RESISTANCE OF SPRING BARLEY GENOTYPES TO *BIPOLARIS SOROKINIANA*

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Resistance of 43 spring barley genotypes to *Bipolaris sorokiniana* was evaluated using three methods. Three distinct isolates of the pathogen were used for inoculation. Two experiments in growth chamber on seedlings or subcrown internodes and one in laboratory conditions on detached leaves were conducted. Disease severity of *B. sorokiniana* on leaves or subcrown internodes differed among the genotypes at the seedling plant stage. Results showed that the cultivar Brise was the most resistant with a mean disease severity of 1.62 and 2.00 in detached leaves and seedling plants, respectively. Cultivar Pax showed resistant reaction to infection of *B. sorokiniana* in all tests. Positive correlation coefficient ($r = 0.39$, $P \leq 0.05$) was found among the tests using the detached leaves and the seedling plants.

Key words: artificial inoculation, *Bipolaris sorokiniana*, disease severity, *Hordeum vulgare* L.

INTRODUCTION

Bipolaris sorokiniana (Sacc.) Shoemaker (teleomorph *Cochliobolus sativus* (Ivo et Kurib.) Drechsler ex Dastur, syn. *Helminthosporium sativum* Pam. King et Bakke) is an important pathogen causing common root rot, crown rot (Wordell et al. 2005) and spot blotch disease in wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) in warmer and humid regions of the world (Saari 1998; Pandey et al. 2005; Joshi et al. 2007). *B. sorokiniana* usually induces symptoms on the leaf, sheath and stem (Chand and Joshi 2004). Yield losses due to spot blotch vary from 20 to 80% in wheat (Duveiller and Gilchrist 1994) and from 16 to 33% in barley (Clark 1979). Severe infections may also reach the spikes, resulting in low-weight, shrivelled grains (Kiesling 1985) with black point at the embryo end of kernels (Chand and Joshi 2004). *B. sorokiniana* induces lesions on the lower leaf sheath and reduces seedling height, seedling emergence, dry and fresh weight of roots and shoots during seeds germination (Gonzalez and Trevathan 2000).

The most important factor for the success of the pathogen has not been discovered yet and there is still no major resistance available. Disease severity, disease reaction, and incidence of *B. sorokiniana* in grains also differ among the cultivars at the adult plant stage (Wordell et al. 2005). Resistance in barley to *B. sorokiniana* is often evaluated at the seedling stage in the greenhouse and at the adult plant stage in the field (Fetch and Steffenson 1994; Steffenson and Fetch 1996; Arabi and Jawhar 2004; Wordell et al. 2005).

There is not any reference to appearance and harmfulness of this pathogen in the area of the Slovak republic. The objective of this study was to evaluate the reaction of some spring barley genotypes to *B. sorokiniana* at the juvenile stages under controlled conditions.

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MATERIAL AND METHODS

Bipolaris sorokiniana isolates sample collection

The *B. sorokiniana* isolates were obtained from diseased barley leaves growing in different geographical regions of Slovakia (Piešťany, Levice and Veľký Krtíš). The isolates were cultivated on Czapek Dox agar medium and grown under controlled conditions at 20–23°C with a 12 h photoperiod (20 W fluorescent lamps emitting 50 µmol m^{-2} s^{-1}). The spore suspension culture was prepared from 14-day old pathogen culture of each isolate with concentration of 8×10^3 ml^{-1} conidia.

Detached leaf test

Resistance to pathogen was tested on 43 barley genotypes (cultivars from "List of registered varieties", resistant control lines CI-9819, CI-5791 and sensitive control SK 13-991; Table 1). The primary seedling leaves were cut and segments of 20–25 mm in length were placed on filter paper moistened with 0.004% solution of benzimidazole. The detached leaves (10 segments per genotype) were inoculated by a drop of each conidial suspension (approx. 0.5 ml) with 100 μl l^{-1} of the surfactant (Tween 80) and incubated under continuous light (20 W fluorescent lamps emitting 50 µmol m^{-2} s^{-1}). The response of the detached leaves was assessed on 4th day after inoculation by rating scale 1–4: 1 – dot necrosis without chlorosis (high resistance); 2 – brown necrosis limited to the diameter of the infection drop with weak chlorosis (resistance); 3 – brown necrosis limited to the diameter of the infection drop and chlorosis on most of the leaf surface or without necrosis (susceptible); 4 – dot necrosis and chlorosis occupied all leaf surface, mycelium tufts can be visible (high susceptibility).

