Online Journal of Biological Sciences

Research Article

Genetic and Environmental Interactions in Spring Wheat Resistance to *Bipolaris Sorokiniana* Root Rot in Kazakhstan

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Article history
Received: March 12, 2025
Revised: May 25, 2025
Accepted: May 29, 2025

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Abstract: Common root rot caused by *Bipolaris sorokiniana* represents a major constraint to wheat production in Kazakhstan. This study evaluated the resistance of 30 spring bread and durum wheat genotypes under diverse environmental and treatment conditions in two contrasting regions (Almaty and Aktobe) during 2024. The experimental design included three treatment regimes: fungicide application, artificial inoculation with the pathogen, and natural infection. Agronomic traits (stem length, spike characteristics, grain weight) and disease parameters (prevalence, severity, yield loss) were assessed using standard phytopathological methods, while molecular markers identified resistance genes *Sb1* and *Sb2*. Results demonstrated that genotypes carrying resistance genes exhibited significantly lower disease incidence and severity under infectious conditions. Genotype #518/Serke, gord. (*Sb2*) showed stable performance with 87.38 cm stem length and 21.67 g grain weight per 50 plants, while line #575 (*Sb1*, *Sb2*) achieved the highest spikelet number (14.27) and grain weight (9.67 g). Fungicide application improved stem length (84.24 cm) and grain weight (16.96 g), whereas natural infection significantly reduced yield-related traits. Significant regional differences emerged: Aktobe plants exhibited higher tillering capacity (2.32) but shorter stems (77.07 cm), while Almaty plants showed longer stems (81.29 cm) and superior spike structure. These findings demonstrate that integrating genetic resistance, strategic fungicide application, and region-specific breeding strategies can effectively mitigate yield losses from common root rot. This research provides a framework for developing climate-resilient wheat varieties combining disease resistance with environmental adaptability, essential for food security in Kazakhstan's diverse agroecological zones.

Keywords: Spring Wheat, *Bipolaris sorokiniana*, Common Root Rot, Disease Resistance, Resistance Genes, Genotype-Environment Interaction, Molecular Markers, Kazakhstan

Introduction

Food security remains a key global concern due to the projected increase in the human population by 2050 (Canton, 2021). Wheat yield losses due to diseases account for up to 10% of potential productivity (Ludemann *et al*., 2024), and in Kazakhstan, one of the most destructive diseases is common root rot caused by *B. sorokiniana* and *Fusarium spp.* (Smiley *et al*., 2009a). These phytopathogens cause stunted plants, white ear, basal stem necrosis, and reduced grain quality (Kumar *et al*., 2002; Al-Sadi, 2021). Low resistance of varieties to these pathogens causes yield losses of up to 35-45% (Bozoğlu *et al*., 2022; Ozer *et al*., 2023a). In addition, *B.*

sorokiniana causes "black spot" that impairs grain quality and reproductive performance. *Fusarium culmorum*, another important root rot pathogen, causes stem basal necrosis, vascular dysfunction, and lodging, which significantly reduces yield and quality (Iwaniuk *et al*., 2021; Gao *et al*., 2024).

International studies confirm the widespread distribution of these pathogens in various geographic areas, including the United States, Canada, China, Turkey, and Azerbaijan (Paulitz *et al*., 2002; Fernandez *et al*., 2014; Moya-Elizondo *et al*., 2015; Zhou *et al*., 2019; Özer *et al*., 2020b). In Kazakhstan, the dominant root rot pathogens are *B. sorokiniana* and *Fusarium* spp., as confirmed in collaboration with CIMMYT scientists (Özer *et al*., 2023a; Alkan *et al*., 2021). In addition to studying the pathogens of root rot, our previous research has focused on the genetic resistance of wheat to other important diseases, such as stem and leaf rust (Kokhmetova and Atishova, 2012; Gultyaeva *et al*., 2020; Olivera *et al*., 2022; Kokhmetova *et al*., 2020a; Kumarbayeva *et al*., 2025). Expanding our understanding of biotic stresses, we have also investigated the genotyping of wheat for resistance to leaf spot diseases (Kokhmetova *et al*., 2021; Kokhmetova and Atishova, 2020) and to toxins produced by Pyrenophora tritici-repentis, the causal agent of leaf spot diseases (Kokhmetova *et al*., 2019; Kokhmetova *et al*., 2020b). The current study on common root rot complements and extends this body of work by addressing another major biotic stress affecting wheat in Kazakhstan. By integrating pathogen diversity assessment, resistance genotyping, and field evaluation, this research aims to fill existing gaps and contribute to the development of wheat cultivars with broad-spectrum and durable resistance.

Despite the scale of the problem, effective solutions to reduce the prevalence of *B. sorokiniana* and *Fusarium spp.* in the field remain scarce. Breeding resistant varieties using molecular markers, as well as evaluating agronomic and chemical protection methods, are important areas of research in the field of phytopathology. In 2023, we studied 80 spring wheat samples, which revealed the dominance of *Fusarium spp.* (49 isolates) and *B. sorokiniana* (27 isolates), with the highest infection intensity observed in the Aktobe region, where prevalence reached 100% and development - 60% (Dutbayev, 2024). Recent studies have identified wheat genotypes resistant *to B. sorokiniana*, using molecular markers for the *Sb1* and *Sb2* genes, which have significant implications for breeding programs (Bolatbekova *et al*., 2026).

Given the relevance of the problem, this study included a comprehensive assessment of the resistance of 30 varieties and lines of spring bread and durum wheat to common root rot in two regions of Kazakhstan - the Almaty and Aktobe regions. The experiment included three background conditions:

(1) Fungicide treatment
(2) Artificial inoculation with *B. sorokiniana*
(3) Natural infectious background

The resistance analysis was carried out taking into account the agronomic indicators of plants and genetic markers associated with resistance to the pathogen.

