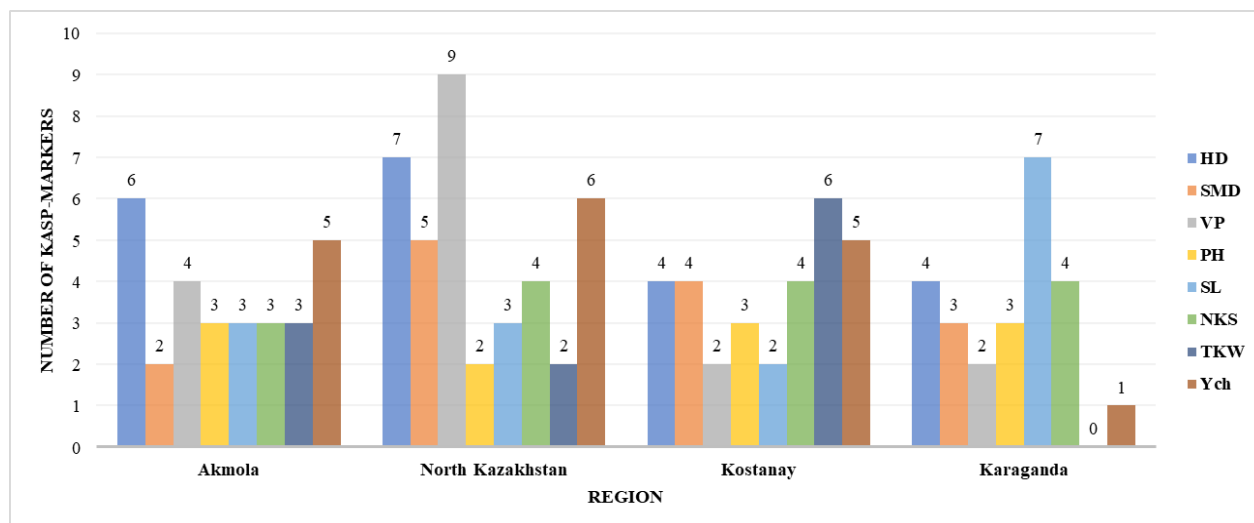


Figure 5. Number of KASP markers significantly associated with adaptation- and yield-related traits in each of four Kazakhstan environments. Associations were determined by two-tailed independent-samples Student's t-tests comparing homozygous allelic classes at $P < 0.05$.

Karaganda collection formed the most distinct group. Despite some overlap among breeding collections, the clustering pattern generally reflected breeding origin.

Marker-Trait Associations Across Environments. Association analysis based on Student's t-test revealed numerous significant relationships between KASP markers and agronomic traits across the four testing environments (Figure 5). The highest number of significant marker-trait associations was detected in North Kazakhstan, where 22 markers showed significant effects, followed by Karaganda (20 markers), while 19 markers were significant in both Akmola and Kostanay regions. The most significant associations were with phenological traits, including HD, SMD, and VP, highlighting the importance of these loci in environmental adaptation.

Five markers, namely *ipbb_ta_152*, *ipbb_ta_219*, *ipbb_ta_223*, *ipbb_ta_183*, and *ipbb_ta_162*, demonstrated stable associations across all four environments. Among them, marker *ipbb_ta_223* exhibited the strongest and most con-

sistent effects, showing significant associations with heading date, vegetation period, spike-related traits, and grain yield across multiple environments. Similarly, markers *ipbb_ta_152* and *ipbb_ta_162* were repeatedly associated with both adaptation- and yield-related traits, suggesting possible pleiotropic effects. Six additional markers (*ipbb_ta_238*, *ipbb_ta_106*, *ipbb_ta_220*, *ipbb_ta_243*, *ipbb_ta_294*, and *ipbb_ta_302*) displayed significant effects in the northern environments (Akmola, Kostanay, and North Kazakhstan), indicating their potential contribution to adaptation under northern Kazakhstan growing conditions. Several markers exhibited environment-specific effects. For example, *ipbb_ta_166* and *ipbb_ta_171* were significant only in Akmola, *ipbb_ta_165* only in North Kazakhstan, and *ipbb_ta_161* only in Karaganda. These region-specific associations suggest the presence of GEI affecting the expression of agronomic traits.

