Cereal Research Communications 44(4), pp. 585–593 (2016)
DOI: 10.1556/0806.44.2016.030
First published online September 29, 2016

Virulence of Wheat Leaf Rust (*Puccinia triticina* Eriks.) in the Years 2013–2015 and Resistance of Wheat Cultivars in Slovakia

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(Received 3 March 2016; Accepted 12 April 2016;
Communicated by J. Kolmer)

In 2013–2015 virulence in the wheat leaf rust population was evaluated on 17 Thatcher near-isogenic lines with leaf rust resistance genes. A total of 110 wheat leaf rust isolates were analyzed. Resistance genes *Lr9* and *Lr19* were effective to all tested isolates. Genes *Lr24* and *Lr28* conditioned resistance to 92% of the tested rust isolates. Thirty-seven winter wheat cultivars registered in Slovakia were analyzed for the presence of *Lr10, Lr24, Lr26, Lr34* and *Lr37* using tightly linked molecular markers. Gene *Lr37* was the most common in the tested cultivars. Leaf rust resistance was also tested in 13 wheat cultivars at the seedling stage with representative pathotypes of leaf rust.

Keywords: leaf rust pathotypes, *Lr* genes, resistance, wheat, Slovakia

Introduction

At present breeding for disease resistance is an important goal in many crops as it is the most economical means of disease control. Breeding for rust resistance has a long tradition in wheat breeding. In Slovakia leaf rust, caused by *Puccinia triticina* Eriks., yellow rust (*Puccinia striiformis* Westend.) as well as stem rust (*Puccinia graminis* Pers.) can cause considerable yield losses. Of the above-mentioned rust species leaf rust is of particular economic importance in the southern and eastern parts of the country in the years when the pathogen appears early and the weather during wheat ripening is hot and dry. Knowledge of virulence in the rust population is necessary for successful resistance breeding. In combination with the data on resistance genes in registered cultivars it also helps wheat breeders to develop germplasm resistant to the present rust pathotypes. Virulence of wheat leaf rust (physiologic races) in Slovakia has been studied since the 1960s. Virulence data from the years 1994–2011 were published by Hanzalová et al. (2008, 2010, 2012). This paper contains results of the wheat leaf rust virulence surveys carried out in the years 2013, 2014 and 2015, as well as data on seedling resistance of selected wheat cultivars grown in Slovakia to 12 different leaf rust races. Molecular markers for five *Lr* genes were applied for analysis of leaf rust resistance in selected wheat cultivars.

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Material and Methods

Collections of wheat leaf rust on dry leaves were obtained from different cultivars from the variety trials located across the country and organized by The Central Institute for Supervising and Testing in Agriculture in Slovakia. The initial source of inoculum was a dry leaf containing one or more pustules. Inoculation was performed by rubbing the second leaves of seedlings of the wheat cultivar Michigan Amber between fingers moistened with a water suspension of urediospores. This removed the waxy layer so that small droplets of water were more likely to adhere to the leaves. Inoculated plants were sprayed with water and incubated in closed glass cylinders for two days. Then plants were kept in open cylinders in the greenhouse at the temperature 18–22 °C with supplemental (16 h/d) illumination. When flecks appeared on inoculated leaves, a leaf segment with one developing uredinium of each rust sample was transferred to Petri dish with 15 ml water and kept in the greenhouse until urediospores have developed. Single uredinial isolates were increased on seedlings of the cv. Michigan Amber using the same methods of inoculation as described above. Near isogenic lines with *Lr* genes were inoculated with the increased spores by the same methods as described above. Differentials (ten plants of each *Lr* NIL) were grown in pots (∅ 15 cm) divided into six cells, with six differentials in each pot. Inoculation of seedlings was carried out with urediospores (ca. 30 µl spore suspension/50 seedlings in one pot). Infection types were basically evaluated according to Stakman et al. (1962). Avirulence was characterized by infection types 0, ; , 1, 1–2, 2, virulence by infection types 3. Frequency of rust isolates virulent to the differentials was expressed in percentages. Thatcher near-isogenic lines (NILs) with single *Lr* genes approved as leaf rust differentials by participants in the international COST 817 Action (Mesterházy et al. 2000) were used as differentials. In addition *Lr10* and *Lr13* were also included. Seed of the NILs was supplied by Dr. J. Kolmer to the Cereal Research Non-Profit Company in