This study aims to evaluate the resistance of spring wheat to common root rot in Kazakhstan by analyzing the influence of genetic factors (*Sb1* and *Sb2* genes) and environmental conditions, and to assess the effectiveness of integrated plant protection strategies in reducing disease impact. The results obtained will substantiate strategies for integrated control of common root rot and increase the productivity of spring wheat.

Materials and Methods

In 2024, the resistance of a collection of 30 best wheat varieties and lines to common root rot was assessed in the field at two sites: The Kazakh Research Institute of Agriculture and Plant Growing in Almaty region (43.237589, 76.692629) and the Kazakh Research Institute of Horse Breeding and Forage Production in Aktobe region (50° 16' 60.00" N, 57° 09' 60.00" E). The experiments were carried out in triplicate, with each plot area being 1 m². Field trials were conducted in three replications, with each plot measuring 3 m². Three experimental backgrounds were tested:

(1) Plots treated with fungicides during the growing season
(2) Plots infected with *B. sorokiniana* conidia after sowing
(3) Plots under natural conditions

Root rot assessment was performed on ten plants per plot (n = 10), with each plot covering an area of 3 m² and arranged in three replications. The following commercial varieties of spring bread wheat were used as standard varieties: Aktobe 39, Alb., Dynastiya, Lut., and Kazakhstanskaya 10. Additionally, spring durum wheat varieties included Kargala 69 and Gordeyforme 254. A total of 30 wheat genotypes were selected based on preliminary field evaluations of productivity and resistance.

The number of replications (n = 10) was determined by field layout constraints and experience, which demonstrated sufficient variability for valid statistical analysis. While a formal power analysis was not

performed, it is planned for future studies to enhance experimental design.

The severity of root rot was assessed on 10 randomly selected plants per plot at the stages of tillering and of full plant maturity by analyzing grain from 50 plants with varying degrees of damage. The degree of damage was assessed visually on a four-point scale: from healthy plants to severely damaged. Several agronomic traits were measured, including the number of stems, plant height, ear length, number of spikelets, grain weight from 50 plants, and 1000-grain weight (calculated from two samples of 500 grains each) (Kharipzhanova *et al*., 2024; Dutbayev *et al*., 2023).

The prevalence of common root rot (P) was calculated using the formula:

$$P = (N / n) \times 100,$$

Where N is the number of diseased plants and n is the total number of plants in the sample.

The development of root rot (R) was determined using the formula:

$$R = (\Sigma a \times b) / (A \times K) \times 100,$$

Where A is the total number of plants in the sample, a is the number of plants with specific damage, b is the corresponding damage score, and K is the maximum possible damage score (Kharipzhanova *et al*., 2024). The harmfulness of root rot (B) was calculated as the percentage reduction in the yield of diseased plants compared to healthy ones using the formula:

$$B = [(a–b) / a] \times 100,$$

Where a is the yield of healthy plants and b is the yield of diseased plants (Kharipzhanova *et al*., 2024).

All data were statistically analyzed using ANOVA and regression models. Before analysis, the assumptions of normality and homogeneity of variance were verified using the Kruskal–Wallis test and Pearson correlation test, respectively. All statistical procedures were performed using R and RStudio software (Aphalo, 2017; Dutbayev *et al*., 2022a). This approach ensured reliable statistical evaluation of the data and provided valuable results on the resistance of wheat genotypes to common root rot.

Molecular Genetic Analysis

Genomic DNA was isolated from plant material from 5-day-old wheat seedlings using the CTAB method (Chen *et al*., 2003). The concentration and purity of the obtained genomic DNA were measured using a NanoDrop One spectrophotometer, and the DNA concentration for PCR was adjusted to 20 ng/µL. Primers associated with *Sb* genes were used according to approved protocols. Polymerase Chain Reaction (PCR) was performed using primers and annealing temperatures specified for each *Sb* gene in the references. To identify carriers of resistance genes, the Polymerase Chain Reaction (PCR) method was used according to the protocol of Chen *et al*. (1998). Amplification was performed in a BioRad amplifier (Eppendorf, Germany), and the amplification programs were selected depending on the identified resistance gene. The PCR reaction mixture (25 µl) contained 2.5 µl genomic DNA, 1 µl of each primer (1 pM/ µl) (SigmaAldrich, USA), 2.5 µl dNTP mixture (2.5 mM, dC, dG, dT, and dATP) (ZAO Sileks, Russia), 2.5 µl MgCl2 (25 mM), 0.2 µl Taq polymerase (5 units in µl) (ZAO Sileks, Russia), 2.5 µl 10X PCR buffer, and 12.8 µl ddH20. The amplification products were separated in a 2% agarose gel in TBE buffer (45 mM Tris-borate, 1 mM EDTA, pH 8 with the addition of ethidium bromide. A 100-bp DNA marker (Fermentas, Lithuania) was used to determine the length of the amplification fragment. The results were visualized using a gel documentation system (Gel Doc XR+, BIO-RAD, Hercules, USA).

Molecular screening of wheat samples for potential root rot resistance genes. Wheat leaves can inhibit infection by various pathogens using similar molecular approaches mediated by resistance genes (Lagudah *et al*., 2006). In this regard, molecular markers for these genes were used in our studies. To identify carriers of the *Sb1* gene, PCR analyses of wheat genotypes were performed using STS marker *csLV34* (Lagudah *et al*., 2009). To determine carriers of the *Sb2* gene, PCR analysis was performed using the SSR marker *Xfcp623* (Gupta *et al*., 2018; Zhang *et al*., 2009).

