Physiological Specialization of Wheat Leaf Rust (*Puccinia triticina* Eriks.) in the Czech Republic in 2009–2011

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Abstract

Hanzalová A., Bartoš P., Sumíková T. (2013): **Physiological specialization of wheat leaf rust (***Puccinia triticina* Eriks.**) in the Czech Republic in 2009–2011**. Czech J. Genet. Plant Breed., **49**: 103–108.

In 2009–2011 virulence of the wheat leaf rust population was studied on Thatcher near isogenic lines with *Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr3a*, *Lr9*, *Lr11*, *Lr13*, *Lr15*, *Lr17*, *Lr19*, *Lr21*, *Lr23*, *Lr24*, *Lr26* and *Lr28.* Samples of leaf rust were obtained in different parts of the Czech Republic. A total of 164 wheat leaf rust isolates were analysed. Resistance gene *Lr9* was effective to 98% of all tested isolates. No virulence to *Lr19* was found. Gene *Lr24* was effective to 93% of isolates. A lower frequency of virulence to *Lr2a, Lr2b* and *Lr28* was also observed. Recently registered cultivars were tested with six older and five most widespread leaf rust pathotypes at present. Winter wheat cultivars Carroll and Citrus were resistant to all tested older pathotypes at the seedling stage and they were also resistant to almost all pathotypes widespread at present. They displayed resistance also in official yield trials. Genes *Lr34*, *Lr37*, *Lr10*, *Lr24* were determined in the tested cultivars by molecular markers.

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

Breeding for resistance is an important part of integrated leaf rust control together with fungicides. Due to a large number of different pathotypes of leaf rust the knowledge of virulence in the leaf rust population is necessary for successful resistance breeding. It enables to choose sources of resistance effective against local leaf rust pathotypes and can substantially contribute to epidemic studies on rust. The importance of international surveillance of wheat rust pathotypes was recently underlined by Park *et al.* (2011). It is one of the most important topics in the Borlaug Global Rust Initiative (McInhtosh & Pretorius 2011). In the former Czechoslovakia physiologic races (pathotypes) of *P. triticina* were studied in virulence surveys since the sixties of the last century (Šebesta & Bartoš 1968, 1969; Bartoš & Šebesta 1971). The present contribution contains results of virulence surveys carried out in the years 2009–2011 and data on leaf rust resistance of recently registered winter wheat cultivars.

MATERIAL AND METHODS

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, Czech Republic. 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 the cultivar Michigan Amber for tests on differentials. Inoculation of seedlings was carried out with water suspension of urediospores. Inoculated plants were kept in closed glass cylinders to provide high air humidity for 24 hours. Infection types were basically 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, 2, virulence by infection types 2–3 ,3. Frequency of virulence 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) and in addition NIL *Lr13* were used in the tests. Most of the resistance genes in the applied differentials were derived from bread wheat; however the following *Lr* genes are of alien origin: *Lr9* – *Aegilops umbellulata*, *Lr19* – *Thinopyrum ponticum* (*Aegilops squarrosa* var. *meyeri*), *Lr23* – *Triticum turgidum* var. *durum*, *Lr24* – *Thinopyrum ponticum*, *Lr26* – *Secale cereale* cv. Petkus, *Lr28* – *Aegilops speltoides.* Seed of the NILs was supplied by Dr. J. Kolmer to the Cereal Research Non-Profit Company in Szeged, Hungary, where it was subsequently increased. The pedigree of NILs was described by Mesterházy *et al.* (2000). In 2009 46 single pustule isolates from 15 localities, in 2010 64 single pustule isolates from 23 localities, and in 2011 54 single pustule isolates from 15 localities were analysed.

Two greenhouse trials were carried out. In the first trial 22 winter wheat cultivars registered in 2009–2011 were tested for resistance to six older leaf rust pathotypes differing in virulence to *Lr1*, *Lr26*, *Lr9*, *Lr23*, *Lr26* and *Lr28* as a tool for the phenotypic analysis of resistance genes. In the second trial 28 cultivars mostly identical with the cultivars tested in the first trial were tested with the most widespread pathotypes at present. Data on resistance originate from official yield trials carried out by the Central Institute for Supervising and Testing in Agriculture (9 – high resistance, 1 – susceptibility).

