doi: 10.17221/121/2016-CJGPB

Pathotypes of Wheat Leaf Rust (*Puccinia triticina* Eriks.) and Resistance of Registered Cultivars in the Czech Republic in 2012–2015

Alena HANZALOVÁ, Pavel BARTOŠ and Taťána SUMÍKOVÁ

Department of Genetics and Plant Breeding Methods, Crop Research Institute, Prague-Ruzyně, Czech Republic

**Corresponding author: hanzalova@vurv.cz*

Abstract

Hanzalová A., Bartoš P., Sumíková T. (2017): Pathotypes of wheat leaf rust (*Puccinia triticina* Eriks.) and resistance of registered cultivars in the Czech Republic in 2012–2015. Czech J. Genet. Plant Breed., 53: 122–126.

In 2012–2015 the virulence of the wheat leaf rust (*Puccinia triticina* Eriks.) population was studied on Thatcher near-isogenic lines with *Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr3a*, *Lr9*, *Lr10*, *Lr11*, *Lr13*, *Lr15*, *Lr17*, *Lr19*, *Lr21*, *Lr23*, *Lr24*, *Lr26* and *Lr28.* Samples of leaf rust were obtained from different parts of the Czech Republic. A total of 163 wheat leaf rust isolates were analysed. No virulence for the resistance gene *Lr9* was found. Virulence for *Lr19* was found only in one isolate in 2015. A lower frequency of virulence to *Lr24*, *Lr2a*, *2b*, *2c* and *Lr28* was also observed. The presence of *Lr10*, *Lr24*, *Lr26*, *Lr28* and *Lr37* in registered cultivars was detected by polymerase chain reaction (PCR) molecular markers.

Keywords: leaf rust pathotypes; *Lr* genes; resistance; wheat cultivars

The main objective of virulence surveys in the wheat leaf rust population is to present data useful for resistance breeding. In former Czechoslovakia physiologic races (pathotypes) of *Puccinia triticina* Eriks. on wheat (*Triticum aestivum* L.) had been studied in virulence surveys since the sixties of the last century. Results of the race surveys till 2011 were summarized in two papers (Hanzalová & Bartoš 2014a, b). The present contribution contains results of virulence surveys carried out in 2012–2015 and data on leaf rust resistance genes of recently registered winter wheat cultivars.

Collections of wheat leaf rust on 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. Rust was inoculated on the susceptible cultivar Michigan Amber. When flecks appeared on inoculated leaves, a leaf segment with one developing uredinium of each rust sample was transferred to Petri dish with water and kept in the greenhouse until urediospores developed. Single pustule isolates were increased on cv. Michigan Amber for tests on differentials. Inoculation of seedlings was carried out by rubbing the leaves of seedlings with fingers moistened in a water suspension of urediospores. Inoculated plants were kept in the greenhouse closed glass cylinders to provide high air humidity for 24 h. Infection types were evaluated according to Stakman *et al.* (1962) 10–14 days after inoculation when plants were kept in a greenhouse at 18–22°C. Avirulence was characterized by infection types 0; 1 and 2, virulence by infection types 2–3 and 3. Frequency of virulence to the resistance genes 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) and in addition NIL *Lr10* and *Lr13* were used in the tests.

In the molecular analysis DNA for polymerase chain reaction (PCR) assays was extracted from the second wheat leaves by a commercial kit (Qiagen,

doi: 10.17221/121/2016-CJGPB

Table 1. Polymerase chain reaction (PCR) conditions and primers for *Lr* gene diagnostics

Gene	Chromosome location	Amplification conditions	PCR product (bp)	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	Gultyaeva *et al.* (2009)
Lr19	7DL	95°C for 2 min; 35 cycles of 94°C for 60 s, 60°C for 60 s, 72°C for 60 s; 72°C for 7 min	512	Gupta *et al.* (2006a)
Lr24	3DL	95°C for 2 min, 36 cycles of 94°C for 60 s,	607	Gupta *et al.* (2006b)
Lr28	4AL	60°C for 60 s, 72°C for 60 s; 72°C for 7 min	570	Cherukuri *et al.* (2005)
Lr26	1BS	94°C for 3 min; 35 cycles of 94° for 30 s,	412	de Froidmont (1998)
Lr37	2AS	58°C for 30 s, 72°for 45 s; 72°C for 10 min	262	Helguera *et al.* (2003)
Lr34	7DS	5 cycles of 94°C for 1 min, 55°C for 1min, 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	Lagudah *et al.* (2006)

