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

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Abstract: 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 from different parts of the Slovak Republic. A total of 122 wheat leaf rust isolates were analysed. Resistance gene *Lr19* was effective to all tested isolates. Virulence to *Lr9* was found, however only in one isolate. Gene *Lr24* conditioned resistance to almost all rust collections. A lower frequency of virulence to *Lr2a* and *Lr28* was also observed. Nineteen winter wheat cultivars grown in Slovakia were tested with 8 leaf rust isolates. Winter wheat cultivar Bona Dea was resistant to all isolates applied in the greenhouse test. Presence of *Lr* genes was estimated according to the reactions of the tested cultivars. Presence of *Lr10*, *Lr26*, *Lr34* and *Lr37* was studied by molecular markers.

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

In Slovakia wheat leaf rust caused by *Puccinia triticina* belongs to the most important wheat diseases particularly in the southern part of the country. Breeding for resistance is the most economic control. Due to the large number of different pathotypes of leaf rust and their changes resistance breeding is a continuous process. For successful resistance breeding the knowledge of virulence in the leaf rust population is necessary because it enables the choice of sources of resistance effective against local leaf rust pathotypes. Physiological races (pathotypes) have been studied in Slovakia since the sixties of the last century (Šebesta & Bartoš 1968, 1969; Bartoš & Šebesta 1971). Results of the surveys were published first together with the data from Bohemia and Moravia, later on since 1994 separately (Hanzalová *et al.* 2008, 2010). The present contribution contains results of virulence surveys carried out in the years 2009–2011. Selected pathotypes from the surveys were used to study seedling reactions of 19 winter wheat cultivars grown in Slovakia. An attempt was made to estimate resistance genes in the studied cultivars by comparing reactions of the tested cultivars with selected Thatcher near-isogenic lines possessing leaf rust resistance genes (*Lr*). Results of the resistance gene estimation were supported and supplemented by molecular marker analyses.

MATERIAL AND METHODS

Collections of wheat leaf rust on leaves were obtained from different cultivars, mainly from the variety trials located across the country and organized by the Central Controlling and Testing Institute in Agriculture in Slovakia. 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 a Petri dish with water and kept in the greenhouse until ure-

Table 1. Prevailing pathotypes of *Puccinia triticina* in the Slovak Republic in 2009–2011

Year	Virulence on NILs	Occurrence (%)*	Locality
2009	*Lr1*, *Lr3a*, *Lr11*, *Lr13*, *Lr15*, *Lr17*, *Lr21*, *Lr26*	27.6	Beluša, Malý Šariš, Veľké Ripňany, Víglaš
2010	*Lr1*, *Lr3a*, *Lr11*, *Lr13*, *Lr15*, *Lr17*, *Lr21*, *Lr26*	35.0	Radošiná, Spišské Vlachy
2011	*Lr1*, *Lr3a*, *Lr11*, *Lr13*, *Lr15*, *Lr17*, *Lr21*, *Lr26*	14.6	Báhoň, Jakubovany, Spišské Vlachy, Veľký Meder, Želiezovce
	Lr1, *Lr3a*, *Lr11*, *Lr13*, *Lr15*, *Lr17*, *Lr21*, *Lr23*, *Lr26*	10.4	Báhoň, Jakubovany, Veľký Meder, Želiezovce

*Percentage of occurrence out of total of tested rust samples in the relevant year

diospores 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 closed glass cylinders to provide high air humidity for 24 h. 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 2^+, virulence by infection type 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 with *Lr13* were used in the tests. 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. Pedigree of NILs was described in the paper by Mesterházy *et al.* (2000). In 2009 47 single pustule

Table 2. Reactions of differentials (NILs) to eight leaf rust isolates used in the variety test (Table 5)

