



Efficiency of SNP Mutations in Genes for Grain Weight and Yield in Spring Wheat

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ABSTRACT

Improving grain yield in common wheat (*Triticum aestivum* L.) remains a paramount challenge for safeguarding global food security. This study conducted an analysis of the contribution of several productivity-associated genes (TaGS5, TaGW6, and TaKAO) within hybrid wheat populations. The study examined 250 hybrid lines of three populations of Chinese and Kazakh varieties Xn-10×Karagandinskaya 22, Xn-08×Karagandinskaya 29, and Xn-10×Karagandinskaya 30. These combinations showed the highest level of productivity in studies in two agroecological zones during the period 2018-2022. They were selected as promising samples for further study. Sequencing revealed one SNP in each gene. It was determined that the allelic variants TaGS5-A and TaGS5-C of the TaGS5 gene were significantly associated with enhanced grain yield, providing yield increments of +32.7 g/m² (P=0.0031) and +26.7 g/m² (P=0.033), respectively. However, no significant effects were observed in the Xn-10×Karagandinskaya 22 population. The effect of TaGW6 was found to be less pronounced and statistically non-significant. Conversely, the TaKAO-C allele exhibited a strong positive correlation with productivity (+36.2 g/m², P=0.0012), thereby supporting its crucial role in grain mass formation, likely mediated by gibberellin biosynthesis. These findings are consistent with data from previous research, which highlights the variable contribution of distinct loci to yield traits. Consequently, TaKAO and TaGS5 are proposed as promising targets for marker-assisted selection (MAS), while TaGW6 could serve as a complementary locus. The present data advance our understanding of the genetic architecture underpinning wheat productivity and facilitate the development of more efficacious breeding strategies.

Keywords: Genotyping, SNP-markers, Spring wheat, *Triticum aestivum* L., Yield-related genes.

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INTRODUCTION

Spring wheat (*Triticum aestivum* L.) is one of the world's most vital cereal crops, occupying up to 222 million hectares of arable land globally, according to CIMMYT data (Farouk et al., 2024). While the gradual increase in wheat yield is achieved through continuous advancements in agronomy, breeding, and molecular biology, its productivity

remains significantly dependent on global climatic changes. Research has demonstrated that abiotic stresses, such as high temperature and water deficit, reduce the technological properties of the grain by 10-30%, negatively impacting both protein and starch quality. Furthermore, high-quality genotypes often exhibit instability when exposed to stress conditions, making abiotic factors a key challenge in ensuring stable wheat production (Filip et al., 2023).

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The three primary yield components in wheat are thousand-grain weight (TGW), the number of spikes per unit area, and the number of grains per spike. Among them, increasing grain weight represents one of the most effective pathways to enhance yield potential. Grain morphology traits such as length, width, and thickness directly determine grain size and consequently influence TGW (Xie & Sparkes, 2021). Wheat yield is a complex polygenic trait that needs to be studied in an integrative framework, as strong negative correlations have been reported among yield components due to both environmental and genetic variation (Huang et al., 2012).

As is well known, the three principal components of wheat yield are thousand-grain weight (TGW), the number of spikes per unit area, and the number of grains per spike. Among these, an increase in grain mass represents one of the most effective avenues for enhancing yield potential. The increase in productivity attributed to genetic modifications is primarily associated with the enhancement of grain productivity, which is determined by both the number and the mass of the grains (Devkota et al., 2023). The rapid development and application of molecular marker analyses have facilitated genomic selection and genome-wide linkage and association studies in wheat breeding. Although PCR-based markers and genotyping methods have significantly contributed to gene discovery and marker-assisted selection (MAS), the release of a more accurate and comprehensive common wheat reference genome has led to the development of Single Nucleotide Polymorphism (SNP) arrays based on varying densities or application targets (Sun et al., 2020).

Understanding the genetic and molecular mechanisms that control grain size and mass is a prerequisite for enhancing grain yield potential. Significant progress has been achieved over the past decade in genomic research concerning the regulation of these traits in wheat (Gasparis & Miłoszewski 2023). The study by Tian et al., (2023), based on an analysis of 192 spring wheat cultivars phenotyped across six distinct environments, utilized Genome-Wide Association Study (GWAS) for yield-related traits. The authors successfully identified 47 significant Marker-Trait Associations (MTAs), including stable associations for thousand-grain weight, grain size, and spike number. The phenotypic variation explained by these associations reached up to 18.7%, underscoring the value of the detected SNPs for the subsequent breeding of high-yielding wheat genotypes.

Li et al. (2021) investigated the genetic architecture of yield using 583 spring wheat genotypes, analyzing over 40,000 high-quality SNP markers. They successfully identified 96 significant SNP associations with yield components and determined lines carrying up to 8 to 10 favorable alleles linked to spike number, grain number, and grain mass. Liu et al. (2023) investigated a Recombinant Inbred Line (RIL) population comprising 262 lines derived from the cross (Zhongmai 578 × Jimai 22). Utilizing a 50K SNP array, the authors identified 53 Quantitative Trait Loci (QTLs) controlling yield-related traits. Of these, 23 stable QTLs were detected in three or more environments, including novel loci associated with thousand-grain weight, grain size, and grain filling rate.

Among the most effective yield-related gene families are TaGW and TaGS, which have demonstrated significant contributions to grain width and TGW (Nadolska-Orczyk et al., 2017; Yang et al., 2019; Yang et al., 2022; Tillett et al., 2022). In Chinese wheat germplasm, SNPs in the promoter region of TaGW2-A1, a well-studied homolog on chromosome 6A encoding a RING-type E3 ubiquitin ligase, were associated with increased TGW, reduced indole-3-acetic acid (IAA) content, and decreased grain number per spike (Zhai et al., 2018). Similarly, two SNPs forming the haplotype Hap-6A-A of TaGW6 were shown to regulate seed size by encoding a novel indole-3-acetic acid glucose hydrolase, primarily expressed in young spike tissues, and negatively regulating grain width and weight. Moreover, TaGW2-6A and TaGW2-6B haplotypes were significantly associated with increased grain size and TGW, with Hap-6B-1 exerting a stronger effect than Hap-6A (Su et al., 2011). Bazhenov et al. (2025) demonstrated that the application of a customized panel comprising approximately 3,500 SNP markers facilitated the identification of associations with key genes controlling agronomic traits in wheat. Significant SNPs were detected at the Rht-B1 (IAB60001), Rht-D1 (IAB07969), Ppd-D1 (IAB04062), and Vrn-D1 loci, which influence plant height, photoperiod sensitivity, and developmental habit (or growth habit). Furthermore, the lack of variability was noted for the TaGW2-A1 and Gpc-B1 genes, suggesting the necessity of their introgression. The investigation by Jaiswal et al. (2015) revealed four SNPs in the promoter region of the TaGW2-6A gene across 207 wheat genotypes, including two novel variants (at -988 and -494 bp). The most significant was SNP-494, which demonstrated a clear association with thousand-grain weight (TGW): genotypes carrying the A allele exhibited an average ≈ 41.1 g, whereas those with the G allele showed ≈ 38.6 g, reflecting an increase of over 6%. Furthermore, SNP-494 was found to correlate with awn length, spike length, grain length-to-width ratio, protein content, and plant height. It was demonstrated that carriers of the favorable A allele are characterized by downregulated expression of TaGW2-6A, which is correlated with an increase in grain size and mass.

