Short Communication

Use of Detached Seedling Leaf Test to Evaluate Wheat Resistance to Septoria Tritici Blotch

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Abstract: Two experimental sets of selected winter wheat cultivars, breeding lines, old Czech and Slovak landraces and wheat wild relatives were infected with three isolates (R-116, UH-105, BR-331) of *Mycosphaerella graminicola* (anamorph *Septoria tritici*) isolated in the Czech Republic. Groups of cultivars with different disease severity to all three isolates of the pathogen were found. Differences between old Czech and Slovak landraces and wild wheat relatives were larger than between modern wheat cultivars and breeding materials. In experiment one the isolate BR-331 differed significantly from the other two isolates in virulence to the tested modern wheat cultivars and breeding lines. The method is considered as suitable for preliminary screening in wheat resistance breeding or phytopathological testing and as a complement to field tests.

Keywords: *Mycosphaerella graminicola*; wheat; test of resistance; detached leaf technique; isolates

The fungus *Mycosphaerella graminicola* (sexual stage *Septoria tritici*) is currently a major disease in most European countries and it is important in many other regions worldwide (Eyal *et al*. 1987; Polley & Thomas 1991). European breeders have increased their efforts to select cultivars resistant to this disease (Johnson 1992). Populations of *M. graminicola* display high levels of genetic variability, as shown by molecular markers (Owen *et al*. 1998; Schneider *et al*. 1998; McDonald *et al*. 1999). Besides the availability of suitable resistance sources, the success of breeding for resistance to septoria tritici blotch (STB) undoubtedly depends on an appropriate method of testing for resistance. However, resistance to *M. graminicola* is a difficult phenomenon that is highly dependent on environmental conditions (Shaw & Royle 1993). Most of currently grown wheat cultivars are more or less susceptible to *M. graminicola*. Chartrain and Brown (2003) reported that twenty-four cultivars used as sources of resistance in world wheat breeding programmes were screened with a range of 12 isolates of the STB pathogen, *M. graminicola*. Several cultivars such as Kavkaz-4500 (KK), TE9111, Olaf and Veranopolis display high levels of resistance to STB in the field and have been durable sources of resistance. Zadoks (2003) showed that many older cultivars were medium resistant to *Septoria tritici*.

The aim of this study was to determine resistance or susceptibility of modern wheat cultivars, old cultivars and wheat wild relatives to three isolates of *M. graminicola*.

MATERIALS AND METHODS

The method of detached seedling leaf tests (Arraiano *et al*. 2001) was verified for use in resistance

wheat breeding or phytopathological testing. Two experimental sets of wheat cultivars with different reactions to *M. graminicola* were used: (1) seventeen modern wheat cultivars and breeding materials (Table 1); (2) eight old cultivars and five wheat relatives (*Triticum araraticum* JAKUBZ. var. *thumanianii* (JAKUBZ.) JAKUBZ., ECN:01C0105085; *Triticum monococcum* L. var. *sofianum* STRANK., ECN:01C0101086; *Triticum urartu* THUM. ex GANDIL., ECN:01C0104213; *Triticum boeoticum* BOISS. var. *boeoticum*, ECN:01C0104086; *Triticum dicoccoides* KOERN. ex ASCHERS. var. *kotschyi* JAKUBZ., ECN:01C0204539) (Table 2). Three *M. graminicola* isolates (R-116, UH-105 and BR-331) from different parts of the Czech Republic were used for infection. Disease tests were performed in the seedling stage on primary detached leaves. Each cultivar was tested in four replications. The inoculum was produced from sporulating cultures of *M. graminicola*, grown on potato dextrose agar for 7 days under near-ultraviolet light. The concentration of the conidial suspension was adjusted to 10^6 spores per ml. Wheat seedlings were sprayed using a Paint Spray Gun Kit with a spore suspension 20–30 ml.

