https://doi.org/10.17221/86/2019-CJGPB

Wheat leaf rust (*Puccinia triticina* Eriks.) virulence frequency and detection of resistance genes in wheat cultivars registered in the Czech Republic in 2016–2018

Alena Hanzalová[1], Veronika Dumalasová[1], Ondřej Zelba[1,2]*

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

[2]Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, Prague, Czech Republic

**Corresponding author: hanzalova@vurv.cz*

Citation: Hanzalová A., Dumalasová V., Zelba O. (2020): Wheat leaf rust (*Puccinia triticina* Eriks.) virulence frequency and detection of resistance genes in wheat cultivars registered in the Czech Republic in 2016–2018. Czech J. Genet. Plant Breed., 56: 87–92.

Abstract: In 2016–2018 virulence of the Czech wheat leaf rust population was studied on Thatcher near-isogenic lines, carrying different *Lr* genes, and 130 leaf rust isolates. Virulence to *Lr9* was found only sporadically. Virulence frequency to *Lr2a*, *Lr2b*, *Lr2c* and *Lr28* was lower than in previous years. All tested isolates were avirulent to *Lr19*. *Lr24* conditioned resistance to majority of isolates. Nineteen recently registered Czech cultivars were tested with six isolates of the pathogen and *Lr* genes were postulated. Presence of genes *Lr1*, *Lr10*, *Lr19*, *Lr24*, *Lr26*, *Lr28*, *Lr34* and *Lr37* was tested by molecular markers. *Lr37* prevailed, followed by *Lr* genes *10*, *24*, *28*, *1* and *26*; genes *Lr19* and *Lr34* were not determined.

Keywords: leaf rust pathotypes; *Lr* genes; gene postulation; multi-pathotype tests; *Triticum aestivum*

Wheat leaf rust caused by *Puccinia triticina* Eriks. is a serious disease worldwide. The fungus is highly variable for virulence. Knowledge of virulence in the rust population is therefore 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. To date, 76 *Lr* genes have been formally catalogued in the wheat genome (McIntosh et al. 2016). Most of these are seedling resistance genes or major genes that are presumed to interact with leaf rust on a gene-for-gene basis (McIntosh et al. 1995). However, host resistance conferred by major genes have a tendency to be easily overcome by new virulent rust pathotypes (McDonald & Linde 2002). Therefore, pyramiding several *Lr* genes into a single cultivar is a useful strategy as the combined effects of several genes give the cultivar a wider base of disease resistance, thereby extending the period of effectiveness (Roelfs et al. 1992). In recent years, only a few *Lr* genes, *Lr9*, *Lr19* and *Lr24* are resistant against prevalent leaf rust pathotypes in Czech Republic (Hanzalová et al. 2017).

Though methods of molecular biology are applied in the studies of specialization of cereal rusts (e.g. Kolmer et al. 2013), virulence studies on wheat near isogenic lines (NILs) possessing different resistance genes remain useful for resistance breeding and may contribute to epidemiologic studies (e.g. Huerta-Espino et al. 2011).

Identifying of *Lr* genes in the currently released Czech wheat cultivars is important for sustainable

Funded by European Union, Horizon 2020, Grant agreement No. 773311. Supported by the Ministry of Agriculture of the Czech Republic, Project No. MZE RO0418 and Project NAZV QK1910041.

https://doi.org/10.17221/86/2019-CJGPB

gene deployment and for breeding new resistant cultivars. This contribution presents a continuation of long - lasting surveys on wheat leaf rust pathotypes started in the sixties of the last century in former Czechoslovakia. It contains data on virulence to *Lr* differentials as well as results of the seedling tests of resistance and data on *Lr* genes analysed by molecular markers in the majority of cultivars registered in the period 2016–2018 and seedling and field rust resistance genes in 19 wheat cultivars.

