BRAZILIAN
JOURNAL OF
BIOLOGY®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Studying the influence of *TaGW8* and *TaGS5-3A* genes on the yield of soft spring wheat in arid climate conditions of the Republic of Kazakhstan

Estudo da influência dos genes *TaGW8* e *TaGS5-3A* no rendimento de trigo mole de primavera em condições climáticas áridas da República do Cazaquistão

L. Zotova[a], A. Zhumalin[a], A. Gajimuradova[a], I. Zhirnova[b], A. Nuralov[a], M. Zargar[c], D. Serikbay[a], L. Chen[d], T. Savin[b], A. Rysbekova[a]* and Z. Zhao[d]

[a]S. Seifullin Kazakh Agrotechnical Research University, Faculty of Agronomy, Department of Plant Production, Astana, Kazakhstan
[b]A.I. Barayev Research and Production Centre for Grain Farming, Shortandy, Kazakhstan
[c]RUDN University, Institute of Agriculture, Department of Agrobiotechnology, Moscow, Russia
[d]Northwest A&F University, College of Life Science, State Key Laboratory of Crop Stress Biology for Arid Areas, Yangling, China

Abstract

Drought is a primary ecological stress limiting wheat yield in water-deficient regions. Conducting targeted genetic selection of wheat cultivars can expedite the adaptation process of wheat to the climatic conditions of the region, allowing for the identification of high-yielding varieties with stable genetic traits. This study investigated the impact of the TaGW8 and TaGS3A genes, known for their contribution to wheat productivity. The effective productivity genes TaGW8-B1b/B1a and the TaGS5-3A-T genome exert a 32.8% influence on the variability of the 1000 grain weight (TGW) trait. This influence stems from both individual genes and their interactions, with at least 17.5% of TGW variability explained by the gene combinations examined in the study. Notably, the TaGS5-3A-T gene exhibits a significant positive correlation with total yield, exceeding 63%. The integration of these productivity genes, based on field phenotypic data, has resulted in an overall yield increase of selected samples by 0.8 tons/ha compared to the country's average multi-year indicator.

Keywords: spring wheat, drought conditions, grain weight genes, selection.

Resumo

A seca é um estresse ecológico primário que limita o rendimento do trigo em regiões com deficiência hídrica. A realização de seleção genética direcionada de cultivares de trigo pode agilizar o processo de adaptação do trigo às condições climáticas da região, permitindo a identificação de variedades de alto rendimento com características genéticas estáveis. Este estudo investigou o impacto dos genes TaGW8 e TaGS3A, conhecidos por sua contribuição para a produtividade do trigo. Os genes de produtividade efetiva TaGW8-B1b/B1a e o genoma TaGS5-3A-T exercem uma influência de 32,8% na variabilidade da característica de peso de 1000 grãos (TGW). Essa influência decorre de genes individuais e de suas interações, com pelo menos 17,5% da variabilidade do TGW explicada pelas combinações de genes examinadas no estudo. O gene TaGS5-3A-T exibe uma correlação positiva significativa com o rendimento total, excedendo 63%. A integração desses genes de produtividade, com base em dados fenotípicos de campo, resultou em um aumento geral de rendimento de amostras selecionadas em 0,8 toneladas/ha em comparação com o indicador médio multianual do país.

Palavras-chave: trigo de primavera, condições de seca, genes de peso de grãos, seleção.

1. Introduction

Wheat yields face numerous challenges worldwide, in particular the effects of climate change. In recent years, global warming and climate change have dramatically affected crop yields, including wheat (Langridge et al., 2022). Despite large areas used for wheat cultivation, its productivity is significantly lower than that of rice and corn.

Kazakhstan's average annual wheat production ranges from 12 to 12.5 million tons, of which only 6 million tons are used for domestic consumption, and the remainder is exported. Wheat productivity is more constrained by abiotic stresses than by biotic ones. An increase in the average daily temperature, drought, and salinization are

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*email: rysbekova.alua@yandex.ru; aiman_rb@mail.ru
Received: May 2, 2024 – Accepted: June 11, 2024

the main abiotic stresses that harm wheat productivity and quality (Karatayev et al., 2021).

During breeding work, it is also essential to consider the presence of the TaGW and TaGS genes, which determine the size of wheat grains and ensure potential yield. In Chinese wheat lines, the single nucleotide polymorphism (SNP) in the TaGW2-A1 promoter region, the most studied homolog of the A genome, is located on the 6A chromosome and encodes RING-type ubiquitin ligase E3. It is responsible for an increased mass of 1000 grains, a lower content of indolyl acetic acid (IAC), and the number of grains per ear.

TaGW2-B1 and -D1 also affect the protein content in the grain and the weight of grains and modulate the number and length of cells in the outer pericarp of developing grains. Some research claims that the TaGW2-B1 trait is stronger; however, mutants lacking B1 and D1 have more protein in their seeds. Two SNPs forming a haplotype Hap-6A-A for the TaGW6 gene, the rice ortholog GW6, regulate seed size, encode a new indole-3-acetic acid-glucose hydrolase mainly expressed in the tissues of young spikelets, and negatively regulate grain weight and width. At the same time, TaGW2-6A is associated with wider grains and a larger mass of 1000 grains. An additional haplotype, Hap-6B-1 for TaGW2-6B, has a stronger effect on grain weight than TaGW2-6A (Zhang et al., 2020; Govta et al., 2022).

The TaTGW-7A gene (TaTGW-7Ab, TaTGW-6) on chromosome 7A, encodes IAA-glucose hydrolase, increases the mass of 1000 grains and grain length. TaGW8B1 was positively correlated with the grain size of soft wheat. The TaGW8-B1a gene has an InDel length of 276 bp in the first intron, which is absent in the TaGW8-B1b gene. The TaGW8-B1a gene is associated with a significantly larger core width, a larger number of grains in the ear, a longer core length, a larger mass of thousands of grains, and a large number of spikelets per ear (Yan et al., 2019).