Genes such as TaGS-D1 and TaGS5-3A have also been associated with TGW and grain length. For example, the TaGS3-7A-A allele significantly increases TGW, while the TaGS5-3A-T haplotype, defined by a SNP (T/G) in the sixth exon, was linked to larger grain size, higher TGW, and shorter plant height, spike length, and peduncle length (Zhang et al., 2014). Mroz et al. (2023) conducted a Genome-Wide Association Study (GWAS) on a panel of 301 spring wheat cultivars cultivated in Norway during the period of 2015-2021. This analysis facilitated the identification of 12 key loci associated with yield, phenology, and adaptation to Northern conditions. The most significant regions, specifically QYld.nmbu-5A and QHd.nmbu-1B, were found to govern adaptation to a short growing season and substantially influence productivity. The authors demonstrated that modern breeding progress was linked to a shift in the frequencies of favorable alleles, including TaGS5-3A and TaCol-5 rendering the identified loci promising markers for future selection.

In addition, genes such as TaKO (ent-kaurene oxidase), TaKAO (ent-kaurenoic acid oxidase), and TaSPS (sucrose phosphate synthase) contribute to grain weight, with expression detected in leaves, developing spikes, peduncles, and upper internodes (Huang et al., 2012). Recent meta-QTL studies have further confirmed TaKAO-4A as a novel candidate gene strongly associated with grain size (Yang et al., 2021; Liu et al., 2023). Khlestkina et al. (2010) successfully identified a series of SNPs within the investigated genes encoding the enzyme ent-kaurenoic acid oxidase (KAO). These SNPs exhibited differentiation across the homeologs KAO-A1 (on chromosome 7AS), KAO-B1 (4AL), and KAO-D1 (7DS). The majority of the detected substitutions were localized in the coding regions and predominantly represented by synonymous (silent) SNPs, which do not alter the amino acid sequence. This observation suggests high conservancy of the enzyme and its inherent functional significance. Nevertheless, the presence of intergenomic differences in the SNP profile confirms the divergence of the homeologs and creates an opportunity to utilize these variants as specific markers for allelic differentiation and the analysis of their respective contributions to gibberellin biosynthesis. These polymorphisms may serve as the foundation for the development of gene-specific markers and for the subsequent elucidation of the KAO's pivotal role in the regulation of wheat growth and productivity.

Taken together, SNPs in yield-related genes such as TaGW and TaGS have been shown to play a crucial role in increasing grain weight. In contrast, SNP variation in TaKAO remains largely unexplored, and its relationship with TGW has not yet been clarified. Since many of the reported alleles are prevalent in Chinese wheat germplasm, this study aims to investigate hybrids of Chinese and Kazakh wheat varieties to identify the most effective SNPs. The most precise and efficient approach to evaluate such polymorphisms is the development of gene-specific markers, enabling large-scale selection of donor genotypes carrying valuable mutations.

The aim of this study is to identify, analyze, and select promising hybrids for the presence of high-value SNPs in TaGW, TaGS, and TaKAO genes, with the ultimate goal of improving wheat productivity by enhancing spike yield components.

MATERIALS & METHODS

The study utilized parental lines of wheat, sourced from Chinese and Kazakh breeding programs, which demonstrated the highest yield potential during the 2018-2022 growing seasons under the dry-steppe conditions of Northern and Central Kazakhstan (Serikbay et al., 2024; Zotova et al., 2024). These were studied alongside their F3-F4 hybrid populations (Xn-10×Karagandinskaya 22, Xn-08×Karagandinskaya 29, Xn-10×Karagandinskaya 30) and 250 F5-F6 lines resulting from individual selection, which were evaluated in the field during the 2023-2024 periods.

Climatic Conditions across two Agroecological Locations

For the accurate interpretation of the generated phenotypic data, it is crucial to account for the climatic specificities of the regions where the field trials were conducted. Below is a comparative analysis of the weather conditions in the Akmola and Karaganda regions during the 2018-2024 growing seasons, which reflects the differences in temperature regimes and moisture availability (Fig. 1).

The comparative analysis revealed that the Akmola region experienced more pronounced climatic contrast during the 2018-2024 period compared to the Karaganda region. Mean temperatures during the growing season almost annually exceeded the established norm, reaching 19.7-20.0°C, particularly in June-July. Conversely, temperature deviations in the Karaganda region were less severe, though isolated anomalies were recorded (e.g., a cold May in 2018, 6.8°C; a warm May in 2020, 19.0°C). The primary difference, however, was associated with moisture availability. The Akmola region exhibited a severe precipitation deficit,

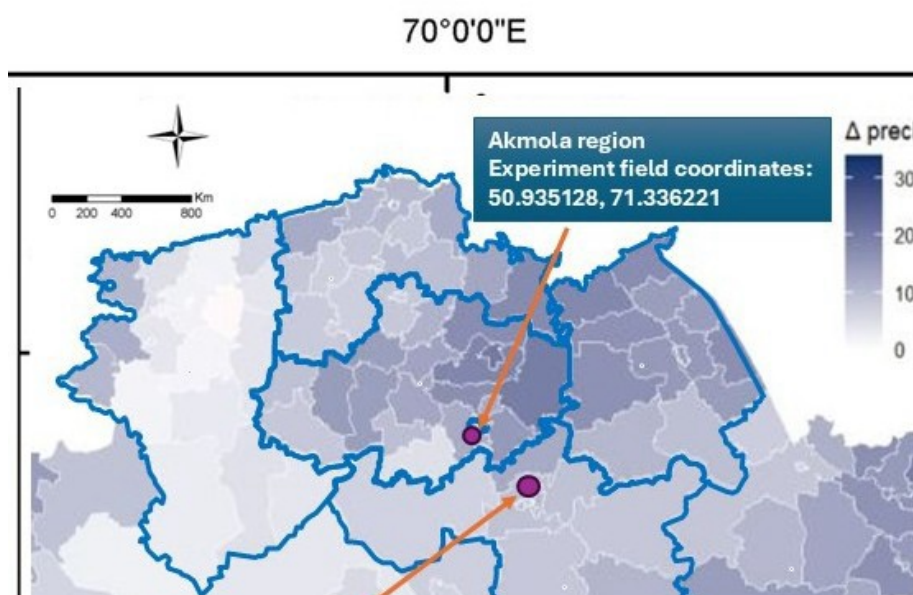


Fig. 1: Geographical Location of the Agroecological Locations (Abdrzakuly, 2025).

with rainfall sums ranging from 39-117 mm against the established norm of 168.7 mm in most years. In contrast, total precipitation in the Karaganda region was moderately reduced (93-126 mm against a norm of 140-150 mm). This divergence is reflected in the values of the Hydrothermal Coefficient (HTC): in the Akmola region, it varied from 0.34 to 0.89, indicating more severe aridity, while in the Karaganda region, the range was 0.39 to 0.72, suggesting dry but less extreme conditions. Overall, the Akmola region demonstrated harsher environmental stress conditions for vegetation, whereas the Karaganda region was characterized by milder climatic fluctuations.