The same methods as in the virulence analysis were used in the variety resistance trials.

DNA for PCR assays was extracted from the second wheat leaves by a commercial kit (Qiagen, Hilden, 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/*Hin*dIII (Fermentas, Vilnius, Lithuania). The genes *Lr10*, *Lr24*, *Lr26*, *Lr28*, *Lr34*, and *Lr37* were tested by PCR with published primers marking these genes according to de Froidmont (1998), Helguera *et al.* (2003), Cherukuri *et al.* (2005), Gupta *et al.* (2006), Lagudah *et al.* (2006), Gultyaeva *et al.* (2009). The reactions were carried out in the Veriti thermal cycler (Applied Biosystems, Foster City, USA). 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, Vilnius, Lithuania) was used as a molecular weight marker. *T. aestivum* NILs containing the corresponding *Lr* genes were included as positive controls.

RESULTS

None of the isolates was virulent to the line with *Lr19* and only 2% isolates were virulent to *Lr9* (Table 1). Very few isolates were virulent to *Lr24* (on average 7%) and not many to *Lr28* (on

Table 1. Virulence frequency of *Puccinia triticina* isolates to Thatcher NILs with *Lr* genes in 2009–2011

Lr genes	Virulent isolates (%)			Average (%)
	2009	2010	2011	
Lr1	96	92	67	85
Lr2a	35	5	9	16
Lr2b	39	5	7	17
Lr2c	41	13	12	22
Lr3a	76	89	64	76
Lr9	2	3	2	2
Lr11	93	95	93	94
Lr13	100	95	100	98
Lr15	85	97	91	91
Lr17	100	97	86	94
Lr19	0	0	0	0
Lr21	100	95	88	94
Lr23	87	98	84	90
Lr24	4	8	9	7
Lr26	67	78	60	68
Lr28	33	13	7	18
No. of tested isolates	46	64	54	total 164

average 18%). In addition to the above-mentioned *Lr* genes virulence below 50% of isolates was found to *Lr2a* (16%), *Lr2b* (17%), *Lr2c* (22%). The average frequency of virulence to other genes was over 50%. Virulence frequency over 80% was revealed to *Lr1* (85%), *Lr11* (94%), *Lr13* (98%), *Lr15* (91%), *Lr17* (94%), and *Lr21* (94%) and *Lr23* (90%). In all three years of the survey the pathotype virulent to *Lr1*, *Lr3a*, *Lr11*, *Lr13*, *Lr15*, *Lr17*, *Lr21*, *Lr23* and *Lr26* prevailed. Because many pathotypes, namely 29 in 2009, 26 in 2010 and 26 in 2011, were determined, only reactions of the pathotypes determined at least three times in the relevant year were summarized in Table 2.

In the first trial in which variety resistance of registered cultivars was tested, cultivars Bagou, Carroll and Citrus were highly resistant to all applied leaf rust isolates. Cultivar Seladon was also resistant in the greenhouse, however it displayed higher infection types and was scored in the field by 6. High seedling resistance of cultivars Carroll and Citrus corresponds to high field resistance scored 8. In the second trial resistant reaction to all pathotypes was also observed in cv. Carroll and to all but two pathotypes in cv. Citrus. Cultivars Bagou and Feria though resistant in the field (scored 7) were susceptible to all five applied pathotypes. Other cultivars scored 7, Beduin, RW Nadal and Aladin, were resistant at least to one of the applied pathotypes. In most cultivars there was no clear relation between the reactions in the greenhouse and in the field.

No definite similarity in reactions of the tested cultivars and reactions on the lines possessing different *Lr* genes was observed. Hence the phenotypic analysis of resistance genes was not possible.