The TaGS-D1 and TaGS5-3A genes are associated with the mass of 1000 grains and grain length size. The TaGS3-7A-A allele significantly increased the mass of 1000 grains. TaGS5-3A-T The TaGS5-3A-T haplotype, based on the SNP T/G in the sixth exon, significantly correlates with a larger grain size, a higher mass of 1000 grains, and a smaller plant height, ear length, and internode length under the ear. A study by Zhang et al. (2014) showed that the DNA locus of the TaGS-D1 gene has one SNP in the first intron and identified 30 SNPs, a 40 bp InDel, and a 3 bp InDel, in the second intron between genotypes with a larger and smaller mass of thousand grains (TGW).

Our research aims to select varieties and hybrids obtained by the hybridization of Kazakh and foreign varieties (Australia, Africa, Russia, and China) to coarse-grained forms based on phenotypic and genetic indicators. However, it is impossible to select hybrids under the changing harsh continental climate conditions of Kazakhstan only on the basis of phenotypic indicators. We also studied the genetic potential of varieties and hybrids using coarse-grained genes (TaGW, TaGS) to obtain more reliable data during selection. Among the most studied productivity-related genes are *TaGW* (*Triticum aestivum* grain weight) and *TaGS* (*Triticum aestivum* grain size), determining wheat grain size and ensuring potential yield. In Chinese wheat lines, a single nucleotide polymorphism (SNP) in the promoter region of *TaGW2-A1*, the most studied homolog of the A genome on chromosome 6A, encodes a ring-type E3 ubiquitin ligase. It leads to increasing of TGW, lowering of indoleacetic acid content (IAC), and the number of grains per spike variety.

The aim of this study is to assess the effectiveness of the influence of productivity genes TaGW8-B1a/B1b, TaGS5-3A-T, and their combinations on the phenotype (TGW, GS) and total yield (TY) of spring soft wheat varieties and hybrids under drought conditions.

2. Materials and Methods

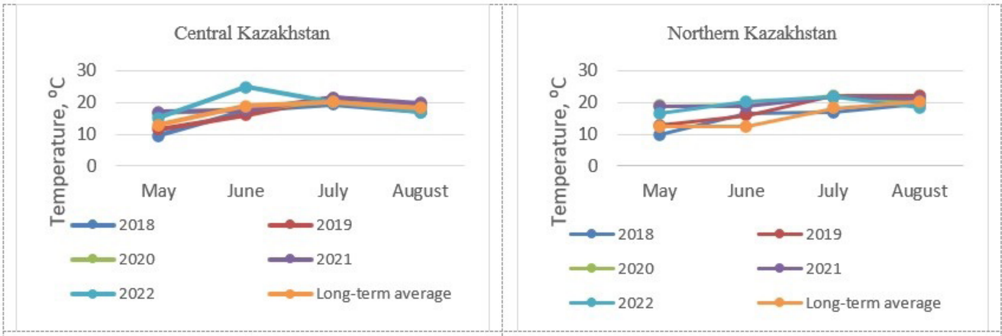
The studies were conducted between 2018 and 2022. The laboratory experiments were conducted at the Research Platform of Agricultural Biotechnology of the S. Seifullin Kazakh Agrotechnical Research University (Astana, Kazakhstan); field experiments were carried out at the A.F. Khristenko Karagandinskaya Agricultural Experimental Station LLP (Central Kazakhstan) and Farm “Niva” (Northern Kazakhstan).

All samples were collected from 2 m2 area and were repeated twice. The hybrid nursery was laid according to the scheme of maternal, hybrid, and paternal forms, and the plot area for each sample was 1 m2 as described earlier (Zotova et al., 2019). A total of 124 parental forms and 167 hybrids were studied during this period, of which 20 hybrids were selected as having promising phenological characteristics during the first three generations. Semi-dwarf wheat varieties of Chinese selection (Xn03, Xn04, Xn08, Xn09, Xn10, Xn13), Australian selection (Gladius, RAC 875, Wyalkatchem, Kite), African selection (MMF-044), tall varieties of Russian selection (Saratovskaya 66, Lutestsens 141), and Kazakh selection were selected as parent forms (Erythrospermum 35, Tauelsizdyk 20, Astana, Akmola 2, Aktyubinka, Karabalykskaya 25, 90, Karagandinskaya 22, 25, 29, 30, 31, Shortandinskaya 2012, 2017). Hybridization was carried out by “tvel”-N. Borlaug’s method of limited free pollination (Urazaliev, 2019; Zotova et al., 2019).

2.1. Weather conditions

Weather conditions in Central (Figure 1A, C) and Northern (Figure 1B, D) Kazakhstan differ in average monthly temperatures and precipitation, especially during the growing season.

In both regions, over the past five years, there has been a shortage of moisture during the growing season. Over the past three years, the lack of humidity has particularly affected wheat yields. At the same time, in Central Kazakhstan, over the past five years, there has been an increase in temperature indicators compared to the long-term average and a low level of precipitation. In the country’s northern region, the average temperatures for five years are 3–5 degrees above the average annual values, and the average precipitation values are 20–26 mm less than in the central part of the country.