Overall, both stable and environment-specific marker-trait associations were identified, highlighting the complex genetic control of adaptation and productivity traits in spring wheat.

Table 3. Stable and environment-specific KASP markers associated with adaptation- and yield-related traits across environments.

Marker	Main associated traits	Environment
<i>ipbb_ta_152</i>	HD, SMD, VP, TKW, Ych	KB, KA, SH, NK
<i>ipbb_ta_219</i>	HD, VP, TKW, Ych	KB, KA, SH, NK
<i>ipbb_ta_223</i>	HD, VP, SL, NKS, Ych	KB, KA, SH, NK
<i>ipbb_ta_183</i>	HD, VP, SL, NKS, Ych	KB, KA, SH, NK
<i>ipbb_ta_162</i>	HD, VP, TKW, Ych	KB, KA, SH, NK
<i>ipbb_ta_238</i>	HD, PH, SL, NKS, TKW, Ych	KB, SH, NK
<i>ipbb_ta_106</i>	SL, NKS	KB, SH, NK
<i>ipbb_ta_220</i>	SMD, VP, PH, TKW, Ych	KB, SH, NK
<i>ipbb_ta_243</i>	PH, NKS, TKW	KB, SH, NK
<i>ipbb_ta_294</i>	HD, PH, TKW	KB, SH, NK
<i>ipbb_ta_302</i>	HD, SMD, VP, TKW, Ych	KB, SH, NK
<i>ipbb_ta_166</i>	SMD, WKP	SH
<i>ipbb_ta_171</i>	TKW	SH
<i>ipbb_ta_165</i>	WKS, TKW	NK
<i>ipbb_ta_161</i>	HD, PH	KA

Notes: HD – heading date, SMD – seed maturation date, VP – vegetation period, PH – plant height, SL – spike length, NKS – number of kernels per spike, TKW – thousand kernel weight, Ych – yield quintals per hectare (q/ha), KA – Karaganda region, KB – Kostanay region, SH – Akmola region, NK – North Kazakhstan.

