https://doi.org/10.17221/219/2024-PPS

Assessing stem rust tolerance in commercial wheat varieties: Insights from field trials in Kazakhstan

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Citation: Maulenbay A., Rsaliyev A. (2025): Assessing stem rust tolerance in commercial wheat varieties: Insights from field trials in Kazakhstan. Plant Protect. Sci., 61: 333–349.

Abstract: This study provides the first comprehensive assessment of stem rust tolerance in commercial wheat varieties from Kazakhstan and Russia, including spring and winter varieties. Field trials were conducted to compare yield and agronomic traits between stem rust-inoculated and fungicide-treated plots, providing a practical framework for assessing tolerance. Key indicators such as disease severity, area under the disease progress curve, thousand kernel weight, and the stress tolerance index were evaluated to gauge variety resilience under stress. Significant variations in tolerance were observed, with varieties such as 'Pamyat' 47', 'Nadezhda', 'Lyubava 5', 'Tselinnaya 3s', 'Severyanka', 'Egemen-20', 'Zhemchuzhina Povolzh'ya', 'Dimash', 'Serke' and 'Korona' maintaining yield potential despite high disease pressure. Correlations revealed that traits such as flag leaf area, vegetative period, and plant height were associated with greater tolerance, highlighting their potential in breeding. With the expected increase in stem rust outbreaks due to climate change and the evolving virulence of stem rust pathogens, these findings emphasise the need for breeding programs incorporating resistance and tolerance, offering a sustainable alternative to fungicide use. This study provides critical insights for breeders and plant pathologists seeking to enhance wheat resilience in regions prone to rust epidemics.

Keywords: field trials; stress tolerance index; resistance; yield

Wheat has been the cornerstone of global agriculture for over 10 000 years, serving as one of the earliest domesticated crops (Sousa et al. 2021). Wheat remains a critical food source, accounting for 18% of global dietary caloric intake (Bakala et al. 2021; Stukenbrock & Gurr 2023). With annual production surpassing 600 mil. t (Dixon et al. 2009), wheat is the third most important cereal, after maise and rice. However, the rising global demand for wheat, driven by population growth, climate change, and geopolitical instability, underscores the need for improved crop productivity and resilience (Shiferaw et al. 2013).

In Central Asia, particularly in Kazakhstan and western Siberia, wheat is cultivated on more than 15 mil. ha, with an average yield of 1.4 t/ha (Amalova et al. 2023a). This region is critical to global food security, with Kazakhstan alone producing over 85% of the region's cereal crops. However, wheat production is frequently limited by abiotic stressors, such as drought, and biotic stressors, such as fungal diseases, including leaf blotch, tan spot, and rust (stem, leaf and stripe). In northern Kazakhstan, abiotic and biotic factors have reduced the average wheat yield to 1.7 t/ha (Morgounov et al. 2020). Moreover, over the last 30 years, increasing temperatures and precipitation have created favourable conditions for the spread of rust diseases (Morgounov et al. 2018).

Supported by the Ministry of Science and Higher Education of the Republic of Kazakhstan, Project No. IRN AP14870987 "Determination and conservation of wheat accessions with tolerance mechanisms to major fungal diseases".

https://doi.org/10.17221/219/2024-PPS

Biotrophic phytopathogenic fungi, such as stem rust (*Puccinia graminis* f. sp. *tritici*), are obligate parasites that penetrate plant cells without destroying them to access nutrients (Glazebrook 2005; Karelov et al. 2022). The fungus has a complex life cycle, with five spore stages, including an asexual uredinial stage in wheat and a sexual stage involving alternate hosts such as barberry. It forms urediniospores on leaves and stems, utilising specialised structures (appressoria, hyphae, and haustoria) to infiltrate plant cells. The disease reduces nutrient flow to kernels, shrivels grains, and weakens stems, leading to yield losses and wheat lodging, particularly in regions with mild winters and wet springs (Leonard & Szabo 2005; Karelov et al. 2022).

Stem rust has recently re-emerged as a significant threat to wheat production in the region. Epidemics in Kazakhstan's wheat-growing regions have occurred periodically, with outbreaks in the 1960s causing up to 50% yield losses (Rsaliyev & Rsaliyev 2019). Although stem rust occurred sporadically between 1990 and 1999, more recent outbreaks in northern Kazakhstan, particularly between 2006 and 2009, reported infection rates of 20–40% (Kokhmetova et al. 2011). Since 2010, the incidence of stem rust has increased, with severe epidemics affecting over 1 million hectares annually between 2015 and 2019, causing yield losses of up to 35% (Shamanin et al. 2016, 2020; Skolotneva et al. 2020; Olivera et al. 2022). Epiphytotics occur in wheat crops approximately every 3–4 years, while stem rust is observed 2–3 times less frequently (Koyshybayev 2018). The disease spreads rapidly across wheat-growing areas due to its airborne nature, making it difficult to maintain wheat productivity (Skolotneva et al. 2013; Rsaliyev et al. 2020).

Fungicides are commonly used to control disease development and reduce crop damage, but their application has significant downsides, such as environmental contamination and residue in agricultural products (Jin et al. 2007; Duarte Hospital et al. 2023). Additionally, the widespread use of fungicides targeting specific cellular processes (monosites) has rapidly increased resistance. About 75% of fungicide action groups, as identified by the Fungicide Resistance Action Committee (https://www.frac.info/knowledge-database/downloads, accessed November 22, 2024), have reported cases of resistance, posing a major challenge to sustainable disease management in agriculture (Wang & Scherm 2023). Rust pathogens were initially categorised as low risk for developing fungicide resistance. However, it has been observed that rust species share life cycle traits with pathogens with a high fungicide resistance risk (Oliver 2014; Grimmer et al. 2015).

Breeding programs have traditionally focused on developing resistant varieties to address this growing threat. Developing varieties with effective disease-resistance genes is a sustainable and environmentally responsible strategy for managing plant diseases (Hiebert et al. 2020). Notably, over 240 rust-resistance genes have been identified in wheat, with *Sr31* being one of the most widely recognised and utilised genes for combating stem rust (Bakala et al. 2021). Some of the *Sr* genes have been identified in bread wheat (wheat's genes), while others have been introgressed from related species. Most *Sr*-genes provide seedling resistance, also known as all-stage or juvenile resistance. Fewer genes are non-race specific and provide adult plant resistance (APR) (Karelov et al. 2022).