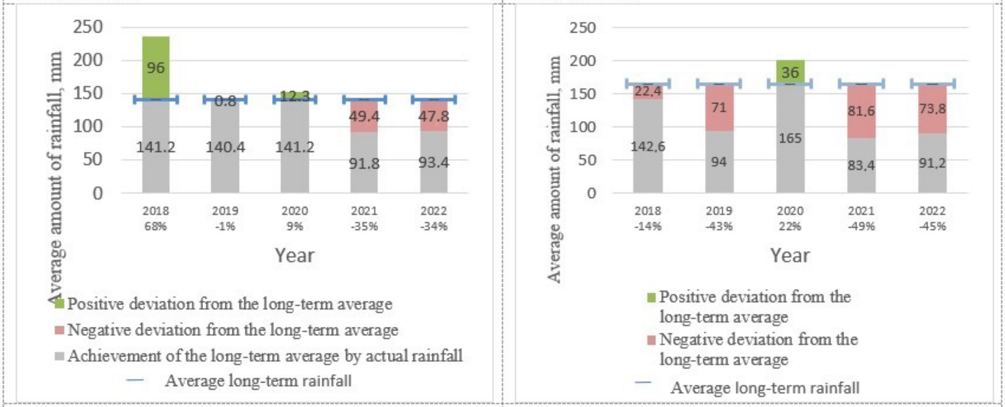


A

Temperature indicators in Central Kazakhstan

B

Temperature indicators in Northern Kazakhstan



C

Precipitation indicators in Central Kazakhstan

D

Precipitation indicators in Northern Kazakhstan

Figure 1. Weather conditions in the period 2018-2022 in the Northern Kazakhstan (B) temperature parameters, (D) precipitation level during vegetative period, and Central Kazakhstan regions (A) temperature parameters, (C) precipitation level.

2.2. Phenotype parameters of parental form and hybrids

2.2.1. Measurement of GS and Grain number (GN)

For measuring GL and grain width (GW), 100 seeds were randomly selected and their GL and GW were measured with a ruler (accuracy 0.05 mm). The mean value of each parameter was taken as the main indicator (Borlaug, 1968). The GN in each spike was determined as the average GN in 50 wheat plants after harvest and at full ripeness.

2.2.2. Measurement of TGW

The TGW was calculated according to ISO 12042-80 Seeds of agricultural crops. Methods for determining the TGW. Two samples, each containing 500 seeds, were taken from the clean seed fraction. The seeds were thoroughly mixed, two samples of 500 seeds each were counted without selecting grains and weighed with an accuracy of one-tenth of a gram. The average sum of the weighing results of the two samples of 500 seeds was calculated. The weights of the first and second samples were combined, and the final value was taken as the TGW.

2.2.3. Yield parameter

The TY was determined by collecting plants from a 1 m^2 plot in duplicate. The grains were separated from the spikes using the WinterSteiger LD350 thresher. Subsequently, the threshed and cleaned seeds were weighed on OHAUS Pioneer PA114C scales with an accuracy of 0.1 g. The average weight from the two plots was considered as the TY value.

2.3. Identification of genes by PCR

All genes were amplified using a VeritiPro amplifier (Applied Biosystems, USA). Primers used are listed:

TaGW8 F: CGTCATCCATTCCTTCATCG
R: GCTATATGGGTTGGTGTCGC;
TaGS5-3A-AGACATGGTGGAGCAAGAGATG
TaGS5-3A-GAACAACCTAATCCTCCTCCTGA.

The PCR reaction mixture was prepared as follows (total volume 20 µl):1 × DreamTaq Buffer, 100 ng DNA matrix, 4 nmol dNTPs, 10 pmol of direct and reverse primers, 1.25 U DreamTaq Hot Start DNA Polymerase (Thermo Fisher Scientific, USA). PCR condition for TaGW8 included the following cyclic conditions: 95 °C for 5 min, 40 cycles at 95 °C for 20 s, 60 °C for 15 s, 72 °C for 30 s, and a final extension at 72 °C for 10 min. The reaction mixture (total volume 20 µl): 1 × DreamTaq Buffer, 100 ng DNA matrix, 4 nmol dNTPs, 10 pmol of direct and reverse primers, 1.25 U DreamTaq Hot Start DNA Polymerase (Thermo Fisher Scientific, USA) (Yan et al., 2019).

For the TaGS5-3A gene, PCR was performed in a total volume of 15 µl, with 50 ng of DNA, 10 mM of direct and reverse primers, 4 nmol DNTP, 1xDreamTaq Buffer, and 1.25 U DreamTaq Hot Start DNA Polymerase (Thermo Fisher Scientific). Amplification was carried out at 94 °C for 4 min, then 35 cycles at 94 °C for 35 s, annealing for 35 s, and 72 °C for elongation for 30 s, with a final elongation for 10 min. PCR products were separated on a 2% agarose gel and visualized after ethidium bromide staining using the Doc-Print imaging system (Vilber Lourmat, France) (Zhang et al., 2020).

2.4. Statistical analysis

The gathered data underwent scrutiny to assess normality and consistent variance. All experiments were carried out in three replications. Data analyses were conducted using the GLIMMIX procedure of SAS (Version 9.4, SAS Institute Inc., Cary, NC) using the mixed procedure with block as the random factor. The least squares mean statement in SAS with the Tukey adjustment at P=0.05 was used for comparison of means. The p-value was calculated relative to the mother and father of the hybrid. Pearson's correlations were used to analyze the relationships between crop components and structural indicators. The phenotypic and genetic indicators were analyzed using the SPSS version 22.0 program with a nonparametric (Man-Whitney test). A Spearman correlation analyze was conducted to evaluate the relationships between the productivity genes and total yield parameters. Ordinary Least Squares (OLS) regression analyze was conducted to evaluate the relationships between phenotypic and gene correlation.

3. Results

3.1. Phenotype analyses

3.1.1. Measurement of GS

The GS indicators significantly differed between hybrids and parental forms. The average GS for the maternal form was 22.29 mm^2, for the paternal form - 21.91 mm^2, and for the hybrid forms - 24.39 mm^2. The results are presented in Figure 2.

