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The Ability of *Bipolaris Sorokiniana* Isolated from Spring Barley Leaves to Survive in Plant Residuals of Different Crops

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Abstract: Spring barley is an important high-value crop globally. However, it is highly susceptible to soil-borne diseases in Kazakhstan and the world. *Bipolaris sorokiniana* (Sacc.) Shoemaker is the most important soil-borne pathogen that causes root rot and spot blotch diseases in cereal crops. This study aimed to evaluate the ability of *B. sorokiniana* isolated from spring barley leaves to survive in plant residuals of different crops. The fungus was isolated from spring barley leaves from the Zholbarys agro farm of Kerbylak district of Almaty, southeastern Kazakhstan, in 2021. Laboratory experiments were conducted at Kazakh National Agrarian Research University. The study was conducted on 10th days of seedlings of cereal, legume, and oilseed crops. The obtained results showed that seedlings of spring barley, spring wheat, and oats retained the B. sorokiniana infection. Interestingly, the infection of B. sorokiniana isolated from leaves of spring barley statistically significantly increased symptoms of spot blotch on spring wheat and spring barley seedlings, while symptoms decreased on oats (P-value <0.01). The introduction of rapeseed, chickpeas, peas, and oats into the crop rotation system in southeastern Kazakhstan could limit the volume of the *B. sorokiniana* infection in spring wheat and spring barley. On rapeseed, peas, and chickpeas seedlings no symptoms of spot blotch were observed (P-value <0.01).

Keywords: Spot Blotch, Wheat, Rapeseed, Chickpea, Peas

Introduction

Barley (*Hordeum vulgare* L.) in Kazakhstan is cultivated on an area of 1.5 million hectares with gross production of 1.9 million tons with a yield of 1.3 tons/ha, which is almost three times less than the global average (Rsaliyev *et al.*, 2017). The soil fungus *Cochliobolus sativus* Drechsler ex Dastur. of the anamorph *Bipolaris sorokiniana* (Sacc.) Shoemaker causes root rot and leaf spot on wheat, barley, rye, triticale, and other cereals. The fungus *B. sorokiniana* has a wide geographical distribution area (Guest, 2017; McDonald *et al.*, 2018; Özer *et al.*, 2020). The low yield of barley in Kazakhstan is largely due to the susceptibility of the cultivated varieties to leaf spotting, while the yield and productivity of plants are significantly reduced by 30-40% (Cegiełko *et al.*, 2018, 2019; Haas *et al.*, 2018; Pathak *et al.*, 2020). Barley strips are covered with dark spots with a brown or dark gray coating, photosynthesis is disrupted, the growth and development of plants slow down and they form fewer primary and secondary roots. Under favorable conditions, similar spots appear on the internodes, which leads to lodging. When the plant roots rot, the mycelium of *B. sorokiniana* causes its underdevelopment and produces toxins that worsen the quality of malting barley varieties (Göksel *et al.*, 2020a; Haas *et al.*, 2018).

Recent global research is dedicated to studying *B. sorokiniana* on barley. Such research has been focused on the influence of fungal infection on indicators such as tyramine, serotonin, and other amines (Ishihara *et al.*, 2017); the influence of the ToxA gene on the virulence of the spot blotch pathogen on barley and wheat (McDonald *et al.*, 2018); the influence of the fungus on the quality of barley grain (Cegiełko *et al.*, 2018); change in the structure of RNA in the barley varieties susceptible and resistant to *Helminthosporium* blotch (Haas *et al.*, 2018);

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mapping of a dominant gene resistance to the new pathotype fungus in barley (Wang *et al.*, 2019); assessment of the biological effectiveness of the types of *Trichoderma* spp. against *B. sorokiniana* (Singh *et al.*, 2018); determination of the location of the loci of resistance to the pathogen of *Helminthosporium* leaf spots caused by *B. sorokiniana* in the genome of barley (Novakazi *et al.*, 2020).