Despite this low-to-medium risk classification for fungicide resistance, *P. graminis* poses a significant threat to wheat due to the polymorphism and high evolutionary potential, which allows for the rapid emergence of new races (McDonald & Linde 2002; Hiebert et al. 2020; Cook et al. 2021). Shortly after the widespread deployment of a resistance gene, a virulent race of the pathogen often emerges, leading to significant agricultural losses in some countries (Pretorius et al. 2000; Singh et al. 2011). This rapid adaptation undermines the long-term effectiveness of the resistance gene, making the control of stem rust particularly challenging (Karelov et al. 2022).

The rapid evolution of new virulent races, such as those in the Ug99 lineage, has raised concerns about the durability of resistance genes. Almost none of the spring wheat varieties cultivated in northern Kazakhstan exhibit resistance to rust diseases (Babkenov et al. 2023). Adult plant resistance to stem rust has been identified in only 16.5% of wheat varieties, with just eight (5%) confirmed to carry the *Sr57* gene. The narrow range of stem rust resistance genes in widely grown varieties increases the region‘s vulnerability to the emergence of new virulent races and potential stem rust epidemics (Olivera et al. 2022). In Kazakhstan, the genetic basis of stem rust resistance in wheat is limited to a few key genes, including *Sr25, Sr31, Sr36, Sr6Ai*, and *Sr6Ai#2* (Shamanin et al. 2016). This narrow genetic base increases the region's vulnerability to the

https://doi.org/10.17221/219/2024-PPS

emergence of new virulent races, potentially leading to future stem rust epidemics (Olivera et al. 2022).

There is an ongoing need for innovative solutions to control plant pathogenic fungi (Lamberth 2022). It is now widely recognised that plants employ two primary defence strategies: resistance and tolerance (Pagán & García-Arenal 2020). Resistance reduces the pathogen's fitness, potentially influencing epidemic dynamics by exerting selection pressure that can lead to the breakdown of resistance over time (Vanderplank 2012; Pagán & García-Arenal 2020). In contrast, tolerance allows the plant to endure the presence of the pathogen without imposing selection pressure, making it a more stable defence strategy. Wheat breeding programs increasingly focus on tolerance traits (Kadkol et al. 2021), highlighting the long-term value of tolerance over resistance. Tolerance offers greater stability, as it is less likely to result in resistance breakdown (Caldwell et al. 1958; Van den Bosch et al. 2006; Vitale & Best 2019), a common issue with resistance-based strategies (Pagán & García-Arenal 2020).

Tolerance – the ability of a plant to maintain productivity despite infection – is an equally crucial trait, especially for minimising yield losses under disease pressure (Vanderplank 2012; Pagán & García-Arenal 2020). Tolerance is typically assessed by comparing the yield performance of wheat varieties under both high and low disease pressures, highlighting its value in breeding programs aiming to enhance long-term stem rust resilience (Pagán & García-Arenal 2020). Despite the importance of stem rust tolerance, there is limited published research on the genetic variation in this trait in wheat germplasm (Maulenbay & Rsaliyev 2024).

This study investigated stem rust tolerance among commercial wheat varieties from Kazakhstan and Russia by comparing yield reduction in stem rust-inoculated plots to that in non-inoculated plots. This approach enabled an evaluation of two critical aspects: the maximum yield potential of each genotype under disease-free conditions and its ability to maintain yield under stem rust pressure.

A key element of the methodology involved using a disease-free control to estimate each variety's potential yield. However, achieving completely disease-free conditions in field trials can be challenging. To address this issue, fungicide-treated plots were utilised as the control, ensuring minimal disease impact. By establishing consistent disease pressure in inoculated plots and comparing them to treated, non-inoculated controls, the study developed a practical and scalable method for assessing stem rust tolerance.

This research aims to support long-term stem rust management and promote yield stability in regions susceptible to rust outbreaks.

MATERIAL AND METHODS

Plant material. This study comprised a set of 67 spring and 15 winter commercial varieties from Kazakhstan and Russia, totalling 82 varieties (Table 1).

The varieties were sourced from 17 breeding stations in Kazakhstan and 10 in Russia, developed independently or through collaborative efforts between stations [Electronic supplementary material (ESM) Table 1]. All varieties are widely grown and well-known commercial varieties with approved cultivation zones in the Republic of Kazakhstan (ESM Table 2)

Field trials. Experiments were conducted in the experimental fields of the Research Institute for Biological Safety Problems (RIBSP) in the Zhambyl region, Southeast Kazakhstan (43.576476 N, 75.213618 E), in a temperate, irrigated environment during the 2023 and 2024 growing seasons. The 2023 growing season was characterised by unusually dry conditions, with an average temperature of 21.1 °C and total precipitation of 144.4 mm. In contrast, the 2024 growing season had an average temperature of 22 °C and significantly higher annual rainfall, measuring 331.3 mm.

The study employed a randomised complete block design with two independent replications

Table 1. Distribution of spring and winter wheat varieties by number of samples, country of origin, and wheat type

Type of wheat varieties	Number of samples	Country of origin		Bread wheat	Durum wheat
		Kazakhstan	Russia		
Spring wheat varieties	67	47	20	62	5
Winter wheat varieties	15	12	3	15	0
Total	82	59	23	77	5

https://doi.org/10.17221/219/2024-PPS

(Lozada et al. 2019; Kelly et al. 2021; Mikaberidze & McDonald 2020; Pandey et al. 2021). Each variety was sown in 0.4 m^2 plots, with two rows per plot, spaced 20 cm apart. The rows were 100 cm long, containing 80 seeds per row. The varieties 'Saratovskaya 29' (for spring wheat) and 'Steklovidnaya 24' (for winter wheat) were used as susceptible checks. To facilitate disease spread, these susceptible varieties were planted after every 20 varieties (Genievskaya et al. 2020).

All 82 commercial wheat varieties were sown in two different sets as follows (Pandey et al. 2021; Zhou et al. 2022):

(i) Control plots (sprayed with fungicide and not inoculated with disease) were treated with the systemic fungicide "Kolosal Pro" (Avgust, Russia), which is approved for use in Kazakhstan. The active ingredients of the fungicide are propiconazole (300 g/L) and tebuconazole (200 g/L), designed to protect plots from natural stem rust infections, effectively applied before the rust is present. Subsequent applications (second or third) were carried out if rust began to develop approximately 4 to 6 weeks after the previous treatment. All field operations were conducted using locally established, highly effective management practices.