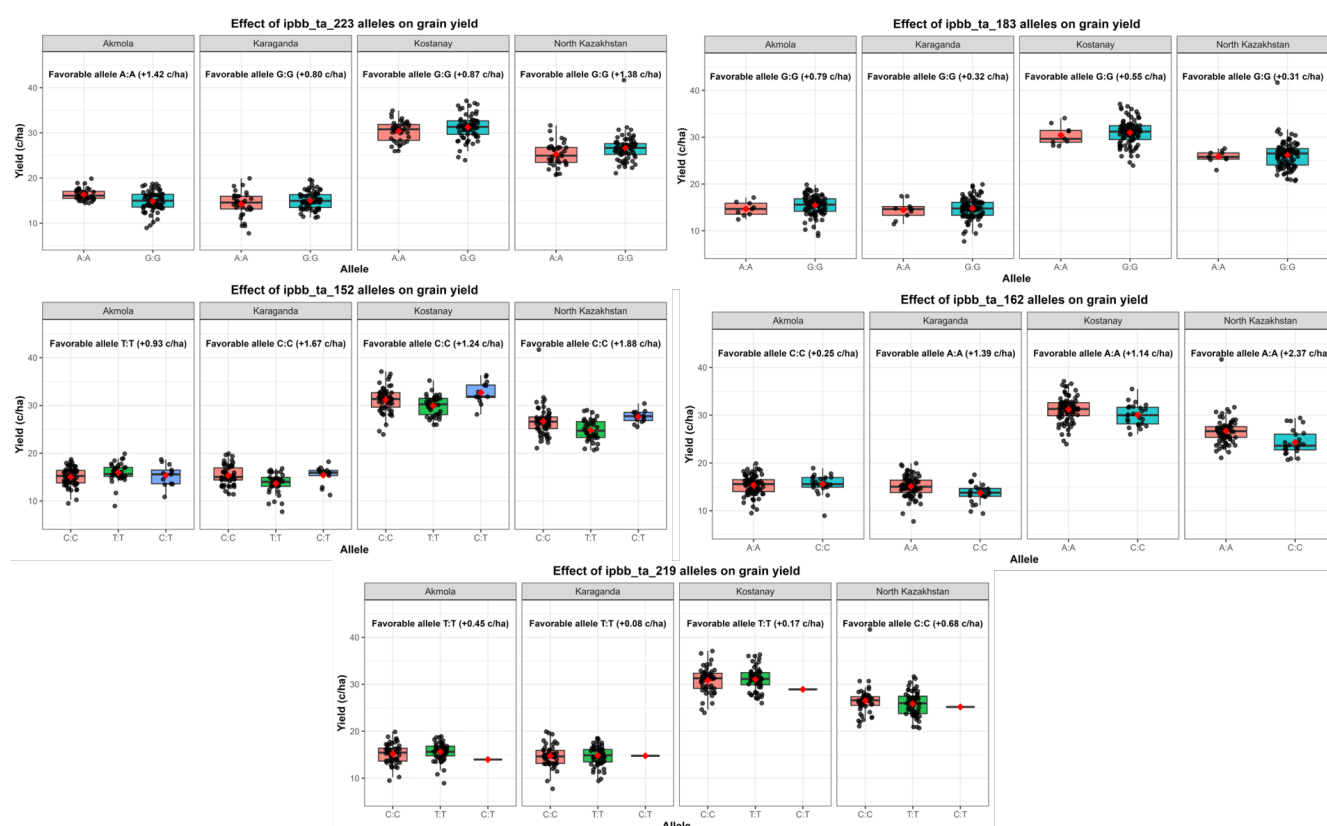


Figure 6. Grain-yield distributions of contrasting homozygous allelic classes for five stable KASP markers in four wheat-growing regions of Kazakhstan. Red diamonds indicate group means. Labels identify the favorable allele and the difference between allelic-class means expressed in c/ha.

Identification of Favorable Alleles. To validate the practical value of the identified marker–trait associations, the effects of contrasting alleles of the most significant KASP markers were evaluated across four wheat-growing regions of Kazakh-

stan. Boxplot analysis revealed considerable variation in grain yield between allelic classes and demonstrated both stable and environment-specific marker effects (Figure 6). Marker *ipbb_ta_223* exhibited a pronounced GEI. In Akmola, the A:A al-

lele increased grain yield by 1.42 c/ha relative to the G:G allele, whereas in Kostanay, North Kazakhstan, and Karaganda, the G:G allele was favorable, providing yield advantages of 0.87, 1.38, and 0.80 c/ha, respectively. Similarly, marker *ipbb_ta_152* showed environment-dependent effects: the T:T allele increased yield by 0.93 c/ha in Akmola, whereas the C:C allele was favorable in Karaganda, Kostanay, and North Kazakhstan, with yield gains ranging from 1.24 to 1.88 c/ha. Among all evaluated loci, marker *ipbb_ta_162* displayed the largest positive effect on grain yield.

The A:A allele increased productivity by 1.14 c/ha in Kostanay, 1.39 c/ha in Karaganda, and 2.37 c/ha in North Kazakhstan, whereas the C:C allele showed a slight advantage of 0.25 c/ha in Akmola. In contrast, marker *ipbb_ta_183* exhibited a stable, favorable allele across all environments. The G:G allele consistently outperformed the A:A allele, increasing grain yield by 0.31-0.79 c/ha depending on the region. Marker *ipbb_ta_219* showed comparatively small allelic effects, ranging from 0.08 to 0.68 c/ha across environments.

Overall, the evaluated markers displayed three distinct patterns of allelic effects: stable effects across environments (*ipbb_ta_183*), strong GEI (*ipbb_ta_223* and *ipbb_ta_152*), and pronounced positive effects under northern Kazakhstan conditions (*ipbb_ta_162*).

DISCUSSION

The successful implementation of MAS in wheat breeding depends on identifying reliable marker-trait associations and validating favorable alleles under target environmental conditions. In the present study, KASP markers previously identified through GWAS and QTL analyses were evaluated using a diverse collection of spring bread wheat germplasm from Kazakhstan's major breeding centers. The results demonstrated that a substantial proportion of the analyzed markers remained polymorphic and retained significant associations with agronomic traits under field conditions, confirming their practical value for breeding applications.