Hilden, Germany). DNA quality was verified by electrophoresis in 0.8% agarose gel, stained with ethidium bromide, visualized under UV light and compared with the Lambda DNA/*Hin*dIII ladder (Fermentas, Vilnius, Lithuania). The genes *Lr10*, *Lr19*, *Lr24*, *Lr26*, *Lr28*, *Lr34*, and *Lr37* were studied by PCR with published primers marking these genes (Table 1). The reactions were carried out in the Veriti thermal cycler (Applied Biosystems, Foster City, USA). The PCR conditions are shown in Table 1. The amplification products were separated by electrophoresis in 2% agarose gels, stained with ethidium bromide, and visualized under UV light. The GeneRuler^{TM} 100 bp DNA Ladder (Fermentas, Vilnius, Lithuania) was used as a molecular weight marker. *T. aestivum* Thatcher NILs containing the corresponding *Lr* genes were included as positive controls.

Virulence frequency. In 2012–2015 the total of 163 leaf rust isolates were tested on 17 differentials. In 2012 fifty-five samples from 13 localities, in 2013 twenty-three samples from 3 localities, in 2014 thirty-six samples from 9 localities and in 2015 forty-nine samples from 15 localities were analysed (Table 2). No virulence was recorded to *Lr9* and only 0.5% (1 isolate) to *Lr19*. Low virulence was registered to *Lr2a* (11%), *Lr2b* (13%), *Lr2c* (14%), *Lr24* (15%) and *Lr28* (21%). Virulence to *Lr3a* was 74%, to *Lr26* it was 79%, to *Lr1* it was 86% and to the remaining *Lr* NILs it was 90–100%.

In 2005–2015 virulence frequency to *Lr24* increased. It was 1% in 2005–2008, 7% in 2009–2011 and 15% in 2012–2015. In 2011 the cultivar Carroll possessing *Lr24* was registered in the Czech Republic. Other cultivars possessing *Lr24* registered in 2012–2015 were Athlon (*Lr24*), RGT Matahari (*Lr24*) (not included in Table 4) and Gordian (*Lr24*, *Lr28*). Genes *Lr9* and *Lr19* remain the most effective leaf rust resistance genes. However, only one of the registered cultivars Citrus (registered in 2011, not included in Table 4) has *Lr19* gene. In the neighbouring Slovakia *Lr19* was recorded in registered cultivars Brejk, Bona Dea

Table 2. Virulence frequency of *Puccinia triticina* isolates to Thatcher near isogenic lines (NILs) with *Lr* gene in 2012–2015

Resistance genes	Virulent isolates (%)				Average (%)
	2012	2013	2014	2015	
Lr1	72	100	94	76	86
Lr2a	9	0	14	20	11
Lr2b	7	0	17	29	13
Lr2c	11	0	14	31	14
Lr3a	69	100	90	37	74
Lr9	0	0	0	0	0
Lr10	–	100	97	96	98
Lr11	100	100	100	100	100
Lr13	100	100	100	97	99
Lr15	100	100	94	86	95
Lr17	91	100	100	88	95
Lr19	0	0	0	2	0.5
Lr21	94	96	97	100	97
Lr23	91	96	100	96	96
Lr24	11	22	19	10	15
Lr26	70	100	75	71	79
Lr28	6	39	11	32	21
No. of tested isolates	55	23	36	49	total 163

doi: 10.17221/121/2016-CJGPB

and Bona Vita (Hanzalová *et al*. 2016). Whereas in the years 2009, 2010 and 2011 a decrease of virulence frequency to *Lr28* was registered (33%, 13% and 7%, respectively), in 2012–2015 virulence frequency to *Lr28* varied. It reached 39% and 32% in the years 2013 and 2015, respectively. In 2012 it was only 6% similar to the previous year, and 11% in 2014. Four registered cultivars Tobak (*Lr28*), SY Passport (*Lr28*, *Lr37*), Gordian (*Lr24*, *Lr28*) and Frisky (*Lr10*, *Lr28*, *Lr37*) possess *Lr28*. An increase of virulence frequency to *Lr28* can be expected because cv. Tobak has the largest seed area increase 6.4 % of the cultivars registered and increased in the Czech Republic (Horáková *et al.* 2015; Horáková & Dvořáčková 2016). Whereas in 2011–2014 in the field trials average leaf rust resistance in cv. Tobak was scored resistant (7), in 2015 an average score of 11 field trials revealed cv. Tobak as susceptible (2.7) (Horáková *et al.* 2015; Horáková & Dvořáčková 2016). On the other hand, cv. Frisky possessing also *Lr28* in addition to *Lr10* and *Lr37* was the most resistant cultivar in the same set of field trials and was scored 8.1 probably due to other additional genes. Another cultivar Gordian possessing *Lr28* and *Lr24* was scored 6.8. Data on SY Passport were not