Seedling test

The seedlings of spring barley genotypes were inoculated at growth stage 13 on Zadoks' scale (three completely expanded leaves, 12–14 days of age) (Zadoks et al. 1974), by spraying a conidial suspension of each isolates (5 ml of conidial suspension per 25 plants in a pot) with 100 μl l^{-1} of Tween 80. Inoculum was prepared, quantified, and applied to plants following the method of Fetch and Steffenson (1999). The disease evaluations were assessed 9 days after inoculation using the scale of Fetch and Steffenson (1999). The disease reaction scores varied from 0 to 9, where 0–3 were considered resistant, 4 and 5 moderately resistant, and 6–9 susceptible.

Pot experiment

Seeds of the spring barley genotypes (Table 1) without disease symptoms were surface-sterilized with 4% NaOCl solution for 5 min and then soaked three times in sterile distilled water. They were inoculated by immersion in conidial suspension of the mixture population of pathogen with 100 μl l^{-1} of Tween 80 for 2 h. Inoculated seeds were sown in pots filled with garden soil. The depth of seeds in soil was 6 cm (Kokko et al. 1993). The cultivars were arranged in a randomized complete block design in two replicates. All pots were placed in a growth chamber under the same conditions as for seedling experiment (see above). To determinate the plant responses to pathogen infection, seven plants were removed 3 weeks after inoculation from the pots. The percentage of infected area of subcrown internodes was evaluated using a 0–5 scale, where 0 is considered immune, no lesions; 5 is considered very susceptible according to Arabi and Jawhar (1999).

Statistics

Statistical analyses were carried out using the SPSS Base 13.0. Significance of difference between data was estimated by Analysis of Variance (ANOVA). Variation among means was analysed using Duncan's test ($P \leq 0.05$).

RESULTS

The response of tested detached leaves of 43 genotypes after inoculation by suspension of pathogen differed. Significant differences ($P \leq 0.05$) in mean severity values were detected. Six genotypes showed susceptible reaction and 37 genotypes resistant reaction (Table 1). Disease severity ranged from 1.62 to 2.78. The most resistant response was detected in the cultivar Brise and the genotype Jubilant showed the most susceptible reaction. The cultivars Celinka and Progres also belong to the most resistant genotypes to spot blotch, according to this test.

In seedling tests, no genotype was found to show sensitive reaction. Continuous range of responses from resistant (Brise) to moderately resistant (Saloon) was observed, but none was immune against the disease (Table 1). The most resistant reaction showed cultivars

T a b l e 1

Disease severity of the 43 barley genotypes inoculated with three isolates of *Bipolaris sorokiniana* under controlled conditions

