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Evaluation of Lithuanian winter wheat breeding lines for *Bipolaris sorokiniana* resistance by detached leaf technique

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The research was conducted at the Lithuanian Institute of Agriculture (LIA) during the period 2005–2008. Resistance of advanced winter wheat breeding lines to *Bipolaris sorokiniana* monoconidial isolates obtained from wheat straw and grain was evaluated under laboratory conditions using the detached leaf technique. A total of three checks with a known resistance level and 104 advanced breeding lines were investigated using four *B. sorokiniana* isolates.

The screening technique used revealed a low resistance of the test material. The resistant cultivar BR8 and the moderately resistant cultivar BH1146 were found to be moderately susceptible (6.1 and 7.0 scores, respectively). The line 'Zentos / Lut97-4' was slightly more resistant (5.9 scores) than BR8 (6.1 scores). The ten lines possessed a resistance level similar to that of the cultivars BR8 and BH1146. The rest of the lines (79.4%) were susceptible or very susceptible. Analysis of the pedigree of the lines did not reveal any clear impact of parental cultivars on the resistance of lines. Only the line WW2498 conferred resistance as all the four breeding lines possessing it were given 6.8 to 7.0 scores. Pathogen isolates with a lower aggressiveness could be more adequate for testing low-resistant wheat.

Key words: *Triticum aestivum*, spot blotch, isolates, susceptibility

INTRODUCTION

Spot blotch caused by *Bipolaris sorokiniana* (Sacc.) Shoem. (syn. *Helminthosporium sativum*, teleomorph *Cochliobolus sativus*) is one of the most important foliar diseases limiting wheat production in warmer, non-traditional growing areas. The pathogen has a worldwide distribution, but is particularly aggressive under conditions of high relative humidity and temperature associated with imbalanced soil fertility [1]. *Bipolaris sorokiniana* is seed-born, and black point and seedling blights are different forms of the disease. Although spot blotch, common root rot and black point are caused by the same pathogen and may occur in combination, one disease form usually prevails over the others, depending on the environmental conditions [2]. Yield losses are variable, but are important in fields with low inputs and under late-sown conditions. Diseased plots yielding by 60% and 20% less than fungicide-protected plots of susceptible and resistant cultivars, respectively, have been found in Nepal [3, 4] and up to 22% in Bangladesh [5]. Diseased wheat plots in the dry country Mexico without fungicides yielded 43% less [6]. In some locations, the disease prevents wheat from becoming a commercial crop. Grain infection by this fungus in a year favourable to the disease was up to 70% in the study of Sharma-Poudyal et al. 2005 [7].

At higher latitudes such as the Canadian and USA prairies [8, 9], and in parts of Australia [10], southern Brasilia [11], *B. sorokiniana* is the dominant pathogen among fungi causing common root rot, resulting in up to 19% losses. A similar situation has been reported in Nepal [12]. Under dry conditions of Turkey, *B. sorokiniana* was widespread on wheat subcrown inter-node and crowns [13].

In the Russian Federation, this foliar disease is encountered especially often in the Far East (Primorskii Krai); also, some records show that this pathogen was highly harmful to wheat in the west-north area [14]. The harmfulness of *B. sorokiniana* in the west-north of the Russian Federation suggests that this fungus can become a serious wheat pathogen in Europe. It is likely that the fungus as a wheat pathogen moves to more northern areas. The inoculum of the fungus is widely spread and persistent [15]. The situation is also complicated by the fact that barley is frequently infected by

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B. sorokiniana in Europe [16], and isolates of this fungus from remote places and plant species genetically differ insignificantly [17, 18].

Under European conditions, *B. sorokiniana* causes yield losses mostly due to root rot [19] and seed black point which negatively affects seed germination and cause root rots in seedlings [20–23]. So far, no significant negative effects on the foliage of winter wheats were reported. Only some data have been presented about this fungus on wheat leaves [24, 25].

The control strategy for the disease caused by *B. sorokiniana* is based on an integrated approach where genetic resistance is a major element. It is generally accepted that the resistance is not satisfactory. However, recent studies show that after several decades of intensive breeding efforts some progress has been achieved in Bangladesh [5], USA [26], India [27], Australia [28], Mexico [29] and in other countries where this pathogen causes yield losses. Also, a broad range of resistance donors are available [30–32].

Research on the resistance of European winter wheat material to *B. sorokiniana* is scanty. Therefore, the present study was aimed to determine the resistance of advanced Lithuanian winter breeding lines developed basically using the European germplasm.

