

Physiologic Specialization of Wheat Leaf Rust (*Puccinia triticina* Eriks.) in the Slovak Republic in 2005, 2006 and 2008

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Abstract: In 2005, 2006 and 2008 the virulence of wheat leaf rust population was studied on Thatcher near-isogenic lines with *Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr3a*, *Lr9*, *Lr10*, *Lr13*, *Lr15*, *Lr17*, *Lr19*, *Lr21*, *Lr23*, *Lr24*, *Lr26* and *Lr28*. Samples of leaf rust (141 in total) were obtained from different parts of Slovakia. Resistance gene *Lr9* was effective to all tested isolates except three isolates from 2008. No virulence was found to *Lr19* and genes *Lr24* and *Lr28* were also highly effective. Low incidence of virulence to *Lr2a* was observed. Sixty-five winter wheat cultivars registered in Slovakia were tested with seven leaf rust isolates in the greenhouse. Cultivar Bona Dea was the most resistant of all.

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

In the present trend to reduce costs of crop production disease resistance of crops is an important trait. Disease resistance not only reduces costs of the chemical control but also contributes to the protection of environment. Wheat is a key crop in our agriculture and leaf rust is an important disease of wheat that is most economically controlled by breeding for resistance. Knowledge of virulence in the leaf rust population is a prerequisite for successful resistance breeding. Pathotypes in the leaf rust population are surveyed in many countries where deliberate breeding for leaf rust resistance is carried out. In former Czechoslovakia pathotypes (physiologic races) were studied since the sixties of the last century (ŠEBESTA & BARTOŠ 1968, 1969; BARTOŠ & ŠEBESTA 1971) and the studies included data also from Slovakia. Results of the pathotype survey from the years

1994–2004 in Slovakia were summarized in the paper by HANZALOVÁ *et al.* (2008). Reactions of registered winter wheat cultivars to leaf rust were usually included in the reports on leaf rust virulence. Also data on *Lr* resistance genes (if available) were presented. This contribution contains results of virulence surveys from the years 2005, 2006 and 2008, as well as results of greenhouse tests for resistance of winter wheat cultivars registered in Slovakia.

MATERIAL AND METHODS

Samples of wheat leaf rust were obtained mainly from trials of the Central Controlling and Testing Institute in Agriculture in Slovakia from different cultivars and locations (Figure 1). They and



Figure 1. Localities for sampling of leaf rust in 2005–2008 in Slovakia

later single pustule isolates from them (mostly two of each increased sample) were propagated on the susceptible cv. Michigan Amber. Inoculation of seedlings was carried out with water suspension of urediospores. Inoculated plants were kept closed in 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. Frequency of virulence to the differentials was expressed in %. Thatcher near isogenic lines (NILs) with single *Lr* genes *Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr3a*, *Lr9*, *Lr10*, *Lr13*, *Lr15*, *Lr17*, *Lr19*, *Lr21*, *Lr23*, *Lr24*, *Lr26* and *Lr28* 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 propagated and sent to us. The pedigree of NILs was described in the paper by MESTERHÁZY *et al.* (2000). In 2005 43 samples from 5 localities, in 2006 62 samples from 12 localities, and in 2008 36 samples from 8 localities were analyzed (Table 1). Leaf rust samples obtained in 2007 did not germinate because of a storage failure. As the number of determined virulence combinations was large (21 pathotypes in 2005, 14 pathotypes in 2006 and 25 pathotypes in 2008), only the pathotypes representing at least 4% of the total number of determined pathotypes in the particular years were summarized in Table 2. Description of isolates is

based on the set of differentials agreed in COST 817 action (MESTERHÁZY *et al.* 2000; HANZALOVÁ 2010). Reactions of 65 out of 75 registered winter wheat cultivars (List of registered cultivars in Slovakia 2007) were tested at the seedling stage with 7 leaf rust isolates using the same method as in the pathotype analysis. These tests comprised 7–10 plants of each cultivar and were repeated twice. If the results varied, additional tests were carried out. Leaf rust isolates were selected from our previous pathotype surveys and originated from various years and locations except the isolate we called ASU that was received by courtesy of Dr. J. Kolmer. They were used because of their ability to differentiate resistance genes of wheat cultivars. Seedling reactions and data on leaf rust resistance in the description of cultivars by the Central Controlling and Testing Institute in Agriculture, Slovakia, were compared.