Amplification of all target genes was performed using the VeritiPro (Applied Biosystems, USA) thermal cyler, the results presented in Table 1.

Table 1: Nucleotide sequences of the oligonucleotides used in the experiments

Gene	Nucleotide sequence of the primer	Product size
TaGS5-3A-F	5'-AGACATGGTGGAGCAAGAGATG-3'	237 bp
TaGS5-3A-R	5'-GAACAACCTAATCCTCCTCTGA-3'	
TaGW6-F	GGACATCTAGCCACCTCCCT	809 bp
TaGW6-R	CGCCGTTCGGGTATGTCA	
TaKAO-F	F_63-CCCGGCAGTTCGGATTTTC	973 bp
TaKAO-R	CTGTGTTGTTGTCCAGGC	

Sanger sequencing was carried out using the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, USA) on a SeqStudio system (Thermo Fisher Scientific, USA), utilizing the Medium Seq protocol. The resulting gene sequence data have been registered in the GenBank database with the following accession numbers: TaKAO (Xn-08 – PV550151, Karagandinskaya 22 – PV550150, Karagandinskaya 29 – PV550152), TaGS5 (Xn-08 – PV554171), and TaGW6 (Xn-10 – PV698420, Karagandinskaya 22 – PV698421, Karagandinskaya 30 – PV698422).

Genotyping using the Amplifluor-like method (Jatayev et al., 2017) was performed according to optimized, gene-specific protocols on the QuantStudio-7 Real-Time PCR system (qPCR) (ThermoFisher Scientific, Paisley, UK) and a FLUOstar Omega microplate reader (BMG LabTech, Ortenberg, Germany). The total reaction volume of 10 µL contained the PCR mixture: 2X Master-mix (1 x PCR Buffer, 1.8 mM MgCl₂, 0.2 mM of each dNTP, 0.25µM of each fluorescent probe, 0.15 µM of each forward primer, 0.78 µM of reverse primer, and 0.1 units of Maxima Hot-Start Taq polymerase (ThermoFisher, USA)). Half of the PCR volume was genomic DNA adjusted to 10ng/µL, as shown in Table 2.

Statistical analysis was conducted to test for normality and homogeneity of variances. All experiments were performed with three biological replicates. The p-values were calculated using the GraphPad Prism 8.0.1 software. Statistical processing and the analysis of associations between genotypic and phenotypic data were carried out using the RStudio software package. Statistical significance was set at p≤0.05.

RESULTS

A comparative sequence analysis of the spike productivity genes TaGS5, TaGW6, and TaKAO was performed using the newly generated sequencing data

Table 2: Protocols and cycling conditions for target gene genotyping

Gene	PCR Cycling Conditions	Master-mix	
TaGS5	Hold – 95°C – 2 min	MM 5X – 2 µL	
	20 Cycles:	Primer Mix – 1 µL	
	95°C – 15 sec	Probe 2024 – 1 µL	
	58°C – 20 sec	Water – 4 µL	
	95°C – 15 sec	DNA – 2 µL	
	54°C – 20 sec	Rox	
	Post-read		
	62°C – 5 min		
	TaGW6	Pre-read – 72°C-30 sec	MM 2X – 5 µL
		Hold – 95°C – 5min	Primer Mx – 1 µL
25 Cycles:		Probe 2024 – 1 µL	
95°C – 15 sec		Polymerase – 0.1 µL	
58°C – 20 sec		Water – 0.9 µL	
72°C – 20 sec		DNA – 2 µL	
95°C – 15 sec		Rox	
55°C – 20 sec			
72°C – 20 sec			
Post-Read 72 – 2 min			
TaKAO	4 – ~ min		
	Pre-read – 72-30sec	MM 2X – 5 µL	
	Hold – 95 – 5min	Primer Mx – 1 µL	
	25 Cycles:	Probe 2024 – 1 µL	
	95°C – 15 sec	Polymerase – 0.1 µL	
	58°C – 20 sec	Water – 0.9 µL	
	72°C – 20 sec	DNA – 2 µL	
	95°C – 15 sec	Rox	
	55°C – 20 sec		
	72°C – 20 sec		
Post-Read 72°C – 2 min			
4°C – ~ min			

from the Chinese and Kazakh wheat cultivars. Our findings demonstrated cultivar-specific polymorphisms distinguishing the investigated lines (Fig. 2).

Overall, the analysis of three productivity genes showed that the identified SNP polymorphisms have a pronounced variety specificity: unique variants characteristic of Chinese lines Xn-08 and Xn-10 are absent in Kazakh varieties and reflect the introgression of new alleles during hybridization. Polymorphisms in TaGS5, TaGW6, and TaKAO affect functionally significant gene regions, including coding regions, which potentially influence grain size and weight, as well as ear morphology. These differences confirm the genetic divergence of the parental forms and emphasize the high value of the discovered SNPs for subsequent breeding use. The final characteristics of the discovered polymorphisms are presented in Table 3.

Table 3: Results of polymorphism analysis in the investigated productivity genes

Parental Genotypes	SNP TaGS5-3A	SNP TaGW-6	SNP TaKAO
Xn-10 × Karagandinskaya 22			
Xn-10	TaGS5-C	TaGW6-A	–
Karagandinskaya 22	TaGS5-A	TaGW6-C	–
Xn-08 × Karagandinskaya 29			
Xn-08	TaGS5-C	–	TaKAO-C
Karagandinskaya 29	TaGS5-A	–	TaKAO-A
Xn-10 × Karagandinskaya 30			
Xn-10	TaGS5-C	TaGW6-A	–
Karagandinskaya 30	TaGS5-A	TaGW6-C	–

Based on the sequencing results, a polymorphism was identified in the TaGS5 gene at position 103bp: the Chinese varieties carried the C nucleotide, while the Karagandinskaya varieties carried the A nucleotide. Analysis of the TaGW6 productivity gene sequence across the five parental lines revealed multiple polymorphisms