Table 1. Experimental set 1 – average percentage of leaf areas covered by lesions bearing pycnidia of *M. graminicola*

Cultivars	Isolate			Cultivar mean
	R-116	UH-105	BR-331	
Ordeal	14.50	13.60	4.38	10.83
Samanta	17.50	6.00	12.88	12.13
SG-S1 990	19.70	14.55	2.75	12.33
SG-U 7029	9.90	27.25	0.00	12.38
Hereward	21.50	10.75	11.13	14.46
Milan	17.95	22.75	4.00	14.90
Reaper	15.15	25.50	7.38	16.01
Galaxie	23.80	17.75	11.25	17.60
Vlasta	20.05	23.75	9.25	17.68
SG-S1 880	25.80	20.25	9.25	18.43
Arina	26.25	21.20	9.38	18.94
Kavkaz	24.20	28.25	5.00	19.15
NSL 92/57	22.85	30.20	6.25	19.77
Batis	21.00	26.25	14.25	20.50
Estica	28.95	14.35	18.75	20.68
Verna	30.85	19.85	16.63	22.44
CWW 93/58	31.80	32.80	7.38	23.99
Isolate mean	21.88	20.84	8.82	17.19

Table 1a. Paired *t*-test for *M. graminicola* isolate differences (experimental set 1)

Isolates	Isolate difference	*t*-value
R-116 and UH 105	0.9824	0.4620
R-116 and BR-331	13.0494	10.7381**
UH-105 and BR-331	12.0671	4.8758*

*$P < 0.05$; **$P < 0.01$

Table 2. Experimental set 2 – average percentage of leaf areas covered by lesions bearing pycnidia of *M. graminicola*

Cultivars	Isolates			Cultivar mean
	R-116	UH-105	BR-331	
Hanácká Bělka	1.00	0.00	0.00	0.33
Slovenská B	27.50	0.00	0.00	9.17
T. araraticum	0.00	30.00	5.00	11.67
Kostomlatská Sametka	16.67	0.00	20.00	12.22
T. monococcum	0.00	20.00	20.00	13.33
T. urartu	8.75	7.50	32.50	13.58
Česká Přesívka	40.00	5.00	0.00	15.00
Dobrovická 10	51.25	0.00	0.00	17.08
T. boeoticum	27.50	27.50	5.00	20.00
Vousatka z Třebíče	26.67	17.50	40.00	28.06
Chlumecká 12	16.67	50.00	25.00	30.56
Slovenská 777	25.00	40.00	27.50	30.83
Pyšelka	48.75	53.33	0.00	34.03
Slovenská 2	27.50	30.00	60.00	39.17
Bílá od Dukovan	30.00	70.00	20.00	40.00
T. dicoccoides	0.00	40.00	80.00	40.00
Rokycanská Sametka	41.67	37.50	42.50	40.57
Isolate mean	22.88	25.20	22.21	23.27

Table 2a. Paired *t*-test for *M. graminicola* isolate differences (experimental set 2)

Isolates	Isolate differences	*t*-value
R-116 and UH 105	–2.3176	–0.3685
R-116 and BR-331	0.6724	0.0859
UH-105 and BR-331	2.9900	0.4663

The leaves were left to dry for 30 min and then they were cut into 3 cm segments and placed on benzimidazole water agar (100 mg/l benzimidazole) in plastic boxes (19.0 × 10.4 cm, with twelve boxes 5.0 × 3.0 cm). Rectangular sections (5 × 1 cm) were cut from the centre of the agar. The seedling leaf sections were laid, top surface uppermost, across the gap so that the ends rested on the agar. The gap below the leaves helped to prevent water soaking and contamination by other microorganisms. Strips of agar were then put over the cut edges of the leaf sections so that they were not exposed to air, thereby delaying senescence. The boxes were closed and covered with black plastic foil to keep them in darkness. The incubation temperature in a climate chamber was 20°C, light period 16 h; then the boxes were transferred to conditions with near-ultraviolet light at 15°C. The disease severity was scored as the percentage of leaf area covered by necrotic lesions bearing pycnidia, twice during the period of 20–26 days after inoculation. Aggressiveness of each isolate to all tested cultivars was expressed as mean percentage of leaf area covered by necrotic lesions bearing pycnidia.

Seeds of all cultivars were obtained from the Gene Bank in Research Institute of Crop Production, Prague-Ruzyně. The paired *t*-test (available in software STATISTICA) was used to separate the effect of isolates on disease severity of cultivars.