RESULTS

Determined virulence of leaf rust isolates from the years 2005, 2006 and 2008 is summarized in Table 1. No virulence was found to the gene *Lr19* and only 1% of isolates on the average of 3 years were virulent to *Lr9*. Very low incidence of virulence was recorded to *Lr24* (4.3%), low incidence of virulence to *Lr2a* (21.3%) and *Lr28* (10%). In-

Table 1. Virulence of leaf rust isolates on *Lr* NILs (%) in 2005, 2006 and 2008

<i>Lr</i> genes	Virulent isolates (%)			Average (%)
	2005	2006	2008	
<i>Lr1</i>	70	58	75	67.6
<i>Lr2a</i>	14	16	34	21.3
<i>Lr2b</i>	72	45	39	52
<i>Lr2c</i>	88	82	32	67.3
<i>Lr3a</i>	93	90	94	92.3
<i>Lr9</i>	0	0	3	1
<i>Lr10</i>	44	41	100	61.6
<i>Lr13</i>	98	91	94	94.3
<i>Lr15</i>	67	86	97	83.3
<i>Lr17</i>	63	86	100	83
<i>Lr19</i>	0	0	0	0
<i>Lr21</i>	100	100	94	98
<i>Lr23</i>	84	65	63	70.6
<i>Lr24</i>	7	0	6	4.3
<i>Lr26</i>	72	85	69	75.3
<i>Lr28</i>	5	3	22	10
No. of samples	43	62	36	141 in total

cidence of virulence to other genes exceeded 50% and reached 98% to the resistance gene *Lr21*.

The most frequently occurring pathotypes are listed in Table 2. In 2005 pathotypes with virulence to eleven *Lr* genes prevailed, followed by pathotypes with virulence to nine *Lr* genes. In 2006 pathotypes with virulence to nine *Lr* genes prevailed followed by pathotypes virulent to eleven *Lr* genes. Unlike in 2005 and 2006, in 2008 pathotypes virulent only to seven *Lr* genes prevailed followed by pathotypes virulent to eight *Lr* genes. In 2005 and 2006 three prevailing pathotypes were identical. Pathotype A represented race 73-62, pathotype B race 53-62, pathotype C race 13-62. In 2008 different races prevailed, namely 12-22 D, 12-62 E and 43-60 F (Table 2). Summarized data of 2005 and 2006 show the average frequency of pathotype A – 17.3%, B – 12.9% and C – 9.8%.

Reactions of winter wheat cultivars to 7 leaf rust isolates are summarized in Table 3. Of winter wheats only cultivars Bona Dea and Petrana were resistant to all seven applied rust isolates. Because cv. Petrana was characterized as medium susceptible to susceptible to leaf rust by the author of this cultivar, we tried to clarify this discrepancy. For this reason cvs. Bona Dea and Petrana were