The genetic diversity analysis revealed moderate levels of polymorphism among the evaluated breeding collections. The highest level of diversity was observed in the Karabalyk breeding collection, whereas the Karaganda collection showed comparatively lower diversity (Table 2). These findings are consistent with previous studies of Kazakhstan wheat germplasm, which reported genetic differentiation among breeding materials originating from different ecological zones and breeding programs [39]. The PCoA further demonstrated clear genetic differentiation among breeding centers (Figure 4). The clustering of accessions from the Aktobe and Karabalyk breeding programs suggests a common genetic background or the utilization of related germplasm resources, whereas the Baraeva collection formed a more distinct group (Figure 4). Similar patterns have been reported in previous SNP-based studies of Kazakhstan wheat germplasm, in which breeding origin was one of the major factors influencing population structure [9, 40].

One of the important findings of this study was the large number of significant marker-trait associations detected across environments. Phenological traits, including HD, SMD, and VP, were particularly enriched for significant associations. Previous GWAS and QTL analyses have repeatedly identi-

fied major loci that control flowering and maturity as key determinants of adaptation and yield stability in wheat [9, 17, 19, 20, 23, 24]. Therefore, the strong representation of phenology-related markers among significant associations supports the biological relevance of the identified loci. Several markers showed stable associations across all testing environments, including *ipbb_ta_152*, *ipbb_ta_219*, *ipbb_ta_223*, *ipbb_ta_183*, and *ipbb_ta_162*. Among these loci, *ipbb_ta_223* displayed the broadest spectrum of associated traits, including HD, VP, SL, NKS, and Ych. Such multi-trait associations may reflect pleiotropy or tightly linked genes that control multiple components of plant development and productivity. Similar relationships between flowering time loci and yield-related traits have been reported in wheat and other cereal crops, where adaptation genes indirectly influence grain productivity by modulating developmental timing [41].

At the same time, several markers exhibited pronounced environment-specific effects. The favorable allele of *ipbb_ta_223* differed between Akmola and the other regions, while *ipbb_ta_152* showed contrasting allele effects among environments. These results indicate the presence of GEI, which are common for quantitative traits such as yield. Under the highly variable environmental conditions of Kazakhstan, the expression of many loci may depend on local climatic factors, including temperature regime, moisture availability, and growing-season duration [42]. Consequently, the effectiveness of some markers may be region-specific, requiring careful consideration when implementing MAS. Among the evaluated markers, *ipbb_ta_162* demonstrated the strongest positive effect on grain yield, particularly in North Kazakhstan, where the favorable allele increased productivity by more than 2 c/ha. In contrast, *ipbb_ta_183* exhibited a stable, favorable allele across all environments, indicating its potential value for broad adaptation breeding strategies.

From a practical breeding perspective, the validated KASP markers can be incorporated into marker-assisted selection at several stages of spring wheat improvement. The stable favorable allele of *ipbb_ta_183* can be used for early-generation screening of crosses intended for broad adaptation across Kazakhstan. The favorable allele of *ipbb_ta_162* can be prioritized in breeding populations developed for the northern wheat-growing regions. In contrast, *ipbb_ta_152* and *ipbb_ta_223* should be applied within region-specific breeding strategies because the direction of their favorable allelic effects varied among environments. These markers may also be used to pyramid complementary favorable alleles; however, marker-assisted selection should be combined with multi-environment phenotypic evaluation.

The effects of several markers on both phenological traits and grain yield further emphasize the close relationship between developmental timing and productivity. Wheat adaptation to local environments is largely determined by the synchronization of flowering and grain-filling periods with favorable climatic conditions. Therefore, markers that influence heading date and maturation may indirectly affect yield by modulating stress avoidance and resource utilization [9, 20, 39]. The observed associations between phenology-related markers and grain productivity support this concept and highlight the importance of integrating adaptation and yield traits within marker-assisted breeding programs.

Several limitations of this study should be considered. The validation panel included 107 genotypes, and the five breeding organizations were represented by unequal numbers of accessions. Therefore, the estimated allele frequencies and marker effects may partly reflect the genetic composition of individual breeding collections. In addition, the study evaluated a targeted set of 43 previously selected markers rather than genome-wide variation. Although the genotypes were tested in four environments, further validation across additional years and locations is required. The most promising markers should also be evaluated in independent breeding populations and segregating progenies before their routine application in marker-assisted selection.

The results demonstrate that KASP markers derived from previous GWAS studies can be successfully applied to validate marker-trait associations under Kazakhstani conditions. The identification of stable markers, environment-specific loci, and favorable alleles provides valuable tools for developing high-yielding, locally adapted wheat cultivars.