MATERIAL AND METHODS

Collections of wheat leaf rust on leaves were obtained from various cultivars, mainly from the variety trials located across the country and organized by the Central Institute for Supervising and Testing in Agriculture in Czech Republic. Rust was inoculated on the susceptible cultivar Michigan Amber. The initial source of inoculum was one or more pustules on dry leaf containing. Inoculation was performed by rubbing the leaves between fingers moistened with water suspension of urediospores. This also 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. When flecks appeared on inoculated leaves, a leaf segment with one developing uredinium of each rust sample was transferred to a Petri dish with water and kept in the greenhouse until urediospores have developed. Single pustule isolates were increased on cv. Michigan Amber for tests on differentials.

Inoculation of seedlings was carried out with water suspension of urediospores. Inoculated plants were kept in a greenhouse in closed glass cylinders to provide high air humidity for 24 h at 18–22 °C. Infection types were evaluated according to Stakman et al. (1962) 10–14 days after inoculation. Avirulence was characterised by infection types 0; 1 2 2+, virulence by infection type 3. The isolates were stored in the collection under standard codes in the Czech National Programme on Conservation and Utilization of Microbial Genetic Resources Important for Agriculture. Frequency of virulence to the resistance genes was expressed in percentages. Thatcher near-isogenic lines (NILs) with single *Lr* genes (Mesterházy et al. 2000) and an additional NILs with *Lr10* and *Lr13* were used in the tests. Nineteen winter wheat cultivars registered in the Czech Republic in 2016–2018 were tested for resistance to the given pathogen. Their seedlings were inoculated and evaluated in the same way as in virulence analyses. Rust pathotypes prevailing in 2018 were used for the inoculation. Field resistance tests were carried out in the locality Prague-Ruzyně in an artificially infected field, with a mixture of leaf rust isolates, symptoms were evaluated by field scale 1–9 (1 – susceptible, 9 – resistant).

Genomic DNA was isolated from the youngest leaves of 20–30 plants per cultivar. Leaf tissue was frozen in liquid nitrogen, ground to a powder and used for DNA extraction by commercial kit (Qiagen, Germany).

The polymerase chain reaction (PCR) protocols used for corresponding markers for genes *Lr1*, *Lr10*,

Table 1. PCR conditions and primers

Gene	Chromosome location	PCR product (bp)	Reference	Amplification conditions
Lr1	5DL	760	Qiu et al. (2007)	94 °C for 5 min; 35 cycles of 94 °C for 60 s, 65 °C for 60 s, 72 °C for 60 s; 72 °C for 10 min
Lr10	1AS	310	Gultyaeva et al. (2009)	95 °C for 3 min; 35 cycles of 94 °C for 45 s, 57 °C for 45 s, 72 °C for 30 s; 72 °C for 3 min
Lr19	7DL	512	Gupta et al. (2006a)	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
Lr24	3DL	607	Gupta et al. (2006b)	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
Lr28	4AL	570	Cherukuri et al. (2005)	
Lr26	1BS	412	de Froidmont (1998)	94 °C for 3 min; 35 cycles of 95 ° for 45 s, 62 °C for 45 s, 72 °C for 45 s; 72 °C for 10 min
Lr37	2AS	259	Helguera et al. (2003)	
Lr34	7DS	150	Lagudah et al. (2006)	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

https://doi.org/10.17221/86/2019-CJGPB

Table 2. Virulence frequency of *Puccinia triticina* isolates to near isogenic lines (NILs) with single *Lr* genes in 2016–2018 (seedling test) (in %)

Lr genes	Virulent isolates			Average
	2016	2017	2018	
Lr1	53	82	98	78
Lr2a	0	20	2	7
Lr2b	32	18	0	17
Lr2c	32	22	0	18
Lr3a	97	51	96	81
Lr9	0.3	0.4	0	0.2
Lr10	88	84	100	91
Lr11	100	100	100	100
Lr13	100	100	100	100
Lr15	100	84	100	95
Lr17	100	82	100	94
Lr19	0	0	0	0
Lr21	100	100	100	100
Lr23	91	80	96	89
Lr24	15	11	6	11
Lr26	79	89	92	87
Lr28	35	31	23	30
No. of isolates	34	45	51	130
No. of localities	15	16	12	–