The presence of the genes *Lr10*, *Lr24*, *Lr26*, *Lr28*, *Lr34* and *Lr37* was tested by PCR assays (Table 3). Only the genes *Lr10*, *Lr24*, *Lr34* and *Lr37* were determined in the tested cultivars. Ten cultivars possessed *Lr37*, seven *Lr10*, one *Lr24* and another one *Lr34*. High field resistance of the cv. Carroll can

Table 2. Prevailing leaf rust pathotypes in 2009–2011

Year	No. of isolates	Virulence on NILs	Locality
2009	7	*Lr1*, *Lr3a*, *Lr11*, *Lr13*, *Lr15*, *Lr17*, *Lr21*, *Lr23*, *Lr26*	Chrlice, Lípa, Věrovany, Lípa, Svitavy
	4	*Lr1*, *Lr3a*, *Lr11*, *Lr13*, *Lr15*, *Lr17*, *Lr21*, *Lr23*, *Lr26*, *Lr28*	Blížkovice, Lípa, Chrlice, Lípa
	4	*Lr1*, *Lr11*, *Lr13*, *Lr15*, *Lr17*, *Lr21*, *Lr23*	Chrastava, Dyjákovice, Zadní Arnoštov
	3	*Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr3a*, *Lr11*, *Lr13*, *Lr15*, *Lr17*, *Lr21*, *Lr23*, *Lr26*	Věrovany, Chrastava, Břeclav
2010	27	*Lr1*, *Lr3a*, *Lr11*, *Lr13*, *Lr15*, *Lr17*, *Lr21*, *Lr23*, *Lr26*	Kroměříž, Pusté Jakartice, Lípa, Stupice, Chrlice, Uherský Ostroh, Domoradice, Jaroměřice, Chrastava, Bílovice u Děčína, Bílovec, Věrovany, Stupice, Velké Pavlovice, Hradec nad Svitavou, Humpolec
	5	*Lr1*, *Lr3a*, *Lr11*, *Lr13*, *Lr15*, *Lr17*, *Lr21*, *Lr23*	Branišovice, Chrlice, Stupice, Žatec, Kroměříž
	4	*Lr1*, *Lr2c*, *Lr3a*, *Lr11*, *Lr13*, *Lr15*, *Lr17*, *Lr21*, *Lr23*, *Lr26*	Hrubčice, Stupice, Věrovany, Pusté Jakartice
	3	*Lr1*, *Lr3a*, *Lr11*, *Lr13*, *Lr15*, *Lr17*, *Lr21*, *Lr23*, *Lr26*, *Lr28*	Hrubčice, Branišovice, Věrovany
2011	11	*Lr1*, *Lr3a*, *Lr11*, *Lr13*, *Lr15*, *Lr17*, *Lr21*, *Lr23*, *Lr26*	Hradec nad Svitavou, Pusté Jakartice, Chrlice, Čáslav, Jaroměřice, Úhřetice, Oldřichovice
	6	*Lr11*, *Lr13*, *Lr15*, *Lr17*, *Lr21*, *Lr23*	Čáslav, Úhřetice, Stařîč
	4	*Lr1*, *Lr3a*, *Lr11*, *Lr13*, *Lr15*, *Lr17*, *Lr21*, *Lr23*	Chrlice, Úhřetice, Krukanice, Uherský Ostroh
	3	*Lr1*, *Lr3a*, *Lr11*, *Lr13*, *Lr15*, *Lr17*, *Lr21*, *Lr23*, *Lr26*, *Lr28*	Chrlice, Branišovice, Hradec nad Svitavou
	3	*Lr1*, *Lr11*, *Lr13*, *Lr15*, *Lr17*, *Lr21*, *Lr23*, *Lr26*	Pusté Jakartice, Úhřetice

Table 3. Reactions of selected winter wheat cultivars to six older leaf rust pathotypes, results of molecular marker analysis and field scoring

