



## Impact of Leaf and Stem Rust Resistance Levels on Productivity Traits of Spring Bread Wheat Varieties

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### ABSTRACT

Wheat rust, both leaf and stem rust, is a major threat to global food security because of its severe effect on crop yields. Although considerable advances have been made in its epidemiology, resistance mechanisms, and pathogen biology, many wheat varieties do not express the same degree of resistance under natural field conditions as under artificial inoculation. This research aimed to evaluate the structural and molecular resistance traits of 11 Kazakh-selected wheat varieties under natural infection in the field. These varieties are widely cultivated in Kazakhstan and play a significant role in regional food production. The study assessed resistance levels and productivity traits of the selected wheat varieties under natural infection conditions. Environmental factors and infection pressure were analyzed in relation to structural plant traits. Correlations between plant height and productivity components such as yield, number of grains per spike, grain weight per spike, and thousand kernel weight were evaluated. Molecular markers for resistance genes *Lr21*, *Lr24*, and *Lr35* were also examined in relation to yield-related characteristics. Findings indicated inconsistent resistance levels to leaf and stem rust among varieties despite similar numbers of resistance genes in their genomes. Environmental conditions and infection pressure influenced structural traits, with negative correlations observed between plant height and yield (-0.54), grains per spike (-0.33), grain weight per spike (-0.60), and thousand kernel weight (-0.41). The resistance genes *Lr21*, *Lr24*, and *Lr35* were associated with important yield traits such as grain weight, spike length, and grain number. These results emphasize the importance of resistance evaluation under natural infection, especially for varieties critical to food security. While further research with larger sample sizes is needed, the preliminary screening revealed variability in resistance levels and their association with yield formation. This study suggests the potential to reduce chemical control by selecting naturally resistant varieties adapted to field environments.

**Keywords:** Spring bread wheat; Leaf rust; Stem rust; Resistance genes; *Lr*; *Sr*; Genetic markers

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### INTRODUCTION

Wheat is one of the world's most significant cultivated food crops, according to the FAO (Rehman et al., 2024). It provides nearly one-fifth of the calories consumed worldwide and is cultivated across diverse agroclimatic zones (Bracho-Mujica et al., 2024). As the demand for food continues to rise with population growth and climate uncertainty, maintaining wheat productivity has become a critical challenge for global agriculture (Erenstein et al., 2022). Wheat is planted every year on around 219 million hectares, producing more than 760 million tons of grain.

Pests and bacterial and fungal diseases, however, result in massive yield losses annually (Zhang et al., 2022). Pests and diseases are accountable for 20–40% of global crop losses and an estimated USD 220 billion in agricultural trade losses annually (Subedi et al., 2023; Singh et al., 2024). Rust diseases, such as leaf and stem rust, continue to pose a significant threat to farmers globally, resulting in considerable losses in one of the world's staple crops (Shafi et al., 2022; Guégan et al., 2023). One of the foremost answers to future food needs is the identification of new wheat varieties that are suited to new environmental conditions (Lidwell-Durnin & Lapthorn, 2020).

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The Green Revolution achieved success in part through the deployment of wheat cultivars with genetic resistance to stem rust. However, the emergence of a new stem rust strain in Uganda (*Ug99*), first identified in 1998, has expanded the threat of stem rust epidemics beyond its original distribution in Africa and the Middle East (Lidwell-Durnin & Laphorn, 2020). Despite more than two decades of research and breeding efforts, most wheat grown today still lacks resistance to *Ug99*, while yellow, stem, and leaf rust races continue to pose similar challenges to market demand (Flavell, 2017). The history of wheat rust spans centuries, with documented severe epidemics leading to sharp declines in yield and food shortages (Dubin & Brennan, 2009). The economic consequences are especially significant in countries where wheat is a major agricultural commodity (Meyer et al., 2021).

Wheat rust is extremely dynamic, continually developing ways to break varietal resistance and thwart the success of traditional breeding strategies, thereby requiring continuous research and adaptation (Babu et al., 2020). To date, nearly 80 leaf rust resistance genes (*Lr* genes) have been discovered and characterized; a number of them have been used in breeding resistant wheat varieties. In research on rust infection of winter wheat in the southeastern United States, the most useful genes for resistance to leaf rust (*LR*) were *Lr9*, *Lr10*, *Lr18*, *Lr24*, *Lr37*, *LrA2K*, and *Lr2K38* (Kokhmetova et al., 2021).

Wheat stem rust can cause a decrease in grain yield by as much as 90%. The genes that are still effective against the most virulent race, *Ug99*, as per International Maize and Wheat Improvement Center (CIMMYT), are *Sr28*, *Sr29*, *SrTmp*, *Sr2*, *Sr13*, *Sr14*, *Sr22*, *Sr35*, *Sr36*, *Sr37*, *Sr32*, *Sr39*, *Sr47*, *Sr33*, *Sr45*, *Sr40*, *Sr24*, *Sr25*, *Sr26*, *Sr43*, *Sr44*, *Sr27*, and *1A.1R*. Some of these genes have already lost their capacity to provide resistance to the pathogenic fungus, though. For pyramiding resistance genes for the management of stem rust in Egyptian wheat, *Sr2*, *Sr13*, *Sr22*, and *Sr24* are suggested as the best (Cat, 2024).

The use of resistant varieties is often the most cost-effective method, especially when compared to the expenses of chemical control and the scale of preventable yield losses at the farm level. Therefore, evaluating the gene pool and identifying donors of resistance to leaf and stem rust is essential, and should consider the agroecological context of each region. Updating information on resistance genes in modern wheat cultivars is particularly important, as these varieties ensure the country's food security. However, evidence on the impact of resistance categories and their association with wheat yield components remains limited (Srinivas et al., 2024).