MATERIALS AND METHODS

The research was conducted at the Lithuanian Institute of Agriculture (LIA) in 2005–2008. The resistance of advanced winter wheat breeding lines to *B. sorokiniana* monoconidial isolates obtained from wheat straw and grain was evaluated under laboratory conditions using the detached leaf technique.

Monoconidial isolates. The fungus was isolated from grain and straw samples randomly collected from winter wheat plots at the LIA winter wheat breeding nurseries at the seed ripening stage in 2005. After sterilization in 1% sodium hypochlorite and rinsing in sterile distilled water, straw pieces were placed in Petri dishes on sterile-water moistened filter paper. Sterilised grains were placed in Petri dishes on water agar (1.5%). Traced spores were transplanted on 15% V8 agar (150 ml mix of vegetable juice, 850 ml water, 2 g $CaCO_3$, 20 g agar) to multiply spores. Monoconidial cultures were produced for each isolate as follows: a dilute spore suspension was prepared from the pure cultures obtained on V8 and then plated on fresh water agar. Single conidia were individually transferred to new V8 medium plates with the help of stereo binoculars and a sterile needle. Cultures were grown at 20 °C in the dark. Monoconidial cultures were stored at 4 °C in the dark.

Selection of isolates. Isolates were planted on potato dextrose agar (2%) and grown at 20 °C in the dark for 5 days. Four isolates were selected because of a different colony growth rate and mycelium colour [15, 18, 33].

Preparation of spore suspension. The inoculum was prepared as follows: after 14 days of growth on V8 agar medium in an incubator at 22 °C in the dark, conidia were collected by flooding the Petri dishes with sterile distilled water and scraping the agar surface with a spatula to dislodge the conidia. The conidial suspension was filtered through a double layer of cheesecloth. The concentration was adjusted to 5000 conidia per ml with the help of a haemocytometer. One drop of Tween 20 per 100 ml of the prepared suspension was added as a surfactant.

Plant material growing and preparation for inoculation. Breeding lines were seeded with surface-sterilized seeds in seedling growing blocks in commercial soil substrates. Wheat seedlings were grown in growth chambers under a 16 / 8 h day / night photoperiod and in a 16 / 20 °C temperature regime for 10 days. Primary leaves were detached to 4 cm segments and placed into plastic boxes on filter paper moistened with water supplemented with 100 mg l^{-1} benzimidazole. Four leaf segments were used per one replication. The Lithuania-registered cultivar 'Zentos' was used as a susceptible check. Two cultivars widely used in researches on *B. sorokiniana* – 'BR8' and 'BH1146' – were used as resistant and moderately resistant checks, respectively [34]. The check cultivars were placed twice per box. The test was made in three replicas and repeated twice.

Inoculation, maintenance and scoring of test material. The prepared leaves were inoculated with a spore suspension by spraying it until the run of drops occurred. The inoculated plant material was incubated at 20 °C in the dark for 24 hours. Afterwards, the plant material was kept in growth chambers under a day / night 16 / 8 h photoperiod and in a 18 / 20 °C temperature regime until scoring. The evaluation of resistance was done when at least 90% of all susceptible checks scored 9. The scoring was done using the scale as follows. Score 1: infection 0%, very resistant (VR); 1.1–3.0: >0–10.0%, resistant (R); 3.1–5.0: 10.1–50.0%, moderately resistant (MR); 5.1–7.0: 50.1–75.0%, moderately susceptible (MS); 7.1–8.0: 75.1–90.0%, susceptible (S); 8.1–9.0: 90.1–100%, very susceptible (VS).

RESULTS AND DISCUSSION

In total, three checks with the known resistance level and 104 advanced breeding lines were investigated (Fig. 1). The winter wheat genotypes differed significantly in spot blotch resistance. However, the screening technique used revealed a low resistance of the test material. Cultivar BR8 in the literature mentioned as resistant and cultivar BH1146 as moderately resistant were evaluated as moderately susceptible (score 6.1 and 7.0, respectively) (Table 1). Only the line 'Zentos / Lut97-4' was slightly more resistant (score 5.9) than BR8 (score 6.1). The rest of the 10 moderately susceptible lines were evaluated by scores (6.4–7.0) ranging within the values of the abovementioned checks. Of the test lines, 73 (70%) were susceptible. Only the line 'Biscay / Dream' (score 8.8) was slightly more susceptible than the susceptible check cultivar 'Zentos' (score 8.6). Moreover, 20 lines were very susceptible.