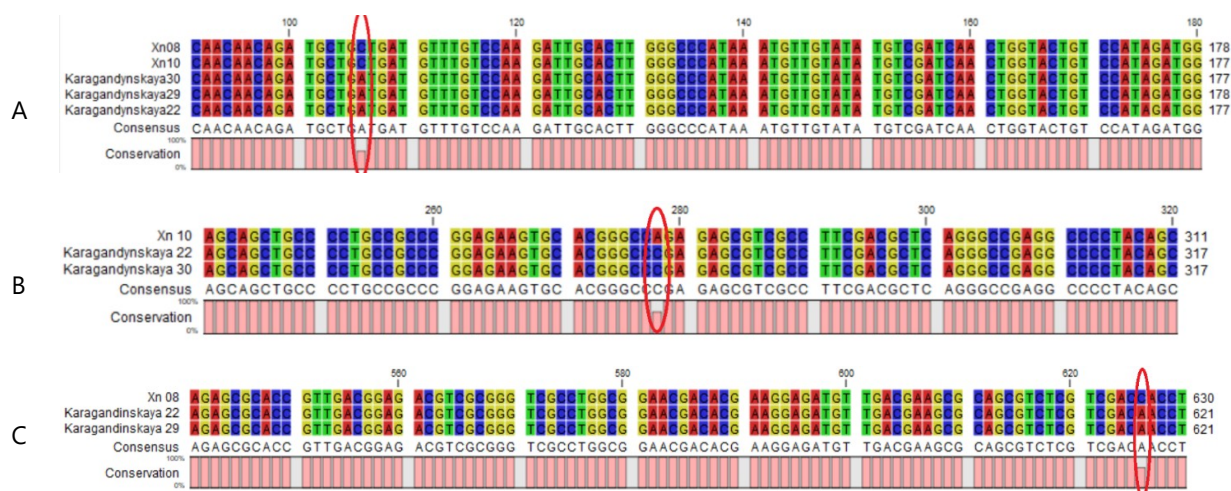


Fig. 2: Sequence polymorphism analysis: A – TaGS5 gene in five wheat varieties (Xn-08, Xn-10, Karagandinskaya 22, 29, 30); B – in the TaGW6 gene in three wheat varieties (Xn-10, Karagandinskaya 22, 30); C – in the TaKAO gene in three wheat varieties (Xn-08, Karagandinskaya 22, 29).

distinguishing the parents. The analyzed TaGW6 sequence showed several polymorphisms among variety Xn-10 and varieties Karagandinskaya 22 and Karagandinskaya 30. However, due to high site variability, the A/C polymorphism at position 278 bp was selected for primer design. Specifically, the Chinese variety Xn-10 possessed the A nucleotide, and the Karagandinskaya varieties (22 and 30) carried the C nucleotide at this position. For the TaKAO gene, polymorphism was identified at position 670bp when analyzing the nucleotide sequence of Xn-08, Karagandinskaya 29, and Karagandinskaya 22. Variety Xn-08 carried the C nucleotide, while the Karagandinskaya varieties possessed the A nucleotide at this position. These selected parental forms were utilized in crosses, resulting in 250 promising hybrid lines.

A crucial phase of this work involved the development of SNP markers and the subsequent genotyping of the five parental lines, and 250 hybrid lines derived from these combinations. Allele-specific SNP markers were designed based on the sequencing results:

TaGS5-F1: GAAGGTGACCAAGTTCATGCTCTTTGTCAACAACAGATGCTGA

TaGS5-F2: GAAGGTCGGAGTCAACGGATTCCTTTGTCAACAACAGATGCTGC

TaGS5-R: TGAACCACTCTATGGACAGTA

TaGW6-F1: GAAGGTGACCAAGTTCATGCTGCCCGGAGAAGTGCACGGGCCA

TaGW6-F2: GAAGGTCGGAGTCAACGGATTGCCCGGAGAAGTGCACGGGCC

TaGW6-R: ATGCGCCGCTCGGAGACGCC

TaKAO-F1: GAAGGTGACCAAGTTCATGCTCGCAGCGTCTCGTCGACC

TaKAO-F2: GAAGGTCGGAGTCAACGGATTCGACGCTCTCGTCGACA

TaKAO-R: GTCTGATGAACAATGCTCCTC.

The developed markers provided selective discrimination of the alleles in the parental lines and were subsequently used for the high-throughput analysis of the hybrid plants. This allowed us to assess the allele distribution in the progeny and identify promising combinations for breeding selection. Genotyping of the hybrid lines was performed using the Amplifluor-like system according to the established methodology (Fig. 3).

Genotyping of the hybrid combinations demonstrated the high efficiency of the synthesized SNP markers for allelic differentiation across the three genes studied. For the TaGS5 locus in the Xn-08×Karagandinskaya 29 cross, the majority of samples clustered into homozygous classes: 52.5% were homozygous for the TaGS5–C allele

(Chinese allele), 27.5% were homozygous for the TaGS5–A allele (Kazakh allele), and heterozygosity was detected in only one sample (2.5%). Conversely, 17.5% of the samples remained undetermined. This distribution indicates a prevalence of homozygous genotypes and an extremely low frequency of heterozygotes in this population.

Analysis of the TaGW6 locus in the Xn-10×Karagandinskaya 30 cross revealed a different pattern: 61.4% of the samples were homozygous for the TaGW6–A allele (Chinese allele), 17.0% were homozygous for the TaGW6–C allele (Kazakh allele), and 8.0% were heterozygotes, with 13.6% remaining undetermined. The presence of a noticeable proportion of heterozygotes reflects the expected segregation in a hybrid population and confirms the marker's informativeness for this locus.

For the TaKAO locus in the Xn-08×Karagandinskaya 29 cross, a more balanced segregation was observed: 34.1% of samples were homozygous for the TaKAO–A allele (Kazakh allele), 31.7% were homozygous for the TaKAO–C allele (Chinese allele), 22.0% were heterozygotes, and 12.2% remained undetermined. This highlights the high efficiency of the marker in separating allelic classes and indicates a significant proportion of heterozygotes within the population structure.

Overall, the results across the three loci confirm the reliability and informativeness of the developed SNP markers, revealed locus-specific genotypic distribution patterns, and demonstrated their practical value for analyzing segregation and utilizing the identified polymorphisms in breeding programs aimed at enhancing wheat productivity.

The effectiveness of the identified SNPs was assessed by comparing the genotyping results with the phenotyping data of the F5 segregating hybrid generation grown under the field conditions of the dry-steppe zone of Kazakhstan. Genetic data were validated by comparison with previously published results from yield trait evaluations in spring bread wheat obtained under similar arid climate conditions (Zotova et al., 2024). This approach allowed us to assess the utility of the markers for selecting favorable productivity-associated allele combinations and define their potential for use in breeding programs (Fig. 4).