(ii) An experimental plot (without fungicide treatments, a disease-inoculated group) was exposed to stem rust infection (Ziv & Eyal 1976; Pandey et al. 2021). Artificial epiphytotic conditions were established by inoculating the field with a virulent mixture of stem rust races, which had been collected from spring wheat varieties of Kazakhstan between 2015 and 2018 (Rsaliyev et al. 2020) and stored in the RIBSP microorganism collection. Spore samples stored at low temperatures were subjected to a heat-shock treatment at 50 °C for 30 min to reactivate them (Rsaliyev & Rsaliyev 2019). Seedlings of the wheat varieties were inoculated by spraying suspended urediniospores in a solution of 3M™ Novec™ 7100 (3M, USA). The suspension was applied using an airbrush spray gun (Revell GmbH, Germany) for uniform distribution over the plants (Patpour et al. 2022). Inoculation was carried out at the seedling stage during evening hours (Roelfs et al. 1992), followed by immediate irrigation to maintain the humidity necessary for spore germination and disease development (Pandey et al. 2021).

Disease assessment in the field and yield-related traits. Disease severity (DS), measured as the percentage of infected stem leaf sheath and true stem using the modified Cobb scale (Peterson et al. 1948), and plant response to stem rust, based on the reaction type (RT) (Roelfs et al. 1992), were assessed 4 times during the growing season: at the early booting stage (GS 41–45), during ear emergence (GS 51–59), at flowering (GS 65–69), and at milk development (GS 75) (Zadoks et al. 1974). The types of reactions were categorised as resistant (R), moderately resistant (MR), moderately susceptible (MS), and susceptible (S) (Roelfs et al. 1992).

The corresponding DS percentages for each disease score were used to compute the area under the disease progress curve (AUDPC), applying the following formula (Wilcoxson et al. 1975):

$$AUDPC = \sum_{i=1}^{n-1}\left[\left(\frac{DS_i + DS_{(i+1)}}{2}\right)\times\left(t_{(i+1)} - t_i\right)\right] \quad (1)$$

where: DS_i – the disease severity percentage at the time t_i; $t_{i+1} - t_i$ – the days between two disease percentage scores; n – the total number of observations

Before harvest, key phenological traits, including days to heading (DH), days to maturity (DM), and vegetation period (VP), were recorded for each variety. Additionally, important agronomic and yield-related traits were measured, such as flag leaf area (FLA), plant height (PH), upper internode length (UIL), and spike length (SP) (Pask et al. 2012).

After the kernels were naturally dried, measurements were taken for plot yield (PY) and thousand kernel weight (TKW) following CIMMYT protocols (Pask et al. 2012). The overall workflow is shown in Figure 1, which was created using Biorender.com (accessed on October 18, 2024).

Yield performance is assessed using two key metrics: yield response (%) (Zhou et al. 2022) and the stress tolerance index (STI) (Fernandez 1992; Pandey et al. 2021). In this study:

(i) nonstress conditions Y_p – control plots, where wheat varieties were sprayed with fungicide and not inoculated with the disease;

(ii) stress conditions Y_s – experimental plots, where wheat varieties were not treated with fungicide and were inoculated with the disease.

$$\text{Yield response (\%)} = \frac{Y_p - Y_s}{Y_p} \times 100 \quad (2)$$

where: Y_p – control plots; Y_s – experimental plots

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To evaluate the ability of different genotypes to tolerate stress while maintaining yield potential, the STI is used:

$$STI = \frac{Y_s \times Y_p}{(\bar{Y}_p)^2} \quad (3)$$

where: Y_p – control plots; Y_s – experimental plots; $\bar{Y}_p$ – the average yield under nonstress conditions

A higher STI value for a wheat variety signifies greater stress tolerance.

Furthermore, wheat varieties were classified into four groups based on their performance in the experimental and control plots. Group A included varieties that consistently produced high yields in both the control plots (sprayed with fungicide and not inoculated with disease) and the experi-