Regarding GS, notable distinctions were detected in only a quarter of instances. In the remaining 75% of cases, no significant differences in GS were obtained. At the same time, 20% of hybrids have a larger GS than both parents. Despite slight differences in GS in parental forms, hybrid forms showed a higher result - 10.4%. Meanwhile, the highest values were observed in Chinese, Kazakhstani hybrids, and one hybrid, *WH131*. The obtained data strongly exceed the results reported by Acosta et al. (2017), who showed that the GS of soft wheat ranges from 15.41 to 18.37 mm^2. Furthermore, these outcomes exceed the metrics of durum wheat varieties, where this parameter ranges from 17.82 to 19.6 mm^2 (Beral et al., 2020).

The reliability of the GS value was limited, as no noteworthy distinctions were observed between hybrids and parental forms. Monitoring this trait perceptibly enhances success of hybrid selection. Thus, considering F4, hybrids with visible differences in GS, such as *WH 131, WH189, WH190*, and *WH191*, were identified.

3.1.2. Measurement of TGW

The final grain yield is a complex trait often heavily influenced by both genetic and environmental factors. In cereal crops, thousand grain weight (TGW) is an important component of yield, and increasing TGW is key to further yield enhancement. Additionally, TGW is more stably inherited than overall final yield. In the study, TGW parameters were examined in both parental forms

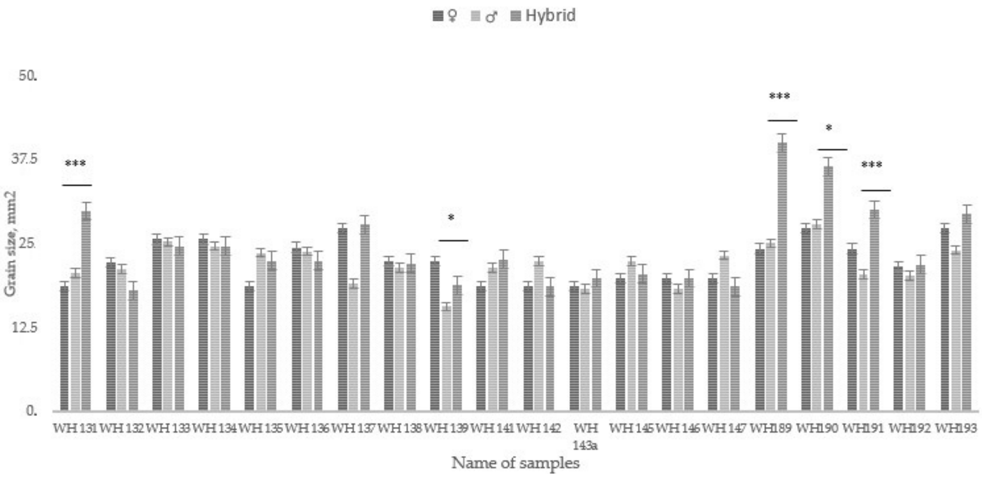


Figure 2. Average parameters of parental and hybrids forms wheat GS, 2018-2022. *$p<0.05$ ***$p<0.001$. Not marked samples have $p \geq 0.05$.

and hybrids, and Figure 3 shows the mean TGW values for 60 samples.

The hybrids WH136, WH137, WH145, WH190, and WH192 exhibited a higher TGW level compared to both parents, ranging from 1 to 27.04%. In 75% of hybrids, this indicator was significantly lower than that of either the maternal or paternal form. As this trait is more genetically determined than others, it is noted that Chinese and Kazakh breeding lines with TGW values exceeding 40 g produced hybrids with high TGW. For example, based on linear regression analysis applied to more than 1850 Chinese wheat varieties, the average TGW increased from 30.16 g in the 1920s to 38.43 g in the 2010s. Correspondingly, grain yield increased from 2.01 to 6.58 t/ha, with selection for higher TGW contributing significantly to yield improvement (Duan et al., 2020). Recently identified QTLs allow narrowing the search zone for promising genes for marker-assisted selection (Miao et al., 2022).

3.1.3. Measurement of GN

Assessing the yield of agricultural crops is an important scientific problem, with the number of grains per spike being one of the most critical parameters (Xu et al., 2023). In the past, grain yield (GY) has been consistently increasing due to a higher grain number (GN) per unit area; however, the trade-off between thousand grain weight (TGW) and GN has become a bottleneck for improving total yield (TY). Increasing individual TGW has the potential to enhance wheat yield; however, attempts to increase TY by increasing grain size have been hindered by the negative relationship between TGW and GN (Calderini et al., 2021). The research results for the GN parameter are presented in Figure 4 and Table S1.

The average GN among maternal forms was noted at 34.55, among paternal forms – 32.49, while among hybrids – 29.23. Correspondingly, the average TGW values were 38.9 g, 35.5 g, and 36.5 g, respectively. In the case of