Table 1. **Winter wheat advanced breeding lines most divergent by resistance to *B. sorokiniana***

Line (cultivar)	Catalogue number	Mean	Isolates			
			1	2	3	4
		Disease severity, scores				
Most resistant						
Zentos / Lut 97-4	6100-2	**5.9**	7.3 a-d*	5.7 a	5.0 a	5.7 ab
BR-8 – R check		**6.1**	**6.7 ab**	**6.7 a-d**	**6.0 a-c**	**5.0 a**
Zentos / Lut 9-371	6090-4	**6.4**	7.7 b-e	7.3 c-f	5.7 a-c	5.0 a
Pobeda / Lut 9-321	5047-1	**6.5**	6.3 a	6.3 a-c	6.7 c-e	6.7 b-c
Dream / Pesma	5368-2	**6.7**	8.3 d-g	6.3 a-c	5.7 a-c	6.3 b-c
Flair / Lut 9-329	5060-47	**6.7**	7.3 a-d	6.3 a-c	6.3 b-d	6.7 b-c
Dream / 91002 G 2.1	5017-1	**6.8**	6.3 a	6.3 a-c	8.0 e-g	6.3 b-c
Flair / Ansgar	6094-1	**6.8**	8.0 c-g	7.7 d-h	5.3 ab	6.3 b-c
WW 2498 / Sj 965491	5059-2	**6.8**	6.3 a	6.0 ab	7.3 d-g	7.7 cd
WW 2498 / Sj 965491	5059-4	**6.9**	7.0 a-c	7.0 b-e	7.3 d-g	6.3 b-c
WW 2498 / Aspirant	5060-2	**7.0**	6.7 ab	7.7 d-h	7.3 d-g	6.3 b-c
WW 2498 / Aspirant	5060-1	**7.0**	6.3 a	7.3 c-f	7.7 d-g	6.7 b-c
BH1146 – MR check		**7.0**	**7.3 a-d**	**8.0 e-i**	**7.3 d-g**	**5.9 ab**
Most susceptible						
Biscay / Pobeda	5028-5	**8.4**	8.3 d-g	8.7 g-i	8.7 f-g	8.0 d-f
STH 1096 / Bussard	6031-3	**8.5**	8.7 e-g	9.0 i	8.7 f-g	7.7 cd
Zolotava / Lut 9-365	6057-3	**8.5**	9.0 fg	8.7 g-i	7.7 d-g	8.7 d-f
Zentos - S check		**8.6**	**9.0 fg**	**8.0 e-i**	**8.7 f-g**	**8.7 d-f**
Maverich / Savannah	6047-4	**8.6**	9.0 fg	8.7 g-i	8.3 f-g	8.3 d-f
Biscay / Pobeda	5028-1	**8.6**	8.3 d-g	8.3 f-i	8.7 f-g	9.0 ef
Biscay / Dream	5025-4	**8.8**	9.0 g	8.3 f-i	8.7 g	9.0 f

* Means followed by the same letters do not differ according to Duncan's Multiple Range Test at 1% of significance.