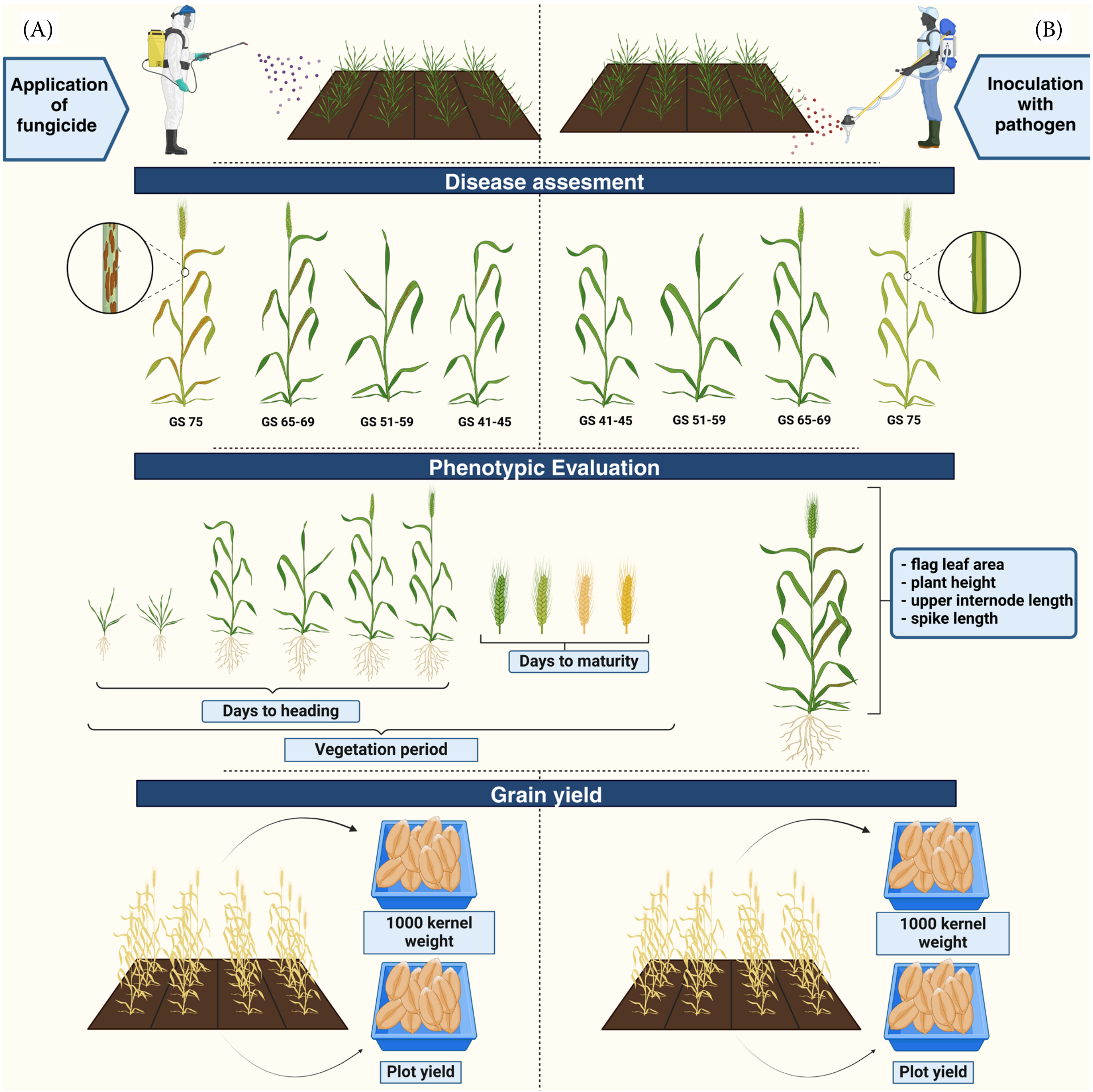

Figure 1. Schematic overview of the experimental design (simplified)

(A) Control plot (no pathogen exposure) – fungicide application: fungicides are applied to control disease and establish a disease-free plot; (B) experimental plot – inoculation with pathogen: wheat varieties are exposed to the pathogen; disease assessment: disease severity and the type of reaction were assessed 4 times during the growing season for two groups of plants, each with a different variety; phenotypic evaluation: days to heading, days to maturity, and vegetation period were recorded for each variety, and flag leaf area, plant height, upper internode length, and spike length were measured for two groups and each variety; grain yield: measurements for plot yield and thousand kernel weight were taken

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mental plots (without fungicide treatment and inoculated with disease). Group B comprised varieties that performed well only in the control plots, whereas Group C included varieties that exhibited relatively higher yields only in the experimental plots. Group D consisted of varieties with low yields in both plot types. The optimal selection criterion focused on distinguishing Group A from the other three groups, as these varieties demonstrated superior adaptability and stability across conditions (Fernandez 1992).

Correlation and principal component study. The significance of differences between the treatment and control groups and among the varieties was evaluated using an Analysis of variance (ANOVA). Additionally, correlation analyses were conducted to assess the relationships between various traits under study. To further investigate the relationships between yield and other yield-related traits, a principal component analysis (PCA) was employed. The PCA results were visualised in Biplots, which were constructed based on each treatment's first two principal components. All analyses were performed using GraphPad Prism (version 10.0.0).

RESULTS

Disease assessment in the field and yield-related traits. The findings presented in this study synthesise two years of field trial data to systematically evaluate stem rust tolerance and yield performance across 82 wheat varieties. ESM Table 3 summarises disease metrics and yield-related traits under inoculated and fungicide-treated conditions. This comparative analysis reveals distinct resilience patterns, with select varieties maintaining yield stability despite high disease pressure. Table 2 further delineates disease severity and reaction types in wheat varieties exposed to stem rust inoculation, categorising responses from moderate resistance (MR) to severe susceptibility (90 S).

In the stem rust-inoculated plots without fungicide treatment, wheat varieties exhibited varying levels of disease severity and reaction types. Most spring bread wheat varieties showed susceptibility, varying from 30 S to 50 S, while a few demonstrated moderate resistance (30 MR, 30 MS). Spring durum wheat varieties were predominantly susceptible, with 'Bezenchukskaya 139' classified as 40 S and others as 50 S. Winter bread wheat varieties displayed the highest susceptibility, with disease severity reaching 90 S in some genotypes. 'Dimash' and 'Karlygash' exhibited 60 S severity, while 'Almaly', 'Bogarnaya 56', and 'Mironovskaya 808' showed even higher susceptibility (70 S). The most severely affected varieties included 'Sapaly' and 'Steklovidnaya 24' (80 S) and 'Egemin-20', 'Farabi', 'Grom', and others, reaching 90 S. Notably, no spring durum or winter wheat varieties exhibited moderate resistance and moderate susceptibility, highlighting their heightened vulnerability compared to spring bread wheat.

The evaluation of TKW under both control and stress conditions revealed considerable variation in stress responses across different wheat varieties ($P < 0.001$).

In the spring bread wheat group, several varieties demonstrated resilience under stress. 'Pamyat 47' showed a notable increase in TKW, rising from 42.60 g in control to 44.40 g under stress, representing a 4.2% increase. 'Lyutescens 521' exhibited a similar positive response, with TKW increasing from 40.60 g to 43.30 g (6.7%). 'Lyutescens 32' also improved under stress, with TKW increasing from 40.00 g to 41.20 g, marking a 3.0% gain. In contrast, some varieties experienced reductions in TKW under stress. For instance, 'Avgustina' showed a significant decrease, with TKW dropping from 40.20 g to 33.10 g, a reduction of 17.6%. 'Karabalykskaya 20' also exhibited a minor decline, with TKW decreasing from 32.10 g to 31.86 g (0.7%).

Among the winter bread wheat varieties, several showed improved TKW under stress. 'Egemin-20 'experienced a substantial increase, with TKW rising from 42.40 g to 44.40 g, indicating a 4.7% improvement. 'Karlygash' showed an even more pronounced response, with TKW increasing from 38.40 g to 43.00 g, a 12.0% increase. However, some winter bread wheat varieties showed reduced TKW under stress. 'Dimash' experienced a slight reduction, with TKW decreasing from 40.80 g to 39.80 g (2.5%). 'Konditerskaya yarovaya' saw a marked decrease in TKW, dropping from 35.60 g to 30.20 g, a loss of 15.2%.

For the durum wheat varieties, responses to stress varied. 'Serke' demonstrated exceptional resilience, with TKW increasing from 42.20 g to 46.60 g, representing a 10.4% increase. 'Bezenchukskaya 139' showed a modest improvement, with TKW rising slightly from 40.20 g to 40.70 g (1.2%). Conversely, some durum wheat varieties showed decreased TKW under stress. 'Lan' experienced a reduction,

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Table 2. Disease severity and reaction type of wheat varieties in a stem rust inoculated plot without fungicide treatment