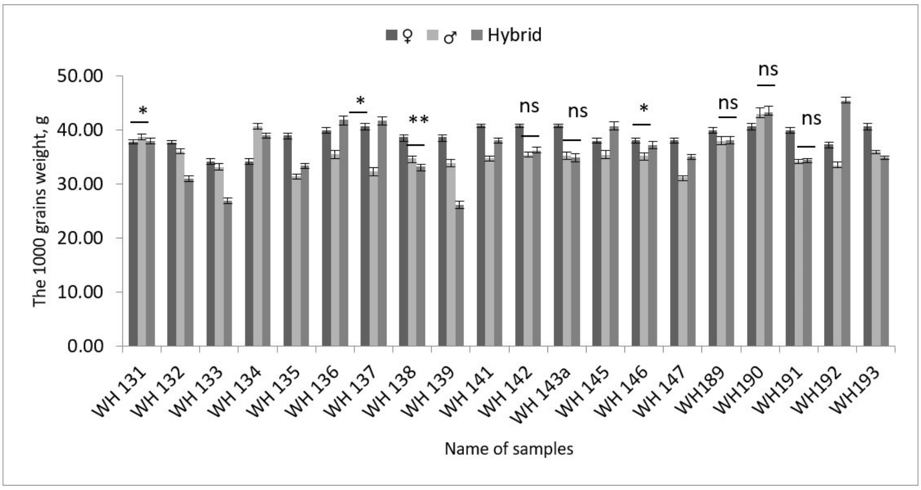


Figure 3. TGW of parental and hybrids forms wheat, 2018-2022. nsp≥0.05, *p<0.05, **p<0.01. Non marked samples have P value 0.001.

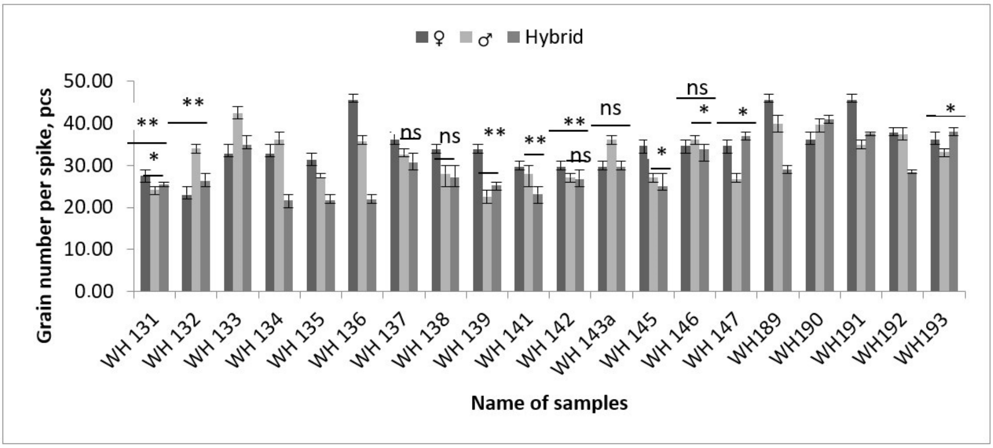


Figure 4. GN of parental and hybrids forms wheat, 2018-2022. nsp≥0.05, *p<0.05, **p<0.01. Non marked samples have P value 0.001.

these 60 samples, a reliable dependence between TGW and GN was not observed. Among maternal lines, the Chinese selection variety Xn-08 exhibited the maximum grain count at 45.6±0.4 grains. Meanwhile, for the varieties Karabalykskaya 25, Saratovskaya 66, Xn-10, Xn-13, Xn-04, Xn-02, the GN value exceeded 31 grains. Paternal forms had significantly lower GN levels. Only varieties MMF 044 and Karagandinskaya 29 had GN values of 42.4 and 40, respectively. Varieties Xn-09, Xn-10, Shortandinskaya 2017, Astana, Lyutescens 141, Karagandinskaya 22, Karabalykskaya 90, Karagandinskaya 31, and 30 had GN levels ranging from 32 to 39.8 grains. It is worth noting that the GN level in hybrids was significantly lower than in parental forms by 10-15%.

3.1.4. Yield parameters

The genetic makeup governing grain TY is intricate, relying on multiple interconnected traits. One of the pivotal factors contributing to the sustenance of high yields is the attributes linked to spike productivity, TGW and GS. Furthermore, the high level of genotype interaction with the environment and the low heritability of this ultimate trait, in most cases, render the selection process highly challenging. In such a situation, indirect selection of TY based on highly heritable correlated traits (Jaenisch et al., 2022) would be more practically feasible. Figure 5 presents the results of TY for parental forms and hybrids.

Samples WH137, WH139, WH142, WH145, as well as samples WH190, WH192, carrying the TaGW-B1a/TaGS-3A-T genes (Supplementary Material Table S8), exhibited TGW values ranging from 30.85 to 45.42 g. This measurement is deemed sufficiently elevated for areas characterized by a dry climate. The GN in these samples ranged from over 25 to 41. Samples with parental forms carrying both productivity genes showed the highest results in TY. For the WH190 hybrid, TY reached 2.5 t/ha, which is 80% higher than the country's average multi-year TY indicator.

4. Genetic Parameters

Samples were scrutinized at the genetic level with the aim of investigating parental forms and hybrids, specifically focusing on coarseness traits. To accomplish this, we examined genes such as *TaGW8-B1a*, responsible for coarse grain determination, and *TaGS5-3A*, governing GS.

4.1. TaGW and TaGS genes

Productivity analysis was conducted using the *TaGW8-B1a* and *TaGS5-3A-T* genes, which reliably showed higher values of GS and GN (Supplementary Material Table S1 and Figure S1). Among maternal forms, the average TGW was 38.85±0.5 g ($p<0.001$), for paternal forms - 35.5±0.6 g ($p>0.05$), and for hybrids - 36.5±1.03 g ($p>0.05$), with TY having an average value of 1.9±0.32 for paternal forms ($p<0.001$), 1.8±0.14 for maternal forms ($p<0.001$), and 1.76±0.06 for hybrids ($p<0.001$) (Supplementary Material Table S8). The TY in hybrids WH190 and WH192 was significantly higher than in both parents by 19% and 17.6%, respectively. One of the hybrids, WH137, exhibited a high TY of 2.1 t/ha. In the genome of the three recognized hybrids, the presence of either one or both genes was detected. Hybrid WH192, for instance, had a TGW level of 45.42 g. Optimal productivity indicators in combination were observed in varieties of Chinese selection (Xn-08, Xn-10). Australian varieties Kite, Gladius, and Wyalkatchem did not show high adaptation in our climatic conditions, despite the presence of productivity genes in their genome.