CONCLUSION

This study validated the effectiveness of KASP markers previously developed from GWAS and QTL analyses for characterizing spring bread wheat germplasm and identifying marker-trait associations under the environmental conditions of Kazakhstan. Among the 43 evaluated KASP markers, 33 were polymorphic and successfully differentiated the studied wheat genotypes, demonstrating their suitability for genetic analysis and breeding applications. Genetic diversity analysis revealed a moderate level of variation among breeding collections originating from five major wheat breeding centers of Kazakhstan. The PCoA further confirmed genetic differentiation among breeding programs, reflecting differences in breeding history and germplasm composition. Association analysis identified numerous significant marker-trait relationships across four contrasting environments. Five markers (*ipbb_ta_152*, *ipbb_ta_219*, *ipbb_ta_223*, *ipbb_ta_183*, and *ipbb_ta_162*) showed stable associations with adaptation- and yield-related traits across all testing locations, indicating their robustness and potential value for marker-assisted selection. Several additional markers showed significant effects in northern environments, highlighting their potential contribution to regional adaptation. Evaluation of allelic effects demonstrated that marker *ipbb_ta_162* possessed the strongest positive influence on grain yield, particularly under North Kazakhstan conditions, whereas *ipbb_ta_183* exhibited the most stable favorable allele across all environments. In contrast, markers *ipbb_ta_223* and *ipbb_ta_152* showed pronounced genotype × environment interactions, indicating that their effectiveness depends on local growing conditions. Overall, the identified favorable alleles and validated marker-trait associations represent valuable tools for wheat improvement programs in Kazakhstan. The integration of these KASP markers into MAS schemes will facilitate the development of high-yielding and environmentally adapted wheat cultivars. Future studies involving larger breeding populations and additional environments will further improve marker validation and accelerate their practical implementation in wheat breeding.

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AUTHOR CONTRIBUTIONS

Conceptualization, Y.K.T.; methodology, S.I.A. and Y.K.T.; investigation, A.Y.A., T.V.S. and Y.K.T.; validation, A.Y.A. and Y.K.T.; formal analysis, A.Y.A.; data curation, A.Y.A. and T.V.S.; writing original draft preparation, A.Y.A., T.V.S., S.I.A. and Y.K.T.; writing and editing, A.Y.A., T.V.S., S.I.A. and Y.K.T.; visualization, A.Y.A.; supervision, Y.K.T.; project administration, S.I.A. All authors have read and agreed to the published version of the manuscript.

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ВАЛИДАЦИЯ KASP-МАРКЕРОВ, СВЯЗАННЫХ С ПРИЗНАКАМИ АДАПТАЦИИ И УРОЖАЙНОСТИ, В КОЛЛЕКЦИИ ЯРОВОЙ МЯГКОЙ ПШЕНИЦЫ КАЗАХСТАНААмалова А. Ы.¹, Савин Т.В.², Абугалиева С. И.¹, Турусбеков Е.К.^{1*}¹ Институт биологии и биотехнологии растений, Алматы 050040, Казахстан² Научно-производственный центр зернового хозяйства имени А.И. Бараева, Акмолинская область 020000, Казахстан

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АННОТАЦИЯ

Валидация молекулярных маркеров в целевых условиях выращивания является необходимым этапом для эффективного внедрения маркер-ассоциированной селекции в селекцию пшеницы. Целью данного исследования была оценка генетического разнообразия коллекции яровой мягкой пшеницы Казахстана, валидация KASP-маркеров, связанных с признаками адаптации и урожайности, а также идентификация благоприятных аллелей для практического использования в селекции. В исследование были включены 107 генотипов яровой мягкой пшеницы из пяти селекционных организаций Казахстана, изученные в четырех контрастных экологических условиях и генотипированные с использованием 43 KASP-маркеров, разработанных на основе предыдущих исследований по полногеномному поиску ассоциаций (GWAS) и картированию локусов количественных признаков (QTL). Из анализируемых маркеров 33 оказались полиморфными и эффективно дифференцировали исследуемый материал. Анализ генетического разнообразия выявил умеренный уровень изменчивости между селекционными коллекциями, при этом наибольший уровень полиморфизма был отмечен в линиях из Карабалыкской СХОС (100%). Анализ главных координат показал четкую генетическую дифференциацию между селекционными центрами. Наибольшее число значимых ассоциаций маркер–признак было выявлено в Северо-Казахстанской области. Пять маркеров (*ipbb_ta_152*, *ipbb_ta_219*, *ipbb_ta_223*, *ipbb_ta_183* и *ipbb_ta_162*) продемонстрировали стабильные ассоциации с признаками адаптации и урожайности во всех условиях испытаний. Маркер *ipbb_ta_162* показал наиболее выраженное положительное влияние на урожайность, тогда как *ipbb_ta_183* характеризовался наиболее стабильным благоприятным аллелем. Полученные результаты подтверждают перспективность использования KASP-маркеров в маркер-ассоциированной селекции пшеницы в Казахстане.