Table 2. Prevailing leaf rust pathotypes in 2005, 2006, 2008

Year	Virulence on NILs	Occurrence in the particular years (%)	Locality
2005	<i>1, 2a, 2b, 2c, 3a, 13, 15, 17, 21, 23, 26</i> (A)	23.3	Jakubovany, Spišská Belá, Veľký Meder, Víглаš, Želiezovce
	<i>1, 2b, 2c, 3a, 13, 17, 21, 23, 26</i> (B)	20.9	Jakubovany, Spišské Vlchy, Veľký Meder, Víглаš, Želiezovce
	<i>1, 2c, 3a, 13, 15, 17, 21, 23, 26</i> (C)	6.7	Spišská Belá, Spišské Vlchy
	<i>2c, 3a, 13, 15, 17, 21, 23</i>	4.7	Spišská Belá, Veľký Meder
	<i>1, 2a, 2b, 2c, 3a, 13, 21, 26</i>	4.7	Spišské Vlchy, Želiezovce
	<i>2b, 2c, 3a, 13, 15, 17, 21, 23</i>	4.7	Víглаš, Želiezovce
2006	<i>1, 2c, 3a, 13, 15, 17, 21, 23, 26</i> (C)	12.9	Beluša, Jakubovany, Želiezovce
	<i>1, 2a, 2b, 2c, 3a, 13, 15, 17, 21, 23, 26</i> (A)	11.3	Jakubovany, Veľký Meder, Vrankuňa
	<i>1, 2b, 2c, 3a, 13, 17, 21, 23, 26</i> (B)	4.8	Jakubovany, Želiezovce
	<i>1, 3a, 15, 17, 21, 26</i>	4.8	Jakubovany
2008	<i>1, 3a, 13, 15, 17, 21, 26</i> (D)	13.8	Veľký Meder, Vrankuňa
	<i>1, 3a, 13, 15, 17, 21, 23, 26</i> (E)	11.1	Haniska, Michalovce, Vrankuňa
	<i>2b, 2c, 3a, 13, 15, 17, 21, 23</i> (F)	8.3	Spišská Belá, Víглаš
	<i>1, 3a, 13, 15, 17, 21, 23</i>	5.6	Veľké Ripňany, Haniska,
	<i>1, 2a, 2b, 2c, 13, 15, 17, 21, 23, 26</i>	5.6	Vranov nad Topľou, Haniska

Table 3. Reactions of winter wheat cultivars registered in Slovakia to 7 leaf rust isolates in the greenhouse tests

Cultivar	<i>Lr</i> genes		Isolates							
	published (citation)	postulated	registered	ASU	4332	333	347/6	4332/3	4003/4	628A
Alacris		<i>Lr3a</i>	2005	0	3	3	3	3	3	3
Alana		–	1997	3	3	3–	3–	3	3	3–
Alka	<i>Lr10, Lr13</i> (1)		1997	;1–3	3+;	3–	;	2	3	2
Arida		<i>Lr3a</i>	2001	;	3–	3	3	3	3–	3
Armelis		<i>Lr3a</i>	2002	0;	3	3	3	3	3	3
Astella		<i>Lr3a</i>	1995	;	3	3	3	3	3	3
Axis		<i>Lr3a</i>	2003	0;	3	3	3	3	3	3
Balada		–	1999	3	3	3	3	3	3	2–3
Bardotka		<i>Lr3a</i>	2004	;1–2	3	3	3	3	3	3
Barroko		–	2006	3	3	3	3	3	3	3
Bazilika		–	2007	3	3	3	3	3	3	3
Bety		–	1999	3	3	3	3	3	3	3
Blava	<i>Lr3ka</i> (2)		1992	;1	3	3–	3	3	3	2
Boka	<i>Lr13</i> (2)		1996	3	3	3	3	3	3	3
Bona Dea		<i>Lr19</i>	2006	0	;	;	;	;	;	;
Bonita		<i>Lr26+</i>	2003	;1	;	0;	0;	3	;1	3
Bosorka		–	2007	3	3	3	3	3	3	3
Brea		<i>Lr3a</i>	1998	;	3	3	3	3	3	3
Bruta	<i>Lr14a</i> (2)		1994	;1	3	3	3–	3	3	2
Caphorn	<i>Lr37+</i> (3)		2005	2–3	2–3	2–3	2–3	0;1	2–3	;1–2
Clever	<i>Lr37+</i> (3)		2004	3	3	2–3	3	3	3–	2–3
Corsaire	<i>Lr37+</i> (3)		2003	2–3	3	3	3	3	3	2–3
Ebi		–	2006	3	3	3	3	3	3	3
Elpa		<i>Lru</i>	2001	3	3–	3	2–3	3	3	2–3
Estica	<i>Lr13, Lr14a</i> (2)		1996	3	3	3	3	3	3	3–
Eva		<i>Lr3a</i>	2001	;	3	3	3–	3	3	3
Evelina		<i>Lru</i>	2004	;	3	;+3	;	3	;	;
Genoveva		<i>Lr26+</i>	2005	;1	;	0;	0;	3	;1	3
Hana		<i>Lr3a</i>	1985	;	3	3	3	3	3	3
Charger		<i>Lru</i>	2003	3–	3	3	2+	2+	3	;
Ignis		<i>Lr3a</i>	2004	0;	3	3	3	3	3	3
Ilias		–	2005	3	3	3–	3–	3	3	2+
Ilona		–	1989	3	3	3	3	3	3	3
Ines		<i>Lr3a?</i>	2004	2–3	3	3	3	3	3	3
IS Karpatia		<i>Lr3a</i>	2007	0	3	3	3	3	3	3
Klea		<i>Lr3a</i>	1998	;	3	3	3	3	3	3
Košútka		<i>Lru</i>	1981	2–3	3	2	3–	2–3	2	3
Lívia		<i>Lr26,</i> +	1991	;	0;	0	0	3	3	3–
Malvina		<i>Lr26</i>	1998	;1–2	0;	0;	0;	3	3	3