The objective of this research is to examine the genetic spectrum of functional *Sr* and *Lr* genes in extensively grown varieties and their effect on yield components in the field in Northern Kazakhstan, to choose the most resistant genotypes and remove highly susceptible ones to leaf and stem rust.

### Highlights of the Study

This study provides an integrated field-molecular assessment of leaf and stem rust resistance in eleven widely cultivated spring bread wheat varieties from

Northern Kazakhstan, emphasizing the practical importance of evaluating resistance under natural infection pressure rather than controlled inoculation. While numerous *Lr* and *Sr* resistance genes have been characterized globally, their effectiveness often declines under field conditions affected by excessive moisture, temperature fluctuations, and evolving pathogen races. The present research bridges this gap by linking the structural traits (plant height, spike length, grain weight, and thousand-grain weight) with the molecular background of resistance genes *Lr21*, *Lr24*, and *Lr35*. Comprehensive field trials during the exceptionally humid 2024 season revealed considerable variability in rust prevalence and productivity across cultivars. Statistical analyses demonstrated that infection pressure not only reduced yield but also inverted the usual positive correlation between plant height and productivity traits, indicating genotype-specific adaptation to stress. Molecular screening identified that although most varieties contained 72–100% of the tested resistance genes, field performance was inconsistent, highlighting the need to evaluate gene effectiveness under dynamic agroecological conditions. The *Lr24* gene showed a statistically significant influence on spike length, confirming its role in yield component formation under disease stress. Among all genotypes, the Taimas variety demonstrated superior resistance and stable productivity, validating its suitability for large-scale cultivation in Northern Kazakhstan. The findings contribute to regional breeding programs by offering empirical evidence on gene-trait relationships and emphasizing the integration of molecular diagnostics with field observations. Ultimately, this work provides a foundation for the development of wheat cultivars that combine durable genetic resistance with high yield stability, thereby supporting national food security and reducing dependence on chemical disease control measures.

### MATERIALS & METHODS

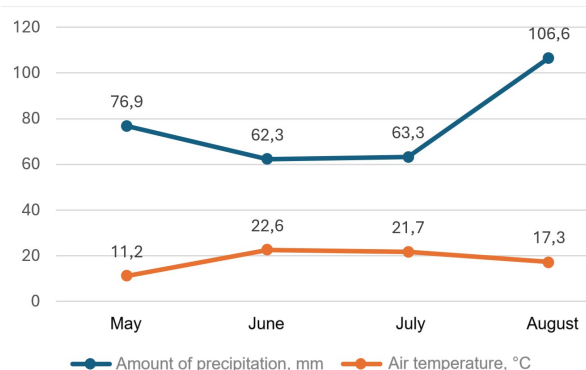
The research objects were regionally adapted standard wheat varieties which are extensively grown in Northern Kazakhstan: Astana, Astana 2, Akmola, Shortandinskaya 95 Improved, Shortandinskaya 2012, Asyl Sapa, Taymas, Shortandinskaya 2014, Shortandinskaya 2007, Tselinnaya Yubileynaya, and Tselina 50. The main agronomic characteristics of these varieties are summarized in Table 1. The causative agents of leaf and stem rust were isolates of *Puccinia triticina* (*P. triticina*) and *Puccinia graminis* f. sp. *tritici* (*P. graminis* f. sp. *tritici*), characterized using microbiological techniques under field conditions on the experimental plots of the investigated varieties. Every variety was planted on an equal 1-hectare plot. For rust prevalence determination, ten 1 m<sup>2</sup> sampling plots were designated on each field at equal distances from each other. In these sampling plots, infection severity and development of rust were evaluated. The data obtained were averaged to ensure a true reflection of disease development and damage to the crop. Infection type (IT) was assessed on a predefined 0–4 scale: 0 – immune, 1 – resistant (R), 2 – moderately resistant (MR), 3 – moderately susceptible (MS), 4 – susceptible (S).

**Table 1:** Characteristics of wheat varieties

Variety Name	Description
Astana	This variety combines earliness with high yield. Maximum yield reached 3.71 t/ha. It is drought-tolerant and resistant to loose smut. Approved for use in Akmola and North Kazakhstan regions since 2004.
Akmola 2	A mid-season variety combining high productivity and drought tolerance. Maximum yield – 4.22 t/ha. Lodging resistant. Recognized as the standard for the Akmola region. Approved for use in Akmola and North Kazakhstan regions.
Taymas	A mid-season, high-yielding variety (up to 3.85 t/ha), drought- and disease-resistant. Recommended for cultivation in Northern Kazakhstan.
Asyl Sapa	Mid-season and drought-tolerant. Lodging resistant. Maximum yield – 3.10 t/ha. Disease and pest resistance is comparable to the standard variety Akmola 2.
Shortandinskaya-2012	Early-mid-season variety (vegetation period 84–90 days). Maximum yield – 3.3 t/ha. Protein content – ~14%, gluten – 28%. Drought-tolerant, environmentally adaptable. 1000 kernel weight – 36 g. Moderately resistant to loose smut. Approved for use in four regions of Kazakhstan.
Shortandinskaya 2014	Mid-season variety (90–95 days). Maximum yield – 4.44 t/ha. Lodging resistant. Drought-tolerant. Disease and pest susceptibility at the level of Akmola 2.
Shortandinskaya 95 Improved	Late-mid-season (95–100 days). Highly resistant to lodging, shattering, spike breakage, and drooping. Maximum yield – 4.2 t/ha. 1000 kernel weight – 38–42 g. Drought-tolerant. Resistant to loose smut.
Shortandinskaya 2007	Mid-season variety (90–95 days). Maximum yield – 2.8 t/ha. Drought tolerance – 4.5 points. 1000 kernel weight – 35.5 g. Lodging resistant.
Tselina 50	High-yielding variety (2.44 t/ha), especially productive in dry and extremely dry years. Drought-tolerant at all development stages. Lodging resistant. Susceptible to major diseases and pests. 1000 kernel weight – 32–34 g.
Astana 2	Mid-season variety with plant height of 95–120cm. Average yield in variety trials – 2.99 t/ha. Resistant to brown and stem rust, relatively resistant to loose smut.
Tselinnaya Yubileinaya	Produces high-quality grain with raw gluten content of 30–32.4%. Yield – 200 g/m <sup>2</sup> . 1000 kernel weight – 30–34 g.

