Brazilian Journal of Biology®

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

Original Article

Identification and management of *Bipolaris sorokiniana* in wheat and barley in Southeast Kazakhstan

Identificação e manejo de *Bipolaris sorokiniana* em trigo e cevada no sudeste do Cazaquistão

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Abstract

Wheat and barley serve as significant nutrient-rich staples that are extensively grown on a global scale, spanning over 219 million hectares. The annual combined global yield is 760.9 million tons, with Kazakhstan contributing 14.3 million tons of wheat and 3.83 million tons of barley to this total. The productivity of grain crops has declined annually due to fungal disease, especially root and crown rot caused by *Bipolaris sorokiniana* and *Fusarium spp*. Research has focused on pinpointing the pathogens responsible for common root rot in various types of wheat and barley grown in Southeast Kazakhstan. The main goal was to examine the efficacy of certain chemical and biological substances in safeguarding barley seedlings during the early growth stage against root rot root rot. Moreover, this study sought to gauge their effects on seed quality by examining aspects such as germination rates, the colonization of seeds by particular fungal pathogens, and the overall vitality of seeds and seedlings. Visual inspection of the plants revealed that the prevalence of *B. sorokiniana* was an average of 51.8%, and that of *Fusarium* species was 58.6%. Three isolates were obtained from the roots of the winter wheat promising line 231, three from the spring wheat roots of the Kazakh variety 10, four from the winter wheat variety Steklovidnaya variety 24, fourteen from the spring barley variety Symbat, and fourteen from the winter barley variety Aidyn variety 2. The external spread of common root rot on spring wheat and spring barley varieties reached 50% and 53%, respectively. Promising line 231 of winter wheat and variety Kazakh 10 of spring barley were affected by the disease by 60%, whereas the winter wheat Steklovidnaya 24 was impacted by 67%. Molecular analysis of *B. sorokiniana* isolates via species-specific primers (COSA_F/COSA_R) from infected plant tissues confirmed their identification. Koch postulates were fulfilled for *B. sorokiniana* isolates Kz 48, 60, and 82 on Steklovidnaya 24 winter wheat and Symbat spring barley varieties. Biological products such as Phytosporin-M and Sporobacterin-Rassada significantly reduced the level of fungal infection, confirming their potential as environmentally safe plant protection agents.

Keywords: *Triticum aestivum*, *Hordeum vulgare*, common root rot, soilborne pathogen, species-specific PCR.

Resumo

O trigo e a cevada são alimentos básicos ricos em nutrientes, cultivados extensivamente em escala global, abrangendo mais de 219 milhões de hectares. A produção global combinada anual é de 760,9 milhões de toneladas, com o Cazaquistão contribuindo com 14,3 milhões de toneladas de trigo e 3,83 milhões de toneladas de cevada para esse total. A produtividade das culturas de grãos diminui anualmente devido a doenças fúngicas, especialmente podridões de raízes e copas causadas por *Bipolaris sorokiniana* e *Fusarium* spp. A pesquisa se concentrou em identificar os patógenos responsáveis pela podridão radicular comum em vários tipos de trigo e cevada cultivados no sudeste do Cazaquistão. O objetivo principal era examinar a eficácia de certas substâncias químicas e biológicas na proteção das mudas de cevada durante o estágio inicial de crescimento contra a podridão das raízes. Além disso, o estudo procurou avaliar os seus efeitos na qualidade das sementes, examinando aspectos como as taxas de germinação, a colonização das sementes por determinados fungos patogênicos e a vitalidade geral das sementes e mudas. A inspeção visual das plantas indicou prevalência média de *B. sorokiniana* de 51,8% e de espécies de *Fusarium* de 58,6%. Três isolados foram obtidos a partir de raízes da linha promissora de trigo de inverno 231, e três de raízes

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Received: July 23, 2024 – Accepted: October 24, 2024

de trigo de primavera da variedade cazaque 10, quatro de trigo de inverno variedade Steklovidnaya 24, quatorze de cevada de primavera variedade Symbat e quatorze de cevada de inverno variedade Aidyn 2. A disseminação externa da da podridão radicular comum nas variedades de trigo de primavera e cevada de primavera foi de 50% e 53%, respectivamente. A linha promissora 231 de trigo de inverno e a variedade cazaque10 de cevada de primavera foram afetadas pela doença em 60% das unidades, enquanto o trigo de inverno Steklovidnaya 24 foi afetado em 67% das unidades. A análise molecular de isolados de *B. sorokiniana*, utilizando primers específicos da espécie (COSA_F/COSA_R) de tecidos vegetais infectados, confirmou sua identificação. Os postulados de Koch foram cumpridos para os isolados de *B. sorokiniana* Kz 48, 60 e 82 em variedades de trigo de inverno Steklovidnaya 24 e cevada de primavera Symbat. Produtos biológicos como Fitosporin-M e Sporobacterin-Rassada reduziram significativamente o nível de infecção fúngica, confirmando seu potencial como agentes de proteção de plantas ambientalmente seguros.

Palavras-chave: *Triticum aestivum*, *Hordeum vulgare*, podridão radicular comum, patógeno do solo, PCR específico da espécie.

