SHORT COMMUNICATION

Expression of Resistance to Ramularia Leaf Spot in Winter Barley Cultivars Grown in Conditions of the Czech Republic

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Abstract: The fungus *Ramularia collo-cygni* is increasingly important as the causal agent of Ramularia leaf spot (RLS), a novel leaf spot disease of barley. The work aimed to identify gene resources suitable for developing new breeding lines of winter barley with improved resistance. During the first experimental period (2001–2005), RLS incidence was monitored in 711 cultivars and advanced breeding lines. Differences were detected in the intensity of symptomatic expression, but no material showed high resistance. During the second experimental period (2006–2009), response to natural RLS infection was evaluated in 19 winter barley cultivars (12 six-row and 7 two-row) registered in the Czech Republic. Highest susceptibility was detected in 6-row cvs. Luran, Laverda and Wendy while cvs Breunskylie (2-row), Merlot and Highlight (both 6-row) showed relatively lower disease incidence. High resistance was not detected. On average, 2-row cultivars showed lower intensity than 6-row cultivars and significant variation was observed among years.

Keywords: cultivar resistance; natural infection; *Ramularia collo-cygni*

The occurrence of *Ramularia collo-cygni* (Sutton & Waller) (RCC) has been reported on barley in the Czech Republic (Minaříková & Mařík 2001; Minaříková *et al.* 2009), Slovakia (Gubiš *et al.* 2008), and other European countries as well as in New Zealand, Mexico, Argentina and elsewhere. It is increasing in importance as a pathogen of barley, with yield losses ranging from 15% to 25% (Cromey *et al.* 2004). The fungus causes necrotic lesions and could be easily confused with other symptoms caused by abiotic stress and by other pathogens. The rapid leaf senescence, which is probably connected with toxins (rubellins) produced by RCC, results in premature green leaf area browning. In addition to yield reduction, grain quality is also decreased. Infection severity in barley varies significantly by individual year, and most barley cultivars are sensitive to RCC infection. Nevertheless, it has been reported that some cultivars possess moderate levels of resistance (Leistrumaite & Liatukas 2006; Pinnschmidt *et al.* 2006; Gubiš *et al.* 2009). Significant differences in disease levels between cultivars were detected by Makepeace *et al.* (2008). In comparison with other cultivars, cv. Merlot shows the lowest infection severity (Hess *et al.* 2006). That is probably due to its longer vegetation period.

The aims of the study were to monitor Ramularia leaf spot (RLS) infection over a 9-year period, to evaluate the environmental effects, and to detect materials having relatively higher resistance levels.

In the first experimental period (2001–2005), a set of 711 winter barley materials including

registered cultivars and advanced breeding lines was evaluated for resistance to RLS. In the second experimental period (2006–2009), natural infection by RLS was examined in a set of 19 cultivars registered in the Czech Republic (12 six-row and 7 two-row). To obtain data that could be statistically analysed, 4-year experiments were conducted. All plots (1 × 10 m) were arranged in randomized blocks with three replications. Mineral fertilizers (regeneration and production rates of 64.4 kg N/ha in the form of ammonium nitrate with limestone) were applied to the stands. Treatment with herbicides was carried out in autumn. Neither fungicides nor growth regulators were applied.

Experiments were located in Lužany, West Bohemia, Czech Republic. This is a cereal production region (altitude: 360 m a.s.l., annual mean temperature: 8.0°C, and total annual precipitation: 571 mm). The soil at the site according to FAO classification is clay loam, and pH averages 6.3.

Disease severity was measured at the milk-waxy growth stage (BBCH 75) using a 9–1 scoring scale according to the CISTA methodology, where:
9.0 = no infection;
8.0 = maximum 5% of leaf area of flag leaf and upper leaf layers infected on sporadic plants;
7.0 = up to 15% of leaf area of flag leaf and upper leaf layers infected on sporadic plants;
6.0 = up to 25% of leaf area of upper leaf layers infected;
5.0 = up to 50% of leaf area of upper leaf layers infected, first symptoms also occur on leaf sheaths;
4.0 = infection spreads to lower leaf layers, tips of infected leaves begin to wither away on the most infected plants;
3.0 = nearly the whole stand area is infected, up to 15% of the most infected leaves are entirely dead and first symptoms occur in the ear;
2.0 = up to 50% of the most infected leaves die on most plants, ear and awn infection is visible;
1.0 = more than 50% of leaves on plants are dead, total withering of the stand.

Statistica 7.0 software was used for statistical evaluation.

