Assessment of juvenile resistance of barley and wheat accessions to dark brown leaf spot

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Abstract. Dark brown leaf spot of cereal crops caused by the Bipolaris sorokiniana fungus causes significant crop losses worldwide. To create new resistant varieties, it is necessary to use sources with a high level of resistance. In the laboratory of physiology and biotechnology of the Krasnoyarsk Research Institute of Agriculture, the juvenile resistance of the breeding material of spring barley and spring soft wheat of competitive variety testing to dark brown spot was evaluated. The most toxigenic isolates of Bipolaris sorokiniana were preliminarily selected for the production of inoculum, which was used to treat horizontally placed seedlings of the studied cereal samples in the 1-2 leaf phase. The development of the disease was assessed on a scale from 0 to 6. The sources of resistance included highly resistant samples, the damage of which did not exceed 2 points. These are samples of spring barley V19-6718, V21-6723, B25-6260, B33-6315, V33-6767, V34-6713, V40-6828, K-91-2, K104-1, S-106, K-134- 3 and spring soft wheat Altaiskaya 75, K-613-2, K-654-1, K-229-9, K-693-2, K-780-1, K-696-7, KSI-10, of interest for selection for fungal diseases.

1 Introduction

Barley and wheat are the main crops in world agricultural production. The damage of these cereals by widespread leaf spots negatively affects the productivity of plants and the quality of the seeds obtained. The use of modern agrotechnological methods and the use of fungicides can partially mitigate the situation, but at present the development of resistant varieties is considered the most effective, cost-effective and environmentally friendly way to combat the disease.

Dark brown leaf spot is caused by an imperfect micromycete-hemibiotroph with a wide habitat *Bipolaris sorokiniana*, which is also one of the causes of diseases such as root rot and black germ of cereals [1-4]. Fungi genus *Bipolaris* are producers of hydrolytic enzymes and phytotoxic substances. The most common and active compounds secreted by *B. sorokiniana* are helminthosporal, prehelminthosporol, and victoxin toxins [5].

B. sorokiniana has a wide host range and is capable of infecting all of their organs. The primary symptoms of the disease appear as small dark brown spots, which merge during the years of epiphytotics, covering 50-90 percent of the leaf area, which subsequently dies off.

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Yield losses from dark brown spotting can reach 100%, depending on the variety and region of growth [6].

The degree of *B. sorokiniana* damage to plants of different genotypes can be affected by the density of stomata and trichomes, as well as their morphological and anatomical features, lignin content in leaves, relative humidity and temperature regime of the growing season, etc. Resistance to dark brown spotting is under polygenic control. This factor, as well as a number of others, such as the lack of sources of resistance, the peculiarities of assessing plant damage in the field and laboratory conditions, and adaptation of the pathogen to the host, are the reason for slow selection for immunity to this disease [7–9].

The aim of this study was to form a collection of sources of a high level of resistance to regional populations of the dark brown leaf spot pathogen *B. sorokiniana* based on the breeding material of barley and wheat of the Krasnoyarsk Research Institute of Agriculture.

2 Materials and results

Juvenile resistance of accessions of spring barley and spring soft wheat from competitive variety testing 2020-2021. Breeding units of the Krasnoyarsk Research Institute of Agriculture to dark brown leaf spot were evaluated in laboratory conditions. To do this, the seeds were sown on moistened cotton wool and placed on a light installation (2500 Lx, 22-25ºC). 7 days after sowing, plants in the 1-2 leaf phase were used for inoculation. The seedlings were evenly sprayed with a suspension of conidia of the most toxigenic *B. sorokiniana* isolates with a titer of $50x10^3$conidia/ml and placed under conditions of 100% humidity [10].

After 5-7 days, at the time of death of susceptible control plants (wheat variety Uyarochka, barley variety Biom), the development of the disease was assessed on a scale from 0 to 6, where 0 is the absence of symptoms of damage, 1,2,3,4 - affected 10, 20, 30, 40% of the leaf surface, 5 - more than 50% of the leaf surface is affected, 6 - leaf death (Figure 1). Samples, the defeat of which does not exceed 2 points, are highly resistant and can be considered a source of resistance to the disease; 3-4 points ¬ moderately stable; 5-6 points susceptible.

Fig. 1. Screening of barley for resistance to dark brown blotch in laboratory conditions.

The toxigenicity of *B. sorokiniana* isolates was assessed by the effect of their cultural filtrate (CF) on germinating cereal seeds. To obtain CF, the block with the culture of the isolate was placed on Czapek's liquid nutrient medium and grown for 14 days at a temperature of 25°C. Then it was filtered, freeing from mycelium and conidia, and poured 20 ml into Petri dishes. Seeds of the wheat variety Kuraginskaya 2 and the barley variety Krasnoyarsky 91,

previously washed for 2 hours under running water, were placed in the CF for 24 hours. The experiment was carried out in 5 repetitions, 10 seeds in each. After exposure to CF, the seeds were placed in Petri dishes on moist filter paper discs and germinated for 7 days at a temperature of 25°C. Water was used as a control. The number of germinated seeds was taken into account, and the length of the seedling was measured.

The production of the inoculum of the most toxigenic *B. sorokiniana* isolates was carried out on barley grain [11]. After formation, the spore material was dried and stored in a refrigerator at 3°C.