1. Introduction

Wheat and barley are primary sources of nutrients (Shiferaw et al., 2013); globally, they are cultivated over an area of 219 million hectares, yielding 760.9 million tons, with Kazakhstan producing 14.3 million tons (FAO, 2022) and 3.83 million tons, respectively. Root and stem base rot diseases stand as significant impediments to wheat production, particularly under conditions of heightened pathogenic pressure (Poole et al., 2015). These diseases, which are induced by various fungal pathogens (Bockus et al., 2010), frequently lead to diminished wheat yields, weakened stands, and compromised grain quality. Infection at the root and crown levels results in constriction of the vascular system, impeding the absorption and transport of water and ultimately giving rise to whiteheads during the filling phase. Multiple fungal species typically coexist, exhibiting either synergistic or competitive interactions that influence their progression and disease-causing capabilities. Field assessments of crown and root rot pathogens on wheat are regularly documented in primary wheat-producing countries of global importance in the USA (Paulitz et al., 2002), Canada (Fernandez et al., 2007), Chile (Moya-Elizondo et al., 2015), China (Zhou et al., 2019), Türkiye (Tunali et al., 2008), Kazakhstan (Bozoğlu et al., 2022), and Azerbaijan (Özer et al., 2020a, b). Fernandez et al. (2014) noted that *Bipolaris sorokiniana* Shoem. and *Fusarium* spp. are the most widespread fungi in cereals in western Canada. Özer et al. (2020a) investigated the biological characteristics of root rot in triticale associated with *B. sorokiniana* in Kazakhstan. They reported that this fungus causes growth retardation and necrosis in triticale roots.

The observable signs above ground may not be easily recognizable, but initially, there was the emergence of dark brown to black necrosis on either the entire or a portion of the internode situated just below the crown. This necrosis subsequently extends upward, affecting the plant crown, tiller bases, and leaf sheaths (Mathre, 1997; Mathre et al., 2003; Savin et al., 2024).

Recent studies on cereal soil pathogens have focused on evaluating synthetic wheat nurseries for disease resistance (Morgounov et al., 2018), and a comparative study of barley varieties with leaf spot pathogens under artificial infection conditions was conducted (Dutbayev et al., 2022a). Sultanova et al. (2021) revealed that the most widespread diseases of barley in the southeastern region of Kazakhstan are common root rot and dark brown spot. Dutbayev et al. (2022b) reported that barley, wheat, and oat seeds can defend themselves against *B. sorokiniana* infection. Through joint research collaborations with CIMMYT scientists, *B. sorokiniana* was found to be the most prevalent disease-causing agent in cereals in Kazakhstan (Özer et al., 2020a; Alkan et al., 2021). Also, Qalavand et al. (2023) demonstrated that resistance to *B. sorokiniana* in wheat is associated with enhanced activities of defense-related enzymes like peroxidase, catalase, and β-1,3-glucanase. In our study, we observed similar defense responses in wheat and barley treated with chemical and biological products, which promoted healthy plant growth by suppressing fungal infection.

Seed treatment before sowing with fungicides, nematicides, and proper agronomy practices has proven effective against these pathogens (Dababat and Fourie, 2018). The most resilient technique for controlling plant diseases involves introducing resistant varieties and searching for new, effective sources of disease resistance (Khan et al., 2020).

Therefore, the search for effective methods to protect cereal crops from common root rot has become a priority. In this context, we conducted research from 2022-2023 at Kazakh National Agrarian Research University (KazNARU) in Almaty, Kazakhstan, Bolu Abant Izzet Baysal University (BAIBU) and the International Maize and Wheat Improvement Center (CIMMYT) in Türkiye.

This research focused on pinpointing the pathogens responsible for common root rot in various types of wheat and barley grown in Southeast Kazakhstan. The main goal was to examine the efficacy of certain chemical and biological substances in safeguarding barley seeds against root rot. Moreover, this study sought to gauge their effects on seed quality by examining aspects such as germination rates, the colonization of seeds by particular fungal pathogens, and the overall vitality of seeds and seedlings.

2. Materials and Methods

Plant samples of spring and winter barley, as well as winter and spring wheat, were collected in 2022 from field experiments conducted under the conditions of the Kazakh Scientific Research Institute in the Almaty region, specifically in the village of Almalybak (coordinates 43.237589°N, 76.692629°E). The monitoring of disease

prevalence and development aims to determine the extent of disease spread and progression via standardized methods (Koishybaev and Muminzhanov, 2016).

The plant samples were stored in paper bags labeled with corresponding sample information and transported to the laboratory of Bolu in Türkiye for further studies.

The fungal culture isolation process involved isolating fungi from the roots and subcrown internodes of various types of wheat and barley—winter wheat, spring wheat, winter barley, and spring barley (Figure 1). For isolation, the roots and stem bases were first washed under tap water to remove soil particles and then dried on sheets of absorbent filter paper. Sections of tissue displaying discoloration from the crown and stem were cut into pieces that were 1-2 cm long. The surfaces of these sections were treated with a 1% solution of sodium hypochlorite for 3 min, followed by three rinses with sterile distilled water. These treated sections were dried and then placed onto Petri dishes containing PDA medium, following the method outlined by Shikur Gebremariam et al. (2018).

The root segments were cut into 1 cm pieces and mounted on 1/5-strength potato dextrose agar (PDA) media. To create the PDA medium, 40 grams of potato tubers were boiled in water for 15 min, and the strained broth was adjusted to one liter. Subsequently, 4 grams of dextrose and 15 grams of agar were added to this broth, which was then autoclaved. Additionally, enriched streptomycin (0.1 g/L) was added to the medium to prevent bacterial contamination.

The fungal colonies isolated from the sections were transferred onto new Petri dishes with full-strength PDA (200 g of sliced potato tubers, 20 g of dextrose, 15 g of agar, and 1 liter of distilled water) and purified via the hyphal tipping or single-spore isolation method after five days of incubation in darkness at 23 °C. Cultures were incubated alternatively for 7 days, alternating between 12-hour cycles of light/darkness at 25 °C and 20 °C under cool white and black fluorescent lamps, respectively (Leslie and Summerell, 2006). After 10-14 days, the cultures were transferred to SNA agar (Spezieller Nährstoffarmer) (1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4$ $7H_2O$, 0.5 g KCl, 0.2 g dextrose, 0.2 g sucrose, 20 g agar, and 1 liter of distilled water) to initiate sporulation. This characterization was based on the previously described morphological features detailed in (Duczek et al., 1985; Leslie and Summerell, 2006).