Table 1. PCR conditions, products and primers

Gene	Chromosome location	Amplification conditions	PCR product	Reference
Lr10	1AS	94 °C for 3 min; 40 cycles of 94 °C for 30 s, 58 °C for 30 s, 72 °C for 45 s; 72 °C for 10 min	310 bp	Gultyaeva et al. 2009
Lr24	3DL	95 °C for 2 min; 36 cycles of 94 °C for 60 s, 60 °C for 60 s, 72 °C for 60 s; 72 °C for 7 min	607 bp	Gupta et al. 2006
Lr26 *Lr37*	1BS 2AS	94 °C for 3 min; 35 cycles of 94 °C for 30 s, 58 °C for 30 s, 72 °C for 45 s; 72 °C for 10 min	412 bp 262 bp	de Froidmont 1998; Helguera et al. 2003
Lr34	7DS	5 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min; 30 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 50 s; 1 cycle of 94 °C for 30 s, 55 °C for 30 s; 72 °C for 5 min	150 bp	Lagudah et al. 2006

Table 2. Virulence frequency of wheat leaf rust isolates in Slovakia to Thatcher near isogenic lines with *Lr* genes in 2013–2015

Lr genes	Virulent isolates (%)			Average (%)
	2013	2014	2015	
Lr1	67	90	92	83
Lr2a	20	8	16	15
Lr2b	31	8	12	17
Lr2c	31	8	12	17
Lr3a	82	58	60	67
Lr9	0	0	0	0
Lr10	100	95	100	98
Lr11	100	98	100	99
Lr13	78	100	100	93
Lr15	82	100	88	90
Lr17	78	85	80	81
Lr19	0	0	0	0
Lr21	96	98	92	95
Lr23	51	90	80	74
Lr24	9	8	8	8
Lr26	78	93	92	88
Lr28	7	8	8	8
Number of tested isolates	45	40	25	
Number of localities	11	5	5	

Szeged, Hungary, where it was subsequently increased and distributed to participants of the COST 817 action.

Molecular markers were used to analyze the studied cultivars for the presence of leaf rust resistance genes. DNA was extracted from the second leaf using the Qiagen DNA extraction kit (Qiagen, Germany). DNA quality was verified by electrophoresis in 0.8% agarose gel, stained with ethidium bromide, visualized under UV light and compared with ladder Lambda DNA/HindIII (Fermentas, Thermo Scientific, USA). The presence of genes *Lr10, Lr24, Lr26, Lr34* and *Lr37* were determined with the use of PCR assays with published primers marking these genes (de Froidmont 1998; Helguera et al. 2003; Lagudah et al. 2006; Gultyaeva et al. 2009). The PCR conditions and amplification products indicating the probable presence of analyzed genes of resistance are listed in Table 1. The amplification products were separated by electrophoresis in 2% agarose gels, stained with ethidium bromide and visualized under UV light. GeneRuler™ 100 bp DNA Ladder (Fermentas) was used as a molecular weight marker. Thatcher near-isogenic lines with the

Table 3. Prevailing leaf rust pathotypes in Slovakia in 2013–2015

Year	Virulence on near-isogenic lines	Number	Locality
2013	*Lr1, Lr3a, Lr10, Lr11, Lr13, Lr15, Lr17, Lr26*	11	Báhoň, Beluša, Bučany, Haniska, Jakubovany, Michalovce, Spišské Vlachy, Ripňany, Veľký Meder, Víglaš, Želiezovce
	Lr1, Lr3a, Lr10, Lr11, Lr13, Lr15, Lr17, Lr21, Lr23, Lr26	4	Báhoň, Beluša, Jakubovany
	Lr1, Lr10, Lr11, Lr13, Lr15, Lr17, Lr21, Lr23, Lr26	3	Jakubovany, Veľký Meder
	Lr2a, Lr2b, Lr2c, Lr3a, Lr10, Lr11, Lr15, Lr21, Lr23, Lr24, Lr26	2	Víglaš
	Lr2a, Lr2b, Lr2c, Lr3a, Lr10, Lr11, Lr15, Lr21, Lr23, Lr26	2	Víglaš
2014	*Lr1, Lr3a, Lr10, Lr11, Lr13, Lr15, Lr17, Lr21, Lr23, Lr26*	14	Báhoň, Beluša, Ripňany, Veľký Meder, Želiezovce
	Lr1, Lr10, Lr11, Lr13, Lr15, Lr17, Lr21, Lr23, Lr26	4	Báhoň, Veľký Meder, Želiezovce
	Lr1, Lr3a, Lr10, Lr11, Lr13, Lr15, Lr21, Lr23, Lr26	3	Veľký Meder, Želiezovce
	Lr1, Lr3a, Lr10, Lr11, Lr13, Lr15, Lr17, Lr21, Lr23, Lr24, Lr26	2	Beluša, Veľký Meder
	Lr3a, Lr10, Lr11, Lr13, Lr15, Lr17, Lr21, Lr23, Lr26	2	Želiezovce, Veľký Meder
	Lr1, Lr3a, Lr10, Lr11, Lr13, Lr15, Lr17, Lr21, Lr26	2	Báhoň, Beluša
2015	*Lr1, Lr10, Lr11, Lr13, Lr15, Lr17, Lr21, Lr23, Lr26*	6	Báhoň, Veľký Meder, Želiezovce
	Lr1, Lr3a, Lr10, Lr11, Lr13, Lr15, Lr17, Lr21, Lr23, Lr26	5	Báhoň, Haniska, Ripňany
	Lr2a, Lr2b, Lr2c, Lr3a, Lr10, Lr11, Lr13, Lr21, Lr23, Lr26	3	Veľký Meder
	Lr1, Lr3a, Lr10, Lr11, Lr13, Lr15, Lr17, Lr21, Lr26	2	Haniska, Želiezovce
	Lr1, Lr3a, Lr10, Lr11, Lr13, Lr15, Lr17, Lr21, Lr23, Lr24, Lr26	2	Veľký Meder