Table 3 continued

Cultivar	<i>Lr</i> genes		Isolates							
	published (citation)	postulated	registered	ASU	4332	333	347/6	4332/3	4003/4	628A
Malyska		<i>Lr3a</i>	2001	;1–2	3	3	3	3	3	3
Markola		<i>Lr3a</i>	2005	0;	3	3	3	3	3	3
Meritto		–	2004	3–	3–	3	3	3	3	3
Mladka		<i>Lru</i>	2003	3	3	2–3	3	3	3	3
MV Magvas		–	2003	3	3	3	3	3	3	3–
MV Palotás		<i>Lru</i>	2005	3	;	;	;1+	;1+	2–3	;1
Pavlina		<i>Lr3a</i>	2005	;	3–	3	3	3	3–	3
Pegassos		<i>Lru</i>	2004	3	3–	3	2–3	2–3	3–	3
Petrana		<i>Lru</i>	2002	;	;	;	0;	0;	12	0;
Rapsodia	<i>Lr10, Lr26, Lr37</i> (4)		2005	;1	0	0	0	;1	3	;
Rheia	<i>Lr 37</i> (3)		2007	3	3	3	3	3	3	3
Sana		<i>Lr26,+</i>	1995	;1	0;	0	0	3	3	3–
Solara		<i>Lr3a?</i>	1998	;1	3–	3	3–	3	3	3
Stanislava		–	2005	3	3	3	3	3	3	3
Sulamit			2004	3	3	3	3	3	3	3
Šarlota		<i>Lr3a</i>	2007	0;	3	3	3	3	3	3
Šárka	<i>Lr13, Lr17b</i> (2)		2000	3	3	3	3	3	3	3–
Torysa		–	1992	3	3	3	3	3	3	3–
Vanda		–	2001	3	3	3	3	3	3	3
Veldava			2005	3	3	3	3	3	3	3
Velta		<i>Lr3a</i>	2001	0;	3	3	3	3	3	3
Venistar		–	2002	3	3	3	3	3	3	3
Verita		<i>Lr26+</i>	2005	2	;1	;	0;	3	;1–2	3
Viginta		<i>Lr3a</i>	1984	0	3–	3–	3	3	3–	3
Vláda	<i>Lr1, Lr3a, Lr13</i> (2)		1990	;	3	3	3	0;1	3	2
Zerda		<i>Lr3a</i>	1999	;	3	3	3	3	3	3
NIL (Thatcher)		<i>Lr3a</i>		;1	3	3	3	3	3	3
NIL (Thatcher)		<i>Lr19</i>		0;	;	;	;	;	;	;
NIL (Thatcher)		<i>Lr26</i>		;	;	;	;	3	3	3
NIL (Thatcher)		<i>Lr37</i>		3	3	3	3	3	3	3