In Kazakhstan, studies on soil pathogens have recently been conducted to assess the collection of synthetic wheat for disease resistance (Suleimanova *et al.*, 2016; Kuresbek *et al.*, 2017; Morgounov *et al.*, 2018) and the compatibility of barley varieties with leaf spot pathogens on an artificial infectious background (Pathak *et al.*, 2020). The International Maize and Wheat Improvement Center (CIMMYT) researchers have conducted 2 intensive surveys for soil-borne diseases of grain crops and found that *B. sorokiniana* was the most common pathogen (Göksel *et al.*, 2020). Özer *et al.* (2020) studied the biological features of common root rot on triticale caused by *B. sorokiniana* and found that the fungus caused growth retardation and necrosis of internodes on the roots. Sultanova *et al.* (2021) found that the most common diseases of barley in the southeastern region of Kazakhstan were the common root rot and dark brown spotting (caused by *Bipolaris sorokiniana* Shoem.).

The best measures to combat leaf spotting on barley are the removal of plant residues, crop rotation, cultivation of resistant varieties, and pre-sowing treated seeds with chemicals (Suleimanova *et al.*, 2016; Guest, 2017; Kuresbek *et al.*, 2017; Morgounov *et al.*, 2018). In our study, we have established that the fungus *B. sorokiniana* isolated from the leaves of spring barley can persist in the seeds of spring wheat, spring barley, and oats. In addition, it was found that the fungal infection significantly increased the infection rate of seedlings of spring wheat and spring barley with *B. sorokiniana*, whereas it was decreased in oats. Seedlings of rape seed, chickpeas, and peas are not populated by the pathogen. We assume that the introduction of rapeseed, chickpeas, peas, and oats into the crop rotation of south-eastern Kazakhstan can reduce the infestation of spring wheat and spring barley with helminthosporous spots.

Our study aims to evaluate the ability of *B. sorokiniana* isolated from spring barley leaves to survive in plant residues of various crops.

Materials and Methods

Selection of Barley Leaves

The affected leaves with symptoms of leaf spotting were collected in the fields of the Zholbarys agro farm of the Kerbulak district of the Almaty region (GPS coordinates: 44.376726, 78.573329). The sowing of spring barley of the Arna variety was carried out in the third decade of April 2021. Pure cultures of the pathogen of dark brown spotting of the leaves of spring barley (the *B. sorokiniana* fungus) were isolated in the laboratory of the Kazakh Research Institute of Plant Protection and Quarantine named after Zh. Zhiembayev LLP in 2021 (Fig. 1).

Isolation and Identification of the Pathogen

To obtain *B. sorokiniana* isolate, pieces of barley leaves with symptoms of leaf spotting were sterilized in 90% alcohol for 1 min, then immersed in a 1% solution of sodium hypochlorite for 3 min and washed three times with sterile water (Fig. 1). Next, the affected parts of barley leaves with a size of 1 cm^2 were placed in Petri dishes on a Czapek nutrient medium.

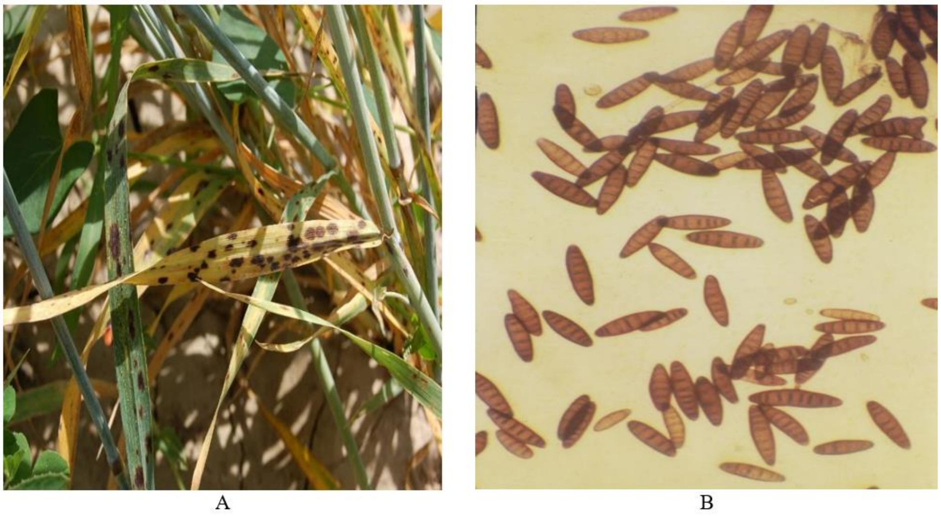

Fig. 1: Symptom and signs of spot blotch on barley leaves (Zholbarys agro farm of Kerbylak district of Almaty region, July 2021)
Note: A: Symptoms of barley leaf spotting, B: Conidia of *Bipolaris sorokiniana* Shoem