Disease severity (DS, %) was estimated by a modified Cobb scale. The area under the Area under Disease Progress Curve (AUDPC) was calculated to describe non-specific resistance of the varieties. The infection coefficient (IC) was determined as the product of the disease severity (DS) and the constant for the type of host reaction. Constants for each type of infection were: immune = 0.0; R = 0.2; MR = 0.4; MS = 0.8; S = 1.0. The employment of two different factors in the calculation can cause the same or similar IC values to be the consequence of two distinct combinations of severity and infection type.

The trials were carried out in the 2024 crop year on the fields of crops in the northern part of Kazakhstan. The climatic conditions in the Akmola Region in 2024 were extremely humid, with a Selyaninov Hydrothermal Coefficient (HTC) value exceeding 2, indicating excessive moisture availability. Fig. 1 illustrates the information on temperature and precipitation over the growing season of the crop in Akmola Region of Northern Kazakhstan.

**Fig. 1:** Temperature and precipitation patterns during the 2024 vegetation season.

As per the (HTC), May and August were excessively wet, with HTC values of 2.21 and 1.99, respectively. Excessive May rainfall had a detrimental effect on the spread of fungal diseases, while the excessively humid August during the grain-filling stage had a negative impact

on grain quality. August temperature conditions, along with heavy rainfall, also negatively affected the ripening of grains and the spread of fungal infections. Plant height during this period was at its highest among varieties; however, grain quality, for all its quantity, was comparatively modest and did not go beyond the second class (Acién et al., 2023). Phenological observations and assessment of plant development stages were conducted according to the official method of state variety testing of agricultural crops (Methodika, 2002; Singh et al., 2025). Accounting for yield was accomplished by the weight method (Bertolini et al., 2025). Statistical analysis of yield data was conducted using analysis of variance (Dospekhov, 1973; Xu et al., 2025).

Plant height was determined by taking the mean of 20 plants measured. The measurements were taken from the soil surface to the spike tip using a ruler with 0.5 mm accuracy (Tao et al., 2020). Productivity traits like thousand grain weight (TGW) were determined according to ISO 12042-80 for agricultural seeds (Savin et al., 2024). Yield was quantified by harvesting plants in 1-hectare plots. Threshing was done with a WinterSteiger LD350 thresher. Seeds that had been cleaned were weighed on an OHAUS Pioneer PA114C balance with an accuracy of 0.1g. The two-plot average weight was used as the final total yield (TY) value.

For molecular genetic analysis, the following procedures were employed: DNA extraction was done by the cetyltrimethylammonium bromide (CTAB) (Doyle & Doyle, 1987), and polymerase chain reaction (PCR) amplification was conducted according to the procedure outlined by Kleppe et al. (1971). Seeds of the spring wheat varieties were germinated through the moist roll method until the emergence of the first leaves. The sprouts were cut and genomic DNA was extracted from them. The integrity of the genomic DNA was checked by electrophoresis in 1.5% agarose gel. Quantification was done on a NanoDrop 2000 spectrophotometer (Eppendorf). The concentrations of DNA varied from 546.26 to 1250.2ng/μL. All samples were diluted to a working concentration of 20ng/μL.

**Table 2:** Field resistance of spring wheat cultivars to stem and leaf rust

No.	Primer Name	Primer Sequences (5' to 3')	Reference
1	<i>Lr19</i>	Forward: CCTGATCACCAATGACGATT Reverse: CCTGATCACCTTGCTACAGA	Gupta et al., 2006
2	<i>Lr21</i>	Forward: CGCTTTTACCGAGATTGGTC Reverse: TCTGGTATCTCACGAAGCCTT	Naz et al., 2021
3	<i>Lr24</i>	Forward: CACCCGTGACATGCTCGTA Reverse: AACAGGAAATGAGCAACGATGT	Schachermayr et al., 1995
4	<i>Lr35</i>	Forward: AGAGAGAGTAGAAGAGCT Reverse: AGAGAGAGAGCATCCACC	Kumar et al., 2009
5	<i>Lr37</i>	Forward: AGGGGCTACTGACCAAGGCT Reverse: TGCAGCTACAGCAGTATGTACACAAAA	Błaszczuk et al., 2004
6	<i>Lr39</i>	Forward: CCTGCTCTGCCCTAGATACG Reverse: ATGTGAATGTGATCGATGCA	Singh et al., 2004
7	<i>Sr2</i>	Forward: AAGGCGAATCAACGGAATA Reverse: GTTGCTTTAGGGGAAAAGCC	Mago et al., 2011
8	<i>Sr32</i>	Forward: GCGGTCAAGACACTCCACTCTCTC	Mago et al., 2013
9	<i>Sr47</i>	Forward: GGCTATCTCTGGCGCTAAAA Reverse: TCCACAAACAAGTAGCGCC	Klindworth et al., 2017
10	<i>Sr35</i>	Forward: ACATGTGATGTGCGGTCATT Reverse: TCCTCAGAACCCATTCTTG	Saintenac et al., 2013
11	<i>Sr21</i>	Forward: ATCGCATGATGCACGTAGAG	Chen et al., 2015