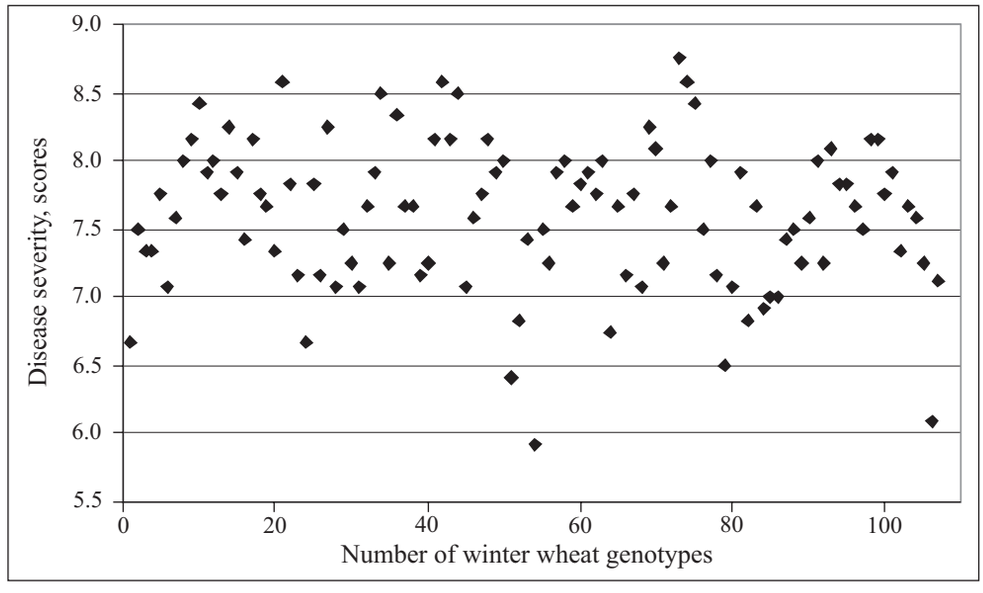

Fig. 1. Distribution of winter wheat genotypes by resistance to *B. sorokiniana*

The first, second and third isolates used were very similar in the mean disease severity score of the test genotypes (scores 7.9, 7.9, 7.8, respectively) (Fig. 2). Only the fourth isolate exhibited a lower aggressiveness (score 7.0). The aggressiveness of the isolates depended on their morphological characteristics in the same manner as reported in [18]. The isolates of *B. sorokiniana* did not differ considerably in virulence on wheat [18, 35, 36]. Therefore, selection of isolates depends on their aggressiveness level. The latter, in turn, is selected according to the resistance level of the available breeding material. If wheat genotypes possess a considerable resistance level, the use of more aggressive isolates highlights the most resistant lines. However, when the breeding material is of a poor resistance level, as was in our case, less aggressive isolates could give a more adequate resistance differentiation of the genotypes. This technique could allow the concent-

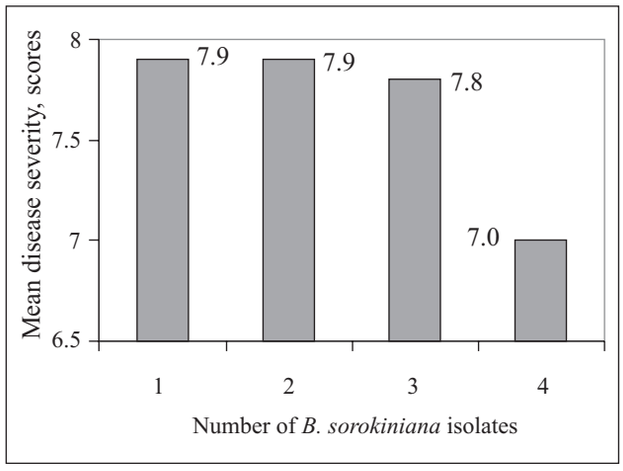

Fig. 2. Mean disease severity score of all winter wheat genotypes by every *B. sorokiniana* isolate

ration of resistance genes with a lower efficiency to develop new breeding lines possessing some resistance [37].

The parental material used for breeding as well as the developed advanced lines were not intensively and purposively selected for spot blotch resistance. Nonetheless, about 10% of breeding lines possessed a similar resistance level as do moderately resistant and resistant check cultivars.

Analysis of the pedigree of the test lines did not reveal any clear impact of parental cultivars on the resistance of lines. Only the line WW2498 conferred resistance as all the four breeding lines possessing it were given 6.8 to 7.0 scores. On the other hand, the cultivars 'Flair' and 'Dream' were widely used as parental material and were present in the pedigree of 19 and 14 lines, respectively (Table 2). These lines expressed almost the whole range of resistance reactions and were evaluated by 6.7 to 8.8 scores. The lines differed in resistance level at the same growth stage and under the same

Table 2. **Resistance to *B. sorokiniana* of winter wheat lines possessing the same cultivars in their pedigree**