Laboratory Experiment

The impact of *B. sorokiniana* isolate was determined at the Kazakh-Japanese Innovation Center of the Kazakh National Agrarian Research University (KazNAIU) in Almaty. Conidia of *B. sorokiniana* were collected by adding distilled water to five-day Potato Dextrose Agar (PDA) dishes and scraping off the surface of the agar with a spatula. The suspension with conidia was purified from mycelium fragments by filtering through two layers of gauze. The concentration of the inoculate was adjusted to 8000 conidia per 1 mL of distilled water using a hemocytometer. Five-day-old seedlings of spring barley, spring wheat, rapeseed, chickpeas, peas, and oats were inoculated with approximately 0.2 mL of conidial suspension each. Seedlings were evaluated by the second leaves of plants 5 days after inoculation (Parikh *et al.*, 2018; Singh *et al.*, 2021).

The fungal mycelium was thoroughly washed with sterile distilled water to remove the remnants of the culture filtrate from the mycelial mat and placed on filter paper to remove excess moisture. The mat was weighed and distilled water was added to it (1 g of mycelial mat/4 mL of water). The mycelium was whipped in a blender at a speed of 5000 rpm for 5 min. The unsettled filtrate was filtered out (pore size: 0.45 Microns), 1 mL was added to 4 mL of sterile distilled water and the resulting suspension was used for seed inoculation.

The seeds of spring barley, spring wheat, rapeseed, chickpeas, peas, and oats were sterilized with 92% alcohol for 1 min and washed twice with distilled water for 2 min. Then the seeds were dried on sterilized filter paper.

For inoculation, the seeds were immersed in a mushroom suspension for 10 min and then left to dry. Evenly distributed seeds were laid out on Petri dishes with water agar in three repetitions. Seeds of spring barley, spring wheat, rapeseed, chickpeas, peas, and oats were placed at a distance of 1.5-2 cm from each other under aseptic conditions in a humid chamber (high humidity). There were 10 seeds in each dish. Then they were incubated at 25°C in a thermostat until the fungal mycelium grows well after 5 days in the dark.

Laboratory germination was evaluated on a scale from 0 to 3 (Patsa *et al.*, 2018; Kang *et al.*, 2021) where: 0 = 100% germination without signs of seed (root) damage; 1 = 70-99% germination with root damage; 2 = 30-69% germination with fused lesions; 3-0-29% germination when all seed tissues are affected. During the experiment, all samples were regularly examined and recorded. Eight seeds were placed on a petri dish with PDA (Sigma Aldrich) with the addition of 0.01% tetracycline (PDAt). Dishes with agar were placed in a thermostat for 7 days at 25°C. The cultures were repeatedly transferred to a new PDA to obtain pure cultures of fungal isolates (Suleimanova *et al.*, 2016; Kuresbek *et al.*, 2017).

To assess the incidence of spotting, the Prevalence (P) by signs was calculated using the formula (Suleimanova *et al.*, 2016; Kuresbek *et al.*, 2017):

$$P = n\text{x } 100 / N, \tag{1}$$

where, P is the spread of the disease; n is the number of affected plants; N is the number of plants in the sample. The development of leaf spotting is calculated by the formula (Suleimanova *et al.*, 2016; Kuresbek *et al.*, 2017):

$$R = ab\text{x } 100 / AK \tag{2}$$

where, a is the number of plants affected by the disease; b is the corresponding lesion score; A is the number of plants in the sample; K is the highest lesion score (Suleimanova *et al.*, 2016; Kuresbek *et al.*, 2017).

Using the wet chamber method, the infestation of seedlings of crops with conidia of *B. Sorokiniana Fusarium*, spp., *Alternaria*, and other fungi was determined.

Statistical data processing was carried out using the R-Studio program according to the non-parametric Mann-Whitney U-criterion for independent samples. The significance of the calculations was evaluated using the P-value (Aphalo, 2017).

Results

The results of the analysis showed that 33.6-46.9% of 5th day seedlings of spring barley, spring wheat, and 18.6% of oat seedlings were infected with the fungus *B. sorokiniana*. The seedlings of rapeseed, peas, and chickpeas showed no signs of infection. On the 5th day, seedlings of crops were also populated with fungi from the genera *Fusarium, Aspergillus* spp*., Mucor,* as well as jointly affected by *Aspergillus,* and *Penicillium* spp. (Table 1, Fig. 2).