In the absence of *TaGS5-3A-T* and *TaGW8-B1a* genes, 65% of hybrids had TGW exceeding 30 g. Hybrids *WH 142, WH 145*, and *WH192*, with the presence of one *TaGW8* productivity gene in their genome, showed TGW of 36.2 g, 40.7 g, and 45.2 g, respectively. The *TaGS5-3A-T* gene was present in the genome of hybrid *WH 137*, while the absence of the *TaGW8-B1a* gene did not affect TGW (41.8 g) and GS (27.8 mm2). Only one of the hybrids, *WH190*, showed the presence of *TaGS5-3A-T* and *TaGW8-B1a* genes, demonstrating TGW of 43.4 g and GS of 36.4 mm2, while

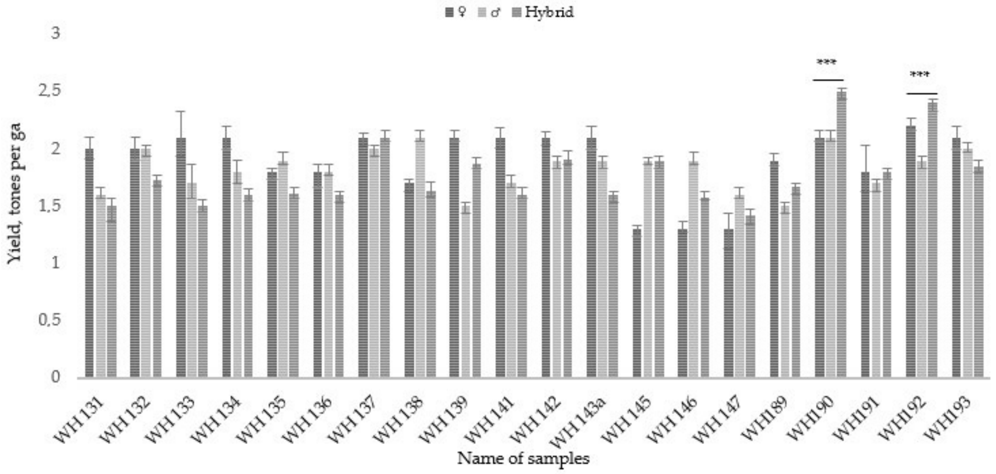


Figure 5. Productivity of parental and hybrids forms wheat, 2018-2022. nsp≥0.05, ***p<0.001.

both genes were present in the genome of both parents. At the same time, the PH of hybrid *WH190* was 62.2 cm. A PH value of 60-62 cm was optimal for TGW exceeding 35 g and GS exceeding 25 mm2. It is well-documented that these two traits exhibit a negative correlation, where an increase in GN is associated with a decrease in GW (Xie and Sparkes, 2021). However, many studies have shown transgressive segregation in yield when crossing varieties with high GN and GW. Larger but smaller-seeded grains indicate a higher allocation toward smaller GN, whereas a greater number of but smaller-seeded grains indicate a lower investment in larger GN. Studies on GN and GW in various cereals and environments have determined that GN is a highly plastic trait, while GW is rather more inheritable.

4.2. Gene correlation

In accordance with the Maine-Whitney correlation analysis, there is a direct correlation between the presence of productivity genes and the phenotypic index of GS and TGW. The seed productivity is directly linked to the presence of the *TaGW8*-gene and TGW. To evaluate the influence of different genetic allelic variations on agronomic traits, information from 33 parental materials was utilized to analyze the genetic effects of *TaGS5-3A-T*, and *TaGW8* (Table 1). Statistical analysis was conducted using SPSS software version 22.0, employing nonparametric tests (Mann-Whitney test).

Thus, the *TaGW8-B1a* gene showed a significant correlation with TY ($p<0.01$). TY in samples with the *TaGW8-B1a*-gene was 9.2% higher than in samples lacking the gene. However, the presence of the *TaGS5-3A-T* gene did not show any visible correlation with TY. It is worth noting that samples carrying both gene variants had TY of 2 tons per hectare or more. For example, the variety RAC 875, carrying 2 genes in its genome, had a yield of 2.0 t/ha, Xn-10 – 2.1 t/ha, Karagandinskaya 22 – 2.1 t/ha, and the hybrid WH190 – 2.5 t/ha. Importantly, samples carrying a single TaGW8-B1a gene variant at 46.6% exhibited yields of 2 t/ha or higher. It is noteworthy that the well-known high-yielding and drought-tolerant variety Wyalkatchem, carrying 2 genes in its genome, had a yield of 1.5 t/ha. This variety did not exhibit its properties in the agroclimatic conditions of Kazakhstan. The results indicated that TaGW8-B1a noticeably increased the width of wheat grains by 13.37%, consistent with the findings of and Yan et al. (2019).

4.2.1. OLS Regression analyzes

During the OLS Regression, the genes were set as the predictor variables(X). “TGW_mean” (TGW) and “Grain_square_mean” (GS) was set as the response variables (Y). The Ordinary Least Squares (OLS) regression analysis for data provides detailed insights into the relationships between the gene types and both TGW and GS (Tables 2-5).

Table 1. Effect of *TaGW8* and *TaGS-3A* on wheat grain morphology.