Infection types: ; chloroses, N = necroses, 0 = no pustules; ;, 1, 1–2, 1–2+, 2, 2+ = resistant; 2–3, 3–, 3 = susceptible (1) – BLAŽKOVÁ *et al.* (2002), (2) – PATHAN and PARK (2006), (3) – BARTOŠ *et al.* (2004), (4) – HANZALOVÁ *et al.* (2009) (see References to the present paper)

tested in an additional test with two isolates from the Czech Republic (Lípa, Praha-Ruzyně) and one isolate from Slovakia (Beluša). Whereas cv. Bona Dea was resistant to all three additionally applied isolates, cv. Petrana was resistant to two Czech isolates but susceptible to the isolate from

Beluša. Obviously, the originally applied 7 leaf rust isolates lack virulence to cv. Petrana. Out of 65 tested winter wheat cultivars only less than one third was susceptible to all 7 rust isolates. Most cultivars were resistant at least to one leaf rust isolate.

A limited number of isolates used in the greenhouse test enables to draw only preliminary conclusions on the presence of *Lr* genes. The most frequent reaction pattern was similar to the reactions of NIL *Lr3a*. The following cultivars belonged to this group: Alacris, Arida, Armelis, Astella, Axis, Bardotka, Blava (*Lr3ka?*), Brea, Eva, Hana, Ignis, Ines(?), IS Karpatia, Klea, Malyska, Markola, Pavlina, Solara, Šarlota, Velta, Viginta, Zerda. By rust reactions the presence of *Lr26* was postulated in cvs. Bonita, Genoveva, Lívia, Malvina, Rapsodia, Sana and Verita. Reactions of cultivars Genoveva, Verita, Rapsodia and Bonita indicate that they possess other additional gene(s) for leaf rust resistance in addition to *Lr26*. The presence of *Lr26* in cv. Rapsodia was confirmed by a molecular marker (HANZALOVÁ *et al.* 2009). Cv. Bona Dea was resistant to all applied leaf rust isolates similarly like NIL possessing *Lr19*. The postulation of *Lr 19* in cv. Bona Dea is supported by the yellow colour of flour caused by a gene closely linked with *Lr19*. It was also confirmed by the author of the cultivar (Zalabai, personal communication). He also defined cv. Bona Dea as highly resistant.

DISCUSSION

In comparison with previous results (HANZALOVÁ *et al.* 2008) the virulence frequency to *Lr1* increased. Virulence frequencies to other *Lr* genes remained similar to those in 2001–2004. Like in previous years no virulence to *Lr19* was identified and only a very low frequency of virulence to *Lr9* was found. Virulence to *Lr24* recorded in 2001 (HANZALOVÁ *et al.* 2008) was found again in 2005 and 2008 whereas virulence to *Lr28* was registered in each year of the period 2005–2008 when the pathotype survey was carried out.

Because of the linkage of *Lr19* with a gene conditioning the yellow colour of flour that gene was not widely used in breeding in Europe. Swedish spring wheat cv. Sunnan, several cultivars from the former USSR, e.g. Saratovskaya 29, Samara, Volgogradskaya (MARTYNOV & DOBROTVORSKAYA 2006) and of the Slovak cultivars tested by us cv. Bona Dea possess *Lr19*. Virulence to that gene was found in Germany already in 1999 (GULTYAEVA *et al.* 2000). However, it was not recorded in earlier ring tests comprising France, Czech Republic, Germany, Italy, Spain, Hungary,