Results

Descriptive Analysis of Common Root Rot in Spring Wheat

Table 1 presents the descriptive statistics of spring bread and durum wheat across two regions of Kazakhstan in 2024, along with the influence of various environmental and experimental factors on plant structural traits. In bread wheat, the prevalence varied from 0 to 100%, and development from 0 to 50%, with medians of 40 and 10%, respectively. The indicators reached 100 and 50% in durum wheat, and the medians were 50 and 15%. In addition, the influence of various factors on the structural indicators of spring bread and durum wheat was established. It was found that regional conditions significantly affected the plant parameters.

Regional conditions significantly influenced plant traits. In the Aktobe region, higher tillering (2.32), but smaller stem length (77.07 cm) and grain weight (28.03 g)

were observed, compared to the Almaty region, where the stem length reached 81.29 cm, and the grain weight was 11.51 g. Differences in all indicators were statistically significant ($P < 0.01$).

Impact of Environmental and Experimental Factors on Disease Severity

The influence of various factors on the infestation of spring bread wheat with common root rot was established. The analysis showed significant differences between the regions: in the Aktobe region, the infestation reached 55.21% (spread) and 18.85% (development), while in the Almaty region, the rates were lower - 23.21 and 6.48%, respectively ($P < 0.01$). The stages of plant development also affected the infestation: the greatest infestation was observed during harvesting (57.92 and 17.43%), and the least at the tillering stage (23.03 and 8.64%, $P < 0.01$). The use of a fungicide background reduced the infestation (24.04 and 7.24%), while the infectious background caused the maximum rates (52.86 and 16.89%, $P < 0.01$). Wheat genotypes with resistance genes such as *Sb1* and *Sb2* showed lower disease incidence, while lines without resistance genes had higher rates of disease prevalence and development. These data highlight the importance of an integrated approach, including regional conditions, growth stages, agronomic backgrounds, and plant genotype for effective protection against root rot (Table 2).

Genotypes with resistance genes demonstrated a lower level of root rot infection: prevalence rates ranged from 33.48 to 42.50%, and development rates ranged from 9.89 to 12.81%. At the same time, genotypes without resistance genes, such as #401/k-52304 WW14753, lut., Stepnaya 2, lut., showed higher rates: prevalence reached 53.33%, and the development rate was 19.58%. This emphasizes the effectiveness of using resistance genes in reducing root rot infection in wheat (Table 2, Figure 1).

Table 1: Descriptive statistics indicators of the indices of infestation of spring bread and durum wheat with common root rot in 2024

Minimal	1st Quantile	Median	Mean	3rd Quantile	Maximal
Bread wheat, prevalence of root rot, %					
0.0	10.0	40.0	39.5	60.0	100.0
Bread wheat, root rot development, %					
0.0	2.5	10.0	12.8	20.0	50.0
Durum wheat, prevalence of root rot, %					
0.0	10.0	50.0	50.0	80.0	100.0
Durum wheat, root rot development, %					
0.00	2.5	15.0	15.5	25.0	50.0
Kazakh Research Institute of Horse Breeding and Forage Production, Aktobe region; Kazakh Research Institute of Agriculture and Plant Growing, Almaty region					
Bushiness (units)					
1.0	1.0	2.0	1.8	2.0	6.0
Stem length cm					
2.0	73.0	80.0	79.9	88.0	112.0
Spike length cm					
3.0	6.0	7.0	7.3	8.0	13.0
Number of spikelet units					
3.0	11.0	13.0	13.3	15.0	22.0

Table 2: The influence of various factors on the infestation of spring bread wheat with common root rot (Kazakh Research Institute of Horse Breeding and Forage Production, Aktobe region; Kazakh Research Institute of Agriculture and Plant Growing, Almaty region, 2024)

Factor	Spreading, %	Development, %
Region		
Kazakh Research Institute of Horse Breeding and Forage Production, Aktobe region	55.21	18.85
Kazakh Research Institute of Agriculture and Plant Growing, Almaty region	23.21	6.48

P - value	<0.01	<0.01	
Time			
Harvesting	57.92	17.43	
Tillering	23.03	8.64	
P - value	<0.01	<0.01	
Background			
fungicidal	24.04	7.24	
infectious	52.86	16.89	
natural	42.38	14.22	
C	<0.01	<0.01	
Line			Presence of genes
#401/k-52304 WW14753, lut.	53.33	19.58	none
#410/k-57729 Celinnaya ubil.lut.	42.50	12.81	*Sb2*
#436/Line 1616 ae 14, lut.	33.48	9.89	*Sb2*
#448/Orenburgskaya 23, lut.	42.12	12.98	*Sb2*
Aktobe 39, alb. Standart	36.52	11.83	*Sb2*
Dynastiya, lut. Standart	37.83	11.63	*Sb2*
Kazakhstanskaya 10, Standart	32.50	10.63	no information
Line 1415м	37.83	12.72	*Sb2*
Line 205м	37.39	13.04	*Sb2*
Line R-1413м	34.78	10.65	*Sb2*
Stepnaya 2, lut.	36.96	11.63	none
Stepnaya 50, alb.	37.39	12.39	*Sb2*
Ekada 113, alb.	33.48	11.41	*Sb2*
P - value	<0.01	<0.01	

The results show that the incidence of common root rot in spring durum wheat was significantly higher in the harvesting phase (69.21% prevalence, 20.62% development) compared to the tillering phase (28.20 and 9.72%, respectively). Interregional differences are also significant: in the Aktobe region, the incidence was 71.48% and development was 23.88%, while in the Almaty region, the figures were lower (32.02 and 8.62%). Backgrounds also had a significant impact: fungicide background reduced the incidence (36.58 and 11.92%), while infectious background contributed to the maximum values (61.94 and 19.03%).