Lr19, *Lr24*, *Lr26*, *Lr28*, *Lr34* and *Lr37* are listed in the Table 1. The PCR was performed in the thermal Labcycler (*SensoQuest GmbH*, Germany). The amplification products were separated by electrophoresis on 1.6% agarose gels, stained with ethidium bromide, and visualised under UV light. GeneRulerTM 100 bp DNA Ladder (Fermentas, Lithuania) was used as a molecular weight marker. *Triticum aestivum* Thatcher NILs containing the corresponding *Lr* genes were included as positive controls.

RESULTS AND DISCUSSION

In the years 2016–2018 *Lr9* and *Lr19* were the most effective genes. No virulence to *Lr19* and only two isolates out of 130 isolates were found virulent to *Lr9*. Relatively low percentage of isolates was virulent *to Lr2a*, *Lr2b* and *Lr2c*, 7%, 17% and 18%, respectively. Only 11% of the isolates were virulent to *Lr24* and 30% to *Lr28*. Average virulence to other *Lr* genes fluctuated between 78% and 100% (Table 2).

The prevailing pathotype (2016 – 29%, 2017 – 37%, 2018 – 63%) was virulent to *Lr1*, *Lr3a*, *Lr10*, *Lr11*, *Lr13*, *Lr15*, *Lr17*, *Lr21*, *Lr23* and *Lr26* (Table 3). The

Table 3. Prevailing leaf rust pathotypes in 2016–2018* (seedling test)

Virulence on *Lr* NILs	Occurrence (%)*	Locality
2016		
1, 3a, 10, 11, 13, 15, 17, 21, 23, 26	29	Branišovice, Čáslav, Hodonín, Hradec n. Svitavou, Lednice, Uh. Ostroh, Znojmo
2b, 2c, 3a, 10, 11, 13, 15, 17, 21, 23, 26, 28	15	Hradec n. Svitavou, Úhřetice, Čáslav
2b, 2c, 3a, 10, 11, 13, 15, 17, 21, 23, 26	9	Pusté Jakartice
2b, 2c, 3a, 10, 11, 13, 15, 17, 21, 23, 24, 26	9	Hradec n. Svitavou, Jaroměřice, Úhřetice
3a, 10, 11, 13, 15, 17, 21, 23	6	Přítluky
2b, 2c, 3a, 10, 11, 13, 15, 17, 21, 23, 24, 28	6	Pusté Jakartice
3a, 10, 11, 13, 15, 17, 21, 23, 26, 28	6	Uherské Hradiště, Vsetín
1, 3a, 10, 11, 13, 15, 17, 21, 23	6	Hradec n. Svitavou, Znojmo
2017		
1, 3a, 10, 11, 13, 15, 17, 21, 23, 26	37	Čáslav, Lípa, Hradec n. Svitavou, Hrubčice, Nechanice, Pusté Jakartice, Kuřim
1, 10, 11, 13, 15, 17, 21, 23, 26	11	Čáslav, Lípa
1, 3a, 10, 11, 13, 15, 17, 21, 23, 26, 28	7	Chrastava, Pusté Jakartice
2a, 2b, 2c, 3a, 10, 11, 13, 15, 17, 21, 23, 26, 28	7	Hradec n.Svitavou
1, 3a, 10, 11, 13, 15, 17, 21, 23, 24, 26	7	Hrubčice, Pusté Jakartice
1, 10, 11, 13, 15, 17, 21, 23	4	Nechanice, Čáslav
2018		
1, 3a, 10, 11, 13, 15, 17, 21, 23, 26	63	Čáslav, Lípa, Hrubčice, Humpolec, Nechanice, Praha, Staňkov, Uh. Ostroh, Úhřetice
1, 3a, 10, 11, 13, 15, 17, 21, 23, 26, 28	20	Čáslav, Humpolec, Podbořany, Praha, Staňkov, Tuněchody, Uh. Ostroh, Úhřetice
1, 3a, 10, 11, 13, 15, 17, 21, 23	4	Čáslav, Staňkov