Table 1: The colonization of 5-day cereal, legume, and oilseed seedlings with fungal infection (Kazakh-Japanese innovation center, KazNAIU. 2021)

		The colonization of seedlings with a fungal infection, %					
5th-day seedlings, crop	Healthy seedlings	*B. sorokiniana*	*Fusarium* spp.	*Aspergillus* spp.	*Penicillium* spp.	*Mucor*	*Aspergillus* spp. + *Pennicilium* spp.
Spring barley	26.6	33.6	11.6	5.0	1.7	0.0	0.0
Spring wheat	36.5	46.9	6.6	10.0	0.0	0.0	0.0
Oats	53.5	18.3	6.6	0.0	0.0	21.6	0.0
Rapeseed	43.2	0.0	3.3	0.0	22.0	0.0	29.9
Peas	64.7	0.0	13.3	22.0	0.0	0.0	0.0
Chickpeas	10.4	0.0	10.0	0.0	0.0	79.6	0.0

$P<0.01$

Spring wheat Spring barley Oats

Rapeseed Peas Chickpeas

Fig. 2: Seedlings of crops on the 5th day after sowing on PDA (KazNAIU. 2021)

Table 2: Indicators of the ability of cereal, legume, and oilseed seeds to retain infection of *Bipolaris sorokiniana* (Kazakh-Japanese Innovation Center, KazNAIU. 2021)

		Infected seedlings. %		
Seedlings	Inoculation with *B. Sorokiniana*	Healthy	*B. sorokiniana*	Another fungal infection
5th day seedlings inoculated with a suspension of *B. sorokiniana* isolated from barley leaves				
Spring barley	Inoculated	26	34.00	40
	Non-inoculated	27	34.00	39
Spring wheat	Inoculated	36	45.00	19
	Non-inoculated	38	44.00	18
Oats	Inoculated	18	53.00	29
	Non-inoculated	21	49.00	30
Rapeseed	Inoculated	2	47.00	51
	Non-inoculated	3	45.00	52
Peas	Inoculated	0	64.00	36
	Non-inoculated	0	61.00	39
Chickpeas	Inoculated	0	11.00	89
	Non-inoculated	0	13.00	87
P-value	Inoculation factor		2.60	
10th days after inoculation with a *B. sorokiniana* suspension				
Spring barley	Inoculated	0	100.00	0
	Non-inoculated	0	45.00	25
Spring wheat	Inoculated	30	100.00	0
	Non-inoculated	0	48.00	14
Oats	Inoculated	38	18.00	55
	Non-inoculated	10	51.00	45
Rapeseed	Inoculated	0	16.00	64
	Non-inoculated	20	0.00	83
Peas	Inoculated	17	0.00	45
	Non-inoculated	55	0.00	40
Chickpeas	Inoculated	60	0.00	100
	Non-inoculated	0	0.00	100
P-value	Inoculation factor	0	<0.01	

5th day seedlings of spring barley, spring wheat, rapeseed, chickpeas, peas and oats inoculated with a suspension of the *B. sorokiniana* conidia. A rank scale was used for the analysis. The results of the analysis showed that the average values in the groups for the inoculation factor were equal (Table 2, P-value = 2.6). However, the process of inoculation of seedlings was carried out in a random order.

After 10th days according to the inoculation factor, the P-value at a 99% confidence interval was small, <0.01. This means that the average values in the groups were unequal and we can correctly speak about the significant impact of *B. sorokiniana* infection on the seedlings (Table 2). The results showed that the seedlings of spring wheat and spring barley infected with the pathogen had an increased level of *B. sorokiniana* infection (100%) compared to the non-infectious background (45-48%). In oat seedlings, there were no significant differences in the fungal population of seedlings between inoculation backgrounds (49-53%). In rapeseed, 16% of the surface of inoculated seedlings was infected with the fungus *B. sorokiniana*, while non-inoculated seedlings showed no symptoms of the disease. Peas and chickpeas were not affected by infection (Table 2). Another fungal infection affected oats (45-55%), rapeseed (64-83%), chickpeas (100%), and peas (40-45%).

Thus, we assume that the introduction of rapeseed, chickpea, pea, and oat seeds into crop rotation limits the infectious load of the fungus *B. sorokiniana* in spring wheat and spring barley crops in the conditions of the Almaty region (Table 2, Fig. 3).