RESULTS AND DISCUSSION

Differences in the level of resistance to three isolates of *M. graminicola* were detected in both experimental sets. In the first experiment (Table 1) the most resistant cultivars were: Ordeal, Samanta, SG-S1990, SG-U7029, Hereward and Milan. On the contrary, the most susceptible reaction on average of all tested cultivars to three isolates was observed in the cultivars: CWW93/58, Verna, Estica and Batis. In previous field experiments Šíp *et al*. (2003) reported that the cultivars NSL9257, CWW93/58, Batis, Verna, Reaper, Estica and Ordeal had high field resistance to septoria tritici blotch. In our experiments the cultivar Ordeal was resistant to all three used isolates. But unlike to field tests the remaining cultivars CWW93/57, Batis, Verna, Reaper and Estica were on average susceptible and they had partial resistance to some isolates only. Chartrain *et al*. (2004) found out by testing twenty-four wheat cultivars and breeding lines for specific resistance to septoria tritici blotch caused by 12 isolates of *M. graminicola* that several cultivars, including Arina and Milan, had high levels of partial resistance to most isolates tested. In our experiments the cultivar Milan had medium resistance.

The results showed significant difference in disease severity between the isolates R-116 and BR-331 and between UH-105 and BR-331. The difference between the isolates R-116 and UH-105 was not significant.

The cultivar Hanácká Bělka was the most resistant in the second experiment with old Czech and Slovak land races and wild relatives (Table 2) and resistant were cultivars Slovenská B, *Triticum araraticum*, Kostomlatská Sametka, *T. araraticum* and *T. urartu*. Vice versa, the most susceptible were cultivars Rokycanská Sametka, *T. dicoccoides*, Bílá od Dukovan, Slovenská 2, Pyšelka, Slovenská 777 and Chlumecká 12. The cultivars Hanácká Bělka, Slovenská B and Dobrovická 10 were completely resistant to the isolates UH-105 and BR-331. High differences in disease severity between the tested land races and wild relatives were found in single isolates. However, no significant difference at the $P = 0.05$ was found between the used isolates.

Our experiments indicate that different isolates of *Mycosphaerella graminicola* are dissimilar in virulence to individual cultivars of wheat. Arama *et al*. (2000) showed that some isolates of *Septoria tritici* collected in Kenya from the same locality differed in virulence. Similarly like Chartrain *et al*. (2004), we can recommend for each tested material at least four replicate leaves and inclusion of different fungus isolates. The evaluation of disease severity is recommended to perform in four or five terms between 2 and 4 weeks after inoculation.

This study indicated that the detached seedling leaf method can be suitable for studying disease resistance of wheat to septoria tritici blotch and looking for new sources of resistance in old wheat land races and bread wheat relatives. Nevertheless, it should be regarded (Arraiano *et al*. 2001) as complementary to field studies of *M. graminicola*.

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Abstrakt

Věchet L., Vojáčková M. (2005): **Použití testu na listových segmentech pro hodnocení odolnosti pšenice k braničnatce pšeničné**. Czech J. Genet. Plant Breed., **41**: 112–116.

Dvě pokusné řady vybraných odrůd pšenice a šlechtitelských linií starých českých a slovenských odrůd a planě rostoucích pšenic byly infikovány třemi izoláty (R-116, UH-105, BR-331) *Mycosphaerella graminicola* (anamorph *Septoria tritici*), izolovanými v České republice. Byly nalezeny skupiny odrůd odlišujících se v závažnosti choroby ke všem třem izolátům patogena. Větší rozdíly byly mezi starými krajovými českými a slovenskými odrůdami a příbuznými planými druhy pšenice než mezi moderními odrůdami a šlechtitelskými materiály. V pokusu 1 se izolát BR-331 průkazně lišil od ostatních dvou izolátů ve virulenci k testovaným moderním odrůdám a šlechtitelským liniím. Metoda je vhodná pro předběžné hodnocení ve šlechtění na rezistenci pšenice nebo pro fytopatologické testy a jako doplněk k polním testům.

Klíčová slova: *Mycosphaerella graminicola*; pšenice; testy na rezistenci; technika listových segmentů; izoláty

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