After sporulation, conidial suspensions were prepared in sterile distilled water (SDW) and transferred onto Petri dishes filled with water agar (WA) to isolate single spores (Shikur Gebremariam et al., 2018).

To identify *B. sorokiniana* isolates, a molecular method was employed with specific COSA_F and COSA_R primers designed previously (Matusinsky et al., 2010). The DNA fragment amplification process was carried out via a T100 thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA). PCR was performed in a 50 µL reaction mixture, which included 5 µL of 10× PCR buffer, 0.4 mM of each primer, 20 ng of DNA template, 0.2 mM of each dNTP, and 1.25 U of Dream *Taq* DNA polymerase (Thermo Fischer Scientific, Waltham, MA, USA).

For the PCR cycling, the annealing temperature was adjusted—initially set at 66 °C for the first five cycles, followed by 64 °C for the subsequent 5 cycles, and finally 62 °C for the remaining 30 cycles. Each cycle included denaturation at 95 °C for 30 s, annealing for 20 s, and extension at 72 °C for 45 s. The PCR process included a final extension step at 72 °C for 5 min, after which the samples were cooled to 10 °C before retrieval.

The PCR products, 10 µL in volume, were subjected to agarose gel electrophoresis at a concentration of 1.4% (w/v) for approximately 1.5 hr. Visualization of the results was accomplished via the G:BOX F3 gel documentation system (Syngene, Cambridge, UK) after the gel was stained with ethidium bromide.

The pathogenicity assessment of *B. sorokiniana* isolates involved evaluating several parameters to gauge their impact. Measurements were taken to assess the percentage of wilted plant seedlings, the spread of common root rot, and the development of fungal infection. To conduct this

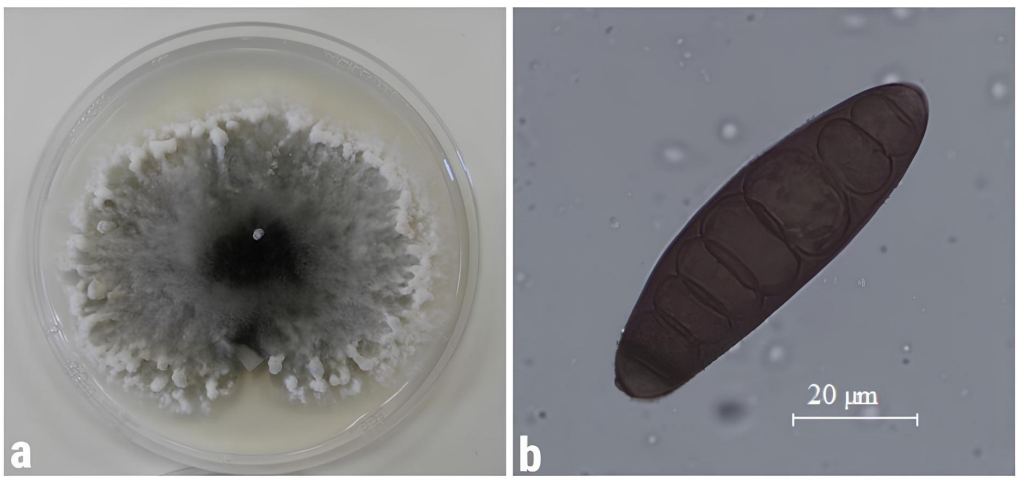

Figure 1. Morphological characteristics of *Bipolaris sorokiniana*. (a) Colony morphology; (b) Conidia of *B. sorokiniana*. Scale bars represent 20 µm.

experiment, a mixture comprising sterilized vermiculite, sand, and soil in equal proportions (1:1:1, v:v:v) was prepared. Each isolate was combined with a conidial suspension at a density of 250 conidia/g of soil and introduced into this substrate mixture. Moreover, control seeds were planted in a substrate devoid of infestation.

Plastic pots, 15 cm in height and 9 cm in diameter, were used to plant five susceptible wheat and barley varieties. The pots containing the isolates were placed in a growth chamber with a 12-hour light cycle, and the temperature was maintained at 24 °C for 7 weeks. Throughout this period, observable symptoms of common root rot manifested in plants exposed to the isolates, whereas no such indications were observed in the control plants. The experiments were conducted in triplicate to ensure the reliability and consistency of the obtained results.

The fulfillment of Koch's postulates — a fundamental principle in establishing the causal link between a pathogen and a disease — was achieved by repetitively isolating and characterizing the pathogen. This characterization was based on the previously described morphological features detailed in Duczek et al. (1985).

2.1. Petri dish experiments

The fungal strain was cultured in solid-strength media in test tubes. Once the fungal mycelium developed in the test tube, 5 mL of ionized water was added to it. The tube was shaken on a shaker for 1 min.

To dilute the inoculum, 1 mL of the previous dilution was added to 9 mL of 0.2% aqueous agar, aiming for a suspension ranging from 10^4 to 10^8 per mL. Each prepared suspension was immersed in a specific quantity of crop seeds, left for 10 min, and then dried on filter paper for 30 min.

The incubation conditions were consistent, maintaining a temperature of 25 °C in the incubator for 7 days. The severity of the disease was subsequently assessed via a scale ranging from 0-3 according to Broders et al. (2007).

0: 100% germination without any symptoms of root infection;
1: Seventy-99% germination with root formation;
2: 30-69% germination with combined lesions;
3: 0-29% germination, indicating that severe infection affects all the seed components.