Based on the literature, primers associated with resistance to leaf and stem rust were selected (Table 2). The PCR amplification programs were optimized as follows: 95°C for 5min; 95°C for 20s; annealing at 60°C (*Lr19*), 58°C (*Lr21*), 60°C (*Lr24*), 60°C (*Lr35*), 62°C (*Lr37*), 58°C (*Lr39*), 60°C (*Sr2*), 56°C (*Sr32*), 58°C (*Sr47*), 60°C (*Sr35*), and 54°C (*Sr21*) for 20s; 72°C for 20s; and a final elongation step at 72°C for 5min.

### Statistical Analysis

Standard statistical analysis of the results was carried out using the Student's t-test (t) and p-values (p) using Microsoft Excel 2019. 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. The impact of resistance genes on yield-related traits was evaluated by grouping variables into clusters using cluster analysis methods in RStudio.

## RESULTS

Under the excess moisture condition, rust infection was seen on all the field plots. The natural background of infection allowed the evaluation of the wheat varieties' real susceptibility level to leaf and stem rust. Some of the varieties were almost fully infected. Table 3 shows the field observation results, which include the evaluations of infection spread and disease severity.

The general situation for leaf rust resistance was better, even though 64% of the samples lacked *Lr21* and *Lr35* genes. Moderate susceptibility was seen in only the Tselinnaya Yubileynaya and Shortandinskaya 95 Improved varieties, with AUDPC values ranging between 350 units. Moderate resistance was seen in 72% of the samples, with Taimas being completely free from infection. On the other hand, stem rust exhibited more pronounced infection and susceptibility across the varieties. Despite the moderate resistance noted for Asyl Sapa, Shortandinskaya 2014, Tselina 50, and Astana 2, the AUDPC varied between 490 and 770 units. Infection coefficient among moderately

resistant varieties varied from 12 to 24. The most susceptible varieties included Astana and Tselinnaya Yubileynaya, which had 50–60% infection. The most resistant variety was Taimas, with 10% infection and AUDPC of 105 units.

With the good weather conditions for the development of rust, the varieties showed an overall low resistance to stem rust. Even though most of the varieties were reported by breeders to be resistant to fungal diseases, such as rust, the samples under study were susceptible in this very suitable year for disease development. Together with rust severity ratings, yield components were measured in the standard cultivars. In the very humid year, measurements of plant height along with spike length and grain number were considerably higher. Data for productivity components are given in Table 4.

The average vegetation period under the weather conditions of 2024 for all varieties was 92–97 days and did not differ significantly from the standard Fig. s for early-, mid-, and late-maturing varieties. The most representative parameter in this too-wet year was plant height, which amounted to 102cm in the variety Tselinnaya Yubileynaya. The varieties Astana 2 and Shortandinskaya 2012 had average heights of 80cm and 88cm, respectively, which are characteristic of tall-statured Kazakh wheat varieties. Taimas, the newest variety developed for cultivation in Northern Kazakhstan, has a shortened stem and achieved an optimal height of 63cm in the wet year, in accordance with local standards (Serikbay et al., 2024).

Under favorable conditions, the normal spike length in control varieties is  $7.3 \pm 0.8$ cm (Zotova et al., 2024). The average spike length in 2024 was  $8.8 \pm 0.4$ cm. Spikelet density was medium in all varieties, but the number of grains per spike was up to  $30.8 \pm 1.2$  relative to the usual  $16.5 \pm 1.1$  grains (Zotova et al., 2024). Average grain weight per spike was  $0.95 \pm 0.3$ g, while in other favorable years it varied from  $0.56 \pm 0.2$  to  $0.64 \pm 0.35$ g. These data indicate that the climatic conditions of 2024 significantly influenced wheat productivity characteristics. One of the major productivity traits the thousand grain weight (TGW) was  $30.2 \pm 2.8$ g and had an effect on the final grain yield, which

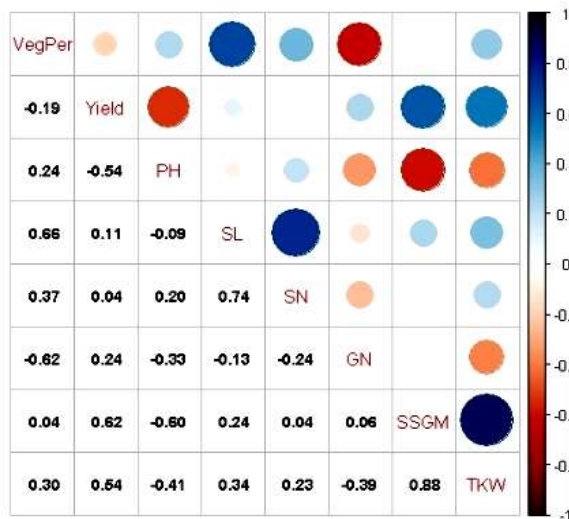
**Table 3:** Field resistance of spring wheat cultivars to stem and leaf rust