Table 3. Combinations of virulence to *Lr* genes in the determined isolates

Year	No. of isolates	Virulence to resistance genes (*Lr*)	Location
2015	16	*1, 10, 11, 13, 15, 17, 21, 23, 26*	Lípa, Uherské Hradiště, Most, Bojanovice, Opava, Stupice, Čáslav, Branišovice
	4	*1, 3, 10, 11, 13, 15, 17, 21, 23, 26*	Branišovice, Čáslav, Hradec nad Svitavou
	3	*1, 10, 11, 13, 15, 17, 21, 23*	Uherské Hradiště, Most, Praha-Ruzyně
	3	*1, 10, 11, 13, 15, 17, 21, 23, 26, 28*	Kroměříž, Stupice, Žatec
	3	*1, 3, 10, 11, 13, 15, 17, 21, 23, 26, 28*	Stupice, Vsetín
	2	*10, 11, 13, 15, 17, 21, 23*	Hradec nad Svitavou, Praha-Ruzyně
	2	*2a, 2b, 2c, 10, 11, 13, 15, 17, 21, 23, 24, 28*	Teplice
2014	15	*1, 3, 10, 11, 13, 15, 17, 21, 23, 26*	Branišovice, Chrlice, Lednice, Spytihněv- Zlín, Trutnov, Žatec
	4	*1, 10, 11, 13, 15, 17, 21, 23, 26*	Chrlice, Litoměřice, Trutnov
	4	*1, 3, 10, 11, 13, 15, 17, 21, 23*	Břeclav, Žatec
	3	*2a, 2b, 2c, 3, 10, 11, 13, 15, 17, 21, 23, 24, 28*	Čáslav
	3	*1, 3,* 10*, 11, 13, 15, 17, 21, 23, 24, 26*	Branišovice, Chrlice, Spytihněv- Zlín
2013	13	*1, 3, 10, 11, 13, 15, 17, 21, 23, 26*	Trutnov, Praha-Ruzyně
	4	*1, 3, 10, 11, 13, 15, 17, 21, 23, 26, 28*	Trutnov
	3	*1, 3, 10, 11, 13, 15, Lr17, 21, 23, 24, 26, 28*	Praha-Ruzyně
2012	14	*1, 3, 10, 11, 13, 15, 17, 21, 23, 26*	Čáslav, Chrlice, Jehnědí, Hradec nad Svitavou, Pusté Jakartice, Oldřichovice
	5	*10, 11, 13, 15, 17, 21, 23*	Čáslav, Frýdek Místek, Pusté Jakartice, Úhřetice
	3	*1, 3, 10, 11, 13, 15, 17, 21, 23, 26, 28*	Branišovice, Chrlice
	3	*1, 10, 11, 13, 15, 17, 21, 23*	Chrlice, Hradec nad Svitavou, Krukanice
	3	*1, 3, 10, 11, 13, 17, 21, 23*	Krukanice, Uherský Ostroh, Úhřetice
	3	*1, 3, 10, 11, 15, 17, 21, 26*	Chrlice, Uherský Ostroh
	3	*1, 10, 11, 13, 15, 17, 21, 23, 26*	Pusté Jakartice, Úhřetice
	2	*1, 2c, 3, 10, 11, 13, 15, 17, 21, 23, 26*	Brno, Pusté Jakartice
	2	*1, 3, 10, 11, 13, 15, 17, 23, 26*	Brno, Pusté Jakartice
	2	*1, 3, 10, 11, 13, 17, 21, 23, 26*	Uherský Ostroh, Úhřetice
	2	*3, 10, 11, 13, 15, 17, 21, 23, 24, 26*	Jaroměřice nad Rokytnou

Pathotypes recorded only once (in 2012 – 16 pathotypes, in 2013 – 5 pathotypes, in 2014 – 12 pathotypes, in 2015 – 22 pathotypes) are not included

doi: 10.17221/121/2016-CJGPB

included (Horáková *et al.* 2016). These data suggest that resistance in the field in the above-mentioned cultivars is not conditioned only by genes that we have determined. Gene interactions may also play a role in the expression of resistance.