Throughout the 7-day incubation period, observations were regularly made for all samples, the results were recorded, and any developments or changes were noted.

Sets of 8 dried seeds were placed in Petri dishes filled with WA. Control plants that were not subjected to inoculation were sprayed with SDW. This experimental setup was replicated three times (in triplicate) (Haas et al., 2018).

2.2. Efficacy of chemical and biological agents on winter wheat and spring barley seedlings

The efficacy of chemical and biological agents on both winter wheat and spring barley seedlings was evaluated through a comprehensive assessment. Various treatments, such as Celest Top 312.5, a suspension concentrate (262.5 g/L thiamethoxam + 25 g/L fludioxonil + 25 g/L difenoconazole, Dividend 030, a suspension concentrate (30 g/L difenoconazole) provided by Syngenta Crop Protection AG, Switzerland, and Raxil Ultra FS (120 g/L tebuconazole), and Redigo Pro, a suspension concentrate (150 g/L prothioconazole +20 g/L tebuconazole) provided by Bayer Crop Science AG, Phytosporin-M, liquid (titer not less than 1 billion viable cells, spores/mL, live spores and cells of *Bacillus subtilis* strain 26-D) provided by NPP BashInkom LLC (Russia), and the plant disease control agent Sporobacterin- Rassada 5 g, containing spores of *Trichoderma viride* and *Bacillus subtilis* (Russia), were applied to the seeds of agricultural crops.

The effectiveness of the preparations was assessed on a variety of winter wheat (Steklovidnaya 24) and on a variety of spring barley (Symbat) under laboratory conditions. The assessment was conducted in humid chambers, and 50 seeds were analyzed in four separate replicates. This included measuring the seed energy germination on the 3rd and 5th days according to State Standard 12038-84, as well as the germination capacity on the 7th day according to State Standard 12044-93 and determining fungal infection (*B. sorokiniana*, *Fusarium* spp., and *Alternaria* spp.) as previously described (Naumova, 1978; Koyshibaev, 2006).

Throughout the study, the numbers of diseased seeds and seedlings were recorded. Furthermore, the species compositions of the fungal and bacterial microflora present in the seeds were determined and documented.

Scoring

0: 100% absence of symptoms of root rot;
1: 70-99% germination with mild symptoms;
2: 30-69% germination with noticeable damage;
3: 0-29% germination with severe damage and a decline in plant health.
4: 0% germination, seedlings perished according to (Naumova, 1978; Koyshibaev, 2006).

Statistical analysis of the data was performed via the R-Studio program, which employs the nonparametric Kruskal-Wallis test. The significance of the calculations was assessed using $P<0.05$ (Aphalo, 2017).

3. Results

Visual analysis, isolation in pure culture, and generation of monosporic cultures revealed that the fungus *B. sorokiniana* was isolated from 51.8% of the plant segments, whereas *Fusarium* spp. fungi were isolated from 58.6% of the samples (Figure 1).

3.1. Molecular analysis

All the isolates obtained from diseased plant tissues were identified as *B. sorokiniana* via molecular tools via the species-specific primers COSA_ F/COSA_R. The primers amplified one sharp band (520 bp) in all the tested isolates (Figure 2).

Isolates of the fungus *B. sorokiniana* (Kz 8, Kz 12a, Kz 15b) were obtained from the roots of the promising line 231 of winter wheat. Isolates (Kz 48, Kz 52, Kz 56) were obtained from the roots of the spring wheat variety Kazakhstan 10-3. The winter wheat variety Steklovidnaya 24 yielded 4 isolates (Kz 60, Kz 69, Kz 70, Kz 78b). The Spring barley variety Symbat provided 14 isolates (Kz 82, Kz 83, Kz 85,

Kz 86, Kz 87, Kz 89, Kz 91, Kz 92, Kz 93, Kz 94, Kz 95, Kz 96, Kz 97, and Kz 98). The winter barley variety Aidyn 2 resulted in 14 isolates (Kz 127, Kz 128, Kz 130, Kz 132, Kz 134a, Kz 137, Kz 139, Kz 141, Kz 144, Kz 145, Kz 147, Kz 148, Kz 149, and Kz 153a).

3.2. Visual analysis

The analysis revealed that the percentage of plant roots affected by common root rot was 67%, whereas the stems and first internode were affected at a rate of 50% (Figure 3).

Visual inspection of the plants revealed that, on average, *B. sorokiniana* fungi were isolated from 51.8% of the samples, whereas fungi belonging to the genus *Fusarium* were isolated at a rate of 58.6% (Figure 4-6).

An evaluation of the pathogenicity of *B. sorokiniana* isolates Kz 48, 60, and 82 on the winter wheat variety Steklovidnaya 24, the spring barley variety Kazakhstan 10 and Symbat revealed that 61-70% of the infected seedlings dried, the spread of common root rot ranged from 43-52%, and disease development ranged from 25-27%. In the uninoculated control varieties (Steklovidnaya 24, Kazakhstan 10, Symbat), these indicators were 10-16%, 13-15%, and 2.5-4%, respectively (Table1).

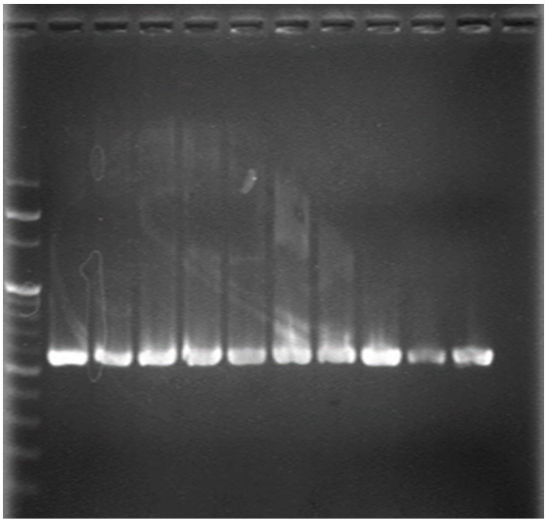

Figure 2. PCR products with primers COSA_F/R both to amplify the DNA of *Bipolaris sorokiniana*.