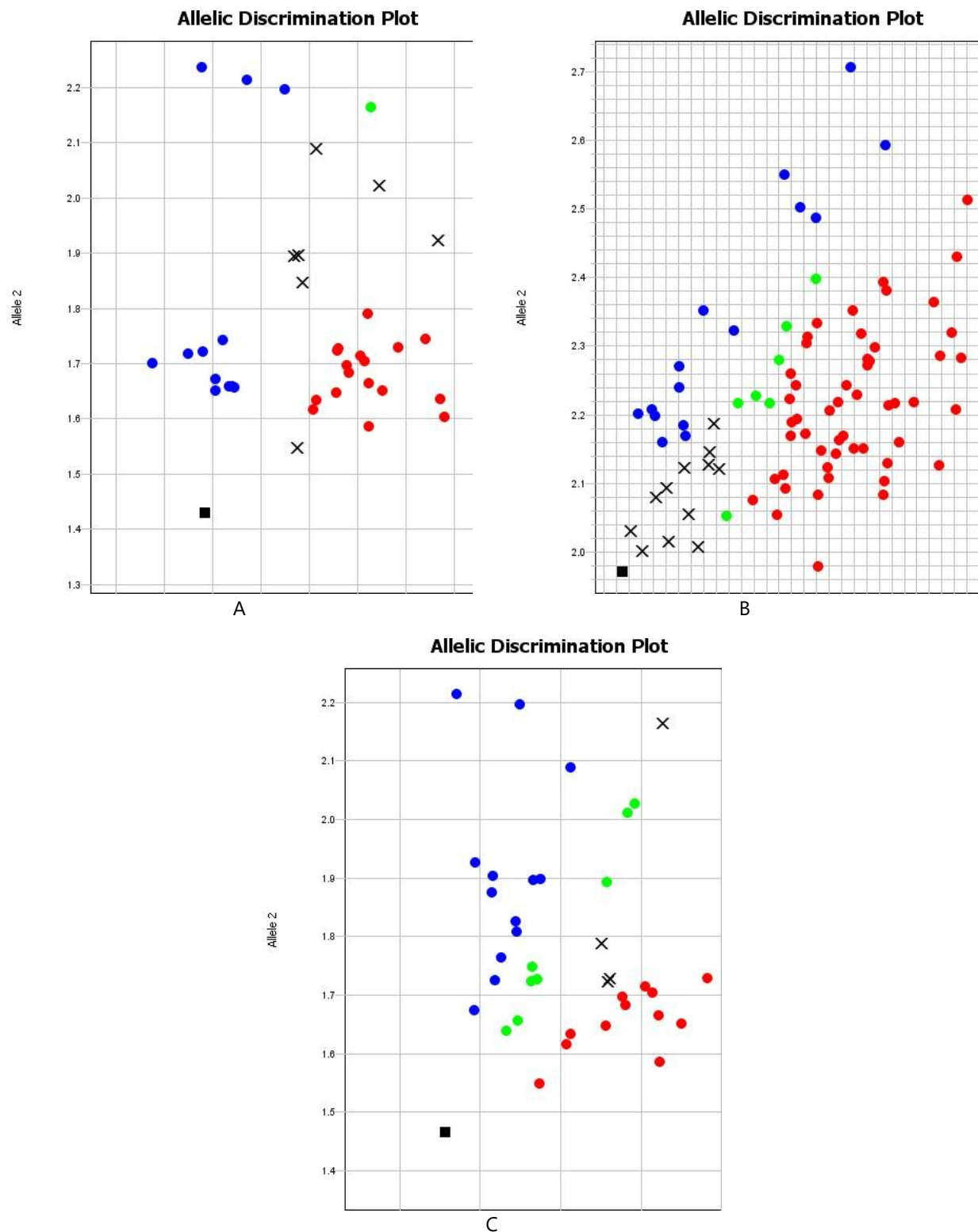


Fig. 3: Genotyping results of 250 hybrid lines derived from selected parental forms for the presence of polymorphisms. (A) Allelic distribution of TaGS5 in the Xn-08 × Karagandinskaya 29 population; (B) Allelic distribution of TaGW6 in the Xn-10 × Karagandinskaya 30 population; (C) Allelic distribution of TaKAO in the Xn-08 × Karagandinskaya 29 population.

In the hybrid samples of the Xn-08×Karagandinskaya 29 cross, the TaGS5–C allele (Fig. 4A) was associated with a significantly higher grain mass per spike, providing a gain of +0.4 g ($P=0.019$) compared to the TaGS5–A allele. In contrast, no significant effect of this gene on

grain mass per spike was observed in the parental lines of the Xn-10×Karagandinskaya 30 cross ($P=0.376$), with allelic differences ranging between -0.1 g and $+0.2$ g. Similarly, differences in the Xn-10×Karagandinskaya 22 cross were also statistically non-significant ($P=0.298$),

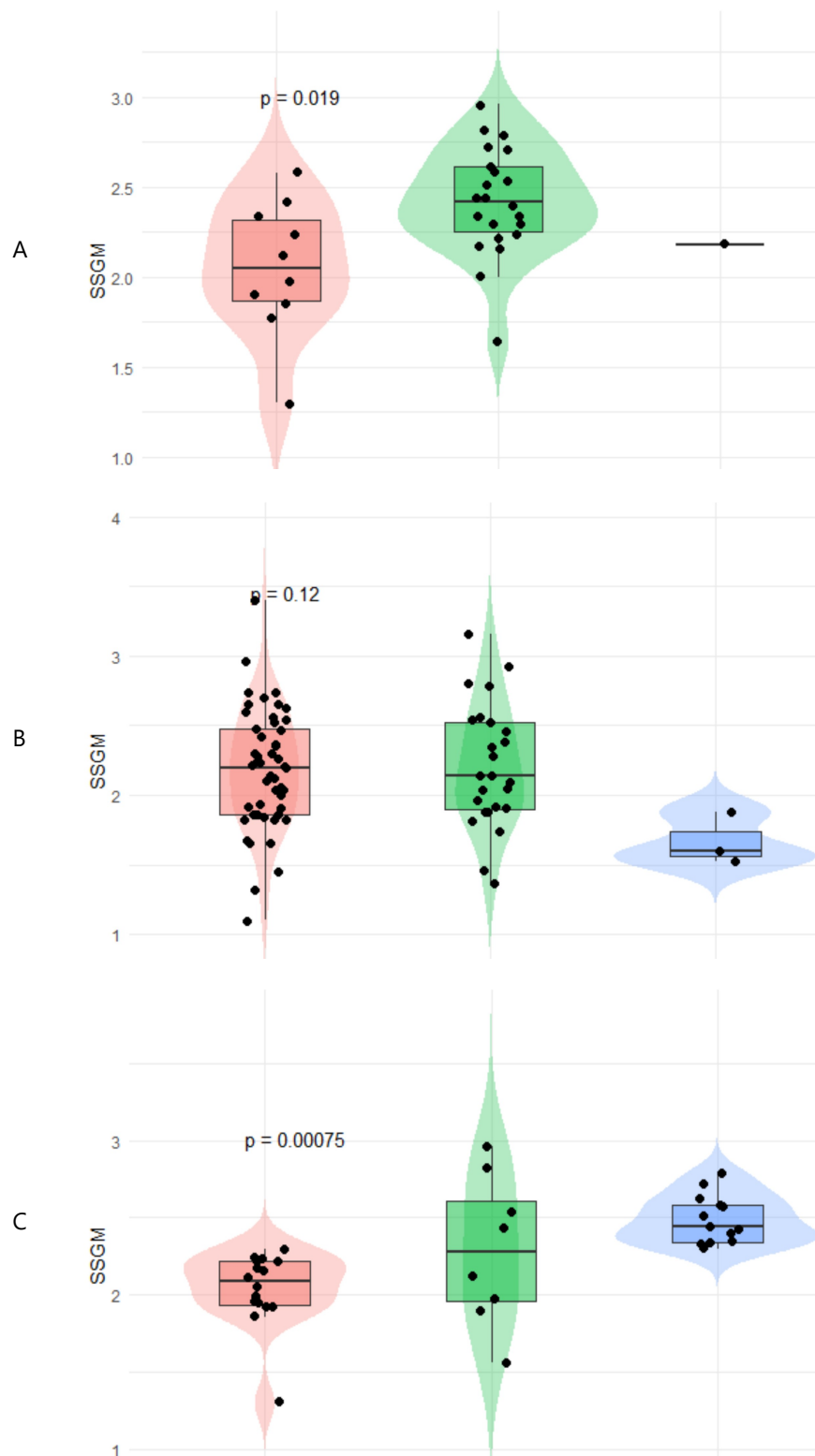


Fig. 4: Distribution of sample grain weight per spike in the population. (A) Xn-08×Karagandinskaya 29 population (TaGS5); (B) Xn-10×Karagandinskaya 30 population (TaGW6); (C) Xn-08×Karagandinskaya 29 population (TaKAO).