Cultivar	Registered	Leaf rust isolate							Field**
		Lr genes*	1887	1947	628	4332/3	333/3	347	
Aladin	2010	*Lr37*	3	3	2,3	3	3	3	7
Altigo	2011	*Lr37*	3	3	3	3	3	3	6
Bagou	2009	*Lr10*	;1	;1	0;	;1	;2N	;1	7
Beduin	2011	*Lr34*, *Lr37*	3	3	;2	3	3	3	7
Bodyček	2010	*Lr37*	3	3	3	3	3	3	6
Brentano	2010		3	3	3	3	3	3	5
Brilliant	2009		0	;1	3	;1	0	0	5
Carroll	2011	*Lr10*, *Lr24*	0;	0;	0;	;	;N	0;	8
Chevalier	2011	*Lr10*, *Lr37*	;2	;1	;1	3	;2	;2	5
Citrus	2011		0;	0	0;	0;	0	0;	8
Elly	2010	*Lr37*	3	3	;2	3	3	3	5
Federer	2009		3	3	3	3	3	3	5
Feria	2011		3	3	0;	3	;2	;	7
Graindor	2010	*Lr37*	3	3	;2	3	;2 N	3	6
Henrik	2010		3	3	3	3	3	3	6
Iridium	2009	*Lr10*	2,3	;1 N	3	3	;2 N	;2	5
Jindra	2010	*Lr10*, *Lr37*	;1	3	;1	3	3	3	5
Matylda	2011	*Lr37*	3	3	3	3	3	3	6
RW Nadal	2011	*Lr37*	3	;2	;1	3	;2	;2 N	7
Sailor	2011		3	3	3	3	3	3	6
Secese	2009	*Lr10*	3	0 ;	3	;2	3	3	6
Seladon	2009	*Lr10*	;2 N	;1	;2	;2	;2 N	2.3	6

**Lr* genes determined by molecular markers, **1 – susceptible, 9 – resistant; infection types: ; – chloroses, N − necroses, 0, 1, 1–2, 2 – resistant, 2–3, 3 – susceptible

be ascribed to the gene *Lr24* as virulence frequency to the gene was very low in our pathotype survey. None of the studied genes was determined in the resistant cv. Citrus. Cultivars Bodyček, Matylda, Altigo and Graindor possessing *Lr37* displayed medium field resistance scored 6. Lower resistance scored 5 was found in cvs Jindra and Elly, which also possess *Lr37.* Cultivars RW Nadal and Aladin with the same gene *Lr37* were scored 7. Resistance scored 7 in the cv. Beduin can be ascribed to the combination of APR genes *Lr34* and *Lr37*. Comparison of field trials with greenhouse tests indicates that other genes than the gene *Lr37* for adult plant resistance and the genes determined by us can be more important for resistance in the field in the studied cultivars.

DISCUSSION

No significant changes occurred in the leaf rust population on the set of differentials in the period 2009–2011. Compared with previous results (Hanzalová 2010) virulence frequencies were similar to those in 2005–2008. Virulence to *Lr19* was not identified; virulence frequency to *Lr19* in the previous four years was only 0.8%. Virulence to *Lr9* was not found in the previous four years; it reached only 2% in 2009–2011. Virulence frequency to *Lr24* increased from 1.5% to 7% in comparison with the period 2005–2008. Virulence frequency to *Lr28* was the same in both time periods, namely 18%. The highest virulence frequency in our survey was to the genes *Lr13* (98%), *Lr11*, *Lr17* and *Lr21* (94% all three NILs). The number of different pathotypes determined in 2009–2011 was relatively high, namely 81 pathotypes from 164 samples.

Virulence to *Lr19* gene, not recorded in 2009 to 2011, was identified in the Czech Republic in 2005 and 2008 but only very rarely (Hanzalová 2010). In the period 1994–2011 virulence to *Lr19* was not recorded in Slovakia (Hanzalová *et al.* 2008, 2012). In Germany virulence to *Lr19* was found in 1999 (Gultyaeva *et al.* 2000). However, it was not recorded in France, Czech Republic, Germany, Italy, Spain, Hungary, Poland, Bulgaria, Romania and Slovakia in the 1996–1999 virulence survey summarized by Mesterházy *et al.* (2000). The gene *Lr19* was used only rarely in the breeding process because of a gene linked with *Lr19* that conditions the yellow colour of flour. In Slovakia the gene *Lr19* is possessed by the cultivar Bona Dea, in Sweden it has been incorporated in the spring wheat cultivar Sunnan.