Near isogenic line	Locality							
	Veľké Ripňany		Jakubovany		Spišské Vlachy		Veľký Meder	
	A	B	C	D	E	F	G	H
NIL *Lr1*	3	3	3	3	0	0	0;	0
NIL *Lr2a*	;	0	0;	;	3	3	3	;2
NIL *Lr2b*	; N	;	0;	;1	3	3	3	3
NIL *Lr2c*	;	0;	;	;	3	3	3	3
NIL *Lr3a*	0;	3	3	3	3	3	3	3
NIL *Lr9*	0	;	;	0	0	0	0	;
NIL *Lr11*	3	3	3	3	3	3	3	3
NIL *Lr13*	3	3	3	3	;2	;2	;1–2	;1–2
NIL *Lr15*	;2	;2	3	3	3	3	3	;2
NIL *Lr17*	3	3	3	3	;	;	3	;
NIL *Lr19*	;	0	;	0;	0;	0;	0	0
NIL *Lr21*	3	3	;1	3	3	3	3	3
NIL *Lr23*	3	;1	0	;1–2	3	3	;2	;1–2
NIL *Lr24*	;	3	0	;	3	3	;	;
NIL *Lr26*	;2	3	;	3	;1–2	;1–2	;2	0;
NIL *Lr28*	;	3	0	0	0	0	;	;

Infection types: ; – chloroses, N – necroses, 1, 1–2, 2 – resistant; 3 – susceptible

Table 3. PCR conditions and primers

Gene	Chromosome location	Name of primer	Amplification conditions	PCR product	Reference
Lr10	1AS	Fl2245 Lr10-6/r2	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)
Lr34	7DS	csLV34F csLV34R	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)
Lr26	1BS	SECA2 SECA3	94°C for 3 min; 35 cycles of 94° for 30 s, 58°C for 30 s, 72°for 45 s; 72°C for 10 min	412 bp	De Froidmont (1998)
Lr37	2AS	URIC LN2	94°C for 3 min; 35 cycles of 94° for 30 s, 58°C for 30 s, 72°for 45 s; 72°C for 10 min	262 bp	Helguera *et al.* (2003)

isolates from 9 localities, in 2010 27 single pustule isolates from 11 localities, and in 2011 48 single pustule isolates from 11 localities were analysed.

Because many pathotypes, namely 38 in 2009, 20 in 2010 and 33 in 2011, were determined, only reactions of the pathotypes representing at least 10% of the total number of determined pathotypes were summarized in Table 1.

Nineteen winter wheat cultivars grown in Slovakia were tested at the seedling stage with eight leaf rust isolates using the same method as in the pathotype analysis. Applied leaf rust isolates were selected from the 2011 pathotype survey. Their reactions on Thatcher near-isogenic lines are described in Table 2. For estimation of resistance genes rust reactions of selected NILs were compared with reactions of the tested cultivars.

Molecular markers were used to support and extend data obtained by phenotypic estimation of resistance genes. DNA was extracted from the second wheat leaves using the Qiagen DNA extraction kit. 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). The genes *Lr10*, *Lr34*, *Lr26* and *Lr37* were identified with the use of PCR with published primers marking these genes (de Froidmont *et al.* 1998; Helguera *et al*. 2003; Lagudah *et al.* 2006; Gultyaeva *et al.* 2009). Genes *Lr26* and *Lr37* were detected using multiplex PCR in one reaction. The PCR conditions and names of used primers are shown in Table 3. Thatcher NILs containing the corresponding *Lr* genes were used as a positive control. The thermal cycler Veriti (Applied Biosystems) was

Table 4. Virulence frequency of *Puccinia triticina* isolates in the Slovak Republic to Thatcher near-isogenic lines with *Lr* genes in 2009–2011

Lr genes	Virulent isolates (%)			Average (%)
	2009	2010	2011	
Lr1	38	59	69	54.9
Lr2a	28	30	19	24.6
Lr2b	49	44	29	40.2
Lr2c	47	63	29	43.4
Lr3a	66	93	81	77.9
Lr9	0.5	0	0	0.2
Lr11	53	100	100	81.9
Lr13	77	96	73	79.5
Lr15	74	100	75	80.3
Lr17	79	93	79	81.9
Lr19	0	0	0	0
Lr21	89	96	94	92.6
Lr23	34	70	33	41.8
Lr24	4	7	10	7.4
Lr26	60	56	75	64.7
Lr28	2	7	8	5.7
No. of tested isolates	47	27	48	122

used for PCR reactions. The amplification products were separated by electrophoresis in 2% agarose gels, stained with ethidium bromide, and visualized under UV light. GeneRuler^{TM} 100 bp DNA Ladder (Fermentas) was used as a molecular weight marker.