Cultivar	Stem Rust		Stem Rust Severity (%)		Stem Rust Severity (%)	
	Stem Rust	Stem Rust Severity (%)	SR IT	SR Coefficient	Leaf Rust	Leaf Rust Severity (%)
Astana	50S	50	515	30MR	12	245
Akmola 2	50MS	40	525	20MR	8	175
Tselinnaya Yubileynaya	60S	60	770	20MS	16	350
Taimas	10R	2	105	0	0	0
Asyl Sapa	30MR	12	280	20MR	8	280
Shortandinskaya 2012	50MS	40	595	10MR	4	105
Shortandinskaya 2014	50MR	20	525	20MR	8	175
Shortandinskaya 95 ul.	30MS	24	315	20MS	16	350
Tselina 50	60MR	24	490	30MR	12	455
Astana 2	40MR	16	420	30MR	12	175
Shortandinskaya 2007	30MS	24	315	20MR	8	140

**Table 4:** Structural characteristics of naturally infected wheat plants (2024)

Cultivar	Vegetation (days)	Period Yield (t/ha)	Plant Height (cm)	Spike Length (cm)	Spikelets per Spike	per Grains per Spike	per Grain Weight (g)	Weight per 1000-Grain Weight (g)
Astana	92.6±1.2	27.6±0.15	99.5±0.3	8.1±0.07	15±0.3	31.7±0.6	0.83±0.01	26.2±0.4
Akmola 2	94±2.3	22.6±0.17	96±0.1	9.2±0.6	16.6±0.2	31±0.7	1.03±0.02	33.2±0.6
Tselinnaya Yubileynaya	95±1.7	17.5±0.14	102±0.3	8.8±0.5	15.2±0.2	29.6±0.5	0.8±0.03	27±0.4
Taimas	93±1.5	31.7±0.15	63.3	8.8±0.7	14.6±0.3	34.2±0.5	1.4±0.03	33.7±0.4
Asyl Sapa	92±1.5	21.8±0.15	99±0.24	8.7±0.4	15±0.10	32.6±0.6	0.94±0.01	27.4±0.5
Shortandinskaya 2012	93±1.2	23.7±0.18	95±0.2	8.6±0.5	15.8±0.3	28.8±0.6	0.95±0.02	33±0.5
Shortandinskaya 2014	92±1.5	16.0±0.17	88±0.2	8.9±0.7	16±0.2	32.6±0.4	0.77±0.03	23.6±0.4
Shortandinskaya 95 ul.	96±1.7	23.3±0.12	95±0.3	9.9±0.6	16.4±0.1	28.8±0.5	0.94±0.02	32.6±0.3
Tselina 50	93±1.3	15.6±0.17	94±0.3	8.2±0.5	14.2±0.2	30.1±0.6	0.9±0.02	29.1±0.4
Astana 2	92±1.5	30.0±0.15	80±0.14	8.8±0.5	15.7±0.1	31.6±0.8	1.0±0.02	33.4±0.6
Shortandinskaya 2007	93±1.2	19.6±0.14	95±0.2	8.5±0.7	15.4±0.2	28.5±0.4	0.95±0.03	32.1±0.5

was on average 22.7c/ha. Considering the long-term mean yield of 12.5c/ha, this is a significant jump (Serikbay et al., 2024). Astana 2 and Taimas varieties were the leaders in terms of major productivity characters.

**Fig. 2:** Pearson correlation of productivity traits in infected plants.

Pearson's correlation analysis indicated that plant height was negatively correlated with yield (-0.54) under pressure of infection. The same tendency was observed in the other productivity traits: number of grains per spike (-0.33), grain weight per spike (-0.60), and thousand grain weight (-0.41), whereas in more favorable conditions, these correlations are usually in the opposite direction (Fig. 2). This confirms the conclusion that shorter-statured types like Taimas, and moderately tall types like Astana 2, had high productivity even in conditions of disease stress. Disease pressure alters the usual correlation between

structural and yield characteristics, making it possible to reveal genotypes adaptive in stress conditions. Given that excessive moistening is the principal cause of rust development, the selection of varieties not only resistant to rust but also able to preserve optimal structural parameters—particularly plant height—which positively influence productivity, is critical.

For studying the molecular basis of resistance in the investigated wheat varieties, PCR analysis was used to identify the presence of *Sr* and *Lr* rust resistance genes in the wheat genome. These genes have been described to have a key role in resistance to rust infection and its spread under field conditions and have revealed the greatest effectiveness in conferring resistance in our previous research (Kokhmetova et al., 2021; Cat, 2024; Savin et al., 2024). Data on the presence of *Sr* and *Lr* resistance genes in the tested wheat varieties are given in Table 5. Agarose gel electrophoresis results after PCR are provided in Appendix 1.

Based on the PCR data, three varieties (Astana, Akmola 2, and Shortandinskaya 2007) had all 11 of the tested resistance genes. The *Lr21* and *Lr35* genes were missing in 7 of the 11 varieties. The *Lr24* gene was missing in just one variety, Shortandinskaya 95 Improved. The varieties tested had between 72 and 100% of the tested resistance genes, even though their efficiency in field resistance is doubtful. Field trials to determine the most resistant genotypes were conducted to evaluate the level of infection and disease spread. Though the varieties under study had some of the best rust resistance genes, their performance decreased considerably under the uncontrolled field environment because of the extremely favorable environment for rust development. These results stress the necessity for more precise evaluation of the level of resistance to select the most resilient varieties capable of guaranteeing food security in the region.