Genotype	Detached leaf[1]		Seedling test[2]		Subcrown internodes[3]	
	Severity ± SD	Disease classification	Severity ± SD	Disease classification	Severity ± SD	Disease classification
Akcent	2.27±0.32 a-g	R	3.33±0.58 ab	R	4.29±1.89 fg	S
Amulet	2.13±0.31 a-g	R	4.00±1.00 ab	MR	2.86±2.67 b-f	MS
Annabell	1.85±0.41 a-e	R	3.67±0.58 ab	MR	4.43±1.51 fg	S
Atribut	1.78±0.33 a-d	R	3.67±1.53 ab	MR	4.14±1.86 fg	S
Biatlon	2.47±0.45 b-g	R	3.67±0.58 ab	MR	4.57±0.79 fg	HS
Bojos	2.38±0.56 a-g	R	4.00±1.00 ab	MR	4.57±0.79 fg	HS
Bolina	1.93±0.25 a-f	R	4.67±2.31 ab	MR	0.29±0.49 a	HR
Breamar	2.13±0.48 a-g	R	4.67±0.58 ab	MR	1.29±1.70 ab	HR
Brise	1.62±0.30 a	R	2.00±1.73 a	R	4.29±1.50 fg	S
Celinka	1.68±0.60 ab	R	2.67±1.53 ab	R	4.57±1.13 fg	HS
CI-5791	2.37±0.71 a-g	R	4.00±1.73 ab	MR	3.00±0.63 c-f	MS
CI-9819	2.02±0.55 a-g	R	4.33±0.58 ab	MR	1.86±1.95 b-d	R
Cyril	1.72±0.28 a-c	R	4.33±1.15 ab	MR	4.57±0.79 fg	HS
Danuta	1.87±0.32 a-f	R	3.67±0.58 ab	MR	5.00±0.00 g	HS
Ebson	1.95±0.61 a-f	R	2.67±0.58 ab	R	5.00±0.00 g	HS
Expres	2.68±0.38 fg	S	3.67±0.58 ab	MR	5.00±0.00 g	HS
Ezer	2.37±0.60 a-g	R	3.00±1.73 ab	R	5.00±0.00 g	HS
Forum	1.90±0.35 a-f	R	4.00±0.00 ab	MR	2.43±2.51 b-e	R
Garant	2.63±0.25 e-g	S	3.67±1.53 ab	MR	2.43±1.51 b-e	R
Hortop	2.38±0.45 a-g	R	3.67±1.53 ab	MR	3.57±2.44 e-g	S
Jubilant	2.78±0.37 g	S	4.67±0.58 ab	MR	5.00±0.00 g	HS
Kompakt	2.52±0.13 c-g	S	4.00±1.73 ab	MR	5.00±0.00 g	HS
Lenka	2.17±0.43 a-g	R	3.67±1.15 ab	MR	3.43±1.51 d-g	MS
Ludan	1.92±0.28 a-f	R	4.00±0.00 ab	MR	4.29±1.89 fg	S
Madonna	1.97±0.36 a-f	R	3.67±1.53 ab	MR	5.00±0.00 g	HS
Malz	2.38±0.40 a-g	R	3.67±2.08 ab	MR	4.14±1.57 fg	S
Margret	1.87±0.21 a-f	R	2.33±0.58 a	R	3.71±1.60 e-g	S
Messina	2.02±0.53 a-g	R	3.67±1.15 ab	MR	4.57±1.13 fg	HS
Nitran	1.97±0.28 a-f	R	4.33±1.53 ab	MR	4.14±1.46 fg	S
Novum	1.98±0.50 a-g	R	3.00±1.00 ab	R	3.86±1.21 e-g	S
Orbit	2.37±0.46 a-g	R	2.33±2.08 a	R	4.86±0.38 g	HS
Pasadena	1.90±0.26 a-f	R	3.00±1.00 ab	R	4.86±0.38 g	HS
Pax	1.72±0.33 a-c	R	3.00±2.00 ab	R	1.71±2.36 a-c	R
Pedant	2.02±0.38 a-g	R	3.67±1.53 ab	MR	4.43±1.51 fg	S
Prestige	2.35±0.38 a-g	R	4.00±1.73 ab	MR	4.00±1.53 e-g	S
Progres	1.68±0.39 ab	R	3.33±1.15 ab	MR	5.00±0,00 g	HS
Radegast	1.85±0.48 a-c	R	2.33±1.52 a	R	3.71±1.98 e-g	S
Saloon	2.50±0.10 b-g	S	5.33±0.58 b	MR	5.00±0.00 g	HS
SK 13-991	2.15±0.35 a-g	R	3.00±2.00 ab	R	4.86±0.3 g	HS
Sladko	2.40±0.17 a-g	R	4.00±2.00 ab	MR	4.43±0,79 fg	S
Svit	2.60±0.31 d-g	S	4.00±1.00 ab	MR	4.43±1.51 fg	S

Continuation of Table 1

Genotype	Detached leaf[1]			Seedling test[2]			Subcrown internodes[3]		
	Severity ± SD		Disease classification	Severity ± SD		Disease classification	Severity ± SD		Disease classification
Tolar	1.88±0.39	a-f	R	3.33±0.58	ab	R	5.00±0.00	g	HS
Vladan	2.10±0.38	a-g	R	3.33±1.15	ab	R	5.00±0.00	g	HS
$\bar{x}$	2.13±0.32			3.59±0.69			4.08±1.13		

[1]Assessed by rating scale 1–4 (see the text).
[2]Assessed by scale of Fetch and Steffenson (1999).
[3]Assessed by scale of Arabi and Jawhar (1999).
Means within columns followed by the same letter do not differ significantly at $P \leq 0.05$ based on the Duncan's test
HR = highly resistant, R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible, HS = highly susceptible.