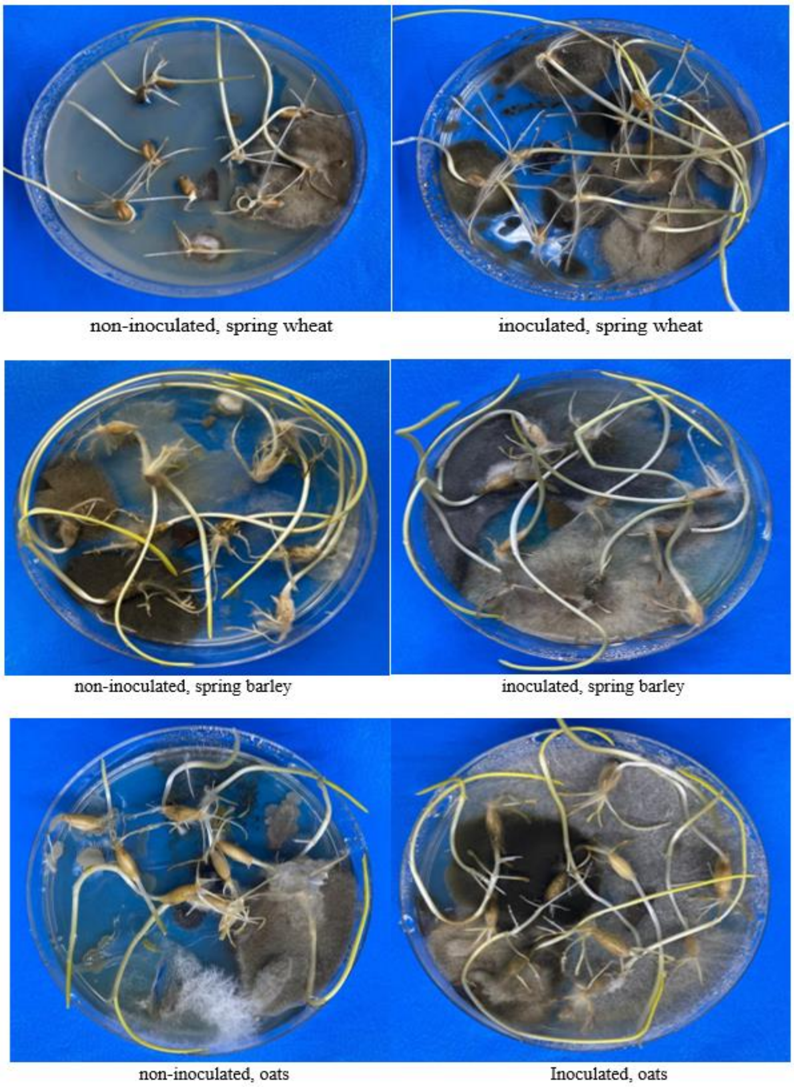
non-inoculated, spring wheat
inoculated, spring wheat
non-inoculated, spring barley
inoculated, spring barley
non-inoculated, oats
Inoculated, oats

Fig. 3: Seedlings of grain crops on the 10th day after inoculation with a suspension of *Bipolaris sorokiniana*

Discussion

Bipolaris sorokiniana is the pathogen responsible for spot blotch disease in cereals. The disease could be carried out by seeds, roots, or air (Matorin *et al*., 2018; Pathak *et al*., 2020; Al-Sadi, 2021). Plowing, removing weeds and cereals, and introducing plants that are not affected by root rot into crop rotation reduces the amount of *Bipolaris sorokiniana* inoculum in the soil (Duveiller and Dubin, 2002).

Recent studies on *B. sorokiniana* in cereals have been focused on developing of control of common root rot as a phytosanitary previous crop (Campanella *et al*. 2020), pesticide research (Wei *et al*., 2021), actinobacterial activity (Campanella *et al*., 2020), antagonistic activity of *Bacillus sp*. (Harba *et al*., 2020) and source of non-fungi toxic wheat-protecting metabolites that induces resistance to tomato wilt (Shcherbakova *et al*., 2018).

The use of *Brassica carinata* as a previous crop of durum wheat as green manure can reduce the number of *B. sorokiniana*, *Fusarium* spp. conidia and common root rot incidence by 49%, and the weight of grain by 48% compared to wheat monoculture (Campanella *et al*., 2020).