RESULTS

None of the isolates was virulent to *Lr19* and only one isolate was virulent to *Lr9*. Very few isolates were virulent to *Lr24* (7.4%) and *Lr28* (5.7%). In addition to the above-mentioned *Lr* genes virulence below 50% of isolates was found to *Lr2a* (24.6%), *Lr2b* (40.2%), *Lr2c* (43.4%) and *Lr23* (41.8%). The average frequency of virulence to other genes was over 50%. Virulence frequency over 80% was revealed to *Lr11* (81.9%), *Lr15* (80.3%), *Lr17* (81.9%) and *Lr21* (92.6%) (Table 4). In 2009, 2010 and 2011 the pathotype virulent to *Lr1*, *Lr3a*, *Lr11*, *Lr13*, *Lr15*, *Lr17*, *Lr21* and *Lr26* prevailed, representing 27.6%, 35.0% and 14.6 % of the total of analysed samples, respectively. In 2011 the second most widespread pathotype (10.4%) differed from the prevailing pathotype only by additional virulence to *Lr23* (Table 1).

Reactions of registered winter wheat cultivars to 8 leaf rust isolates are listed in Table 5. Of 19 cultivars only the cultivar Bona Dea was resistant to all rust isolates like NIL possessing *Lr19*. Most cultivars were resistant at least to one leaf rust isolate. Out of 19 tested winter wheat cultivars only 3 cultivars Pinta, Questor and Mv Palotas were susceptible to all rust isolates. Cultivars Viglanka, Karolinum, Bertold, Petrana and Rapsodia displayed similar reactions resembling those of NIL possessing *Lr26*. Another group of cultivars with mutually similar reaction pattern comprised cultivars Apache, Median, Ezopus, Karpatia, Alacris. Reactions of cultivars Bodyček and Mulan were

Table 5. Reactions of selected winter wheat cultivars to eight different leaf rust isolates and results of the molecular marker analysis

Cultivar	Leaf rust isolate								*Lr* genes determined by molecular markers			
	A	B	C	D	E	F	G	H	*Lr10*	*Lr34*	*Lr26*	*Lr37*
Alacris	;	3	;1	3	3	3	3	;1		+		
Apache	;1	3	0;	3	3	3	3	;2		+		
Bazilika	3	3	3	3	3	;2	;2	;2				+
Bodyček	3	3	3	3	;2	;2	;2	;2				
Bona Dea	;	0	0;	;	0;	0;	0;	0;				
Bonita	;2	3	0;	3	3	3	3	3				
Bertold	;1	3	;1	3	0;	;	;	;1			+	
Caphorn	3	3	3	3	2+	3	3	;2	+			
Ezopus	0;	3	;1	3	3	3	3	;2		+		
Karolinum	;2N	3	;1	3	0	0	0	;1		+		
Karpatia	;1	3	;1	3	3	3	3	;2	+			
Median	0;	3	;	3	3	3	3	;1		+		
Mulan	3	3	3	3	;2	;2	;2	;2	+			
MV Palotas	3	3	3	3	3-	3	3	3	+	+		
Petrana	;1	3	0;	3	0	;1	0;	2+			+	
Pinta	3	3	3	3	3	3	3	3				
Questor	3	3	3	3	3-	3	3-	3				
Rapsodia	;2	3	;1	3	;2	;2	2+	;	+		+	+
Viglanka	;2	3	0;	3	;2	;2	;2	;1			+	

Infection types: ; – chloroses, N – necroses, 0,1, 1–2, 2,2+ – resistant; 3 – susceptible

also similar to each other. However, reactions of the last two groups of cultivars did not correspond with reactions of any *Lr* NIL.