During the first experimental period (2001–2005), cultivars and lines exhibited various intensities of RLS infection symptoms, but a high level of resist-

Table 1. RLS infection severity in registered winter barley cultivars evaluated during 2006–2009

Cultivar	Country of origin	Registered in the Czech Republic	Ear No. of rows	Time of ear emergence	RLS scoring (1–9) 9 = no symptoms	Tukey test at $P < 0.05$
Luran	CZE	1998	6	early	3.08	a
Laverda (SUR.01/3128)	DEU	2007	6	early	3.25	ab
Wendy (NORD 02611/33)	DEU	2009	6	early	3.50	abc
Amarena	FRA	2006	6	late	3.67	abcd
Gilberta	BEL	2006	6	early	3.67	abcd
Reni	DEU	2001	2	medium-early	3.67	abcd
Lomerit	DEU	2002	6	medium-early	3.75	abcd
Caravan (NSL 99-6738)	FRA	2008	2	medium-early	3.92	abcde
Scarpia (BE 141601)	DEU	2008	6	medium-early	3.92	abcde
Campanille (NSL 99-8088)	GBR	2007	2	medium-late	3.92	abcde
Alinghi (LP 6-225)	DEU	2007	6	late	4.08	abcde
Fridericus (LP 6-234)	DEU	2007	6	medium-early	4.08	abcde
Heike (SZD 2109W)	AUT	2009	6	medium-early	4.17	abcde
Florian (KM 999/04)	CZE	2008	2	medium-late	4.25	bcde
Wintmalt (LP 2-345)	DEU	2009	2	late	4.42	cde
Saffron	GBR	2009	2	medium-early	4.50	cde
Breunskylie (Br.4597i)	DEU	2008	2	medium-early	4.75	de
Merlot	DEU	2002	6	late	4.75	de
Highlight (LEU 3034-1)	DEU	2008	6	medium-late	5.00	e

Table 2. Analysis of variance for scoring RLS severity in winter barley cultivars during 2006–2009 with experimental factors (cultivar and year) and comparison of RLS infection severity in winter barley according to experimental year of evaluation and 2- versus 6-row ear type

Source	Sum of squares	df	Mean square	F-ratio	*P*
Main effect					
Cultivar	56.5965	18	3.14425	13.03	0.0000
Year	104.0350	3	34.67840	143.76	0.0000
Interaction					
Cultivar × year	62.6316	54	1.15984	4.81	0.0000
Residual	36.6667	152	0.24122		
Year	count		RLS scoring	Tukey test ($P < 0.05$)	
2009	57		3.25	a	
2006	57		3.47	a	
2008	57		4.49	b	
2007	57		4.86	b	
Ear type					
6-row	144		3.91	a	
2-row	84		4.20	b	

df – degree of freedom

ance was not observed in any of them. Cv. Luran can be included among the most sensitive cultivars, and 6-row late cv. Merlot was relatively resistant. Therefore, these two cultivars were selected as contrast controls for the second experimental period.

The results of the second experiments, performed during 2006–2009, are presented in Table 1. In the group of early cultivars, in addition to Luran, cvs Laverda, Wendy and Gilberta rank among highly susceptible cultivars. It should be stated that among early materials there were none with significantly better symptomatic expression of RLS infection. Among medium-early cultivars at heading, the most sensitive were 2-row cv. Reni and 6-row cv. Lomerit. Two-row cvs Saffron and Breunskylie can be included among cultivars with milder infection symptoms. Among medium-late and late cultivars, the strongest symptomatic expressions of RLS infection were recorded in 6-row cv. Amarena, while the relatively highest resistance was found in medium-late 6-row cv. Highlight, and, in agreement with findings from the first experimental period and literature (Hess *et al.* 2006), in 6-row late cv. Merlot.

Infection severity was significantly influenced by a given year's weather conditions. In 2006 and 2009, infection was significantly higher than in 2007 and 2008 (Table 2). Interaction between years and varieties was statistically significant, and susceptible cultivars manifest higher symptomatic expressions in 2006 and 2009. Stronger symptomatic expression was positively affected by a higher number of rain days in the first two decades after heading and lower average daily temperature in the 2nd and 4th decade after heading. In 2007 and 2008, when the average symptomatic expression was weaker, the average temperature in the 2nd to 4th decade after heading was 1.6– 2.2°C higher and the number of rain days in the first two decades after heading about 5 days lower.

The significant difference in average intensity of symptomatic expression of RLS infection between 2- and 6-row cultivars (Table 2) was due to the fact that in the collection during 2006–2009 there was no early material among the 2-row cultivars, whereas there were four of these among the 6-row cultivars. If we compare only the eight 6-row cultivars from medium-early to late, the mean scores for 2- and 6-row cultivars are practically identical (4.20 and 4.18 scores, respectively).

In conclusion, it should be stated that no line or cultivar resistant to RLS was found. Cultivars exhibiting weaker symptomatic expression of infection at milk-waxy to waxy maturity (slightly resistant) stand mostly among the late cultivars. Only three cultivars with significantly weaker

infection symptoms (medium resistance) from the current collection of registered cultivars in the Czech Republic can be recommended for growing in regions with strong RCC occurrence: 2-row medium-early cv. Breunskylie, 6-row late cv. Merlot, and 6-row medium-late cv. Highlight. Nevertheless, none of the cultivars or breeding lines tested so far has proven to be resistant to RLS on such a level that it could be recommended as a donor of resistance to this disease.

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