Pathotypes recorded only once (in 2013 – 23 pathotypes, in 2014 – 14 pathotypes, in 2015 – 7 pathotypes) are not included.

corresponding *Lr* genes were included as positive controls and sterile water as a negative control (two positive and two negative controls per reaction).

Cultivars not estimated as possessing any of the *Lr* genes analyzed by molecular markers were tested with 12 leaf rust isolates from the Slovak and Czech Republics by the same methods as in virulence analyses. In summary applied isolates were virulent to *Lr1, Lr2a, Lr2b, Lr2c, Lr3a, Lr10, Lr11, Lr13, Lr15, Lr17, Lr21, Lr23, Lr26* and *Lr28*; avirulent only to *Lr9, Lr19* and *Lr24*.

Results

Virulence frequency in the leaf rust population in 2013–2015

In the years 2013–2015 none of the single uredinial isolates were virulent to the Thatcher lines with *Lr9* and *Lr19* (Table 2). Very few leaf rust isolates were virulent on *Lr24*. Most isolates were virulent on *Lr10, Lr11, Lr13, Lr26, Lr21, Lr1, Lr15, Lr17, Lr23* and *Lr3a.* Less than 50% of isolates were virulent to lines with *Lr2a, Lr2b*, and *Lr2c*. The most widespread pathotypes were virulent to eight *Lr* genes in 2013, in 2014 to 10 *Lr* genes, and in 2015 to 9 to 10 *Lr* genes (Table 3). In the years 2013, 2014, and 2015, 28, 17 and 13 pathotypes, respectively, were found in Slovakia.

Postulation of Lr *genes in wheat cultivars by molecular markers*

Results of the molecular marker analysis are summarized in Table 4. Fragments corresponding to molecular markers for the genes *Lr10, Lr26, Lr34* and *Lr37* were amplified in 22 tested cultivars. The marker for *Lr37* was revealed in 16 cultivars – Altigo, Avenue, Beduin, Dagmar, Elly, Evina, Gallio, IS Conditor, IS Gordius, Lukullus, Messi, Midas, Mulan, Pannonikus, Tacitus and Yetti. The marker for *Lr34* was identified in six cultivars – Balada, IS Agape, IS Carnea, IS Ezopus, IS Median and Stanislava. The marker for *Lr10* was detected in five cultivars – Avenue, IS Conditor, Messi, Mulan and Pannonikus and the marker for *Lr26* in four cultivars – Balada, Bertold, IS Conditor and Viglanka. Presence of two or three genes is postulated in five tested cultivars: Balada (*Lr26* and *Lr34*), IS Conditor (*Lr10, Lr26, Lr37*), Messi (*Lr10, Lr37*), Mulan (*Lr10, Lr37*), Pannonikus (*Lr10, Lr37*).

Resistance of selected cultivars to leaf rust

Thirteen cultivars (Table 5) tested for leaf rust resistance were ranked according to the number of avirulent leaf rust isolates. Cultivar Brejk was resistant to all isolates. PS Sunanka was resistant to 10 isolates. IS Escoria was resistant to four isolates, IS Corvinus, Bardotka and Bohemia to three isolates, and Diadem, Forhand and JB Asano were resistant only to one isolate. Gallus, IS Questor, KWS Ferrum and Rupert were susceptible to all 12 isolates. These results indicated that additional genes other than those revealed by the molecular markers are also present in wheat cultivars registered in Slovakia.