Table 4. Reactions of selected winter wheat cultivars to the five most widespread leaf rust isolates

Cultivar	Leaf rust isolate				
	9672	9668	9669	9657	9712
Aladin	;2	3	3	3	3
Altigo	;	;	3	3	3
Bagou	3	3	3	3	3
Beduin	;1	3	3	3	3
Bodyček	2-3	3	3	3	3
Brentano	3	3	3	3	3
Brilliant	0;	;1–2	3	;2	3
Carroll	;	;	;	;	;
Citrus	0;	0;	3	;	3
Dulina	;	;	3	3	3
Elly	;	;	3	3	3
Federer	3	3	3	3	3
Feria	3	3	3	3	3
Fermi	;	;	;	;	;
Fortis	;	;	3	3	3
Golem	;2	3	3	3	3
Graindor	3	3	3	3	3
Henrik	3	3	3	3	3
Iridium	;	;	3	3	3
Jindra	;2	3	3	3	3
Magister	3	;	3	3	3
Matylda	;	;	3	3	3
Preciosa	3	3	3	3	3
RW Nadal	;2	3	3	3	3
Sailor	;	;	3	3	3
Salutos	;	;	3	3	3
Secese	3	3	3	3	3
Seladon	;	;	3	3	3

Infections types: ; – chloroses, 0, 1, 1–2, 2 – resistant, 2–3, 3 – susceptible

Virulence to *Lr9* was found neither in the Czech Republic in 2005–2008 (Hanzalová 2010) nor in Germany and in Russia in 2001–2003 (Lind & Gultyaeva 2007). In Slovakia virulence to *Lr9* was recorded in 2008 (Hanzalová *et al.* 2012). It was recorded only once in the European virulence survey for leaf rust in wheat by Mesterházy *et al.* (2000). The gene *Lr9* has not been widely deployed in breeding, however several mainly US cultivars possess it. Among the *Lr* genes effective in our pathotype survey *Lr24* and *Lr28* belong to genes relatively effective worldwide. However, leaf rust resistance conditioned by *Lr24* has completely broken down in the UK and it is spreading in continental Europe (M. Taylor 2012, personal communication). Though highly effective alien resistance genes do not usually guarantee long-lasting resistance. The main reason why highly effective resistance genes are relatively rarely deployed in the breeding of commercial cultivars is the linkage of genes for resistance with undesired agronomic traits.

In addition to cultivars carrying *Lr37* described in this paper, the gene *Lr37* was determined already earlier in many wheat cultivars grown in the Czech Republic, e.g. Bakfis, Barryton, Biscay, Nikol, Kodex, Mulan, Orlando, Sultan (Hanzalová 2010). The gene *Lr26* is possessed by several cultivars grown in the Czech Republic, e.g. Etela, Orlando (Hanzalová 2010). In the present pathotype survey it was ineffective to 68% of the tested leaf rust isolates. The gene *Lr10* determined e.g. in cultivars Bakfis, Baletka, Barryton, Biscay, Etela, Mulan, Pitbul (Hanzalová 2010) was not included in the set of differentials. Earlier the gene *Lr3a* governed leaf rust resistance in many cultivars grown in the former Czechoslovakia; among the registered cultivars, only cv. Astella carries it at present. The gene *Lr3a* was overcome by 76% of the tested leaf rust isolates.

Though several cultivars possess satisfactory leaf rust resistance in the field, resistance of the most widespread wheat cultivars in the Czech Republic is not sufficient and must be completed by routine chemical control, protecting wheat also from other leaf diseases.

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