Ключевые слова: яровая мягкая пшеница, KASP-маркеры, генетическое разнообразие, маркер-ассоциированная селекция, адаптация, урожайность.

ҚАЗАҚСТАННЫҢ ЖАЗДЫҚ ЖҰМСАҚ БИДАЙ КОЛЛЕКЦИЯСЫНДА БЕЙІМДЕЛГІШТІК ПЕН ӨНІМДІЛІК БЕЛГІЛЕРІМЕН БАЙЛАНЫСТЫ KASP МАРКЕРЛЕРІН ВАЛИДАЦИЯЛАУАмалова А. Ы.¹, Савин Т.В.², Абугалиева С. И.¹, Турусбеков Е.К.^{1*}¹ Өсімдіктер биологиясы және биотехнологиясы институты, Алматы 050040, Қазақстан² А.И. Бараев атындағы астық шаруашылығы ғылыми-өндірістік орталығы, Ақмола облысы 020000, Қазақстан

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АНДАТПА

Бидай селекциясында маркерлік селекцияны тиімді қолдану үшін молекулалық маркерлерді нақты өсіру жағдайларында валидациялау маңызды болып табылады. Зерттеудің мақсаты Қазақстанның жаздық жұмсақ бидай коллекциясының генетикалық әртүрлілігін бағалау, бейімделгіштік пен өнімділік белгілерімен байланысты KASP маркерлерін валидациялау және селекцияда қолдануға болатын тиімді аллельдерді анықтау болды. Қазақстанның бес селекциялық ұйымынан алынған 107 жаздық жұмсақ бидай генотипі зерттеліп, елдің төрт контрастты экологиялық жағдайында бағаланды және бұрын жүргізілген толықгеномдық ассоциациялық зерттеулер (GWAS) мен сандық белгілер локустарын (QTL) карталау нәтижелері негізінде әзірленген 43 KASP маркері арқылы генотиптелді. Талдауға алынған маркерлердің 33-і полиморфты болып, зерттелген генотиптерді тиімді ажыратты. Генетикалық әртүрлілікті талдау селекциялық коллекциялар арасында орташа деңгейдегі өзгергіштікті көрсетті, ал полиморфизмнің ең жоғары деңгейі Карабалық ауыл шаруашылығы тәжірибе станциясының коллекциясында анықталды (100%). Негізгі координаттар талдауы селекциялық орталықтар арасында айқын генетикалық жіктелуді көрсетті. Маркер-белгі ассоциацияларының ең көп саны Солтүстік Қазақстан жағдайында анықталды. Бес маркер (*ipbb_ta_152*, *ipbb_ta_219*, *ipbb_ta_223*, *ipbb_ta_183* және *ipbb_ta_162*) барлық зерттеу орталарында бейімделгіштік пен өнімділік белгілерімен тұрақты ассоциациялар көрсетті. *ipbb_ta_162* маркері өнімділікке ең жоғары оң әсер етсе, *ipbb_ta_183* барлық орталарда ең тұрақты тиімді аллельді көрсетті. Алынған нәтижелер Қазақстан жағдайында жоғары өнімді және бейімделген бидай сорттарын шығару үшін KASP маркерлерін маркерлік селекцияда қолданудың жоғары әлеуетін дәлелдейді.

Түйінді сөздер: жаздық жұмсақ бидай, KASP маркерлері, генетикалық әртүрлілік, маркерлік селекция, бейімделгіштік, өнімділік.