	N	TGW (t/g)	GN (pcs)	GL (cm)	GW (cm)	Grain area (mm²)	TY (t/ga)
TaGW8-B1a	17	37.49±3.29	32.8±7.5	0.61±0.05	0.38±0.04	22.84±3.18	1.9±0.05
Null	16	36.86±2.91	34.91±4.28	0.62±0.05	0.31±0.12	21.3±2.33	1.74±0.04*
TaGS5- 3A-T	7	38.76±3.5	33.34±6.01	0.61±0.06	0.37±0.06	22.69±4.58	1.96±0.12
Null	26	36.76±2.88	33.95±6.3	0.62±0.05	0.34±0.1	21.93±2.31	1.8±0.04
TaGW8-B1a+ TaGS5- 3A-T	5	39.8±1.8*	30.4±5.5	0.64±0.04	0.39±0.03*	24.6±3.7**	2.04±0.16*

*denote significance between genotypes at P-value 0.05. **denote significance between genotypes at P-value 0.01.

Table 2. OLS Regression with TGW.

	Coefficient	Standart error	t	P>|t|	0.025	0.975
Constant	35,4393	1,558	22,753	0.000	32,315	38,563
TaGS5-3A-T	3,2033	1,305	2,455	0.017	0.586	5,820
TaGW8-B1a	1,2025	1,040	1,157	0.253	-0.883	3,288

Table 3. OLS Regression with GS parameter.

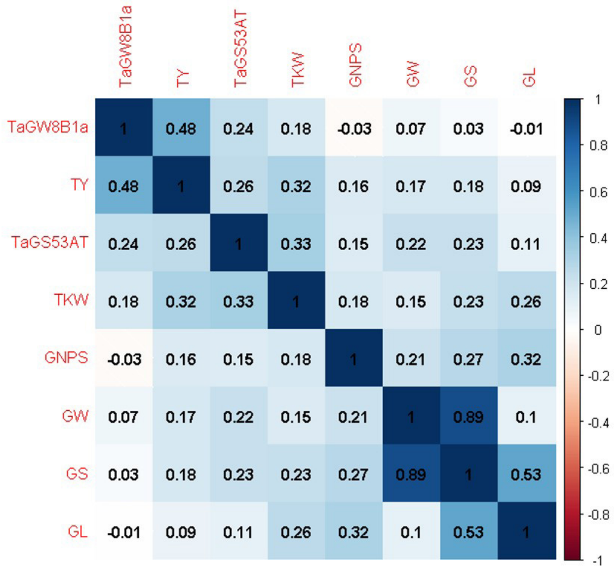
	Coefficient	Standart error	t	P>|t|	0.025	0.975
Constant	23,3563	1,796	13,007	0.000	19,755	26,958
TaGS5-3A-T	3,0570	1,504	2,032	0.047	0.040	6,074
TaGW8	-0.5514	1,199	-0.460	0.647	-2,956	1,853

Table 4. Gene correlation in GW parameter.

	Coefficient	**Standart error**	**t**	**P>|t|**	**0.025**	**0.975**
Constant	35,9138	2,719	13,207	0.000	30,437	41,391
TaGS5-3A-T	-1,0022	5,107	-0.196	0.845	-11,288	9,284
TaGW8-B1a	2,7800	4,003	0.695	0.491	-5,282	10,842

Table 5. Gene correlation in grain area parameter.

	Coefficient	**Standart error**	**t**	**P>|t|**	**[0.025**	**0.975]**
const	17,2821	2,819	6,131	0.000	11,605	22,960
TaGS5-3A-T	-9,7995	5,294	-1,851	0.071	-20,462	0.863
TaGW8-B1a	8,9628	4,150	2,160	0.036	0.605	17,320



	TaGW8B1a	TY	TaGS53AT	TKW	GNPS	GW	GS	GL
TaGW8B1a	1	0.48	0.24	0.18	-0.03	0.07	0.03	-0.01
TY	0.48	1	0.26	0.32	0.16	0.17	0.18	0.09
TaGS53AT	0.24	0.26	1	0.33	0.15	0.22	0.23	0.11
TKW	0.18	0.32	0.33	1	0.18	0.15	0.23	0.26
GNPS	-0.03	0.16	0.15	0.18	1	0.21	0.27	0.32
GW	0.07	0.17	0.22	0.15	0.21	1	0.89	0.1
GS	0.03	0.18	0.23	0.23	0.27	0.89	1	0.53
GL	-0.01	0.09	0.11	0.26	0.32	0.1	0.53	1

Figure 6. Spearman correlation analysis data.

TGW Model suggested that about 17.5% of the variability in TGW is explained by the gene types in this model, indicating a moderate fit. The gene type *TaGS5-3A-T* shows a significant positive relationship with TGW (p<0.05). The other gene types do not show statistically significant relationships at the conventional 0.05 level. GS_Mean Model suggested that about 8.8% of the variance in the GS_ Mean is explained by the genes. This implies a weak fit. *TaGS5-3A-T* again shows a significant positive effect (p<0.05). Other genes do not significantly predict the GS. *TaGS5-3A-T* appears to have a consistent positive effect on both GW and the GS.

GS Model suggested that about 8.8% of the variance in the GS_Mean is explained by the genes. In order to investigate potential synergistic or antagonistic effects between genes.

This time, the R-squared of TGW_Mean Model (contain interaction terms) increased to 0.328, indicating a better fit than the previous model without interaction terms. This suggests that about 32.8% of the variability in TGW is explained by both the individual genes and their interactions. The model shows various coefficients for interaction terms.

4.2.2. Spearman correlation

A Spearman correlation analysis results indicated in Figure 6.

The Spearman correlation analysis between structural parameters and genes showed a high level of positive association both between structural parameters and phenotype with the genes TaGW8B1a and TaGS5-3A-T. This indicates an active influence of the genes in shaping TGW and GN. A correlation of R=0.89 was observed between the parameters GS and GW, as well as between GS and GL (R=0.53). The gene TaGW8B1a significantly influences TY

(R=0.48), whereas the correlation with the gene TaGS5-3A-T is half as much (R=0.26). As previously demonstrated, the gene TaGW8B1a exhibits a negative correlation with GN (R=-0.03), while the gene TaGS5-3A-T shows a positive correlation (R=0.15).