Disease severity and reaction type	Spring bread wheat varieties	Spring durum wheat varieties	Winter bread wheat varieties
30 MR	'Lyutescens 32', 'Lyutescens 521'	–[a]	–
30 MS	'Omskaya 37', 'Saratovskaya 42', 'Stepnaya volna'	–	–
3 0S	'Lyazzat', 'Lyubava', 'Lyubava 5', 'Miras', 'Pavlodarskaya 93', 'Pavlodarskaya yubileynaya', 'Saratovskaya 55', 'Saratovskaya 70', 'Seke', 'Stepnaya 2', 'Tselinnaya yubileynaya'	'Korona', 'Lan'	–
40 MS	'Pamyat' Azieva'	–	–
40 S	'Akmola 2', 'Aktube 39', 'Almaken', 'Altay', 'Altayskaya 325', 'Kazakhstanskaya rannespelaya', 'Nadezhda', 'Nargiz', 'Omskaya 18', 'Omskaya 19', 'Omskaya 20', 'Omskaya 28', 'Omskaya 29', 'Omskaya 36', 'Oral', 'Oskemen', 'Samgau', 'Shortandinskaya 2007', 'Shortandinskaya 2014', 'Shortandinskaya 95 uluchshennaya', 'Tabys 60', 'Tselinnaya 24', 'Tselinnaya 3s', 'Ul'binka 25', 'Yugo-Vostochnaya 2', 'Zhenis'	'Bezenchukskaya 139'	–
50 S	'Alem', 'Altayskaya zhnitsa', 'Aray', 'Astana', 'Astana 2', 'Asyl Sapa', 'Avgustina', 'Baiterek', 'Ertis 7', 'Karabalykskaya 20', 'Karagandinskaya 60', 'Karagandinskaya 70', 'Kazakhstanskaya 15', 'Kazakhstanskaya 25', 'Omskaya 30', 'Pamyat' 47', 'Saratovskaya 29', 'Severyanka', 'Volgouralskaya'	'Serke', 'Seymur 17'	–
60 S	–	–	'Dimash', 'Karlygash'
70 S	–	–	'Almaly', 'Bogarnaya 56', 'Konditerskaya yarovaya', 'Mironovskaya 808', 'Vavilov'
80 S	–	–	'Sapaly', 'Steklovidnaya 24'
90 S	–	–	'Egemin-20', 'Farabi', 'Grom', 'Mereke-70', 'Zhemchuzhina' 'Povolzh'ya', Zhetysu'

[a] absence of varieties in this category

with TKW decreasing from 39.80 g to 36.20 g, marking a 9.0% decrease.Distinct differences in the STI were observed among the evaluated wheat varieties, with specific top-performing genotypes identified in spring bread wheat, winter bread wheat, and durum wheat categories (Table 3). The STI serves as a critical metric for evaluating wheat varieties under disease pressure, with higher values indicating superior stress tolerance and sustained yield potential. By incorporating stress intensity into its calculation, the STI effectively differentiates genotypes exhibiting uniform superiority across both stress and non-stress environments (Group A) from those with environment-dependent performance (Groups B, C, and D). In this study, Table 2 highlights only Group A varieties exhibiting high STI values under severe stem rust infection (disease severity ≥ 30%). To focus on genotypes demonstrating resilience under high disease pressure, varieties with S reactions were prioritised. In contrast, those with MS or MR and lower disease severity (≤ 30%) were excluded.

The highest STI value within the spring bread wheat varieties was recorded for 'Pamyat' 47' (STI = 1.27), followed closely by 'Nadezhda' (STI = 1.20) and 'Lyubava 5' (STI = 1.15). Additional high-performing genotypes in this group included 'Tselinnaya 3s' and 'Severyanka', each with an STI of 1.17, demonstrating strong resilience to stress conditions. These varieties show promise for use in breeding programs

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Table 3. Top-performing wheat varieties with a high stress tolerance index for stem rust tolerance

Wheat varieties	Country of origin	AUDPC	STI
Spring bread wheat			
'Pamyat' 47'	KZ	805.00	1.27
'Nadezhda'	KZ	315.00	1.20
'Tselinnaya 3s'	KZ	665.00	1.17
'Severyanka'	KZ	472.50	1.17
'Lyubava 5'	KZ	280.00	1.15
'Almaken'	KZ	577.50	1.13
'Omskaya 19'	RU	297.50	1.12
'Aktube 39'	RU	595.00	1.11
'Karagandinskaya 60'	KZ	350.00	1.09
'Altayskaya 325'	RU	455.00	1.08
'Nargiz'	KZ	595.00	1.07
'Oskemen'	KZ	595.00	1.07
'Shortandinskaya 95 uluchshennaya'	KZ	630.00	1.05
'Miras'	KZ	420.00	1.04
'Zhenis'	KZ	770.00	1.03
'Samgau'	KZ	455.00	1.03
'Lyazzat'	KZ	262.50	1.02
'Oral'	KZ	595.00	1.02
'Stepnaya 2'	KZ	420.00	1.00
Spring durum wheat			
'Serke'	RU	472.50	1.32
'Korona'	KZ	420.00	1.17
'Bezenchukskaya 139'	KZ	455.00	1.10
Winter bread wheat			
'Egemin-20'	KZ	892.50	1.26
'Zhemchuzhina Povolzh'ya'	KZ	770.00	1.14
'Dimash'	KZ	805.00	1.09
'Almaly'	KZ	682.50	1.05
'Vavilov'	KZ	630.00	1.05
'Farabi'	KZ	1 190.00	1.02
'Sapaly'	KZ	945.00	1.01

AUDPC – area under the disease progress curve; STI – Stress Tolerance Index; KZ – Kazachstan; RU – Russia

focused on stress tolerance enhancement in spring bread wheat.

Within the durum wheat varieties, 'Serke' demonstrated exceptional performance with an STI of 1.32, the highest among all varieties evaluated. 'Korona' followed with an STI of 1.17, also showing strong resilience. These top-ranking durum wheat varieties exhibit substantial tolerance to stress, making them valuable for durum wheat breeding initiatives aimed at improving stress resilience.

In the winter bread wheat group, 'Egemin-20' exhibited the highest STI at 1.26, positioning it as a top candidate for stress resilience. Other notable performers were 'Zhemchuzhina Povolzhya' (STI = 1.14) and 'Dimash' (STI = 1.09), who also displayed considerable stress tolerance. These varieties stand out for their potential adaptability to challenging growing conditions in winter wheat production areas.

Notably, the 'Farabi', a winter wheat variety, showed the highest AUDPC value of 1 190.00, sug-

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gesting a significant susceptibility to stem rust. Farabi maintained a moderate STI of 1.02 despite this high disease severity, reflecting its ability to sustain yield under disease pressure. Similarly, 'Astana', 'Asyl Sapa', and 'Pamyat 47', all spring wheat varieties, also displayed high AUDPC values of 805.00, ranking them among the more susceptible varieties. However, these varieties varied in their stress tolerance, with 'Pamyat 47' exhibiting a high STI of 1.27, suggesting better resilience in yield performance than 'Astana' and 'Asyl Sapa', which had STIs of 0.99 and 0.87, respectively.