varying from -0.02 g to $+0.26$ g. The TaGW6 gene polymorphism (Fig. 4B) showed no significant influence on grain mass per spike in either the Xn-10×Karagandinskaya 30 population ($P=0.122$) or the Xn-10×Karagandinskaya 22 population ($P=0.506$), with value fluctuations ranging from $+0.02$ g to -0.5 g. In the Xn-08×Karagandinskaya 29 population, the TaKAO gene polymorphism showed a highly significant difference between alleles, ranging from

$+0.25$ g to $+0.45$ g ($P=0.0008$) (Fig. 4C). This confirms the substantial contribution of this locus to the formation of grain mass per spike, particularly for the C allele, which is characteristic of the Chinese varieties. Among the studied loci, the polymorphisms of the TaGS5 gene in the Xn-08×Karagandinskaya 29 cross and the TaKAO gene exerted the greatest influence on grain mass per spike, confirming their prospects for future use as markers.

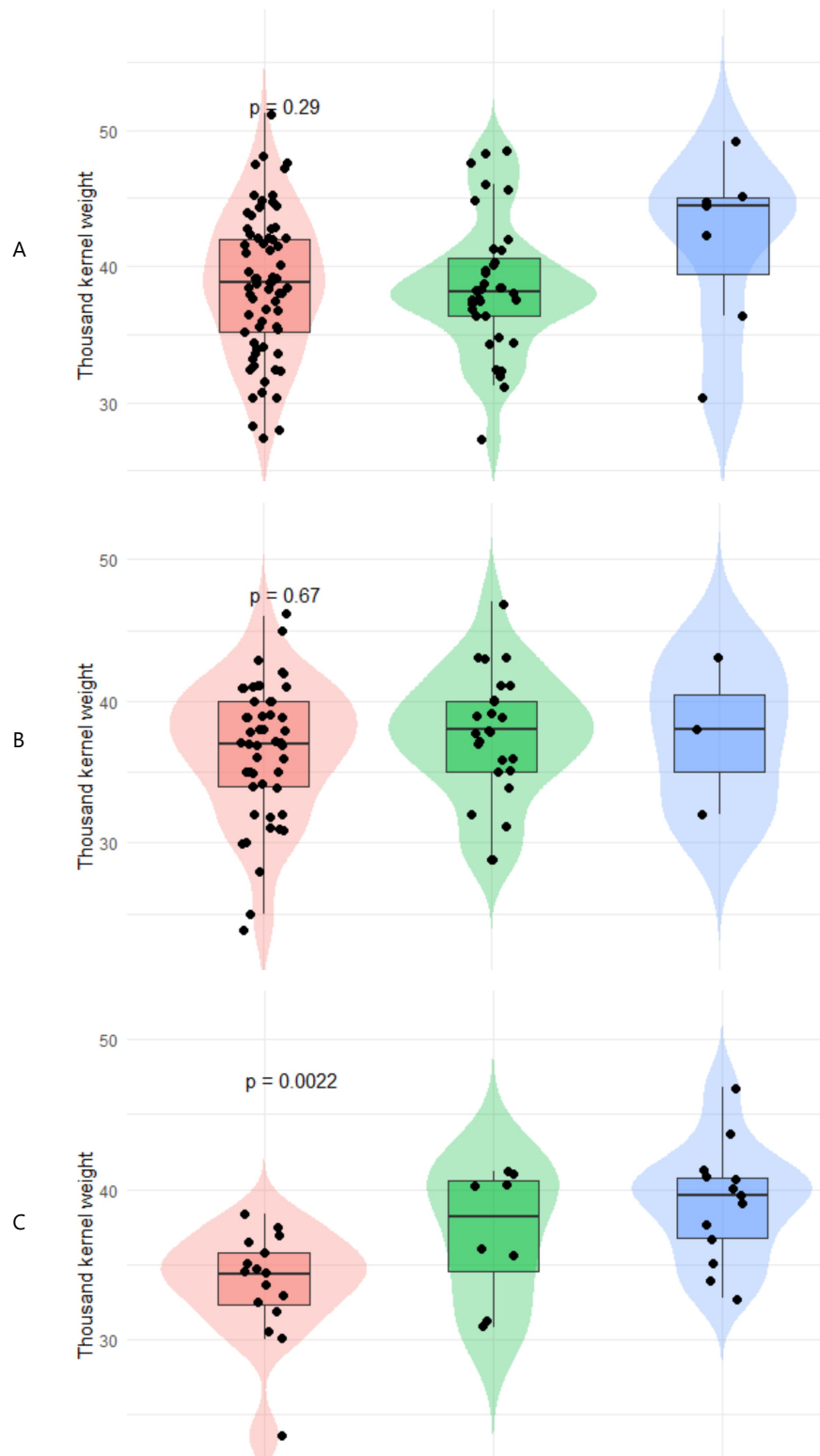


Fig. 5: Distribution of sample thousand-kernel weight in the population. (A) Xn-10×Karagandinskaya 22 population; (B) Xn-10×Karagandinskaya 30 population; (C) Xn-08×Karagandinskaya 29 population.

The distribution of samples during genotyping for the thousand-grain weight (TGW) trait demonstrated a high phenotypic dependency. Fig. 5 illustrated the distribution among 250 samples across the three populations investigated.

In the Xn-10×Karagandinskaya 22 population, no significant difference was observed ($P=0.29$), although the TaGS5-C allele showed a result 0.097g lower than

TaGS5-A. In the Xn-08×Karagandinskaya 29 population, a difference of 2.2g between the TaGS5-A and TaGS5-C alleles was recorded, but it was statistically non-significant ($P=0.318$). Heterozygous alleles were excluded due to small sample size. In the Xn-10×Karagandinskaya 30 population, the TaGS5-C allele exceeded TaGS5-A by 0.86g, but this effect was also statistically insignificant ($P=0.503$). A similar pattern was observed for the TaGW6

gene: differences in thousand-kernel weight (TKW) between the TaGW6-C and TaGW6-A alleles were negligible. An insignificant increase was noted for the TaGW6-C allele in the Xn-10×Karagandinskaya 30 population (+0.99g; $P=0.66$), while the difference in the Xn-10×Karagandinskaya 22 population was only 0.4g. However, the Chinese polymorphism in the TaKAO-C gene demonstrated a significant increase in TKW (+5.45 g; $P=0.002$) compared to the Kazakh polymorphism, showing the greatest contribution to this trait. Under the conditions

of the experiments conducted, the TaGS5 and TaGW6 loci did not significantly influence TKW, whereas the TaKAO-C gene polymorphism showed a high association with this trait, allowing it to be considered a priority marker for future breeding work.