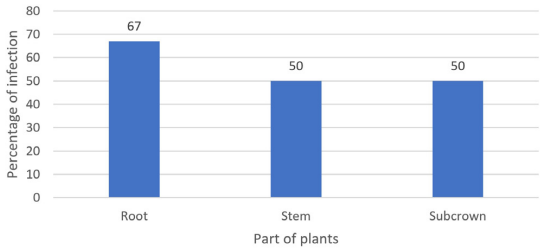

Figure 3. Some plants and morphological identification of cereal crops* Kruskal-Wallis H test <0.05.

These results confirmed the strong virulence of the strains of *B. sorokiniana* isolates Kz 48, 60, and 82 on the winter wheat variety Steklovidnaya 24, the spring barley variety Kazakhstan 10 and Symbat. Additionally, the moderate spread and development of common root rot in the uninoculated control varieties support the hypothesis of maintaining *B. sorokiniana* infection in the plant seeds (Table 1).

The assessment of the winter wheat variety Steklovidnaya 24 and the spring barley variety Symbat with seed treatment agents revealed that this factor does not significantly influence seed germination energy (92.7-93.4%), germination capacity (95.5-96.3%), or seed colonization by fungal infections, such as *B. sorokiniana* (8.1-9.2%), *Fusarium* spp. (8.1-9.2%), and *Alternaria* spp. (2.7-3.7%). The growth vigor of the spring barley seedlings was greater than that of the winter wheat seedlings (Table 2).

The conducted analysis revealed compelling insights into the efficacy of various seed treatments on wheat and barley seeds. The celest top preparation was highly effective at stimulating the growth of both wheat and barley seeds, resulting in a 97.5% increase in seed germination energy compared with the 90.6% increase observed in the control group. Additionally, it notably reduced the level of fungal infection, specifically *B. sorokiniana* infection, to 3.2%, whereas it was 18.8% in the control group. These findings indicate that Celest Top has a remarkable ability to increase seed germination, suppress fungal infection, and promote robust plant growth, as summarized in Table 3.

Moreover, the Dividend 030, Raxil Ultra, and Redigo Pro preparations also had positive effects on seed growth and the inhibition (%) of fungal infection. Detailed results illustrating the impact of these treatments on seed growth and infection levels are provided in Table 3.

Importantly, promising results have been obtained with biological preparations such as phytosporin-M and the plant disease control product Sporobacterin-Rassada. These biological agents significantly reduce the level of fungal infection, confirming their potential as environmentally friendly means of plant protection.

Overall, the findings suggest the potential efficacy of both chemical and biological preparations in enhancing the quality and yield of cereal crops. These studies offer

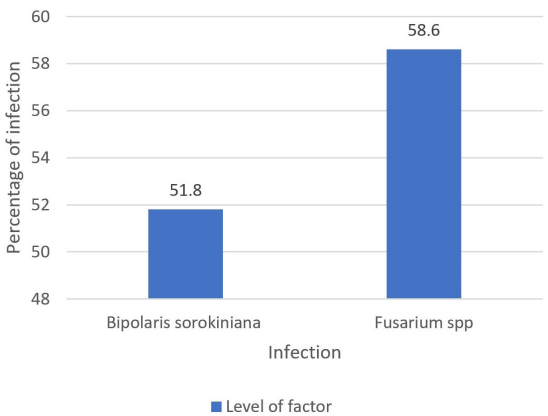

Figure 4. Fungi identification and morphological identification of cereal crops* Kruskal-Wallis H test <0.05.

Figure 5. Infected (a) versus noninfected (b) wheat seedlings.

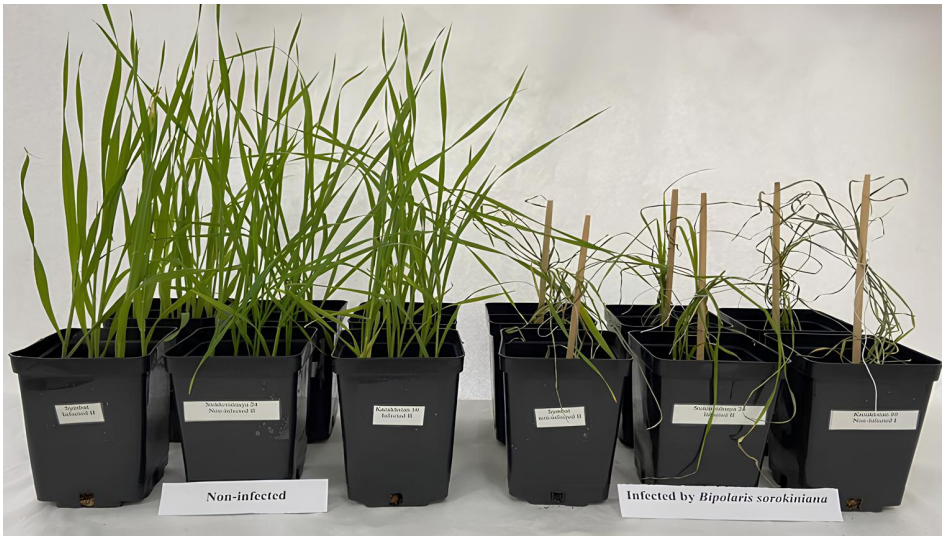

Figure 6. Pathogenicity test of the greenhouse on wheat seedlings. (a) Noninfected wheat seedlings and (b) wheat seedlings infected with *Bipolaris sorokiniana*.