Correlation and principal component study. The correlation between the control and experimental groups was analysed for various traits, including phenological traits, yield-related traits, and stem rust severity. The correlation matrices, shown in Figures 2A and 2B, reveal key relationships between these traits.

In the control group, where the plants were sprayed with fungicide and not inoculated with diseases, significant correlations were observed between various agronomic traits: UIL was positively correlated with FLA (r = 0.22*). FLA positively correlated with spike length SP (r = 0.24*). TKW exhibited a positive correlation with PY (r = 0.22*). PH was positively correlated with SP (r = 0.35***), DH (r = 0.43***), and VP (r = 0.42***). VP was perfectly correlated with DM (r = 1.00***). Significant negative correlations were also observed between various traits: TKW and UIL (r = –0.21*), PY and SP (r = –0.22*), and DM and SP (r = –0.37***).

Under the experimental conditions, where the plants were inoculated with stem rust and not treated with fungicide, significant correlations among agronomic traits were observed, elucidating the interactions between disease progression and plant physiological responses: DS was significantly correlated with the AUDPC (r = 0.68***), PH (r = 0.38***), DH (r = 0.87***), and VP (r = 0.87***). AUDPC showed positive correlations with PH (r = 0.35***), SP (r = 0.23*), DH, and VP (both r = 0.56***). The STI was positively correlated with PY (r = 0.35***) and TKW (r = 0.60***). FLA positively correlated with DH and VP (r = 0.39***). PH correlated positively with UIL (r = 0.26*), SP (r = 0.30**), DH, and VP (both r = 0.50***). SP was also positively correlated with DH and VP (r = 0.24*). DH was per-

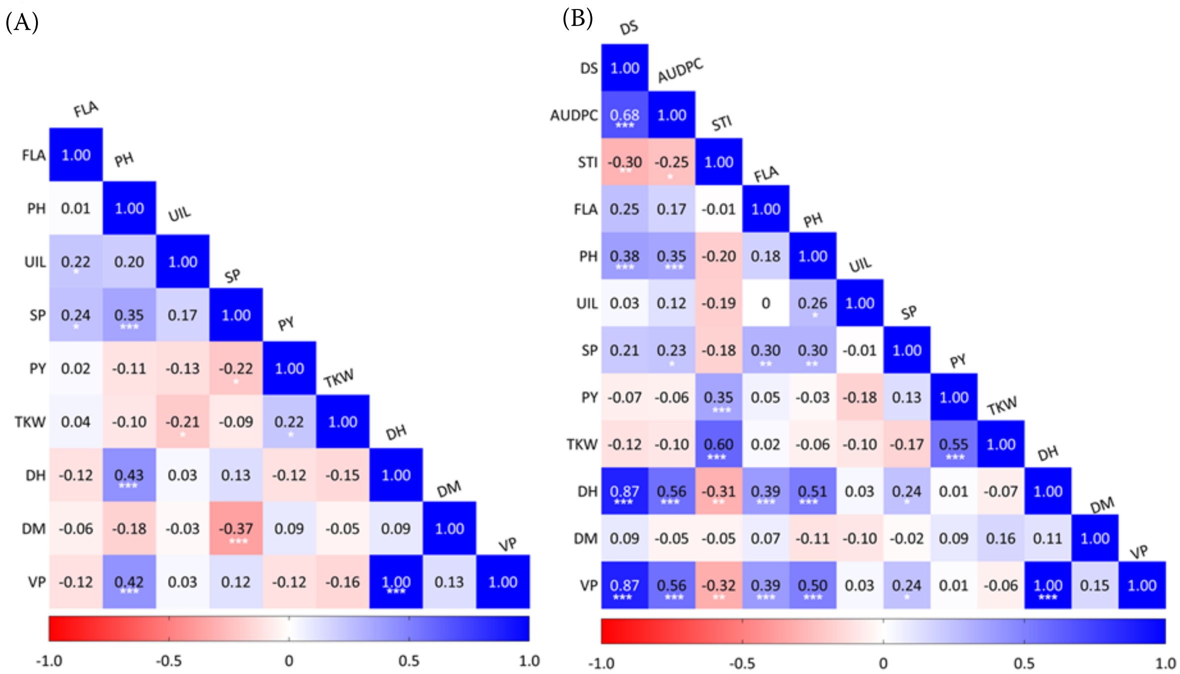

Figure 2. The correlation heatmaps presented in (A) and (B) illustrate the pairwise correlation coefficients between various agronomic traits under control conditions (A) and in the experimental group (B)

Each cell in the heatmap displays the Pearson correlation coefficient, indicating the strength and direction of the relationship between two variables; colour scale: the colour scale ranges from –1 to +1, where 1 (blue) indicates a strong positive correlation, –1 (red) indicates a strong negative correlation, and 0 (white) indicates no correlation; significance levels: statistical significance is denoted by asterisks: ***P < 0.001, **P < 0.01, and *P < 0.05

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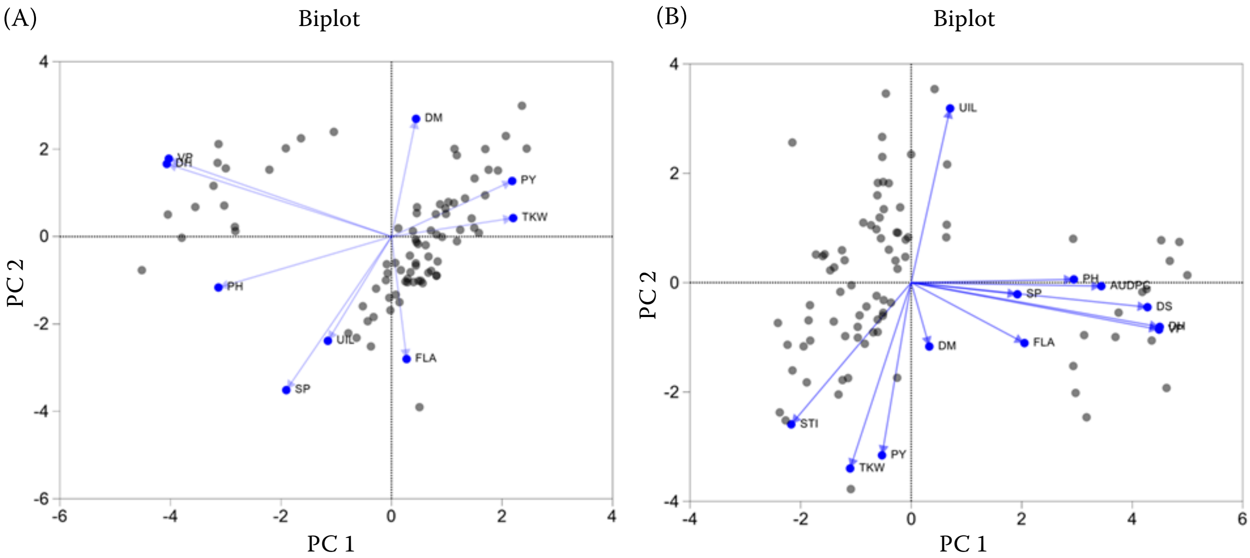