Since the thousand-grain weight (TGW) values in the studied populations represent only a partial genetic effect and may not directly dictate total yield, the subsequent analysis focused on assessing the association between the detected allelic variations and overall plant yield (Fig. 6).

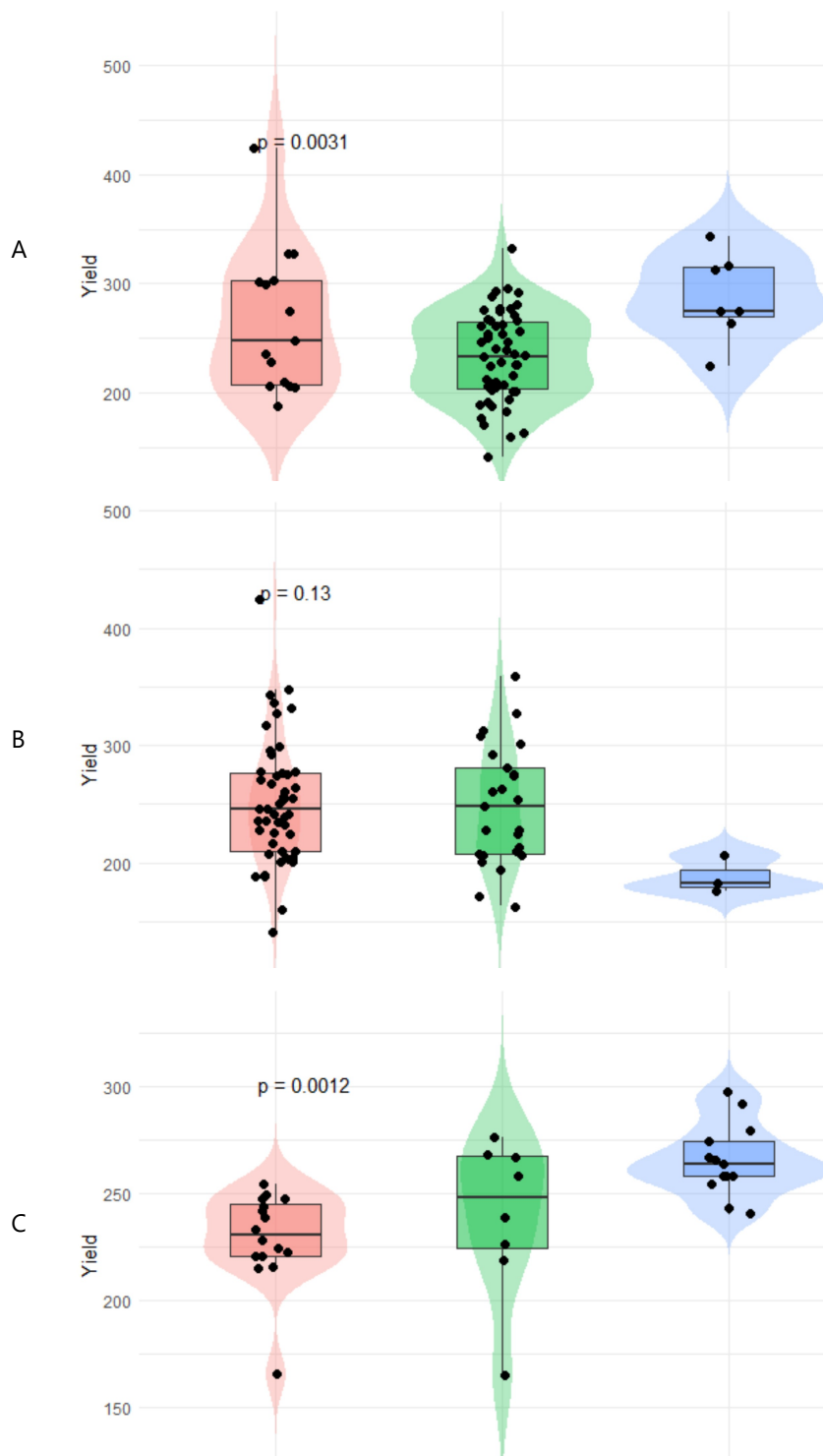


Fig. 6: Distribution of sample thousand-kernel weight in the population. (A) Xn-10×Karagandinskaya 30 population; (B) Xn-10×Karagandinskaya 30 population; (C) Xn-08×Karagandinskaya 29 population.

According to the analysis conducted, the TaGS5 gene demonstrated a pronounced contribution to the formation of overall yield. In the Xn-10×Karagandinskaya 30 population, the presence of the TaGS5A allele was associated with a significantly higher yield (+32.7 g/m²; P=0.0031). Similarly, in the Xn-08×Karagandinskaya 29 population, samples carrying the TaGS5C allele showed a significant yield increase (+26.7 g/m²; P=0.033). Conversely, no statistically significant differences between alleles were found in the Xn-10×Karagandinskaya 22 population (P=0.576). The effect of the TaGW6 gene was less pronounced: in the Xn-10×Karagandinskaya 30 population, the TaGW6C allele was associated with a yield reduction (−3.3 g/m²; P=0.128), and in the Xn-10×Karagandinskaya 22 population, the same allele also showed an insignificant decrease in the trait value (−4.2 g/m²; P=0.534). Consistent with the previous traits, the Chinese polymorphism in the TaKAO gene (TaKAO-C) provided a substantial yield increase (+36.2 g/m²; P=0.0012) across 41 hybrid lines. These results indicate that the TaGS5 gene alleles exert a significant influence on yield in specific populations, while the contribution of TaGW6 is limited and statistically unconfirmed. The greatest effect was demonstrated by the TaKAO-C Chinese polymorphism, making it a promising marker for use in breeding programs aimed at enhancing wheat productivity.

Comparative analysis of the three traits demonstrated that the TaGS5 gene exerts a focused, yet significant influence on overall yield and grain mass per spike in specific crosses. Conversely, the contribution of TaGW6 was limited and statistically unconfirmed. The most stable and pronounced effect was exhibited by the TaKAO-C Chinese polymorphism, which provided a significant increase across grain mass per spike, thousand-kernel weight (TKW), and overall yield. Therefore, TaKAO-C can be considered a priority marker for use in marker-assisted selection (MAS) in wheat, while TaGS5 holds potential within certain genetic backgrounds, and TaGW6 requires further investigation to clarify its role.

DISCUSSION

Global food security in the coming decades largely depends on increasing wheat production (Dadrasi et al., 2023). Due to its broad adaptability, this crop is cultivated across diverse agro-ecological zones and plays a key role in the food system (Langridge et al., 2022). However, climatic instability and the narrowing genetic base of modern varieties are limiting further progress (Walkowiak et al., 2020). Globally, climate variability has profoundly impacted crop productivity and food security, primarily due to alterations in precipitation patterns and temperature regimes (Mao et al., 2023). Among the major agricultural commodities threatened by climate change is wheat (*Triticum aestivum*), which supplies more than 20% of the total protein and caloric intake consumed daily worldwide (Eckardt et al., 2025).