Genotypes with resistance, such as #565/k-65743 Bezenchukskaya zolotistaya, gord. *(Sb2* – 47.69%/13.85%), #575 (*Sb1*, *Sb2* – 54.62/19.04%), and Line 242/243 (*Sb2* – 60.77/19.65%), demonstrated lower or comparable infestation compared to lines without resistance, such as #512/L-3599, eritromelan (48.00/15.25%), and #513/k-65134 Melodiya Dona, gord. (46.92/13.08%). However, some genotypes without resistance had higher infestation, such as the Yantarnaya 150, leukomelan (53.85/18.23%). These data confirm the influence of genotype and environmental factors on infestation (Table 3).

The treatment background also had a significant effect. The best stem length (84.24 cm) and grain weight (16.96 g) were observed against the fungicide background. Against the infectious background, the parameters decreased to 73.55 cm and 14.39 g, respectively, which confirms the negative impact of pathogens. The differences between bread and durum wheat were also significant: bread wheat demonstrated higher tillering (1.91) and grain weight (19.28 g), while durum wheat was superior in stem length (81.98 cm) and the number of spikelets (14.02).

Genetic Resistance and Disease Reduction

Genotypes with resistance, such as *Sb1* and *Sb2,* showed stable plant structure parameters; for example, line #575/k-65351 Voronejskaya 9, gord. (*Sb1*) showed a stem length of 79.00 cm and a grain weight of 9,67 g. However, some genotypes without resistance (e.g., line #513) showed high productivity in some parameters, including grain weight (25.08 g). These results highlight the importance of an integrated consideration of environmental factors, treatment background, and genetic resistance in improving wheat productivity (Table 4).

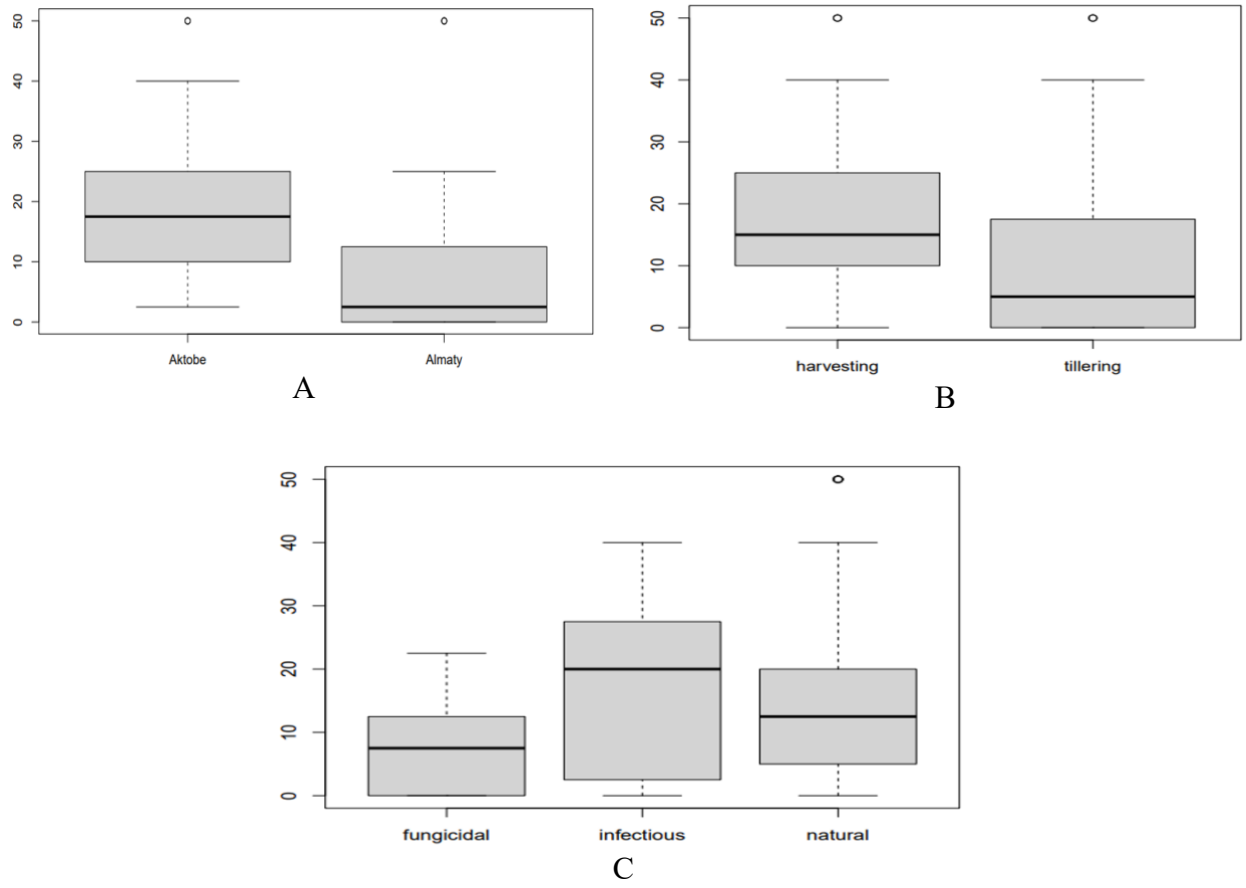


Fig. 1: Boxplot of the influence of various factors on the development of common root rot of spring bread wheat, % (Kazakh Research Institute of Horse Breeding and Forage Production, Aktobe region; Kazakh Research Institute of Agriculture and Plant Growing, Almaty region, 2024)

Note:
A Factor research region
B Factor plant development phase
C Factor background

Table 3: The influence of various factors on the infestation of spring durum wheat with common root rot (Kazakh Research Institute of Horse Breeding and Forage Production, Aktobe region; Kazakh Research Institute of Agriculture and Plant Growing, Almaty region, 2024)