**Table 5:** Presence of rust resistance genes in standardized cultivars

Cultivar	Lr19	Lr21	Lr24	Lr35	Lr37	Lr39	Sr2	Sr32	Sr47	Sr21	Sr35
Astana	+	+	+	+	+	+	+	+	+	+	+
Akmola 2	+	+	+	+	+	+	+	+	+	+	+
Shortandinskaya 95 ul.	+	–	–	–	+	+	+	+	+	+	+
Shortandinskaya 2007	+	+	+	+	+	+	+	+	+	+	+
Shortandinskaya 2014	+	–	+	–	+	+	+	+	+	+	+
Shortandinskaya 2012	+	–	+	–	+	+	+	+	+	+	+
Astana 2	+	–	+	–	+	+	+	+	+	+	+
Tselina 50	+	–	+	–	+	+	+	+	+	+	+
Tselinnaya Yubileinaya	+	–	+	–	+	+	+	+	+	+	+
Taimas	+	–	+	–	+	+	+	+	+	+	+
Asyl Sapa	+	–	+	–	+	+	+	+	+	+	+

One of the most important parts of the research was the determination of the influence of the presence of resistance genes on total yield and its components. To study the connection between rust infection and productivity characteristics, the Mann-Whitney test was used. This non-parametric test is appropriate for the comparison of two independent samples with a small number of samples. All possible combinations of the presence or absence of genes were studied in the 11 varieties of wheat. Statistically significant effects were found only for the *Lr24* gene, which was linked to a decrease in spike length ( $p > 0.01$ ), as presented in Table 6.

**Table 6:** Influence of *Lr21*, *Lr24*, and *Lr35* on spike length (Mann-Whitney Test)

	Residuals:			
	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	9.9000	0.3242	30.535	1.44e-09 ***
Lr21	-0.0381	0.2237	-0.170	0.86902
Lr24	-1.2286	0.3466	-3.545	0.00757 **
Lr35	NA	NA	NA	NA

Without the *Lr24* gene, spike length was decreased by as much as 1.23cm under rust infection, which can have a profound influence on grain number per spike, a key yield component. The *Lr21* gene had a minimal decrease in spike length (0.038cm,  $p > 0.8$ ), whereas the *Lr35* gene had no discernible effect owing to a lack of representation among the genotypes investigated. To visualize the impact of these variable genes more clearly, their effects based on Box-plot analysis (Wilcoxon test) were compared in two groups: Group 1 (gene absent) and Group 2 (gene present), focusing on yield component differences.

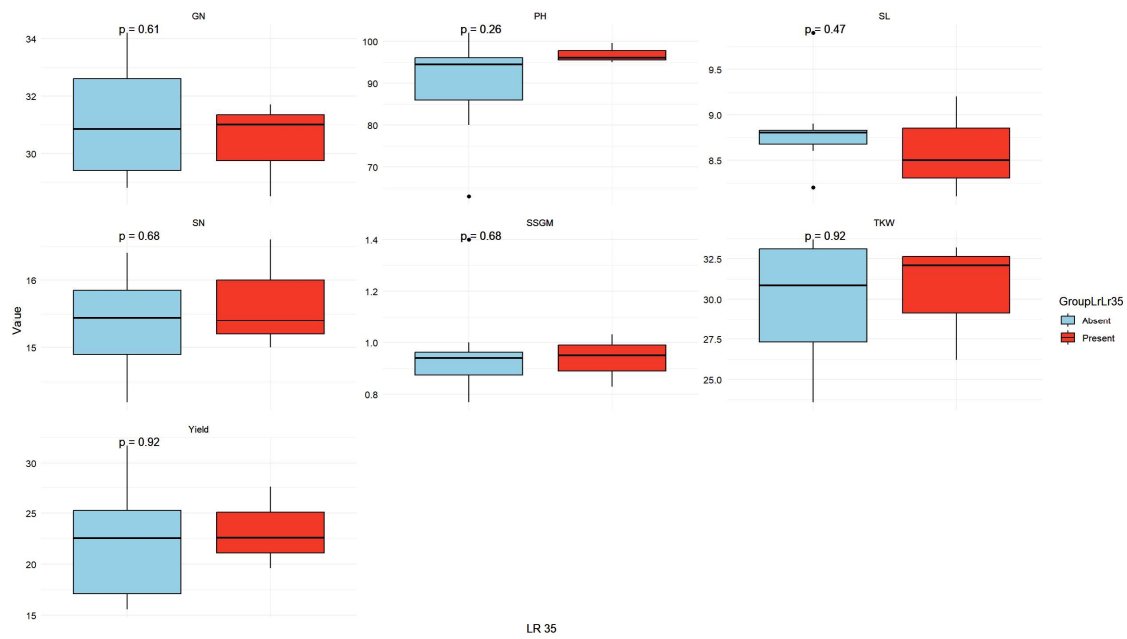
The number of grains per spike in 50% of the sample averaged 32.6 grains. The presence of the gene had a slightly lower average of 31.3 grains in 75% of the samples, with  $p > 0.61$ . This tendency can be related to the features of tall cultivars, which prevailed in the sample. Besides, the impact of the level of moisture on the number of grains cannot be excluded. In the plant height, the presence or absence of the gene did not have a significant impact ( $P > 0.26$ ). Nevertheless, the presence of the gene had a positive influence on grain weight per spike, by increasing its value by 0.4 g ( $P > 0.68$ ). The gene also positively influenced the number of spikelets per plant, by increasing this parameter by 0.28 spikelets ( $P > 0.68$ ). Although statistical significance can be more than 0.05 because of the small sample size, the general trend in the cultivars studied pointed to an impact of the *Lr35* gene on the components of productivity under the conditions of natural rust infection (Fig. 3). Another variable gene was

*Lr21*, which showed a statistically significant association with spike length according to the Mann-Whitney analysis (Table 4). Boxplot analysis of the effect of the *Lr21* gene on productivity components showed the following values (Fig. 4). The data from the analysis indicated an association between the presence of certain resistance genes and plant height: 25% of the genotypes with the *Lr35* gene had a height above 95cm. The same influence of the *Lr35* gene was noted on the number of spikelets per spike ( $P > 0.68$ ) and grain weight per spike ( $P > 0.68$ ). No such significant relation was established between the presence of *Lr35* or *Lr21* and the thousand grain weight (TGW), which indicates that these genes might not be critical for this yield component. Overall, the presence or absence of resistance genes did not have a significant impact on total yield. Multifactorial analysis attested to the statistically significant effect of the *Lr24* gene on spike length in the conditions of natural rust infection and a very favorable environment for disease development.