Line (cultivar)	Catalogue number	Mean	Isolates			
			1	2	3	4
			Disease severity, scores			
Flair / Lut 9-329	5060-47	6.7	7.3 a-d	6.3 ab	6.3 a-c	6.7 a-c
Flair / Ansgar	6094-1	6.8	8.0 c-g	7.7 b-e	5.3 a	6.3 a
Flair / Haldor	6013-2	7.1	8.3 d-g	7.3 b-e	6.3 a-d	6.3 a-d
Flair / Pentium	6008-1	7.1	9.0 fg	7.7 b-e	5.7 ab	6.0 ab
Flair / Bill	6007-1	7.2	8.7 e-g	8.0 c-e	6.3 a-d	5.7 a-d
Flair / Haldor	6013-1	7.3	9.0 fg	7.0 a-c	6.3 a-d	6.7 a-d
Flair / Lut 9-329	5060-15	7.3	8.7 e-g	6.0 a	8.7 g-i	6.0 g-i
Flair / Kris	5043	7.4	7.0 a-c	7.3 b-e	9.0 i	6.3 i
Flair / Ansgar	6094-2	7.4	8.3 d-g	8.3 c-e	6.7 b-e	6.3 b-e
Flair / Lut 3-96	5063-77	7.5	8.0 c-g	7.7 b-e	8.3 f-i	6.0 f-i
Residence / Flair	5067-2	7.5	7.0 a-c	8.7 de	8.3 f-i	6.0 f-i
Flair / Charger	6012-1	7.5	9.0 fg	7.7 b-e	6.7 b-e	6.7 b-e
Elena / Flair	4380-1	7.6	7.7 b-e	7.7 b-e	7.7 d-i	7.3 d-i
Flair / Kris	6014-1	7.7	8.7 e-g	7.7 b-e	7.0 c-f	7.3 c-f
Elena / Flair	4380-2	7.7	7.3 a-d	8.3 c-e	7.7 d-i	7.3 d-i
Emma / Flair	5032-1	8.0	7.7 b-e	8.3 c-e	8.3 f-i	7.7 f-i
Biscay / Flair	5024-3	8.1	7.7 b-e	8.3 c-e	8.3 f-i	8.0 f-i
Flair / Bill	6007-3	8.3	9.0 fg	8.7 e	7.3 c-g	8.0 c-g
Biscay / Flair	5024-2	8.3	8.3 d-g	8.3 c-e	8.3 f-i	8.0 f-i
Dream / Flair	5014-4	7.8	7.7 b-e	8.3 c-e	7.3 c-g	8.0 c-g
Dream / Flair	5014-5	7.9	8.0 c-g	8.0 c-e	7.3 c-g	8.3 c-g
Dream / Pesma	5368-2	6.7	8.3 d-g	6.3 ab	5.7 ab	6.3 ab
Dream / 91002 G 2.1	5017-1	6.8	6.3 a	6.3 ab	8.0 e-i	6.3 e-i
Dream / Bill	5023-4	7.1	7.3 a-d	7.7 b-e	7.7 d-i	5.7 d-i
Dream / Asketis	5020-2	7.2	7.3 a-d	7.0 a-c	7.3 c-g	7.0 c-g
Dream / Pesma	5368-9	7.2	8.0 c-g	7.7 b-e	6.7 b-e	6.3 b-e
Bill / Dream	6062-1	7.3	7.3 a-d	8.0 c-e	7.3 c-g	6.3 c-g
Dream / 91002 G 2.1	5017-2	7.7	6.7 ab	8.0 c-e	9.0 hi	7.0 hi
Biscay / Dream	5025-3	7.7	7.3 a-d	8.3 c-e	8.0 e-i	7.0 e-i
Dream / Convent	5016-1	7.8	7.7 b-e	7.7 b-e	7.7 d-i	8.0 d-i
Dream / Bill	5023-3	7.8	8.0 c-g	8.0 c-e	8.3 f-i	6.7 f-i
Dream / Pesma	5368-3	7.8	7.7 b-e	7.3 b-e	8.7 g-i	7.7 g-i
Dream / Convent	5016-5	8.0	8.3 d-g	7.3 b-e	8.0 e-i	8.3 e-i
Biscay / Dream	5025-4	8.8	9.0 g	8.3 c-e	8.7 g-i	9.0 g-i

conditions. Therefore, they could possess different resistance genes. The resistance of wheat to spot blotch depends on quantitative genes which differ in their effectiveness. Some of them can be responsible for 50% of effectiveness, whereas those least effective can be responsible for only several percent from the total resistance level [30, 38–40]. Such a high variation of gene effectiveness among resistance genes could explain the variability of resistance reaction among the breeding lines.

B. sorokiniana under European conditions is harmful to wheat and causes seedling and adult plant root rots. The other fungi causing root rots are more harmful, therefore they usually mask the damage done to wheat by *B. sorokiniana*. The harmfulness of root rots has been proven to cause considerable damage. However, publications concerning winter wheat cultivars' resistance to a complex of these pathogens in Europe are very rare as compared with papers dealing with resistance to foliar pathogens. The main reason is a much more expensive and longer investigation period. Also, a huge constraint is the impossibility during selection of lines in early generations to evaluate root damage level without destroying a plant. This could be done at harvesting, but there is usually a shortage of time for a detailed screening of thousands of lines. Also, evaluating root health once at ripening is useful when the disease pressure is moderate. The differences among the genotypes will be insufficient under a high disease pressure since roots will be rotten more severely. Wheat genotypes do not differ markedly when the disease pressure is too low. Detailed investigations of root rot resistance can be done with advanced breeding lines. However, the majority of breeding lines in early generations, even those showing resistance to root rots, are discarded if they do not exhibit an adequate yielding capacity. Material valuable for resistance breeding is discarded, too.