Results of the molecular marker analysis are summarized in Table 5 and in Figures 1—3. The analyses revealed *Lr26* in cvs Bertold, Petrana, Rapsodia and Viglanka, *Lr34* in cvs Alacris, Apache, Ezopus, Karolinum, Median and Mv Palotas, *Lr10* in cvs Caphorn, Karpatia, Mulan, Mv Palotas and Rapsodia, *Lr37* in cvs Bazilika, Mulan and Rapsodia.

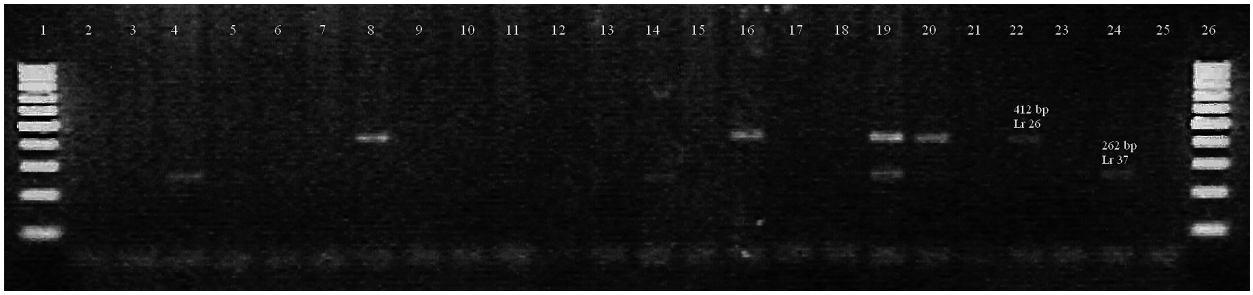

Figure 1. Detection of the *Lr26* and *Lr37* resistance genes in 19 wheat cultivars; 1, 26 – GeneRuler 100bp DNA ladder (Fermentas), 2 – Alacris, 3 – Apache, 4 – Bazilika, 5 – Bodyček, 6 – Bona Dea, 7 – Bonita, 8 – Bertold, 9 – Caphorn, 10 – Ezopus, 11 – Karolinum, 12 – Karpatia, 13 – Median, 14 – Mulan, 15 – Mv Palotas, 16 – Petrana, 17 – Pinta, 18 – Queator, 19 – Rapsodia, 20 – Viglanka, 21 – Lr10, 22 – Lr26, 23 – Lr34, 24 – Lr37, 25 – water