Bacillus spp. has antagonistic activity against the virulent *B. sorokiniana* (isolate CRR16) by 59-92% (Harba *et al*., 2020).

According to Allali *et al*. (2019), the actinobacteria strain LB12 strain has biological activity in vivo against the *Bipolaris sorokiniana* in durum wheat seedlings cv. Vitron. This strain can produce indole-3-acetic acid, siderophores, and hydrogen cyanide by seedlings, which showed chitinolytic activity and solubilized inorganic phosphates, which are useful for stimulating growth and biocontrol of plant activity. In addition, the treatment of wheat seeds with spores of the MB22 strain led to an increase in the dry weight, length of roots, and shoots of seedlings and reduced the damage index of common root rot.

According to Wei *et al*. (2021), the combined application of two fungicides significantly can inhibit the growth of fungal mycelium *B. sorokiniana*-the half of the maximum effective concentration of the mixture contains the active ingredients fludioxonil and difenoconazole in a ratio of 1:4.

Shcherbakova *et al*. (2018) showed that *F. sambucinum* strain FS-94 induces resistance to tomato wilt and has been shown as a source of non-fungi toxic wheat-protecting metabolites, which were contained in a mycelium extract. Pre-inoculation treatments of seeds with PME significantly decreased the incidence (from 30 to 40%) and severity (from 37 to 50%) of root rots on seedlings without any inhibition of the seed germination and Potentiation of Deoxynivalenol (DON), DON monoacetylated derivatives and zearalenon production in FCR agents.

According to Duveiller and Dubin (2002), long-term use of rapeseed has some fungitoxic effects on *helminthosporium* leaf spot in wheat, which is the causative agent. At the same time, wheat and barley must not be rotated in the same fields infected with common root rot. In this current study, it was found that seedlings of spring barley, spring wheat, and oats can retain infection with the fungus *B. sorokiniana*. Infection with *B. sorokiniana* statistically significantly increased the manifestations of spotting on seedlings of spring wheat and spring barley and decreased on oats.

Sultanova *et al.* (2021) found that the most common diseases of barley in the southeastern region of Kazakhstan were common root rot and leaf spot caused by *B. sorokiniana* and *Drechslera graminea*. In this region, *B. sorokiniana* develops mainly in the conidial stage, sometimes the fungus forms the ascigerous stage of *Cochliobolus sativus* (Ito et Kurib.) Drechsler ex Dastur., which is almost lost in the pathogen development cycle. On the primary and secondary roots, as well as on the underground internodes, dark brown elongated ulcers are formed, which often merge and at the end the affected tissue becomes black. The disease can manifest itself as browning, yellowing, and mildew in young leaves (Rysbekova and Sultanova, 2022).

Saad *et al.* (2021) evaluated the ability of *F. pseudograminearum, F. culmorum, F. graminearum, and B. sorokiniana* to cause root rot in winter cereals. It was found that barley and soft wheat plants had the highest rates of disease development on leaf sheaths and root internodes (64.7-99.6%), while these indexes were low on oats (5%).

The fungus *B. sorokiniana* causes symptoms of common root rot and leaf spotting on cereals (Guest, 2017; McDonald *et al.*, 2018; Özer *et al.*, 2020). Infection *with B. sorokiniana* statistically significantly increased the manifestations of spotting on seedlings of spring wheat and spring barley, while it decreased them on oats. We assumed that the introduction of rapeseed, chickpeas, peas, and oats into the crop rotation of southeastern Kazakhstan may limit the volume of *B. sorokiniana* infection in spring wheat and spring barley. There were no signs of spotting on the seedlings of rapeseed, peas, and chickpeas.

Conclusion

In this study, we have evaluated the ability of *B. sorokiniana* isolated from spring barley leaves to survive in plant residues of various crops. It has been concluded that the seeds of spring barley, spring wheat, and oats can retain the infection caused by *B. sorokiniana*. The introduction of rapeseed, chickpeas, peas, and oats into the crop rotation system in southeastern Kazakhstan could limit the volume of the *Bipolaris sorokiniana* infection in spring wheat and spring barley. On rapeseed, peas, and chickpeas seedlings no symptoms of spot blotch were observed.

We plan to devote our future studies to the molecular genetic identification of pathogens of root rot in cereal crops and determine their pathogenicity in other soil and climatic zones of Kazakhstan.

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Author’s Contributions

All authors equally contributed to this study.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues are involved.

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