Combinations of virulence in the tested rust isolates determined at least twice in the given year are summarized on Table 3. In 2012, 2013 and 2014 prevailing isolates possessed virulence to 10 resistance genes, in 2015 to 9 resistance genes. Isolates that possessed virulence to the highest number of resistance genes (13) were determined in 2014, isolates that possessed virulence to the lowest number of resistance genes (7) in 2012 and 2015.

Table 4. Reaction of wheat cultivars registered in 2012–2015 to leaf rust

Cultivar	Registered	*Lr* genes*	Leaf rust isolates/infection types**						Field classification***
			9668	347	4003	9712	628	1947	
Annie	2014	*37*	3	3	;2	3	3	3	6
Athlon	2013	*24*	0;	;1	;N	;1	;1-2	;	5
Artist	2014	*10, 37*	3	;1	;1-2	3	;1	;	5
Avenue	2014	*10, 37*	;	;2	;	3	;	0;	–
Balitus	2015	*37*	;1	3	3	3	3-	3	6
Bernstein	2015	*37*	3	3	;2	;2	;1	3	7.5
Banderola	2015	*37*	3	3	3	3	;2 N	3	–
Bonanza	2015	*10, 37*	;	;1-2 N	;1	3	2	;	6.5
Brokat	2013		;1	3	3	3	3	3	6
Cimrmanova raná	2012		3	3	3	3	3	3	6
Dagmar	2012	*37*	3	3	3	3	;1-2	3	5
Dulina	2013		;1	3	3	3	3	3	–
Elan	2012	*37*	3	3	3-	3	;1-2	3	4
Fabius	2013		3	3	3	3	3	3	6
Etana	2013	*10, 37*	3	3	;2	3	;2	;1 N	5
Fakir	2013	*37*	3	3	3	3	3	3	6
Frisky	2015	*10, 28, 37*	0;	0;	3	0;	0;	0;	8
Genius	2014	*10, 37*	3	;2	3	3	;1	;1	6
Gordian	2014	*24, 28*	0;	;	;	;	;	0;	7
Grizzly	2013	*37*	3	3	3	3	3	3	–
Julie	2014	*37*	3	3	3	3	;2	3	–
KWS Ozon	2012	*37*	0;	3	3	3	3	;2	5
Lavantus	2013	*10, 37*	;1	3	;2	3	;1 N	3	6
Matchball	2013	*26, 37*	3	0;	;1-2	3	3	3	6
Nordika	2014	*10*	0	0	0;	0	0	;2	–
Pankratz	2015	*37*	3	3	;1-2	3	;2	3	7.5
Patras	2013	*10*	3	;1	3	3	;1 N	;1	5
Rumor	2015	*10*	;1	3	3	3	;1-2 N	;1-2	6
Tobak	2013	*28*	0;	;	3	;	0;	0;	3
Tosca	2014	*37*	3	3	;2	3	;1-2 N	3	–
Turandot	2012		3	3	3	3	3-	3	6
SY Passport	2013	*28, 37*	0;	0;	0;	0;	;	0;	–
Vanessa	2013	*26, 37*	3	0;	3	3	;1-2	3	6
Zeppelin	2013		3	3	3	3	3	3	6

*identified by molecular markers; ** – chlorosis; N – necrosis; 0, 1, 2 – resistant; 2–3, 3 – susceptible; ***average evaluation of Central Institute for Supervising and Testing in Agriculture from 2012 to 2015, scale 1–9, 1 – susceptible, 9 – resistant (according to Horáková & Dvořáčková 2016)

doi: 10.17221/121/2016-CJGPB

Molecular marker analysis. Molecular marker analysis (Table 4) revealed the presence of leaf rust resistance genes *Lr10*, *Lr24*, *Lr26*, *Lr28* and *Lr37* alone or in combinations: the genes *Lr19* and *Lr34* were not found in the set of tested cultivars.

Obtained results on the frequency of *Lr37* and *Lr10* genes in the current cultivars registered in the Czech Republic correspond well with data obtained in France and Germany. Goyeau and Lannou (2011) postulated the presence of *Lr37*, *Lr10*, *Lr26* and *Lr24* in multipathotype tests with 275 wheat cultivars grown in France (1983–2007) and recorded their frequency as 45%, 34%, 7%, 1%, respectively. Molecular marker analysis by Serfling *et al.* (2011) with 115 German cultivars revealed *Lr37*, *Lr10* and *Lr26* in 42%, 30% and 12% cultivars, respectively.