Drought and heat stress differentially regulate the accumulation of protein and starch, as well as mineral

metabolism within wheat grains, thereby altering grain quality. These modifications affect grain weight, nutrient and anti-nutrient content, fiber content, protein content and composition, starch granule structure, and the free amino acid composition, all of which are critical determinants for human nutrition (Conceição Gomes et al., 2025).

Adaptation to climate change is emerging as an increasingly critical priority in global agriculture, given the inherent threats it poses to cereal crops. Within this context, landraces of wheat, mixed hybrid populations, and evolutionary populations derived from evolutionary plant breeding are gaining escalating recognition due to their high genetic diversity and their contribution to agrobiodiversity (Babalola et al., 2025).

Currently, less than 10% of natural diversity is involved in breeding efforts (Feuillet et al., 2008), which restricts the potential for sustainable yield improvement. This challenge can be addressed through the application of molecular genetic approaches (Chachar et al., 2024) among which SNP markers are the most promising. To date, over 260 million SNPs have been identified in the wheat genome (Geetanjali & Geetha, 2024), opening up vast opportunities for their use in breeding programs. The conducted analysis revealed that the influence of individual loci on wheat productivity is selective and context dependent. The TaGS5 gene showed significance only in specific genetic combinations, providing a yield increase of +32.7 g/m² for TaGS5A and +26.7 g/m² for TaGS5C. In contrast, differences in grain mass per spike and thousand-kernel weight (TKW) often did not reach statistical significance. These results are consistent with published data confirming the role of TaGS5 in grain size control. For instance, the TaGS5-3A-T allele in Chinese wheat cultivars has been shown to promote increased grain size compared to alternative alleles and is considered a promising marker for high-yield breeding (Ma et al., 2016). Analogous findings were presented in a recent review highlighting the role of TaGS5 in bread wheat grain mass regulation (Tillett et al., 2022). Nonetheless, numerous studies utilizing GWAS and multi-trait analysis report that the TaGS5 effect is moderate and subject to significant genotype × environment interaction (Liu et al., 2018).

In contrast, the TaKAO-C polymorphism exhibited the most stable and significant effect on the productivity traits. It was associated with increases in grain mass per spike (+0.25 to +0.45g; P=0.0008), thousand-kernel weight (+5.45g; P=0.002), and overall yield (+36.2 g/m²; P=0.0012). Information on the TaKAO gene in the literature is extremely limited; it is primarily mentioned within the context of metabolic pathways regulating gibberellin biosynthesis, with its direct effect on wheat yield being virtually undescribed. Therefore, the stable association of TaKAO-C with productivity traits identified in this study represents a novel and particularly significant finding. Recent reviews also emphasize a broader role for TaKAO-related pathways in wheat yield formation (Walkowiak et al., 2020; Chachar et al., 2024).

The TaGW6 locus demonstrated a minimal contribution to productivity formation: trait fluctuations

did not exceed +0.99g for thousand-kernel weight and -3.3 g/m² for overall yield. This is consistent with published data concerning its weak association with grain mass (Tillett et al., 2022). In summary, our findings validate the known role of TaGS5 while significantly expanding the argument for TaKAO-C as a novel and robust marker for selection. The contribution of TaGW6 was minimal. These results support the necessity of further validating TaKAO-C under various genotype × environment conditions and warrant its inclusion in marker-assisted selection panels due to its potentially superior stability. An analysis of the global wheat collection, comprising 397 samples from the National Agrobiodiversity Center of Korea, showed that modern foreign varieties have a higher level of genetic diversity compared to local lines and early deposited materials. The importance of such introgressions is confirmed by the fact that the model built on the basis of KASP markers explains up to 52.7% of the variation in heading date and 42.9% of the variation in 1000-grain weight, which emphasizes the direct role of genetic differences in the formation of productivity (Wang et al., 2025). These data demonstrate that the introduction of foreign varieties with an expanded genetic base can significantly increase the effectiveness of breeding programs and ensure further yield growth in conditions of climatic instability.

Currently, molecular marker-assisted breeding is among the most effective strategies for yield improvement, providing precise genotypic selection for valuable traits (Nadolska-Orczyk et al., 2017). The development of marker panels has been made possible by large-scale GWAS studies and QTL mapping, which have identified hundreds of loci associated with grain weight and number, stress tolerance, and adaptation to different growing conditions. For example, international consortia such as the Wheat Initiative and WheatCAP point to the key role of the TaGNI, TaCKX6, TaGS5, TaDA1, WAPO1, and TaRht1 genes, as well as the Vrn-A1/D1, Ppd-D1, and Eps-D1, which significantly influence productivity and its stability (IWGSC, 2018).

Data from a number of GWAS studies confirm that among these genes, TaGS5 demonstrates the most consistent effect on grain size and weight in various genotypic backgrounds (Zhang et al., 2014; Ma et al., 2016; Tillett et al., 2022), while the effect of TaCKX6 and TaDA1 is variable and depends on environmental conditions (Liu et al., 2018; Wang et al., 2025). At the same time, large-scale QTL meta-analyses (Yang et al., 2021; Liu et al., 2023) show that some metabolic genes involved in hormone biosynthesis are underestimated despite their high potential for regulating productivity. It is in this context that the results obtained in this study on the TaKAO gene are particularly significant. TaKAO is involved in the biosynthesis of gibberellins, a central regulatory system for plant growth. Recent work on functional genomics indicates that variations in GA pathway enzymes can significantly alter plant architecture, internode length, grain filling, and overall productivity (Hedden, 2020), but there are virtually no functionally confirmed alleles of such genes in wheat. Our data demonstrate that TaKAO-C

polymorphism has a stable and highly significant effect on both grain weight per ear and MTZ and total yield-effects that have not been previously described for this gene. This allows TaKAO to be considered a new highly valuable candidate locus that complements existing productivity marker panels. Thus, the functional polymorphism of TaKAO identified by us expands the range of available markers for breeding and confirms the promise of including hormone-regulating genes in wheat improvement programs. Integrating TaKAO into MAS platforms can enhance selection efficiency and contribute to the creation of varieties with increased and stable yields in conditions of increasing climate instability.

Conclusion

The conducted study revealed that the contribution of productivity-related loci in wheat hybrid populations is strictly selective. The TaGS5 gene showed significant effects only in specific genotypic combinations, demonstrating a moderate and context-dependent influence under different environmental conditions. Conversely, the TaKAO-C polymorphism exhibited a strong and stable association with grain mass per spike (+0.25 to +0.45g), thousand-kernel weight (+5.45g), and overall yield (+36.2 g/m²), making it a promising candidate for marker-assisted selection (MAS). The TaGW6 locus demonstrated minimal impact, which confirms its limited breeding value. Thus, the study of productivity gene polymorphisms should be considered a key tool for increasing spring bread wheat yield. Future research, focused on expanding the sample size and validating the identified associations under various ecological conditions, will allow for clarification of the role of these loci and ensure their practical utilization in MAS programs.

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