Factor	**Spreading, %**	**Development, %**
Time		
Harvesting	69.21	20.62
Tillering	28.20	9.72
P - value	<0.01	<0.01
Region		
Kazakh Research Institute of Horse Breeding and Forage Production, Aktobe region	71.48	23.88
Kazakh Research Institute of Agriculture and Plant Growing, Almaty region	32.02	8.62
P - value	<0.01	<0.01
Background		
Fungicidal	36.58	11.92
Infectious	61.94	19.03
Natural	51.30	15.68
P - value	<0.01	<0.01

Line			Presence of gene
#512/L-3599, eritromelan	48.00	15.25	none
#513/k-65134 Melodiya Dona, gord.	46.92	13.08	none
#518/Serke, gord.	48.46	14.23	*Sb2*
#545/Celinnica, melanopus	51.54	15.58	*Sb2*
#546/Melyana, melanopus	51.54	16.35	*Sb2*
#551/Gordeyforme 18585-2, gord.	52.31	17.12	*Sb2*
#565/k-65743 Bezenchukskaya zolotistaya, gord.	47.69	13.85	*Sb2*
#575/k-65351 Voronejskaya 9, gord.	54.62	19.04	*Sb1, Sb2*
Gordeyforme 254, Standard	46.43	15.89	no information
Kargala 69, gord. Standart	46.85	14.69	*Sb2*
Line 242/243	60.77	19.65	*Sb2*
Line 247/257	46.15	13.46	*Sb2*
Line 248/255	43.08	13.27	none
Line P-1409т	43.08	12.50	*Sb2*
Orenburgskaya 10, gord.	52.31	16.92	*Sb2*
Yantarnaya 150, leukomelan	53.85	18.23	none
Yantarnaya 160, gordeyforme	50.77	15.19	none
Yantarnaya 60, gord.	51.54	15.00	*Sb2*
Yantarnaya 70, gordeyforme	53.85	16.73	none
P - value	<0.01	<0.01	

Table 4: The influence of various factors on the structure of spring wheat (Kazakh Research Institute of Horse Breeding and Forage Production, Aktobe region; Kazakh Research Institute of Agriculture and Plant Growing, Almaty region, 2024)

	Bushiness, units	**Stem length, cm**	**Spike length, cm**	**Number of spikelets, units**	**Weight_grain 10 plants, gram**
Region					
Kazakh Research Institute of Horse Breeding and Forage Production, Aktobe region	2.32	77.07	7.31	11.55	28.03
Kazakh Research Institute of Agriculture and Plant Growing, Almaty region	1.53	81.29	7.23	14.14	11.51
P - value	<0.01	<0.01	0.016	0.01	<0.01
Background					
fungicide	1.80	84.24	7.51	13.59	16.96
infectious	1.55	73.55	7.09	12.71	14.39
natural	2.00	81.66	7.16	13.56	19.14
	<0.01	<0.01	<0.01	<0.01	<0.01
Wheat					
bread	1.91	78.71	7.53	12.90	19.28
durum	1.55	81.98	6.76	14.02	12.40
	<0.01	<0.01	<0.01	<0.01	<0.01
Disease degree					
healthy	1.82	85.22	7.52	13.97	
1 degree	1.72	76.31	7.09	12.98	
2 degree	2.00	71.50	6.74	11.27	
healthy	<0.01	<0.01	<0.01	<0.01	

Wheat	**Line**					**Presence of gene**
bread	#401/k-52304 WW14753, lut.	1.88	67.52	7.50	13.70	14.80	*none*
bread	#410/k-57729 Celinnaya ubileinaya., lut.	2.08	79.92	7.65	13.57	15.43	*Sb2*
bread	#436/Line 1616 ae 14, lut.	1.85	78.69	7.70	13.45	22.73	*Sb2*

bread	#448/Orenburgskaya 23, lut.	1.89	77.20	8.31	13.70	18.57	*Sb2*
durum	#512/L-3599, eritromelan	1.80	83.17	7.23	15.70	14.35	*none*
durum	#513/k 65134 Мелодия Дона, горд.	1.90	79.87	6.83	13.90	25.08	*none*
durum	#518/Serke, gord.	1.80	87.38	6.53	14.00	21.67	*Sb2*
durum	#545/Celinnica, melanopus	1.73	81.97	6.50	13.73	20.20	*Sb2*
durum	#546/Melyana, melanopus	1.60	84.15	7.60	14.80	12.27	*Sb2*
durum	#551/Gordeyforme 18585-2, gord.	1.67	81.89	7.18	15.18	13.37	*Sb2*
durum	#565/k-65743 Bezenchukskaya zolotistaya, gord.	1.53	79.40	6.63	13.77	12.27	*Sb2*
durum	#575/k-65351 Voronejskaya 9, gord.	1.57	79.00	6.60	14.27	9.67	*Sb1, Sb2*
bread	Aktobe 39, alb. Standart	2.10	81.60	7.67	12.72	21.92	*Sb2*
bread	Dynastiya, lut. Standart	1.77	75.00	6.85	12.37	11.20	*Sb2*
durum	Kargala 69, gord. Standart	2.00	91.30	6.00	13.40	20.07	*Sb2*
bread	Line 1415м	2.05	79.75	7.25	12.43	18.55	*Sb2*
bread	Line 205м	1.78	81.82	7.52	12.92	8.80	*Sb2*
durum	Line 242/243	1.70	78.30	6.63	14.30	9.10	*Sb2*
durum	Line 247/257	1.40	79.73	6.60	13.40	15.07	*Sb2*
durum	Line 248/255	1.30	78.33	6.67	13.57	8.67	*none*
durum	Line P-1409т	1.23	84.13	6.90	13.37	14.80	*Sb2*
bread	Line P-1413м	1.90	81.18	6.77	12.37	15.43	*Sb2*
durum	Orenburgskaya 10, gord.	1.37	80.60	7.00	13.77	22.13	*Sb2*
bread	Stepnaya 2, lut.	2.03	84.00	7.68	13.22	23.34	*none*
bread	Stepnaya 50, alb.	2.19	76.40	7.03	11.97	20.04	*Sb2*
bread	Ekada 113, alb.	1.87	79.52	7.78	13.50	18.57	*Sb2*
durum	Yantarnaya 150, leukomelan	1.27	83.20	7.67	14.70	10.87	*none*
durum	Yantarnaya 160, gordeyforme	1.20	82.53	6.47	13.67	17.80	*none*
durum	Yantarnaya 60, gord.	1.57	79.43	6.33	12.57	14.35	*Sb2*
durum	Yantarnaya 70, gordeyforme	1.45	86.29	6.13	14.16	25.08	*none*
		<0.01	<0.01	<0.01	<0.01	<0.01	