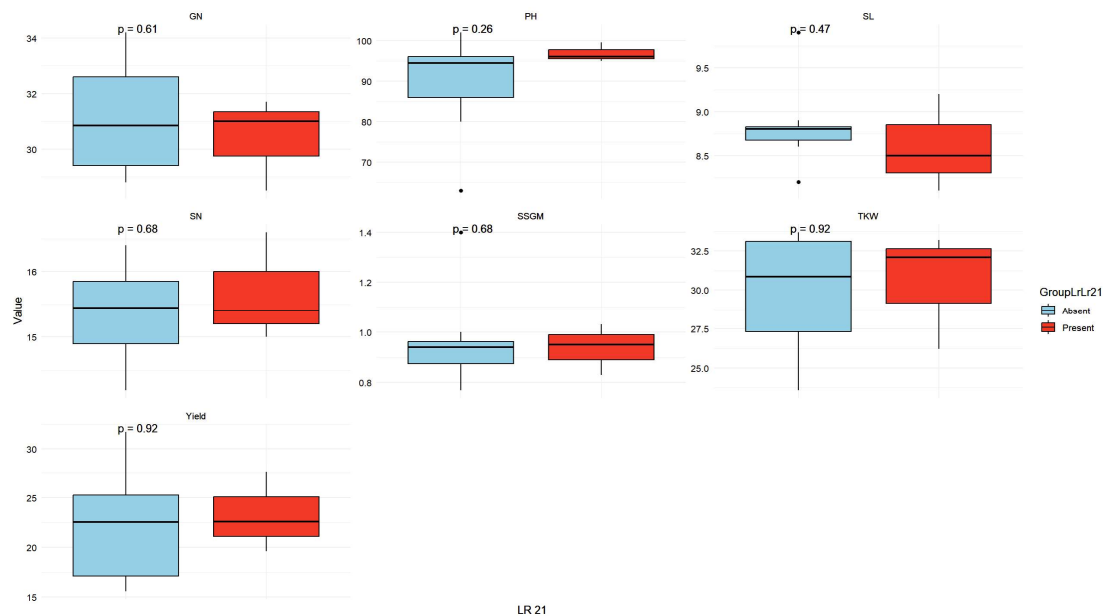
The results show that although the presence of resistance genes adds to the general resistance of varieties, the 2024 season—characterized by high humidity and possibly the appearance of new races of the rust pathogen—manifested low resistance in the majority of varieties overall. In particular, the Taimas variety, which has a shortened stem height and was entered into the State Register of Breeding Achievements in 2022, expressed high resistance to leaf and stem rust. While the genetic foundation of the investigated varieties is comparatively strong and contains as many as 11 resistance genes, their efficiency was nullified in uncontrolled natural conditions. The impact of these genes on the yield components in the presence of natural infection pressure indicates promise for the establishment of statistically strong patterns with an increased and diverse sample size.

## DISCUSSION

Wheat rust continues to pose a major agricultural threat, yet continued research and technological improvement provide optimism for the creation of resistant cultivars amid a changing climate (Hussain et al., 2025; Leharwan et al., 2025). An integrated strategy involving scientific breakthroughs, sustainable methods, and good policy is necessary to counter this menace. Research on wheat rust control in the future should focus on precise and effective areas that can greatly improve our knowledge and management of the disease (Zhang et al., 2019). In addition, knowledge of the long-term



**Fig. 3:** Analysis of the relationship between *Lr35* gene presence and productivity components in wheat cultivars.



**Fig. 4:** Results of the Wilcoxon test for the *Lr21* gene.

evolutionary dynamics between wheat and rust pathogens is key to forecasting future pathogen virulence changes and designing durable resistance strategies (Xia et al., 2022).

The results obtained indicated that under disease-favorable conditions for the development of the disease, the cultivated varieties had a low resistance level to stem rust despite the presence of five effective resistance genes — *Sr2*, *Sr32*, *Sr47*, *Sr21*, and *Sr35*. Meanwhile, 3–6 leaf rust resistance genes — *Lr19*, *Lr21*, *Lr24*, *Lr35*, *Lr37*, and *Lr39* — provided moderate resistance to the disease in 11 Kazakhstan-developed cultivars. Interestingly, the Taimas, Asyl Sapa, and Astana 2 cultivars had high productivity

levels in spite of the disease development. The effectiveness of the resistance genes for stem rust was comparatively low in the varieties tested, which are essential for the country's food security (Karelov et al., 2022; Tolossa et al., 2022; Upadhaya et al., 2024).

Under natural infection conditions and excess moisture, structural components of productivity were determined to be dependent on plant height, with considerable variation in the characteristics of grain weight per spike, grain number, and thousand grain weight (Melash et al., 2023; Sun et al., 2025). All these need to be taken into consideration while choosing varieties to avoid catastrophic wheat yield losses.