Considering the resistance level of the resistant check cultivars BR8 and BH1146, lines characterized as moderately susceptible could be more resistant under field conditions than the ones evaluated by higher disease scores. Comparison of wheat resistance data from laboratory and field shows some inconsistencies. When laboratory and field data are compared, one should bear in mind that the genotypes that are evaluated as resistant in laboratory at seedling stage are characterized only by resistance reaction but not by disease progress as in field conditions. It has been shown in many researches that under a high disease pressure, in field conditions susceptible genotypes are evaluated by a disease severity of 70–90% and the AUDPC value over 2000, whereas resistant ones are characterized by 10–30% of disease severity and the AUDPC value up to 1000 [27, 32, 39].

The resistance reaction of wheat to spot blotch in laboratory and field conditions shows a medium to strong correlation level [14]. Such a correlation level suggests a convenient possibility for searching of resistance sources among thousands of accessions. The same correlation level is obtained when resistance to root rot at seedling and adult plant stages is compared [14]. The correlation is lower when resistance to spot blotch and root rot is compared [14, 41]. However, the resistance of different parts of barley plants to the same pathogen was weak, medium or strong depending on a set of the test cultivars and isolates [16, 42]. Also, the screening techniques differ. Spot blotch resistance at seedling stage is usually tested within several days, whereas screening of root rot resistance takes several weeks. In this case, a higher influence of partial resistance in roots as well as spot blotch progress during vegetation are possible.

Spot blotch is a more theoretically possible problem of wheat in Europe at present. However, the situation with spot blotch can follow the model of spread of tan spot (caused by *Pyrenophora tritici-repentis*) all over the world [43] when during several decades the pathogen from minor has become one of the most harmful diseases of wheat, like spot blotch in Asia region. A similar situation has happened with barley Ramularia leaf spot (caused by *Ramularia collo-cygni*) in Northern Europe and New Zealand [44]. In both cases, it has been suggested that the introduction of varieties with an increased susceptibility to abiotic stresses, coupled with a decreased competition from other foliar pathogens as a result of improved resistance and technological control, are possible reasons for the appearance and increase of tan spot and Ramularia leaf spot.

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LIETUVIŠKŲ ŽIEMINIŲ KVIEČIŲ SELEKCINIŲ LINIJŲ ATSPARUMO *BIPOLARIS SOROKINIANA* VERTINIMAS LAPŲ SEGMENTŲ METODU

Santrauka

Tyrimai atlikti Lietuvos žemdirbystės institute 2005–2008 metais. Perspektyvių žieminių kviečių selekcinių linijų atsparumas buvo įvertintas laboratorinėmis sąlygomis naudojant lapų segmentų metodą ir *B. sorokiniana* monokonidijinius izoliatus. Iš viso tirta trijų žinomo atsparumo kontrolinių veislių bei 104 perspektyvių selekcinių linijų atsparumas keturiems *B. sorokiniana* izoliatams. Naudotas metodas atskleidė nedidelį tirtos medžiagos atsparumą. Atspari veislė 'BR8' ir vidutiniškai atspari veislė 'BH1146' buvo vidutiniškai jautrios – atitinkamai 6,1 ir 7,0 balai. Linija 'Zentos / Lut97-4' buvo nedaug atsparesnė (5,9 balo) nei kontrolinė atspari veislė 'BR8' (6,1 balo). Dešimties linijų atsparumas buvo panašus kaip ir 'BR8' bei 'BH1146' veislių. Likusios linijos (79,4%) buvo jautrios ir labai jautrios. Tirtų linijų kilmės analizė neatskleidė reikšmingos tėvinių veislių įtakos atsparumui. Tarp visų naudotų tėvinių formų tik 'WW2498' linija aiškiai buvo atspari, nes visos 4 selekcinės linijos, turėjusios šią liniją savo kilmėje, buvo įvertintos labai panašiai (6,8–7,0 balai). Patogeno izoliatai, pasižymintys mažesniu agresyvumu, būtų tinkamesni nedidelio atsparumo kviečių medžiagos tyrimui.

Raktažodžiai: *Triticum aestivum*, dėmėtligė, izoliatai, jautrumas