The correlation matrix demonstrates the relationship between the stem length, spike length, and spikelet number of spring wheat. A significant positive correlation was found between the stem length and spikelet length ($r = 0.38$, $P < 0.01$), as well as between the stem length and spikelet number ($r = 0.45$, $P < 0.01$). This indicates that an increase in stem length can be accompanied by an increase in these parameters. A stronger correlation was noted between spike length and spikelet number ($r = 0.61$, $P < 0.01$), which emphasizes the close relationship between these characteristics. The data obtained confirm the importance of taking into account the relationships between structural indicators to improve wheat productivity under different conditions.

To identify carriers of the *Sb2* gene, PCR amplification was performed using *Xfcp-623* primers. The isogenic line Glenlea was used as a negative control. The SSR marker *Xfcp-623* amplified a 380 bp fragment associated with the dominant allele of the *Sb2* gene, which is linked to susceptibility to root rot pathogens, in the following eight genotypes: #401/k-52304 WW14753, lut., #512/L-3599, erythromelan, #513/k 65134 Melodiya Dona, horde., Line 248/255, Stepnaya 2, lut., Yantarnaya 150, leucomelan., Yantarnaya 160, hordeiform., Yantarnaya 70, hordeiform. In the remaining genotypes, this fragment was not amplified, indicating the presence of a null allele, which is associated with the recessive *Sb2* allele conferring insensitivity to the root rot pathogen *B. sorokiniana* (Figure 2).

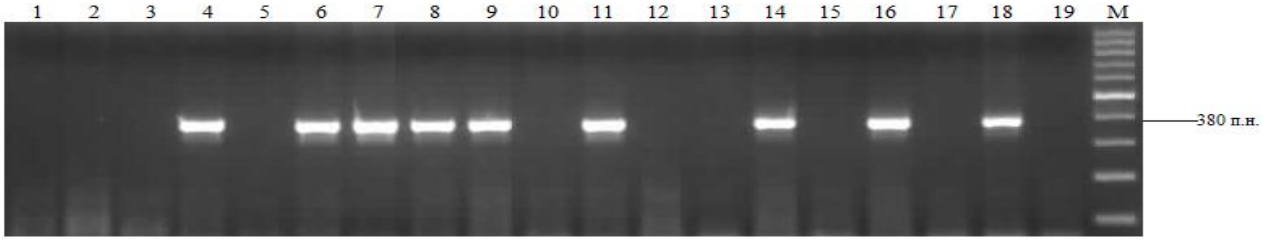
The absence of amplification of the 380 bp fragment in 22 wheat genotypes indicates the presence of a null allele at the *Sb2* locus, suggesting the absence of the dominant susceptibility-associated allele. A similar pattern was observed in the molecular analysis of the following genes: #410/k-57729 Celinnaya ubileinaya., lut., #436/Line 1616 ae 14, lut., #448/Orenburgskaya 23, lut., #518/Serke, gord., #545/Celinnica, melanopus, #546/Melyana, melanopus, #551/Gordeyforme 18585-2, gord., #565/k-65743 Bezenchukskaya zolotistaya, gord.,

#575/k-65351 Voronejskaya 9, gord., Aktobe 39, alb. Standart, Dynastiya, lut. Standart, Kargala 69, gord. Standart, Line 1415м, Line 205м, Line 242/243, Line 247/257, Line P-1409т, Line P-1413м, Orenburgskaya 10, gord., Stepnaya 50, alb., Ekada 113, alb., Yantarnaya 60, gord.

The presence of the *Sb1* gene in wheat genotypes was investigated using STS marker *csLV34*. Of the 30 varieties and lines, one genotype amplified fragments of 150 bp, indicating the presence of the *Sb1* resistance gene. This genotype is #575/k-65351 Voronezhskaya 9.

Discussion

The wide range of prevalence (0–100% for prevalence and 0–50% for development) reflects high variability of wheat resistance to root rot. This is consistent with studies that consider root rot as one of the most widespread and damaging diseases, especially under monoculture conditions and poor crop rotation practices (Smiley *et al*., 2005). The high median values (40–50% for prevalence and 10–15% for development) highlight that even under favorable conditions, the disease remains a significant threat. This confirms the need for the development of resistant varieties and the implementation of integrated crop protection systems (Moya-Elizondo *et al*., 2011). These results underscore the urgency of developing resistant varieties and implementing integrated crop protection strategies.



1 - #410/k-57729 Celinnaya ubileinaya, lut; 2 - #436/Line 1616 ae 14, lut.; 3 - #448/Orenburgskaya 23, lut; 4 - #401/k-52304 WW14753, lut; 5 - #518/Serke, gord; 6 - #512/L-3599, erythromelane; 7 - #513/k 65134 Melodiya Dona, gord; 8 - Line 248/255; 9 - Stepnaya 2, gord; 10 - #545/Celinnisa, melanopus; 11 – Yantarnaya 150, leucomelan; 12 – #546/Meliana, melanopus; 13 – #551/Gordeiforme 18585-2, gord.; 14 – Yantarnaya 160, gordeiforme; 15 – #565/k-65743 Bezenchukskaya zolotistaya, gord.; 16 – Yantarnaya 70, gordeiforme; 17 – #575/k-65351 Voronezhskaya 9, gord.; 18 – Glenlea (negative control); 19 – Salamouni (positive control); M – molecular weight marker (Gene Ruler 100 bp DNA Ladder).