Leaf rust, due to *Puccinia triticina*, continues to be widespread and troublesome, especially in cooler and more humid environments. The pathogen quickly adapts to resistant wheat cultivars, leading to the development of new virulent races that break existing resistance (Kolmer & Fajolu, 2022; Malik et al., 2024). Recent research has emphasized the need to understand the molecular interaction of the pathogen with wheat, both pre-haustorial and post-haustorial resistance mechanisms (Riar et al., 2021; Bellameche et al., 2021). Systemic acquired resistance has been shown, in particular, to be an effective approach to providing long-term resistance to a spectrum of pathogens (Mapuranga et al., 2023; Panthi et al., 2024).

Recent progress has also shown the successful introgression of resistance genes from wild relatives of wheat, including *Lr47* and *Lr42*, into modern wheat varieties (Blower et al., 2025). These genes provide broad-spectrum resistance against leaf rust and have been incorporated through advanced gene-editing tools like CRISPR-Cas9 along with conventional breeding approaches (Li et al., 2023). Research in the future must be directed towards pyramiding these genes with other resistance loci and testing their efficacy under different environmental conditions to ascertain durability (Rollar et al., 2021; Zhang et al., 2024).

Based on the received data, *Lr21*, *Lr24*, and *Lr35* are variable loci in the cultivars under study. The significant influence of *Lr24* on the length of a spike is promising for further examination of its role in yield component formation in the face of infection pressure. In the process of cultivar selection, one should take into account the behavior of resistant and susceptible cultivars in terms of ultimate yield formation in order to reduce the risk of losses in years with extensive outbreaks of the disease.

As revealed through the examination of *Lr24* and *Lr35* effects on yield conservation, and especially on the individual yield components, the presence or absence of these genes affected thousand grain weight, number of spikelets, and grain weight per spike. Even though the limited sample size might have affected the statistical significance, there is no doubt that the genetic background of the cultivar plays an essential part in yield development under disease stress. It is also worth mentioning that the highest yield losses are related to the extensive development of stem rust, to which the cultivars expressed very little resistance. This needs to be considered during the process of choosing effective resistance genes and further strengthening and optimizing breeding practices for the creation of resistant varieties.

Stem rust, incited by *P. graminis*, has long been one of the most destructive wheat diseases (Karelov et al., 2022). Though now less common because of the use of resistant cultivars, it continues to pose a significant threat, especially in warmer climate zones (Tolossa et al., 2022). Recent research has concentrated on detecting and mapping rust resistance loci in wheat genomes, which has enabled the breeding of new, more resistant wheat cultivars (Rollar et al., 2021; Upadhaya et al., 2024; Zhang et al., 2024). The application of functional genomics and genome-wide

association studies (GWAS) has been instrumental in the identification of new sources of resistance. For instance, GWAS on elite durum wheat detected important loci like YrDurum-1BS.1 for yellow rust resistance, and other research uncovered several genomic regions linked to resistance against different rust diseases. The further extension of GWAS and meta-QTL analysis will be essential to incorporate the findings into marker-assisted selection (MAS) and enhance the genetic base of rust-resistant wheat (Ullah et al., 2024). Additionally, the use of high-throughput omics tools, including transcriptomics and proteomics, can reveal further insights into the molecular basis of host-pathogen interactions in rust infections (Gilligan, 2024). Such methods can identify major regulatory genes and pathways involved in rust resistance, thereby enabling the development of novel resistant cultivars (Wang et al., 2024).

As global warming continues to affect agricultural systems, understanding its influence on the epidemiology of rust diseases is crucial. The coupling of climate modeling and rust epidemiology will enable the development of predictive capabilities to inform planting dates, resistance breeding, and disease management strategies, ensuring wheat production remains resilient to environmental changes (Prasad et al., 2021).

As global warming continues to affect agricultural systems, understanding its influence on the epidemiology of rust diseases is crucial. The coupling of climate modeling and rust epidemiology will allow the creation of predictive capacities to inform planting dates, resistance breeding, and disease management strategies so that wheat production can continue to be resilient to changes in the environment (Prasad et al., 2021).

## Conclusion

Molecular screening of 11 varieties of wheat grown in Northern Kazakhstan for leaf and stem rust resistance identified the occurrence of the most effective genes for resistance to both types of rust. In natural infection conditions, the varieties showed low resistance. The study verified the effect of genes *Lr21*, *Lr24*, and *Lr35* on elements of productivity, but the efficacy of *Sr* genes could not be established.

This study constitutes a basis for exhaustive screening of wheat collections, along with newly introduced lines and hybrids, for rust resistance according to the results achieved. It is important to take into account varietal reactions under natural infection and utilize the genetic relationship between the resistance and yield-related characteristics to ensure national food security.

## DECLARATIONS

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**Conflict of Interest:** None.



**Data Availability:** On reasonable request, data will be available from the corresponding author.

**Ethics Statement:** The work involved no human participants, no vertebrate animals, and no endangered or protected plant species, and no transgenic lines were used. Disease assessments relied solely on naturally occurring infections; no quarantine or introduction of non-native pathogens occurred. Field activities were carried out on institutional experimental farms with permission from the host institutions (S.Seifullin Kazakh AgroTechnical Research University and A.I.Barayev Research and Production Center for Grain Farming) and in accordance with national phytosanitary and biosafety regulations.

**Author's Contribution:** LZ: Conceptualization, writing – review and editing, supervision, project administration; AG, AZ, FA, SB and TS: writing – original draft preparation, methodology, formal analysis; AG, DS, SO, LZ, TS and DS: data curation, validation, resources, visualization.

**Generative AI Statement:** The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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