Figure 3. Principal component analysis biplots for traits studied under control (A) and stem rust inoculation conditions (B)

Blue lines (vectors) indicate the contribution of various traits to the principal components; grey points represent an individual wheat variety observation

fectly correlated with VP (r = 1.00***). The STI exhibited a negative correlation with DS (r = –0.30**), AUDPC (r = –0.25*), DH (r = –0.31**), and VP (r = –0.32**).

A principal component analysis (PCA) was conducted to determine the major contributors to phenotypic variation under control and stem rust treatment conditions (Figure 3).

The PCA revealed that the first four principal components had eigenvalues greater than 1 in the control environment. The first two principal components (PC1 and PC2) cumulatively explained 46.78% of the total phenotypic variation, with PC1 accounting for 27.70% and PC2 accounting for 19.08%. The major contributors influencing these components were DH, VP, DM, PH, SP, and FLA, as illustrated in Figure 3A. The first four principal components under stem rust treatment conditions also displayed eigenvalues greater than 1. The first two principal components, PC1 and PC2, explained 33.83% and 16.70% of the phenotypic variation, respectively, totalling 50.53% of the cumulative variation. The traits predominantly contributing to these components were DH, VP, PH, DS, AUDPC, STI, and TWK, as depicted in Figure 3B.

DISCUSSION

In Kazakhstan, bread wheat varieties occupy over 80% of the cultivated wheat. Annually, the country produces between 20 and 25 mil. t of bread wheat, with exports reaching 5–7 mil. t (Turuspekov et al. 2017; Rsaliyev et al. 2020). Our research's limited sample size of durum wheat reflects its restricted cultivation area in Kazakhstan, fluctuating market demand, and significant variations in yield and quality (Gultyaeva et al. 2020; Genievskaya et al. 2022).

A stem rust tolerance assessment was conducted by comparing yield differences between plots inoculated with stem rust and plots sprayed with fungicide without stem rust inoculation (Caldwell et al. 1958; Ziv & Eyal 1976; Parker et al. 2004; Foulkes et al. 2006; Collin et al. 2018; Pandey et al. 2021). In the experimental group, disease severity varied significantly, ranging from 30 MR to 90 S, with these variations correlating strongly with yield changes (P < 0.001). This stark contrast in disease impact underscores some varieties resilience and others vulnerability under pathogen stress. In contrast, the control group exhibited no disease symptoms, providing a baseline of nil-disease conditions, which was essential for comparing the varieties inherent yield potentials without stem rust's confounding effects. This approach allowed us to examine two key traits: the yield potential of each variety under disease-free conditions and the ability to maintain yield under stem rust pressure (Soko et al. 2018; Zhou et al. 2022). By ensuring consistent disease pressure across the inoculated plots and using untreated controls, we were able to develop

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a robust and scalable method for evaluating stem rust tolerance in wheat (Csósz et al. 1999; Pierre et al. 2015; Castro & Simón 2016; Forknall et al. 2019; Kadkol et al. 2021; Kelly et al. 2021).

While critical for wheat pathogen control, Fungicides may act as stressors by disrupting plant physiology. Studies demonstrate that compounds like difenoconazole induce oxidative stress, inhibit chlorophyll synthesis, and impair photosynthesis (Liu et al. 2021; Li et al. 2023; Touzout et al. 2024). Field trials reveal inconsistent yield impacts, with responses varying across seasons (Gaile et al. 2023). Fungicides can disrupt chloroplast function and redox balance, risking oxidative damage and productivity loss (Bailey et al. 2012). These findings underscore the need for judicious, evidence-based fungicide use to balance protection and plant health in wheat systems.

Wheat varieties demonstrating tolerance were identified through detailed examinations and comparisons of both the experimental and control plots. These particular varieties exhibited notable disease symptoms yet sustained minimal losses in yield, highlighting their resilience to the pathogen (Maulenbay & Rsaliyev 2024). In analysing wheat variety responses to stem rust under experimental conditions, certain varieties exhibited notably high AUDPC (Area under the disease progress curve) scores, indicating their susceptibility to disease progression.

The interaction between genotypes and the environment is crucial in enhancing wheat yield and quality (Amanuel et al. 2018; Nehe et al. 2019; Johansson et al. 2020) including proteins, polysaccharides, lipids, minerals, heavy metals, vitamins and phytochemicals, effecting these characters. The genotype and environment is of similar importance for the determination of the content and composition of these compounds. Furthermore, the interaction between genotypes and the cultivation environment may play a significant role. Many studies have evaluated whether the genotype or the environment plays the major role in determining the content of the mentioned compounds. An overall conclusion of these studies is that except for compounds encoded by single major genes, importance of certain factors mainly depend on how wide environments and how diverse cultivars are within these comparative studies. Comparing environments all over, e.g. across Latin America, ends up with a high significance of the environment while large studies including genotypes of wide genetic background result in a significant role for the genotype. In addition, for some technological properties and components, genotype has a higher effect (e.g. grain hardness and gluten proteins. Yield can be assessed by examining related traits, such as the number of productive tillers, spike length, thousand-grain weight, and spikelets per spike (Li et al. 2020). A variety's genetic potential is expressed optimally under favourable environmental conditions, but its performance can vary significantly under stress (Foulkes et al. 2011; Li et al. 2020; Ullah et al. 2021). Wheat yield is primarily determined by three components: spike number per plant, kernel number per spike, and thousand-kernel weight. Among these, spike number per plant and kernel number per spike are more susceptible to environmental factors (Li et al. 2018; Ma et al. 2023). Thousand kernel weight, a key determinant of yield, varies based on genetic and environmental factors. The duration and rate of kernel filling, which is affected by photosynthetic activity, nutrient levels, and water availability, also play a significant role in determining the final kernel weight (Simmonds et al. 2016; Chidzanga et al. 2022; Amalova et al. 2023b; Jabbour et al. 2023).