Table 1. Spread and development of signs of root rot in wheat seedlings.

Isolate	Seedling damping-off spreading (%)			Common root rot prevalence (%)			Development (%)		
	St*	Ka	Sy	St	Ka	Sy	St	Ka	Sy
The text continues here (Table 1)									
B. sorokiniana (Kz 48, 60, 82)*	61.0	70.0	63.0	43.0	47.0	52.0	26.0	25.0	27.0
Control	15.0	10.0	16.0	15.0	13.0	15.0	2.5	2.5	4.0
P value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

*St: Steklovidnaya 24, Ka: Kazakhstan 10, Sy: Symbat.

Table 2. Indicators of the influence of winter wheat and spring barley varieties on the germination capacity and fungal infection of seeds.

Variety	Seed Germination energy (%)	Germination capacity (%)	The growth rate of seedlings (%)			Infection with fungal microflora (%)		
			Low	Middle	High	*B. sorokiniana*	*Fusarium*	*Alternaria*
*St	92.7	95.5	17.2	26.4	40.2	8.2	8.1	2.7
Sy	93.4	96.3	24.3	37.2	46.5	9.4	9.2	3.7
P value	0.8	0.7	0.04	0.01	0.05	0.6	0.5	0.5

*St: Winter wheat, Steklovidnaya 24, Sy: Spring barley, Symbat.

Table 3. Indicators of the effects of seed treatment with different winter wheat varieties on germination capacity and seed infestation by fungal infection.

Seed treatment, rate of usage	Seed germination energy %	Germination capacity, 7-th day	The growth rate of seedlings, %		ANOVA test, *P* value	Infection with fungal microflora,%		ANOVA test, *P* value
Celest Top 312.5, suspension concentrate. (thiamethoxam 262.5 g/L + difenoconazole 25 g/L + fludioxonil 25 g/L), 1.0-1.8 l/T	97.5	100.0	low	21.6	<0.01	*Alternaria*	2.9	<0.8
			middle	34.8		*B. sorokiniana*	2.7	
			high	55.4		*Fusarium*	3.2	
Dividend 030, suspension concentrate (difenoconazole, 30 g/L), 2.0 l/T	92.2	95.7	low	52.1	<0.01	*Alternaria*	0.9	<0.01
			middle	40.9		*B. sorokiniana*	0.9	
			high	26.4		*Fusarium*	0.1	
Raxil Ultra, suspension concentrate (tebuconazole, 120 g/L), 0.2 l/T	92.2	95.0	low	20.5	<0.01	*Alternaria*	3.4	<0.9
			middle	30.9		*B. sorokiniana*	4.0	
			high	44.1		*Fusarium*	3.6	
Redigo Pro, suspension concentrate (prothioconazole, 150 g/L + tebuconazole, 20 g/L), 0.35-0.45 l/T	91.0	94.7	low	14.3	<0.01	*Alternaria*	2.6	<0.05
			middle	26.0		*B. sorokiniana*	1.9	
			high	39.2		*Fusarium*	1.1	
Fitosporin-M, liquid (titer not less than 1 billion viable cells, spores/mL, live spores and cells of *Bacillus subtilis* strain 26-D	94.6	97.2	low	20.2	<0.01	*Alternaria*	6.0	<0.07
			middle	31.8		*B. sorokiniana*	3.8	
			high	44.5		*Fusarium*	5.1	
Remedy for diseases of garden plants 'Sporobacterin -Rassada', 5 g of spores of *Trichoderma viride* and *Bacillus subtilis*	93.2	96.7	low	17.4	<0.01	*Alternaria*	3.8	<0.8
			middle	32.0		*B. sorokiniana*	2.7	
			high	40.9		*Fusarium*	3.6	
Control, without treatment	90.6	91.8	low	24.9	<0.01	*Alternaria*	42.0	<0.01
			middle	26.2		*B. sorokiniana*	44.3	
			high	27.0		*Fusarium*	18.8	

valuable insights into the effects of these treatments on plant growth and health, thereby contributing to the development of more effective plant protection strategies in agriculture.

4. Discussion

The integration of molecular technologies has become integral in identifying and detecting fungal pathogens in plants. Accurate identification is crucial for disease management, plant breeding, and epidemiological studies, as highlighted in the research by Matusinsky et al. (2010). Shimizu et al. (1998) developed a PCR-based diagnostic method specifically for detecting *B. sorokiniana* in barley and wheat tissues. This method employs specific primers derived from the melanin biosynthesis locus, known as COSA_F/R primers, developed by Czech scientists (Matusinsky et al., 2010). Using these primers, the authors successfully detected both *B. sorokiniana* and *Fusarium* spp. fungi in three barley varieties and three wheat varieties.

In our research, all the isolates obtained from affected plant tissues were identified as *B. sorokiniana* via molecular methods with the species-specific primers COSA_F and COSA_R. Specifically, three isolates of this fungus were identified from the roots of the winter wheat promising line 231, three isolates from the roots of the spring wheat variety Kazakhstan 10, four isolates from the winter wheat variety Steklovidnaya 24 (Kz 60, Kz 69, Kz 70, Kz 78b), and fourteen isolates from the spring barley variety Symbat and the winter barley variety Aidyn 2. This comprehensive identification using species-specific primers allowed for a precise determination of the fungal isolates present in different barley and wheat varieties.