Fig. 2: DNA amplification products of wheat samples using primers to the *Xfcp-623* locus linked to the *Sb2* resistance gene

In our research, the regional differences were evident, with higher disease pressure in the Aktobe region (55.21% prevalence and 18.85% development) compared to Almaty (23.21 and 6.48%). These contrasts may be explained by variations in soil and climatic conditions. In Aktobe, arid climate and low soil fertility may contribute to plant stress, increasing susceptibility to pathogens (Cook, 2003). In Almaty, more favourable conditions (high humidity, fertile soils) may reduce infection rates. This is consistent with studies showing that drought and nutrient deficiencies increase root rot incidence (Paulitz *et al*., 2002).

The highest infestation recorded at the harvesting stage (57.92% prevalence, 17.43% development) may reflect cumulative infection over time**,** consistent with previous findings that root rot severity increases with plant age, especially under stress conditions (Smiley *et al*., 2005). Conversely, the lowest levels during the tillering stage (23.03 and 8.64%) may be due to active root system development, which offers stronger early resistance. The reduced infestation observed under fungicide treatment (24.04 and 7.24%) supports the effectiveness of chemical protection. However, the high severity under artificial infection (52.86 and 16.89%) illustrates the limitations of chemical methods alone**,** reinforcing the need for integrated approaches that combine chemical, cultural, and biological controls (Cook, 2003).

The current findings align with recent large-scale surveys in Kazakhstan, which identified *B. sorokiniana* and *Fusarium spp.* as dominant pathogens causing wheat root and crown rot. Bozoğlu *et al*. (2022) reported that among 1221 fungal isolates collected across 65 sites in central, eastern, and southeastern Kazakhstan, *B. sorokiniana* (44.80%) and *F. acuminatum* (20.39%) were the most common, occurring in over 85% of surveyed fields. Several additional pathogens (*F. pseudograminearum*, *C. spicifera*, *C. inaequalis*) were identified for the first time in Kazakhstan, emphasizing the complexity of root rot etiology. In pathogenicity tests, *B. sorokiniana* and *F. culmorum* were among the most virulent species. These findings underscore the urgent need for comprehensive screening and resistance breeding programs adapted to local pathogen populations.

Genotypes with resistance genes (*1* and *Sb2*) showed lower infestation (37,39–48.26% prevalence and 12.39–15.54% development), confirming their potential for breeding resistant varieties. This is consistent with studies where resistance genes have been shown to be effective in reducing root rot infestation (Miedaner *et al*., 2001). However, differences were observed even among resistant genotypes, which may be due to the influence of other genes or environmental factors. This highlights the need for further investigation of the genetic mechanisms of resistance and their interaction with growing conditions (Buerstmayr *et al*., 2012).

The obtained results indicate that among the studied wheat varieties and lines, genotypes carrying the *Sb1* and *Sb2* resistance genes were identified, which is important for breeding work and increasing resistance to diseases. Amplification products characteristic of the *Sb2* gene were detected in 22 varieties, confirming the presence of this susceptibility-associated gene. In contrast, five genotypes showed amplification patterns corresponding to the *Sb1* gene, indicating their potential resistance to pathogens. Voronezhskaya 9 turned out to be the only genotype carrying both genes, which makes it a promising object for further breeding. These data emphasize the importance of molecular genetic analysis in the wheat breeding program aimed at creating highly productive and resistant varieties, which is especially important in the context of a changing climate and increasing pressure from phytopathogens.

The results of the conducted research emphasize the importance of using genetic analysis methods in agriculture to develop high-yielding and disease-resistant wheat varieties. The data obtained confirm the effectiveness of using molecular genetic methods in wheat breeding.

The results of this study reinforce the importance of molecular screening in breeding strategies. This is supported by Qalavand *et al*. (2023), who demonstrated that resistance to *B. sorokiniana* is associated with elevated activity of defense-related enzymes, including peroxidase and β-1, 3-glucanase. Our findings also suggest that wheat and barley treated with chemical or biological agents show enhanced defense responses, supporting healthy growth and limiting fungal colonization. Kharipzhanova *et al*. (2025) confirmed this using Koch's postulates and molecular diagnostics, showing the effectiveness of biocontrol agents such as Phytosporin-M and Sporobacterin-Rassada in suppressing infection.

Additional research in Kazakhstan supports the importance of early-stage protection. For example, Dababat and Fourie (2018) and Khan *et al*. (2020) highlight the importance of seed treatment and the development of resistant cultivars as the most sustainable disease control methods. Moreover, national efforts have focused on root rot management in cereals through artificial inoculation trials (Dutbayev *et al*., 2022a) and disease monitoring across cereal nurseries (Morgounov *et al*., 2018).

Recent advancements in molecular biology and nanobiotechnology highlight the growing potential of biosensing technologies for the early detection and management of diseases. Although these approaches are primarily developed in biomedical contexts, their application in agricultural research is increasingly being explored. For instance, biosensor devices designed for early cancer biomarker detection (Chupradit *et al*., 2022) and bioactive polymeric materials used in regenerative medicine (Atia *et al*., 2023) illustrate how precise molecular diagnostics can be developed. These innovations offer promising directions for creating advanced tools in plant pathology (Dutbayev *et al*., 2020), particularly for studying root rot pathogens such as *Fusarium*. Future research should explore how such technologies could be adapted for field-based monitoring in cereals, contributing to more efficient and timely plant protection strategies.