3 Results and discussion

To determine the juvenile resistance of grain samples to dark brown spotting, the pathogenic properties of 16 local isolates of the causative agent of this infection from our own collection of phytopathogens were previously studied. 7 *B. sorokiniana* isolates were isolated from wheat leaves and seeds, and 9 ones from barley.

Complete inhibition of seed germination of Kuraginskaya 2 after exposure to CF of each of the studied isolates isolated from wheat was not observed. A significant difference from the control in the percentage of germination suppression was found in CF isolates B 4.5.2 and B 11.8, which already indicates their high toxigenicity. No significant effect of CF of *B. sorokiniana* isolates on the length of wheat seedlings was found (Table 1).

Table 1. Influence of cultural filtrates of *B. sorokiniana* isolates on the germination and length of seedlings of seeds of wheat Kuraginskaya 2.

Isolate	Germination, %	Difference with control	Length of seedings, mm	Difference with control
water (control)	80.0	–	77.4	–
V 23.1	83.3	3.3	75.5	-1.9
V 21.4	80.0	0.0	90.6	13.2
V 17ch5	86.7	6.7	72.0	-5.4
V 4.5.2	56.7	-23.3	91.7	14.3
V 17ч3	80.0	0.0	91.6	17.2
V 17ч2	73.3	-6.6	95.3	17.9
V 11.8	60.0	-20.0	92.9	15.5
	$НСР_{0.05}$=19.5		$НСР_{0.05}$=23.0	

Similarly to Kuraginskaya 2, the percentage of suppression of seed germination of the Krasnoyarsky 91 barley variety was quite high. The toxigenicity of the studied isolates of *B. sorokiniana* isolated from barley is also evidenced by the results on the effect of CF on the length of seedlings. A significant negative effect of the impact of CF on the length of the seedling of barley cv. Krasnoyarsky 91 was observed in isolates B 51, B 53, B 57 (Table 2).

Table 2. Influence of cultural filtrates of *B. sorokiniana* isolates on the germination and length of seedlings of barley seeds Krasnoyarsky 91.

CF	Germination, %	Difference with control	Length of seedings, mm	Difference with control
water (control)	65.0	–	38.8	–
B 48	47.5	-17.5	32.8	-6.1
B 49	32.5	-32.5	31.5	-7.4
B 50	27.5	-37.5	33.6	-5.2
B 51	5.0	-60.0	26.3	-12.6
B 53	12.5	-52.5	27.3	-11.6
B 55	30.0	-35.0	34.8	-4.1
B 57	5.0	-60.0	26.6	-12.3
B 59	27.5	-37.5	34.9	-3.9
B 60	22.5	-42.5	29.1	-9.7
	$HCP_{0.05}$=19.8		$HCP_{0.05}$=19.8	

Based on this, isolates of *B. sorokiniana* B 4.5.2, B 11.8, B 51, B 53, B 57 were recognized as the most toxigenic and were subsequently used to produce spore material.

Further, when conducting a laboratory assessment of resistance to dark brown spotting, it was found that out of 56 samples of competitive barley variety testing, the largest number (29) were susceptible to the pathogen (reaction type 5-6). 16 were classified as moderately resistant (reaction type 3-4). 11 samples were highly resistant to *B. sorokiniana* (reaction type 1-2).

Laboratory screening of 22 wheat accessions from the competitive test showed that the Uyarochka variety with reaction type 5 is susceptible to dark brown spotting, 13 were moderately resistant, and 8 accessions had a high level of resistance (Table 3).

Table 3. Resistance of samples of barley and wheat competitive variety testing to dark brown spotting.

Type of resistance	Sample	
	Barely	Wheat
Highly resistant	V 19-6718, V 21-6723, B 25-6260, B 33-6315, V 33-6767, V 34-6713, V 40-6828, K-91-2, K-104-1, C-106, K-134-3	Altayskaya 75, K-613-2, K-654-1, K-229-9, K-693-2, K-780-1, K-696-7, KSI-10
Medium resistant	Abalak, Krasnoyarsky 80, Bakhus, Vulkan, Cedar, B 59-6488, Acha, Krasnoyarsky 91, Uvatsky, C-175, Sobolek, V 89-6975, B 32-6302, V 56-6885, B 51-6431, B 67-6550	Svirel, Chulymskaya, Novosibirskaya 15, Altaiskaya 70, Kuraginskaya 2, Krasnoyarskaya 12, Kanskaya, K-693-1, KSI-21, K-712-4, K-698-2, K-696-6, K-733-3
Receptive	Buyan, Emelya, Olenek, Biom, Takmak, Agul 2, B 10-6180, V 21-6726, V 33-6775, B 6-6146, B 16-6227, E 37-53217, E 76-5695, V 65-6920, V 53-6870, B 70-6465, V 43-6836, C-110, C-14, C-126, C-54, V 46-6850, B 19-6240, B 12-6208, V 33-6770, B 4-6123, B 2-6119, K-91-8, K-101-2	Uyarochka

4 Conclusion

Thus barley samples V 19-6718, V 21-6723, B 25-6260, B 33-6315, V 33-6767, V 34-6713, V 40-6828, K-91-2, K-104-1, S-106, K-134-3 and wheat Altayskaya 75, K-613-2, K-654-1, K-229-9, K-693-2, K-780-1, K-696- 7, KSI-10 can serve as sources of resistance to regional populations of the pathogen of dark brown leaf spot and represent a valuable material for breeding spring barley and soft spring wheat to fungal diseases.

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