According to Kumar et al. (2002), *B. sorokiniana* persists in the soil on infected plant residues, on surfaces, and inside seeds. Its spread primarily occurs through conidia. The disease primarily manifests in seedlings and sprouts and is characterized by specific symptoms. The coleoptiles of affected plants become brown, the leaves turn yellow, and deformation becomes apparent. Additionally, light brown spots emerge on the leaves. Infected plants typically exhibit stunted growth, white heads, reproductive stem death, and brown and wrinkled seeds.

Our findings support Kumar's hypothesis regarding the significant pathogenicity of *B. sorokiniana* isolates, specifically Kz 48, 60, and 82, observed in both the winter wheat variety Steklovidnaya 24 and the spring barley varieties Kazakh 10 and Symbat. Furthermore, our research confirms the ability of *B. sorokiniana* infection to persist within plant seeds. These findings support the notion that these specific isolates have a high capacity for causing disease in these cereal varieties and emphasize the potential of *B. sorokiniana* to remain within plant seeds, potentially impacting future crops.

Our research aligns with and supports the findings presented by Ozer et al. (2020c, 2023), who conducted a comprehensive study on fungal pathogens causing crown and root rot in wheat in northern Kyrgyzstan and Azerbaijan. Similar to our study, Ozer et al. (2020a) identified *B. sorokiniana* as the primary pathogen responsible for common root rot, with a high field incidence. In both studies, *B. sorokiniana* was found to be moderately virulent, posing a significant threat to wheat crops.

While Ozer et al. (2020b) also identified *Fusarium* species as significant contributors to wheat crown and root rot, their study highlights *F. culmorum* and *F. pseudograminearum* as the most virulent species, consistent with our findings on the importance of *Fusarium* spp. However, their identification of additional *Fusarium* species, such as *F. acuminatum*, *F. oxysporum*, and *F. equiseti*, underscores the regional variability in *Fusarium* populations, which may not have been as prominent in our research conducted in Kazakhstan. This suggests that the relative prevalence of specific fungal pathogens may vary significantly based on geographical and environmental factors.

The prevalence of *B. sorokiniana* causing wheat root rot in China is significant, with the reported disease prevalence reaching 82.7%. This fungus has been isolated from roots in 24% and from stems in 33.7% of cases, as documented by Xu et al. (2018). Moreover, globally, *B. sorokiniana* is acknowledged as one of the most destructive pathogens affecting wheat (Xu et al., 2018).

In Türkiye, research conducted by Bozoğlu et al. (2022) aimed to evaluate the distribution of pathogen populations linked to root rot caused by *Fusarium* and *Helminthosporium* fungi in various regions of the country. Their data revealed that the spread and development of root rot in different barley and wheat varieties ranged from 50% to 67%. Notably, the disease affected plant roots in 67% of the cases, whereas stems and basal internodes were affected by 50% of the cases.

Upon visual analysis, the isolation of fungi from pure cultures and the subsequent production of monosporic cultures revealed that *B. sorokiniana* was present in 51.8% of the plant segments examined, whereas fungi from the genus *Fusarium* were present in 58.6% of the samples. This finding highlights the significant prevalence and distribution of both *B. sorokiniana* and the genus *Fusarium* fungi in the affected barley and wheat varieties examined in the present study, shedding light on their involvement in root rot development.

Control methods using chemical agents remain the primary practical approach for protecting wheat from common root rot in cereal crops, as indicated by Al-Sadi (2021) and Zhukova et al. (2019). These methods typically involve seed treatment, soil drenching, and foliar spraying, with seed treatment recognized as particularly effective (Moussa et al., 2013). Also, Gholamaliyan et al. (2021) identified wheat genotypes with varying resistance levels to this pathogen. Similar to their identification of cultivars like 'Alvand' and 'Baharan' with low disease severity, our study also highlights the efficacy of different treatments in managing root rot in barley and wheat. The emphasis on integrating resistant cultivars into management strategies aligns with our findings on the importance of utilizing both biological and chemical control methods for reducing fungal infection in cereal crops.

Kaur (2016) reported the effectiveness of seed treatment with the fungicide Raxil, which contains tebuconazole as an active ingredient. This treatment was found to enhance the effects of *B. sorokiniana* on seed germination. However,

despite this improvement, complete suppression of disease development was not achieved when the seeds were infected with pathogens.

Additionally, Wei et al. (2021) conducted tests with commercially available fungicides against *B. sorokiniana*. They identified a synergistic effect of two fungicides utilized in seed treatment under field conditions to control common root rot. Among the tested fungicides, fludioxonil exhibited the greatest inhibition of *B. sorokiniana*, with an EC50 (effective concentration to inhibit 50% of growth) of 0.0509 mg/L. In addition to fludioxonil, tebuconazole and difenoconazole presented EC50 values of 0.0512 and 0.0627 mg/L, respectively. Although the EC50 values did not significantly differ among fludioxonil, tebuconazole, and difenoconazole, fludioxonil was more effective at inhibiting mycelial growth than the other fungicides were. Therefore, researchers have concluded that fludioxonil has the most effective inhibitory effect on *B. sorokiniana*.

On the basis of our findings, Celest Top, which contains thiamethoxam as its active component, displayed exceptional efficacy in enhancing the growth of wheat and barley seedlings, with a remarkable rate of 97.5% seed germination energy compared with 90.6% in the control group. Notably, Celest Top notably curtailed the prevalence of fungal microflora infection, particularly *B. sorokiniana*, reducing it to 3.2%, in contrast to the control's 18.8%. This underscores the substantial capacity of Celest Top to invigorate seed germination, curb fungal infection, and foster robust plant growth. Additionally, Dividend 030, Raxil Ultra, and Redigo Pro had positive effects on seed development and the inhibition of fungal infections.