*Pathotypes determined only once are not included; NILs – near isogenic lines

https://doi.org/10.17221/86/2019-CJGPB

number of different pathotypes determined in 2016, 2017 and 2018 was 13, 19 and 10 out of 34, 45 and 51 tested isolates, respectively. Similar data regarding virulence were previously recorded for the years 2012–2015 (Hanzalová et al. 2017). Since that time the number of isolates virulent to *Lr28* has slightly increased, probably due to the large growing area of the cultivar Tobak and other cultivars possessing *Lr28*. On the other hand the number of isolates virulent to *Lr24* slightly decreased, possibly to the relatively small growing area of the cultivar Athlon and other cultivars carrying *Lr24*. However, the growing area of the cultivars with specific resistance genes is only one factor affecting the spread of the corresponding virulence, fitness of the pathogen is also very important. Field test indicates that some cultivars (for example cultivar Barracuda) carry other resistance genes that have not been tested by molecular markers.

In the analyses of *Lr* genes by molecular markers the presence of *Lr* genes was as follows: *Lr37* – 13 cultivars, *Lr10* – four cultivars, *Lr24* – three cultivars, *Lr1* – five cultivars, *Lr28* and *Lr26* – one cultivar each out of 19 tested cultivars. Genes *Lr19* and *Lr34* were not identified in the recently registered cultivars (Table 4). Most of the cultivars possessed one *Lr* gene, five cultivars two *Lr* genes, two cultivars even three genes out of the tested *Lr* genes.

Diversity of genes for resistance supports the durability of resistance. Knowledge of the virulence in the rust population in combination with knowledge of resistance genes in wheat cultivars together with data on seedling resistance enables an estimation of leaf rust incidence on the relevant wheat cultivars grown in the field (Table 5). Presence of *Lr24* may indicate a higher average resistance to leaf rust of cultivars possessing that gene. Gene *Lr37* for adult plant resistance as the most frequent gene in recently registered cultivars, as well as *Lr10* can condition leaf rust resistance especially in combination with other *Lr* genes.

Prevalence of *Lr37* and *Lr10* in the recently registered cultivars in the Czech Republic was also described in the cultivars registered in 2012–2015 (Hanzalová et al. 2017).

Similar results were obtained in the study of wheat cultivars grown in France in 1983–2007 by Goyeau and Lannou (2011) and Serfling et al. (2011) in Germany. Resistance genes *Lr37* and *Lr10* also prevailed in the above mentioned studies.

Table 4. Wheat leaf rust resistance genes in 19 cultivars registered in 2016–2018 identified through specific DNA – based markers

Cultivars	*Lr1*	*Lr10*	*Lr19*	*Lr24*	*Lr26*	*Lr28*	*Lr34*	*Lr37*
AF Jumiko KM 178	+							
Atuan	+							+
Barracuda								
Butterfly	+							+
Cecilius								+
Faunus								+
Futurum				+				
Gaudio								+
Johnson					+			
KWS Silverstone	+	+						+
LG Imposanto		+						+
Penelope								+
Proteus		+						+
RGT Cesario				+				
RGT Sacramento								+
Rivero	+							+
Sheriff				+				
Steffi								+
WP8 Calgary		+				+		+

https://doi.org/10.17221/86/2019-CJGPB

Table 5. Reactions of 19 selected winter wheat cultivars to six leaf rust pathotypes and field scoring