A moderate positive correlation between flag leaf area and TKW (0.55) suggests that a larger flag leaf contributes to heavier kernels. Additionally, a longer vegetative period showed a moderate positive correlation with plot yield (0.64) and TKW (0.42), indicating that an extended growth period generally supports higher yields and greater kernel weight. Approximately 95% of the energy in nature is derived from photosynthesis (Zhai et al. 2002), with leaves serving as the primary photosynthetic organs in plants. The flag leaf, located directly beneath the wheat spike, plays a crucial role in photosynthesis, supplying water and nutrients essential for grain development (Yang et al. 2016). Additionally, traits such as the timing of flowering and spike length are strongly associated with grain yield (Woodruff & Tonks 1983; Hedden 2003; Liu et al. 2018; Jin et al. 2020).

Grain yield in crops is determined by various components that form at different growth stages and are influenced by environmental conditions. A variety's overall yield depends on how its genotype interacts with these factors. Therefore, selecting a genotype for a specific environment requires assessing yield component development under various conditions to ensure maximum yield potential. In wheat,

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breeding programs optimise these components to enhance kernel yield (Amalova et al. 2024).

Various tolerance traits may function at different levels of organisation, ranging from the organ level to the crop level (Ney et al. 2013; Mikaberidze & McDonald 2020). Previous studies have extensively discussed candidate traits associated with tolerance (Bingham et al. 2009a, 2009b; Ney et al. 2013; Newton 2016; Pagán & García-Arenal 2018, 2020). Some agricultural crop varieties, even those highly susceptible to infectious diseases, can still produce high yields under optimal conditions, such as during high rainfall seasons or in irrigated trials. These favourable conditions enable crops to reach their yield potential despite disease pressure (Kadkol et al. 2021). In practice, achieving completely disease-free plots in field experiments is challenging, making it essential to use fungicide treatment as an untreated control (Caldwell et al. 1958; Ziv & Eyal 1976; Parker et al. 2004; Foulkes et al. 2006; Collin et al. 2018; Pandey et al. 2021). Moreover, maintaining uniform disease severity across varieties or breeding lines is crucial for accurate tolerance comparisons. This requires the inclusion of multiple reference varieties in each replicate block and accounting for variable gradients across the trial area during variance analysis (Smiley et al. 2005). Tolerant wheat varieties can be identified by thoroughly analysing and comparing experimental and control plots. These varieties exhibit significant disease symptoms but experience minimal yield loss, demonstrating their tolerance to pathogens. The concept of tolerance, which allows plants to minimise yield loss even in the presence of disease, is emerging as a critical trait for wheat farmers. Unlike resistance, which focuses on preventing or restricting pathogen growth, tolerance ensures crops can sustain productivity under disease pressure (Kause 2011).

Our study represents the first comprehensive analysis of approved Kazakhstan wheat varieties from a major wheat-producing region, assessing yield and yield components under both stress and non-stress conditions to evaluate the impacts of disease and the varieties potential. Previous research focused on resistance traits in newly developed varieties (Genievskaya et al. 2020, 2022; Zatybekov et al. 2022; Gultyaeva et al. 2024), but our work provides a broader perspective by examining established varieties in real agricultural settings. However, focusing solely on yield may cause one to overlook the complex relationship between tolerance and resistance, as highlighted in evolutionary genetics studies (Simms & Triplett 1994; Kause & Ødegård 2012). Additionally, practical trials on cereal crops have shown that resistance traits can sometimes come with a yield penalty or, in some cases, offer a yield advantage depending on environmental factors (Smiley et al. 2005; Kazan & Gardiner 2018) including cereals. Fusarium crown rot (FCR. Thus, while yield retention is an important consideration for tolerance, breeders should also account for potential trade-offs between resistance and tolerance when selecting genotypes.

Developing wheat varieties with resistance and tolerance is the most sustainable strategy for managing disease, minimising the need for fungicides and supporting environmentally friendly and cost-effective disease control (Maulenbay & Rsaliyev 2024).

These findings are particularly valuable for breeders, and pathologists focused on producing wheat lines with improved tolerance to stem rust, offering insights to support the development of resilient wheat varieties tailored to Kazakhstan's diverse agricultural regions.

This study highlights the importance of integrating tolerance and resistance traits into wheat breeding programs to ensure sustainable crop production under various disease pressures. While resistance remains vital for preventing pathogen proliferation, tolerance provides a practical means of maintaining yield stability when disease presence is inevitable. Future research should explore the intricate balance between these traits to develop more resilient wheat varieties capable of thriving in diverse and challenging environments (Kelly et al. 2021). Additionally, promoting stakeholder education and adopting these strategies, including using various mixtures, will be essential for improving disease management practices in wheat production.

CONCLUSION

This study provides the first comprehensive assessment of stem rust tolerance in a diverse set of commercial wheat varieties from Kazakhstan and Russia, examining both spring and winter varieties under control and disease inoculation conditions. By analysing the response of 82 varieties to stem rust infection, we identified variations in yield stability and resilience across different genotypes. No-

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tably, the stress tolerance index and thousand kernel weight emerged as valuable indicators for evaluating stem rust tolerance. Among the spring bread wheat varieties, the best genotypes for stress tolerance were 'Pamyat' 47', 'Nadezhda', 'Lyubava 5', 'Tselinnaya 3s', and 'Severyanka'. Egemin-20, Zhemchuzhina Povolzh'ya, and Dimash demonstrated notable resilience in the winter bread wheat group. Serke and Korona emerged as the top-performing varieties for durum wheat, showing substantial tolerance to stress. Despite high disease pressure, these varieties exhibited promising tolerance traits, maintaining yield and kernel weight. Our findings reveal significant correlations between key agronomic traits – such as flag leaf area, vegetative period, and plant height – and tolerance to stem rust, supporting the importance of these traits in breeding programs targeting disease resilience.

Given the increasing threat of stem rust outbreaks due to climate change and the evolving virulence of stem rust, our results highlight the need for breeding programs in Kazakhstan to focus on resistance and tolerance traits. Targeting resistance and tolerance in breeding programs can provide a more sustainable and environmentally friendly alternative to fungicide use, reducing reliance on chemical control methods. This study contributes valuable data for breeders and pathologists seeking to enhance wheat resilience, providing insights that can guide the development of varieties with improved tolerance to stem rust, ensuring stable yields in rust-prone regions and supporting long-term food security in Central Asia.

Acknowledgement: We thank the technical staff from the Laboratory of Phytosanitary Safety for their valuable help with the field studies.

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Received: November 22, 2024
Accepted: April 9, 2025
Published online: June 13, 2025