Thus, a correlation has been identified between genetic and phenotypic parameters in shaping the main components of wheat productivity. Both studied genes contribute to the development of high-yielding specimens.

5. Discussion

Due to the growth of the population (United Nations, 2017), the development of new high-yielding wheat varieties remains the primary goal of wheat breeding programs (Brinton et al., 2017). Yield, a multifaceted metric, is predominantly associated with the number of productive ears per unit area, grains per ear, and thousand grain weight (TGW). However, traits such as growth stage (GS), ear structure, plant height (PH), and flag leaf characteristics can also impact yield by influencing photosynthetic intensity, grain filling, and the movement of dry matter. These traits often exhibit higher heritability than productivity, facilitating their selection in small plots during the early stages of breeding (Li et al., 2019).

To date, many key quantitative trait loci (QTLs) associated with TGW have been mapped on nearly all wheat chromosomes, except for 6D. Due to the large size of the wheat genome, the development and application of these markers in wheat require considerable time and resources. Moreover, the genetic distance between markers on maps is relatively large, complicating gene cloning based on maps (Liu et al., 2018).

However, single nucleotide polymorphisms (SNPs) are the most promising molecular markers, demonstrating high prevalence and uniform distribution throughout the genome, and are increasingly being used in plant genetics. The low mutation frequency and stable genetics are key characteristics of SNPs. QTLs derived from SNPs have high resolution and can be utilized for marker-assisted selection to enhance selection accuracy and breeding efficiency (Liu et al., 2020). For example, according to the findings of Jinping Wang et al. (2023), the identification of the TabHLH123-6B haplotype revealed its association with high root dry weight and TGW, as well as low plant height.

At this research the productivity gene *TaGW8-B1a* showed a significant correlation with TY ($p<0.01$), while the *TaGS5-3A-T* gene did not exhibit a noticeable correlation with TY ($p \geq 0.05$). However, upon examining the correlational interactions between genes and phenotypic traits, it was revealed that the *TaGS5-3A-T* gene, based on Spearman correlation analysis and Ordinary Least Squares (OLS) regression, has a positive impact on both TGW and GS.

According to the results obtained, more than 60% of hybrids had a GN exceeding 20, and in cases of the *TaGW8-B1a* TGW gene, such hybrids had more than 30 g and GS is 25.6 mm^2. The GS in hybrids with the *TaGS5-3A-T*-gene yielded a GS exceeding 27 mm^2 with a GN of 30.8. In these hybrids, TGW surpassed 42 g, establishing itself as a highly effective indicator in drought conditions. For instance, in the *WH190* hybrid, the presence of the *TaGS5-3A-T*-gene resulted in a GS value of 36.4 mm^2, with a TGW of 43 g. Thus, the presence or absence of one of the TGW or GS genes does not guarantee a high GN or bigger GW. Even though both parental forms exhibit outstanding growth and productivity indicators in hybrids due to the presence of *TaGS5-3A-T* and *TaGW8* genes. However, the presence of the *TaGW8-B1a* gene did not demonstrate sufficient reliability in expressing the traits TGW and GS.

The findings led to the identification of potential productive parent forms such as *Saratovskaya 66*, *Karagandinskaya 22*, *Xn-10*, and *Xn-08*, as well as hybrids *WH134*, *WH136*, *WH137*, *WH145*, *WH190*, and *WH192*. These selections displayed optimal PH, CL and GS, thereby contributing to enhanced yields in arid climate regions.

Therefore, an examination of the genetic parameters related to productivity genes will accelerate the selection of parent forms and hybrids, streamlining the development of stable lines through the integration of both field and laboratory data. The results presented highlight the importance of genetically choosing parent forms and hybrids. However, it is essential to note that additional research is needed to identify a set of genes for more efficient sampling.

6. Conclusions

Genetic justification of productivity based on genetic and phenotypic parameters demonstrates the influence of the interaction between the TAGS5-3A-T and TaGW8-B1a genes on TGW and GS. A positive correlation has been identified between the presence of the TaGW8-B1a and TAGS5-3A-T genes and TY formation. According to the results, these presented genes and their combinations account for up to 32.8% of the influence on TGW, leading to an increase in total yield by 13-92% for individual specimens. Field studies conducted under severe drought conditions have shown the impact of the selected genotype on plant development and TY.

Acknowledgements

We would like to express our gratitude to the staff of the Research Platform of Agricultural Biotechnology, as well as to the students of the Faculty of Agronomy of the S. Seifullin Kazakh Agrotechnical Research University. RUDN University Strategic Academic Leadership Program supported this work.

This study was financially supported by the Ministry of Education and Science, Republic of Kazakhstan, within the framework of the Young Scientists Project No. AP13067944 “Molecular SNP-marking of bread wheat by genes *TaGW*, *TaGS*, and *Rht* for grain weight and lodging resistance”.

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Supplementary Material

Supplementary material accompanies this paper.

Table S1. Genetic and phenotypic indicators based on coarse grain.
Figure S1. PCR results to determine the presence of coarse-grained genes.
This material is available as part of the online article from https://doi.org/10.1590/1519-6984.286189
The following supporting information can be downloaded at: www.mdpi.com/xxx/s1
Table S5. PCR condition for the TaGW8.
Table S6. PCR condition for the TaGS5-3A gene.
Table S8. Genetic and phenotypic indicators based on coarse grain.
Figure S3. PCR results to determine the presence of coarse-grained genes.