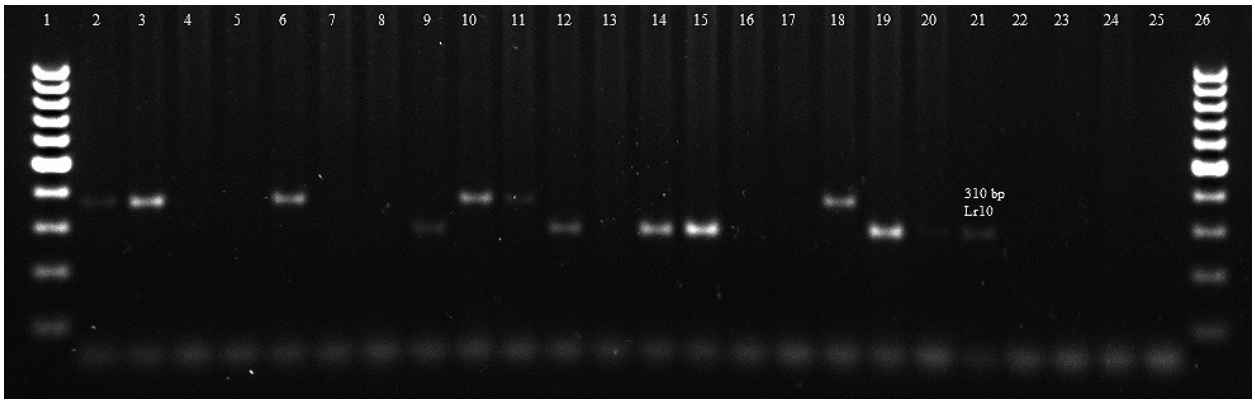

Figure 2. Detection of the *Lr10* resistance gene in 19 wheat cultivars; 1, 26 – GeneRuler 100bp DNA ladder (Fermentas), 2 – Alacris, 3 – Apache, 4 – Bazilika, 5 – Bodyček, 6 – Bona Dea, 7 – Bonita, 8 – Bertold, 9 – Caphorn, 10 – Ezopus, 11 – Karolinum, 12 – Karpatia, 13 – Median, 14 – Mulan, 15 – Mv Palotas, 16 – Petrana, 17 – Pinta, 18 – Questor, 19 – Rapsodia, 20 – Viglanka, 21 – Lr10, 22 – Lr26, 23 – Lr34, 24 – Lr37, 25 – water

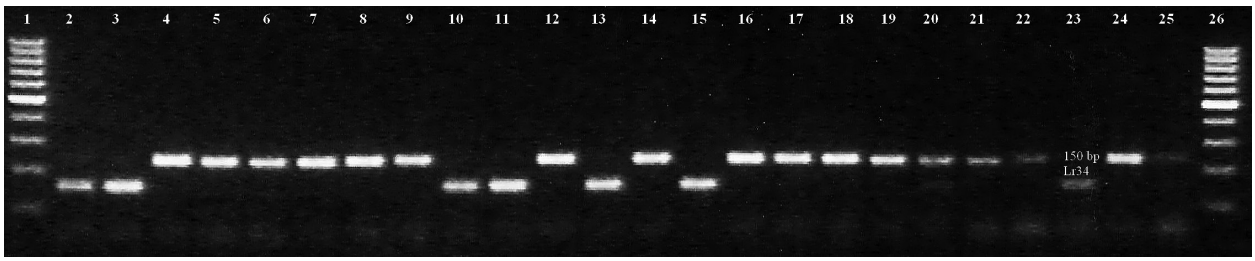

Figure 3. Detection of the *Lr34* resistance gene in 19 wheat cultivars; 1, 26 – GeneRuler 100bp DNA ladder (Fermentas), 2 – Alacris, 3 – Apache, 4 – Bazilika, 5 – Bodyček, 6 – Bona Dea, 7 – Bonita, 8 – Bertold, 9 – Caphorn, 10 – Ezopus, 11 – Karolinum, 12 – Karpatia, 13 – Median, 14 – Mulan, 15 – Mv Palotas, 16 – Petrana, 17 – Pinta, 18 – Questor, 19 – Rapsodia, 20 – Viglanka, 21 – Lr10, 22 – Lr26, 23 – Lr34, 24 – Lr37, 25 – water

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 *et al.* 2010) virulence frequencies had a similar trend like in 2005, 2006 and 2008. Virulence to *Lr9* was not identified in 2 of the 3 years of virulence survey; only in 2009 one virulent isolate was found. Virulence to *Lr24* and *Lr28* was found in each year from 2009 to 2011, however only sporadically. Similarly to previous years virulence to *Lr19* was not found in the Slovak Republic. The most widespread virulence (92.6%) in our survey was to *Lr21.* The number of different pathotypes determined in the Slovak Republic was relatively high, with 34 pathotypes out of 122 samples.