Brise, Radegast, Margret and Orbit. The line CI-5791 which is according to Arabi (2005) considered resistant showed moderate resistance in these experiments. Differences among genotypes were found by Duncan's test ($P \leq 0.05$).

Similarly to the seedling test, the genotypes exhibited a continuous range of responses in the pot experiments. Control uninfected plants did not exhibit disease symptoms on cubcrown internodes. Disease severity on infected plants ranged from 0.29 to 5.00. Susceptible (e.g. Madonna, Danuta, Ezer etc.) to highly resistant (Bolina and Breamar) responses were observed. No genotype was found to be immune (Table 1). The resistant reaction was also observed in genotypes CI-9819, Forum, Garant and Pax (Table 1). Significant differences ($P \leq 0.05$) in mean severity values were detected among different genotypes, with severity values being consistently higher in some genotypes. Highest level of susceptibility when compared with detached leaves and seedlings was observed in 19 genotypes from 43 evaluated.

To conclude results of these three tests, only cultivar Pax showed resistant reaction to infection of *B. sorokiniana* in all tests. Four genotypes (Bolina, Bremar, CI-9819 and Forum) from 43 tested showed moderately resistant to highly resistant reactions in the tests. Cultivars Brise, Margret, Radegast, Akcent and Novum showed resistant reactions in the test with detached leaves and seedling tests but sensitive reaction on subcrown internodes. Following these results, genotypes mentioned above could be recommended as potential source of resistance of barley against *B. sorokiniana*.

DISCUSSION

The reaction of 43 barley genotypes differed significantly for the brown spot severity in reaction of seedlings, subcrown internodes and detached leaves (Table 1). Differential susceptibility of barley genotypes inoculated with isolates from barley leaves or seeds were also reported by some authors (Wilcoxson et al. 1980; Miles et al. 1987; Arabi 2005; Wordell et al. 2005).

The questions of barley resistance to *B. sorokiniana* and the determination of pathotypes were studied by several researchers (Fetch and Steffenson 1994; Meldrum et al. 2004; Arabi 2005). Fetch and Steffenson (1994) found isolates expressing low virulence on genotype ND B112 and stated that it is a primary source of resistance to *B. sorokiniana* in commercial six-row barley germplasm. In addition, Arabi (2005) found three genotypes (cultivar Banteng, Ethiopian line CI-5791 and Syrian line 79-SIO-9) that had partial resistance in all experiments. The Ethiopian line CI-5791 proved to have a resistant to medium sensitive reaction in our experiments. The resistance of the Ethiopian line CI-5791 and the Syrian line 79-SIO-9 to spot blotch confirm that Ethiopia and Syria are sources of resistance to several diseases (Arabi et al. 1990).

In search for control of spot blotch in barley through genetic resistance, the accumulation of information about sources and types of resistance is essential. Therefore, it is important to continue research in this area and to identify new sources with different levels of resistance. This study conducted under controlled conditions at specific developmental stages, demonstrates that using the above-mentioned methods

none of the genotypes tested showed immunity to the disease. However, certain cultivars (i.e. Pax, Brise, Celinka, Radegast, Margret and Novum) showed higher level of resistance in some cases. Cultivar Novum was also recommended by Psota and Bradová (2009) for its acceptable malting quality.

The evaluation of all tested genotypes on detached leaves correlated with seedling plants (r = 0.39, $P \leq$ 0.05). However, resistance of seedling plants showed negative correlation with subcrown internodes. The positive correlation between detached leaves and seedling plants was also reported by Arabi (2005). The cultivar Brise was the most resistant with a mean disease severity of 1.62 and 2.00 in detached leaves and seedling plants, respectively.

CONCLUSION

Our results demonstrated that used methods are simple and rapid. It enables to evaluate the resistance of plants rapidly under uniform conditions. On the other hand, under field testing the inoculum is not uniformly distributed and infection levels may fluctuate widely, requiring evaluation of resistance over several growing seasons.

This methods should be further tested on *B. sorokiniana* and other related pathogens, and if it shows reproducibility, it may then be adopted for routine use and can help the breeder to select resistant genotypes early in a breeding programme.

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