Table 4. Leaf rust resistance genes (*Lr*) postulated by molecular markers in selected cultivars registered in Slovakia

Cultivar	*Lr10*	*Lr24*	*Lr26*	*Lr34*	*Lr37*
Altigo					+
Avenue	+				+
Balada			+	+	
Bardotka					
Beduin					+
Bertold			+		
Bohemia					
Brejk					
Dagmar					+
Diadem					
Elly					+
Evina					+
Forhand					
Gallio					+
Gallus					
IS Agape				+	
IS Carnea				+	
IS Conditor	+		+		+
IS Corvinus					
IS Escoria					
IS Ezopus				+	
IS Gordius					+
IS Median				+	
IS Questor					
JB Asano					
KW Ferrum					
Lukullus					+
Messi	+				+
Midas					+
Mulan	+				+
PS Sunanka					
Pannonikus	+				+
Rupert					
Stanislava				+	
Tacitus					+
Viglanka			+		
Yetti					+

Table 5. Reactions of cultivars recorded in *Table 4* as not possessing the tested *Lr* genes

Cultivar	9804	9784	9803	9821	9785	9793	9833	9838	9823	9845	9836	9829
Bardotka	;1–2	3	3	;1	3	3	3	3	3	3	3	;
Bohemia	;1–2	3	3	;2	3	3	3	3	3	3	3	;1–2
Brejk	0;	0;	0;	0;	;	0;	0;	0;	;	0	;	0;
Diadem	3	3	3	3	3	;	3	3	3	3	3	3
Forhand	3	3	;2	3	3	3	3	3	3	3	3	3
Gallus	3	3	3	3	3	3	3	3	3	3	3	3
IS Corvinus	;1	3	3	;	3	3	3	3	3	3	3	;
IS Escoria	;1	3	3	;1	3	3	3	3	3	3	0	;1
IS Questor	3	3	3	3	3	3	3	3	3	3	3	3
JB Asano	3	3	3	3	3	;1	3	3	3	3	3	3
KW Ferrum	3	3	3	3	3	3	3	3	3	3	3	3
PS Sunanka	;1	;1	;1	3–	;1–2	3	;1–2	;2	;2	;1–2	;2	;2
Rupert	3	3	3	3	3	3	3	3	3	3	3	3

;1, 1–2, 2 – avirulence; 3 – virulence.

Discussion

Results of the 2013–2015 wheat leaf rust virulence survey show some similarities with the data of the previous years (Hanzalová et al. 2012). In the previous years 2009–2011 virulence to *Lr9* was very low (0.2%), and virulence was not found to *Lr19*. In 2009–2011 virulence frequency was above 50% to wheat lines with genes *Lr1, Lr11, Lr13, Lr15, Lr17, Lr21, Lr26* as was found in 2013–2015 except for virulence to *Lr23* with frequency 41.8% in the period 2009–2011.

Molecular markers for *Lr10, Lr26, Lr34,* and *Lr37* were used in the tests as in our previous study (Hanzalová et al. 2012). In addition marker for *Lr24* was also applied because among the cultivars registered in the Czech Republic (Hanzalová et al. 2013) the cultivar Carroll was found to possess *Lr24*. The genes identified in the cultivars Altigo, Bertold, Dagmar, Mulan and Viglanka were recorded already earlier (Hanzalová et al. 2012, 2015). The common *Lr* genes detected with the molecular markers were *Lr37* (43%), followed by *Lr34* (16%), *Lr10* (14%) and *Lr26* (11%). Our results for wheat cultivars grown in Slovakia were similar to the results from Germany and France regarding the prevalence of the gene *Lr37* and a high number of cultivars possessing *Lr10* among European wheat cultivars. Goyeau and Lannou (2011) postulated the frequency of *Lr10, Lr24, Lr26* and *Lr37* using multi-pathotype tests in 275 wheat cultivars grown in France from 1983 to 2007 was 34%, 1%, 7% and 45%, respectively. In the test of Serfling et al. (2011) comprising 115 German cultivars, molecular markers were used to determine the presence of *Lr10* in 35 cultivars, *Lr26* in 14 cultivars, *Lr34* in 2 cultivars, *Lr37* in 48 cultivars.

In the set of cultivars tested with 12 leaf rust races the most resistant cv. Brejk was developed by the plant breeding company RAGT. High bread wheat quality of Brejk is derived from the cultivar Capo, and the leaf rust resistance gene from *Agropyron elongatum (Thinopyrum ponticum)*. Yellow colour of the endosperm conditioned by a gene closely linked with *Lr19* suggets presence of *Lr19*. High resistance was also recorded in cultivar PS Sunanka. Though the name resembles Swedish cultivar Sunnan, possessing *Lr19*, presence of *Lr19* in our tests could not be validated till now.

Virulence analysis of wheat leaf rust in Slovakia in the years 2013 – 2015 confirmed the high effectiveness of the genes *Lr9, Lr19* and to a lower degree the effectiveness of *Lr24* and *Lr28*. Analysis of *Lr10, Lr24, Lr26, Lr34* and *Lr37* by molecular markers revealed *Lr37* as the most frequent gene in the tested cultivars. This was also recorded by other authors for West European wheat cultivars.

Acknowledgements

Supported by Ministry of Agriculture of the Czech Republic, Projects No. MZE ČR RO0416 and No. QJ1210189. Authors thank The Central Institute for Supervising and Testing in Agriculture (ÚKSÚP) in Slovakia for the excellent cooperation.

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