Experience of the recent years has demonstrated the fast breakdown of resistance even if it is conditioned by genes that were not possessed by cultivars grown earlier, and virulence frequency in the local rust population was relatively low. That reminds again of the need for permanent resistance breeding.

Acknowledgements. Supported by Ministry of Agriculture of the Czech Republic, Projects No. RO0416 and No. QJ1210189.

References

Cherukuri D.P., Gupta S.K., Charpe A., Koul S., Prabhu K.V., Singh R.B., Haq Q.M.R. (2005): Molecular mapping of *Aegilops speltoides* derived leaf rust resistance gene *Lr28* in wheat. Euphytica, 143: 19–26.

De Froidmont D. (1998): A co-dominant marker for the *1BL/1RS* wheat-rye translocation via multiplex PCR. Journal of Cereal Science, 27: 229–232.

Goyeau H., Lannou C. (2011): Specific resistance to leaf rust expressed at the seedling stage in cultivars grown in France from 1983 to 2007. Euphytica, 178: 45–62.

Gultyaeva E.I., Kanyuka I.A., Alpateva N.V., Baranova O.A., Dmitriev A.P., Pavlyushin V.A. (2009): Molecular approaches in identifying leaf rust resistance genes in Russian wheat varieties. Plant Industry, 35: 316–319.

Gupta S.K., Charpe A., Prabhu K.V., Haque Q.M.R. (2006a): Identification and validation of molecular marker linked to the leaf rust resistance gene *Lr19* in wheat. Theoretical and Applied Genetics, 113: 1027–1036.

Gupta S.K., Charpe A., Koul S., Haque Q.M.R., Prabhu K.V. (2006b): Development and validation of SCAR markers co-segregating with an *Agropyron elongatum* derived leaf rust resistance gene *Lr24* in wheat. Euphytica, 150: 233–240.

Hanzalová A., Bartoš P. (2014a): The virulence spectrum of the wheat leaf rust population analyzed in the Czech Republic from 2002 to 2011. Czech Journal of Genetics and Plant Breeding , 50: 288–292.

Hanzalová A., Bartoš P. (2014b): Virulence surveys of wheat leaf rust races in the Czech Republic and resistance genes in registered cultivars. Czech Journal of Genetics and Plant Breeding, 50: 241–246.

Hanzalová A., Bartoš P. and Sumíková T. (2016): Virulence of wheat leaf rust (*Puccinia triticin*a Eriks.) in the years 2013–2015 and resistance of wheat cultivars in Slovakia. Cereal Research Communications, 44: 585–592.

Helguera M., Khan I.A., Kolmer J., Lijavetzky D., Zhong-Gi L., Dubcovsky J. (2003): PCR assays for the *Lr37-Yr17-Sr38* cluster of rust resistance genes and their use to develop isogenic hard red spring wheat lines. Crop Science, 43: 1839–1847.

Horáková V., Dvořáčková O. (2016): List of Recommended Cultivars 2016. Brno, ÚKZÚZ. (in Czech)

Horáková V. *et al.* (2015): List of Recommended Cultivars 2015. Brno, ÚKZÚZ. (in Czech)

Lagudah E.S., McFadden H., Singh R.P., Huerta-Espino J., Bariana H.S., Spielmeyer W. (2006): Molecular genetic characterization of the *Lr34/Yr18* slow rusting resistance gene region in wheat. Theoretical and Applied Genetics, 114: 21–30.

Mesterházy A., Bartoš P., Goyeau H., Niks R., Csösz M., Andersen O., Casulli F., Ittu M., Jones E., Manisterski J., Manninger K., Pasquini M., Rubiales D., Schachermayr G., Strzembicka A., Szunics L., Todorova M., Unger O., Vančo B., Vida G., Walther U. (2000): European virulence survey for leaf rust in wheat. Agronomie, 20: 793–804.

Serfling A., Krämer I., Volker L., Schliephake E., Ordon F. (2011): Diagnostic value of molecular markers for *Lr* genes and characterization of leaf rust resistance of German winter wheat cultivars with regard to the stability of vertical resistance. European Journal of Plant Pathology, 130: 559–575.

Stakman E.C., Stewart P.M., Loegering W.O. (1962): Identification of Physiologic Races of *Puccinia graminis* var. *tritici*. Agricultural Research Service E617. Washington D.C., USDA.

Received for publication July 22, 2016
Accepted after corrections March 31, 2017
Published online June 2, 2017