The application of plant growth-promoting rhizobacteria (PGPRs) represents an environmentally safe approach for managing root rot in cereal crops, as highlighted by studies conducted by Mulk et al. (2022) and Castro Tapia et al. (2020). However, despite their potential, their widespread commercial adoption faces constraints due to inconsistent field effectiveness and limited shelf life (Valente et al., 2020). The antagonistic potential of microorganisms plays a pivotal role in shaping the spatial structure of bacterial communities. This phenomenon has been corroborated in diverse communities, such as endophytic bacteria, where antagonism contributes to the structuring of communities in distinct plant compartments (Chiellini et al., 2019). The adaptation of rhizobacteria to the host is a multifaceted process regulated by various factors, including bacterial type, host genotype, and the surrounding rhizosphere environment (Brader et al., 2014).

Rhizobacteria that promote plant growth belong to various genera, such as *Arthrobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Chromobacterium*, and *Caulobacter* (Hakim et al., 2018). A study conducted by Mulk et al. (2022) revealed that various rhizosphere and endosphere bacteria, which were isolated from a wheat-rice cropping system in Punjab, Pakistan, and identified as *Bacillus* spp., significantly inhibited various wheat pathogens, such as *F. oxysporum*, *F. moniliforme*, *R. solani*, and *M. phaseolina*.

Research by Salehpour et al. (2005) revealed that *Trichoderma* isolates could help combat root rot in wheat caused by the fungus *B. sorokiniana*. These isolates produce substances that suppress the growth of the fungus and promote wheat growth. The isolate *T. viride* T112 proved to be particularly effective. Treatment of seeds and soil with these isolates reduced infection and increased wheat yield. According to our findings, biological preparations such as Phytosporin-M (based on *Bacillus subtilis* strain 26D) and Sporobacterin-Rassada, a remedy for garden plant diseases (based on *Bacillus subtilis* 534), also markedly reduce fungal infection levels, confirming their potential as biologically safe plant protection agents.

Articles of Moatamedi et al (2018, 2023) suggest the identification of genetic diversity and resistance genes, such as Cre1, in wheat cultivars in response to Heterodera filipjevi highlights the significance of genetic resistance in combating crop diseases. Although our study did not focus on nematodes, the reliance on genetic markers to evaluate resistance aligns with our approach of identifying the molecular presence of *B. sorokiniana*.

The results indicate the potential of both chemical and biological preparations for improving the quality and yield of cereal crops. These studies could deepen our understanding of how these preparations impact plant growth and health, thus contributing to the development of more effective plant protection strategies in agriculture. Our future research will focus on identifying sources of resistance to root rot caused by *B. sorokiniana* and *F. culmorum* in wheat in Kazakhstan.

5. Conclusions

The implementation of seed treatment technologies presents a promising avenue for improving disease management in agriculture. By fortifying seeds with targeted treatments, farmers gain a proactive approach to combat pathogens and pests right from the start of the growing season. This not only reduces the reliance on potentially harmful chemical interventions but also enhances crop health and resilience against prevalent diseases.

Moreover, seed treatments offer a holistic solution that aligns with sustainable agricultural practices. By precisely delivering protective agents to seeds, resources are utilized efficiently, minimizing the environmental impact while maximizing the yield potential.

The adoption of seed treatment technologies can lead to broader socioeconomic benefits. With healthier crops and increased yields, farmers are better equipped to meet growing global food demands. This can contribute to food security, economic stability, and improved livelihoods within agricultural communities.

Molecular analysis via the COSA_F/COSA_R primer confirmed the identification of all the obtained isolates from affected plant tissues as the fungus *B. sorokiniana*. Three isolates of this fungus were identified from the roots of the winter wheat promising line 231. Three isolates were identified from the roots of the spring wheat variety Kazakhstan 10. From the winter wheat variety Steklovidnaya 24, four isolates were identified (Kz 60, Kz 69, Kz 70, Kz 78b). Additionally, fourteen isolates were identified from the spring barley variety Symbat, and fourteen isolates were identified from the winter barley variety Aidyn 2.

This study reinforces the need for continuous screening and development of resistant cultivars as a core component of disease management.

The external spread of common root rot in spring wheat and spring barley varieties ranges from 50-53%. Wheat promising line 231 and the variety of spring barley Kazakhstan 10 were affected by the disease at 60% units, whereas the winter wheat Steklovidnaya 24 was affected at 67% units. Visual inspection of the plants revealed that, on average, *B. sorokiniana* fungi were isolated from 51.8% of the samples, and fungi belonging to the genus *Fusarium* were present in 58.6% of the samples.

The high virulence of the *B. sorokiniana* Kz 48, 60, and 82 isolates to the winter wheat variety Steklovidnaya 24, spring barley variety Kazakhstan 10 and Symbat has been confirmed, as has the ability of *B. sorokiniana* infection to persist in plant seeds.

Chemical preparations such as Celest Top (active ingredient thiamethoxam), Dividend 030 (difenoconazole), Raxil Ultra (tebuconazole), and Redigo Pro (prothioconazole) demonstrated high effectiveness in stimulating the growth of wheat and barley seeds and suppressing fungal growth.

Biological products such as Phytosporin-M and the Remedy for Diseases of Garden Plants Sporobacterin-Rassada significantly reduce the level of fungal infection, confirming their potential as biologically safe plant protection agents.

These findings suggest that both chemical and biological agents have potential for increasing the quality and yield of cereal crops. These investigations can increase our understanding of how these treatments influence plant growth and well-being, thus assisting in the formulation of more efficient agricultural plant protection methods.

Acknowledgements

The paper is based on the results obtained from a project funded by the Ministry of Science and Higher Education of the Republic of Kazakhstan: AP19676202 – 'Identification of Sources of Resistance to Wheat Root Rot Caused by *Bipolaris sorokiniana* and *Fusarium culmorum* using Phytopathological and Molecular Approaches'.

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