Virulence to *Lr19* was identified in the Czech Republic in 2005 and 2008 but only very rarely (Hanzalová 2010). 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). Breakdown of *Lr19* effectiveness occurred in Volga, Ural region and Central Black Earth and caused considerable yield losses (Gultyaeva 2007). Because of linkage with the gene conditioning yellow colour of flour *Lr19* has rarely been used in the breeding. Swedish spring wheat cv. Sunnan, several cultivars from the former USSR, e.g. Saratovskaya 29, Samara, Volgogradskaya (Martynov & Dobrotvorskaya 2006) and the Slovak cv. Bona Dea, the only cultivar resistant to all rust isolates in our test, possess *Lr19*. The yellow colour of flour from the cv. Bona Dea caused by a gene linked with *Lr19* confirms the presence of *Lr19.* 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). It was registered only once in the European virulence survey for leaf rust in wheat by Mesterházy *et al.* (2000). Virulence to *Lr24* was very rare in Germany, and it was not found in Russia in 2001–2003 (Lind & Gultyaeva 2007). Virulence to *Lr9*, *Lr19*, and *Lr24* was not found in Latvia (Liatukas 2003). Like in Slovakia, in the European virulence survey for leaf rust in wheat virulence frequency to *Lr21* in nine European countries was also recorded as high. It was low only in France (Mesterházy *et al.* 2000).

Average percentage of virulence revealed in the years 2009–2011 is similar to the results from the years 2005, 2006 and 2008, except virulence to *Lr23* that was higher in the previous years. Another difference from earlier results (Hanzalová *et al.* 2010) is switch to avirulence to *Lr2a*, *Lr2b* and *Lr2c* in the prevailing pathotypes.

Virulence in the leaf rust population can be rather ascribed to the fitness of the pathotypes than to the selection pressure due to resistance genes in the grown cultivars because the prevailing cultivars are susceptible to leaf rust.

Comparison of reactions of the tested cultivars with NILs indicated the probable presence of *Lr26* in several cultivars. Estimation of *Lr26* in cvs Bertold, Petrana, Rapsodia and Viglanka was validated by the applied molecular marker. Comparison with data published earlier revealed some discrepancies in results obtained by the phenotyping method. There is a good correspondence between our results and earlier data (Hanzalová *et al.* 2010) in the postulated resistance gene *Lr26* in cv. Rapsodia. However, earlier data on *Lr26* in cvs Bonita and Karolinum (Hanzalová *et al.* 2010) are different from the present results. Discrepancies between earlier and present data in cv. Karolinum can be easily explained because this cultivar is composed of two lines that differ in the presence of *Lr26* (Bradová *et al*. 2009a, b). Obviously, samples from different lines of the cv. Karolinum were used in different experiments. The presence of the gene *Lr26* in cvs Karolinum and Rapsodia, described earlier, was also validated by a gliadin analysis carried out in the Laboratory of Quality of Crop Products of the Crop Research Institute, Prague. Gliadin designated as 1B3 is characteristic of the translocation 1BL.1RS (Bradová & Šašek 2007), which carries *Lr26* and the linked genes *Sr31*, *Yr8* and *Pm8*. A difference between the results in cv. Bonita can have a similar reason. However, up to this time cv. Bonita has not been analysed for the presence of more lines.

By molecular markers *Lr26* in 4, *Lr10* in 5, *Lr34* in 6, and *Lr37* in 3 of the 19 analysed cultivars were determined. Whereas genes *Lr10* and *Lr26* are seedling resistance genes, *Lr34* and *Lr37* are effective only at an adult plant stage and for this reason they are not included in the set of differentials. As shown in Table 4, the majority of analysed rust isolates was virulent to *Lr26*. Gene *Lr10* is described as a gene effective together with other resistance genes, in particular (Mcintosh *et al.* 1995). At present, the gene *Lr37*, which is frequent in West European culti-

vars, is important for adult plant resistance. Besides the highly effective gene *Lr19* (cv. Bona Dea), genes *Lr34* and *Lr37*, though ineffective at the seedling stage, have remained relatively effective at the adult plant stage in the field particularly in combination with other resistance genes. Unfortunately, leaf rust isolates virulent to adult plants possessing *Lr37* were already found in Germany (Lind 2008, personal communication).

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