Regional differences and the effect of treatment backgrounds on structural parameters (stem length, grain weight) confirm that root rot not only reduces yield but also worsens plant quality. In the Aktobe region, higher tillering (2.32) may be associated with compensatory mechanisms of plants in response to stress, while smaller stem length (77.07 cm) and grain weight (28.03 g) may be the result of limited access to nutrients and water (Paulitz *et al*., 2002). Against the fungicide background, the improvement in parameters (stem length 84.24 cm, grain weight 16.96 g) confirms the positive effect of plant protection on their productivity.

The positive correlation between stem length, spike length, and spikelet number ($r = 0.38–0.61$) indicates a relationship between these parameters and plant productivity. This is consistent with studies where improvement of structural parameters contributed to increased yield (Slafer *et al*., 2015). The stronger correlation between spike length and spikelet number ($r = 0.61$) emphasizes that these parameters are closely related and can be used as markers for breeding high-yielding varieties.

These sources confirm the relevance and scientific validity of the obtained results, and also emphasize the need for further research to develop resistant varieties and effective plant protection methods.

Collectively, these findings confirm the scientific robustness of our results and the need for continued research on resistance mechanisms and effective crop protection strategies. In conclusion, the findings demonstrate that resistance to common root rot in

Kazakhstan varies by genotype and region, and is enhanced by the presence of *Sb* genes and integrated management. The study contributes valuable data to the broader effort of protecting wheat and barley yields, ensuring food security under the increasing pressure of phytopathogens and climate change.

Conclusion

This study examines how genetic resistance (*Sb1* and *Sb2*) and environmental factors affect spring wheat's response to root rot in Kazakhstan. The study confirmed the high vulnerability of spring bread and durum wheat to common root rot, with damage reaching 100% in distribution and 50% in development. It was found that damage varies depending on the region, plant development phase, and agronomic backgrounds. The highest damage was observed in the Aktobe region (55.21% distribution and 18.85% development) and during the harvesting phase (57.92 and 17.43%), which emphasizes the influence of soil and climatic conditions and plant growth stage on the development of the disease. The use of a fungicide background significantly reduced damage (24.04 and 7.24%), confirming the effectiveness of chemical methods, but high damage against an infectious background (52.86 and 16.89%) indicates the need for an integrated approach to plant protection.

Genotypes with resistance genes *Sb1* and *Sb2* demonstrated lower infestation (37,39–48.26% prevalence and 12.39–15.54% development), which confirms their potential for breeding resistant varieties. Root rot also negatively affected plant structural parameters, such as stem length and grain weight, especially against the infectious background. Correlation analysis revealed a close relationship between stem length, spike length, and spikelet number (r = 0.38–0.61), which emphasizes the importance of taking these parameters into account to improve productivity.

The study revealed wheat genotypes with resistance genes *Sb1* and *Sb2,* which confirms their potential resistance to phytopathogens and value for breeding work. The molecular screening revealed the presence of *Sb2*-associated alleles in 22 wheat genotypes, and *Sb1*-associated alleles in one genotype. Notably, the variety Voronezhskaya 9 carried both *Sb1* and *Sb2* genes, highlighting its potential value as a donor of resistance traits in breeding programs. The results emphasize the importance of molecular genetic analysis in breeding programs aimed at creating highly productive and resistant varieties, which is especially important in the context of a changing climate and increasing pressure from phytopathogens.

Based on the data obtained, it is recommended to continue work on introducing resistance genes into breeding programs, optimize agronomic practices, and take into account regional characteristics when developing plant protection strategies. An integrated approach combining chemical, agronomic, and biological methods is the most effective way to reduce infestation and increase yields. The results obtained highlight the need for further research to develop resistant varieties and protection methods, which will reduce economic losses and improve food security.

Acknowledgment

We express our gratitude to the master's students of the Department of Fruit and Vegetable Growing, Plant Protection and Quarantine of the Kazakh National Agrarian Research University, Akniet Tursyn and Aigerim Baktygizova, for their assistance in processing field data.

Funding Information

The study was supported by the Ministry of Education and Science of the Republic of Kazakhstan, grant No. AP19676202 “Identification of sources of resistance to wheat root rot caused by *Bipolaris sorokiniana* and *Fusarium culmorum* using phytopathological and molecular approaches”. Study design, data collection, analysis, decision to publish, and manuscript preparation were carried out independently of the sponsors.

Authors' Contributions

Yerlan Dutbayev: Performed data analysis, formulated conclusions, and contributed to writing and editing of the manuscript.

Alma Kokhmetova: Coordinated the research group and authored sections of the manuscript related to phytopathology and genetics.

Ardak Bolatbekova: Assessed plant resistance, performed molecular identification of the pathogen, applied molecular markers *Sb1* and *Sb2*, processed statistical data, calculated disease prevalence and harmfulness, and authored relevant sections of the manuscript.

Aidana Kharipzhanova: Isolated *B. sorokiniana* strains, carried out morphological assessment of the pathogen, performed laboratory screening of wheat samples for resistance, identified pathogen species, and contributed to writing sections on microbiology and phytopathology.

Madina Kumarbayeva: Collected infectious material, isolated pathogen strains, conducted molecular genetic screening of wheat germplasm, worked with molecular markers, and contributed to writing sections on molecular genetics and phytopathology.

Vladimir Tsygankov and Artem Tsygankov: Monitored the prevalence and progression of root rot in the Aktobe region, selected resistant varieties and lines, and participated in field observations and breeding activities.

Zhenis Keishilov and Sholpan Bastaubayeva: Monitored disease development in the Almaty region, selected resistant varieties and lines, and contributed to assessing the impact of agrotechnical factors.

Kanat Bakhytuly and Aidana Sabitova: Conducted fieldwork (sowing and harvesting), collected infectious material in the Almaty region, assessed the phytopathological condition of plants, monitored root rot development in both Almaty and Aktobe regions, selected resistant lines, and contributed to writing sections related to field trials.

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