Cultivar	Registered	Leaf rust isolate*						Field scoring**	
		T 0164	T 0126	T 0111	T 0140	T 0134	T 0171	2017	2018
AF Jumiko	2018	3	3	3	3	3	3	–	–
Atuan	2018	3	3	;2	3	3	3	–	4.5
Barracuda	2017	;1	;2	;	;	;1	;	9	–
Butterfly	2017	3	3	3	3	3	3	–	5.5
Cecilius	2018	3	3	3	3	3	3	9	9
Faunus	2016	3	3	3	3	3	3	5.5	5
Futurum	2016	;	0;	;	0;	;	;	9	9
Gaudio	2017	3	3	3	3	3	3	6.5	5.5
Johnson	2018	3	3	;1	3	3-	3	–	6
KWS Silverstone	2018	3	3	;2	3	3	3	6	4
LG Imposanto	2017	3	3	3	3	3	3	6.5	7.5
Penelope	2016	3	3	;2	3	3	3	6.5	6.5
Proteus	2017	3	3	;2	3	3	3	6.5	9
RGT Cesario	2018	;1	;	;	;	;	0;	8	8
RGT Sacramento	2017	3	3	3-	3	3	3	7	7
Rivero	2016	3	3	3-	3	3	3	6.5	6
Sheriff	2017	;	;	;	; 1-2	;	;	9	9
Steffi	2016	3	3	3	3	3	3-	6	6
WP8 Calgary	2018	0	0;	0;	0;	0;	0;	–	–

*Infections types in seedling test: ; – chloroses, 0, 1, 1-2, 2 – resistant, 3 – susceptible; **field scale 1–9 (1 – susceptible, 9 – resistant); the isolates designation is according with the designation in the collection The Czech National Programme on Conservation and Utilization of Microbial Genetic Resources Important for Agriculture; T – indicates *Puccinia triticina*

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., Sumíková T. (2017): Physiologic specialization of wheat leaf rust and resistance of registered cultivars in the Czech Republic in 2012–2015. Czech Journal of Genetics and Plant Breeding, 53: 122–126.

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.

Huerta-Espino J., Singh R.P., Germán S., McCallum B.D., Park R.F., Chen W.Q., Bhardway S.C., Goyeau H. (2011): Global status of wheat leaf rust caused by *Puccinia triticina*. Euphytica, 179: 143–160.

Kolmer J.A., Hanzalova A., Goyeau H., Bayles R., Morgounov A. (2013): Genetic differentiation of the wheat leaf rust fungus *Puccinia triticina* in Europe. Plant Pathology, 62: 21–31.

Lagudah E.S., McFadden H., Singh R.P., Huerta-Espino J., Bariana H.S., Spielmeyer W. (2006): Molecular genetic

https://doi.org/10.17221/86/2019-CJGPB

characterization of the *Lr34/Yr18* slow rusting resistance gene region in wheat. Theoretical and Applied Genetics, 114: 21–30.

McDonald B.A., Linde C. (2002): Pathogen population genetics, evolutionary potential, and durable resistance. Annual Review of Phytopathology, 40: 349–379.

McIntosh R.A., Dubcovsky J., Rogers W.J., Morris C., Appels R., Xia X.C. (2016): Catalogue of gene symbols for wheat: 2015–2016 Supplement. Available at https://shigen.nig.ac.jp/wheat/komugi/genes/macgene/supplement2015.pdf

McIntosh R.A., Wellings C.R., Park R.F. (1995): Wheat Rusts: An Atlas of Resistance Genes. East Melbourne, CSIRO.

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.

Qiu J.-W., Schürch A.Ch., Yahiaoui N., Dong L.-L., Fan H.-J., Zhang Z.-J., Keller B., Ling H.-Q. (2007): Physical mapping and identification of a candidate for the leaf rust resistance gene *Lr1* of wheat. Theoretical and Applied Genetics, 115: 159–168.

Roefls A.P., Singh R.P., Saari E.E. (1992): Rust Disease of Wheat: Concepts and Methods of Management. Mexico D.F., CIMMYT.

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: September 4, 2019
Accepted: January 13, 2020
Published online: February 10, 2020