Poland, Bulgaria, Rumania and Slovakia summarized by MESTERHÁZY *et al.* (2000). The breakdown of *Lr19* effectiveness occurred in Volga, Ural region and Central Black Earth (GULTYAEVA 2007). Genes *Lr9* and *Lr24* still condition stable resistance in Russia. The virulence frequency to *Lr1* was decreasing in Russia in 2001–2006 in the Northwest and increasing in Central, Central Black Earth, Middle and Low Volga and Ural regions (GULTYAEVA 2007). In Germany an increase in virulence frequencies was detected in 2001–2003 for *Lr1* and *Lr2a*, a decrease for *Lr3*. In Russia virulence for *Lr3* was common (LIND & GULTYAEVA 2007). No virulence to *Lr9*, *Lr19*, *Lr24* was found in Latvia (LIATUKAS 2003). Similarly like in Slovakia, virulence frequency to *Lr1* also increased in Hungary and virulence frequency to *Lr2a* was also low (MANNINGER 2006). Unlike in Hungary, no decrease in the frequency of virulence to *Lr15* was observed in the Slovak Republic and the most striking difference was a high frequency of virulence to *Lr23* in the Slovak Republic and very low in Hungary. In the Czech Republic in 2005–2008 the most effective genes were the same as in Slovakia, *Lr9*, *Lr19*, *Lr24*, *Lr28* and *Lr2a*. A relatively low frequency of virulence to *Lr2b* was also recorded. Unlike in Slovakia, in the Czech Republic no virulence was found to *Lr9* and very low virulence to *Lr19* in recent years (HANZALOVÁ 2010). In the USA leaf rust resistance genes *Lr9* and *Lr24* are effective no more. All the three most widespread pathotypes in the race survey published in 2010 (KOLMER *et al.* 2010) possessed virulence to *Lr24*, and one of them also to *Lr9*. In Canada gene *Lr9* was highly effective in previous years, however virulence to this gene rose dramatically from 2.8% in 2006 to 8.7% in 2007 (MCCALLUM *et al.* 2010)

According to the Description of Registered Bread Wheat Varieties by the Central Controlling and Testing Institute in Agriculture (<http://www.uksup.sk/>) the resistance of cultivars Axis, Bonita, Magvas, Evelina, Markola, Ilias, Rapsodia, Stanislava, Bazilika, Bosorka, Etela, Eurofit, Rheia, Šarlota and MV Palota was described as higher than the resistance of check cultivars. Nine of these cultivars possess specific leaf rust resistance genes. In other six cultivars no specific resistance genes were revealed. Field (partial) resistance is obviously responsible for the low disease severity of the latter group of cultivars. The resistance of cvs. Corsaire and Evelina was described as substantially better

than the resistance of check cultivars, resistance of cv. Clever as good up to very good, resistance of cvs. Caphorn and Barroko as good. Three of these cultivars possess *Lr37* and other gene(s), cv. Barroko seems to have field (partial) resistance. Medium leaf rust resistance was reported in cvs. IS Karpatia, Verita, Genoveva. Two of these cultivars possess *Lr26* and other gene(s), cv. IS Karpatia *Lr3a*. Lower resistance than in check cultivars was described in cvs. Sulamit and Ebi. In these cultivars *Lr* genes were not recorded. The combination of genes *Lr1*, *Lr3a* and *Lr13* in cv. Vlada was reported by PATHAN and PARK (2006) as well as data on *Lr* genes in cvs. Blava, Bruta, Estica and Viginta. Data on the presence of *Lr* genes in cultivar Alka were published by BLAŽKOVÁ *et al.* (2002), in cultivars Caphorn and Rapsodia by HANZALOVÁ *et al.* (2009), in cultivars Clever, Corsaire and Rheia by BARTOŠ *et al.* (2004).

Seedling tests for resistance to selected rust isolates can help to postulate resistance genes in commercial cultivars. However, the number of applied rust isolates in our trials was too low to guarantee that the isolates possess all genes for virulence/avirulence related to the resistance genes in the tested cultivars. For these reasons cultivars showing resistance in the greenhouse tests can possibly suffer from rust in the field, and vice versa, cultivars susceptible in the greenhouse may be resistant in the field. Genes governing resistance only in the field cannot be detected in the greenhouse tests. Results obtained by different sets of rust races may differ. E.g. the genes designated as *Lru* postulated in previous trials (HANZALOVÁ *et al.* 2008) in cvs. Bety and Torysa were not revealed with rust races applied in the present test.

Of *Lr* genes described in wheat cultivars registered in Slovakia, only adult plant resistance gene *Lr37* has remained generally effective in the field until now. Resistance expressed only in the field is common in European cultivars. WINZELER *et al.* (2000) recorded this type of resistance in 50 out of 72 tested cultivars and breeding lines. Breeding for field resistance (partial resistance, adult plant resistance) will belong to the most promising strategies of disease resistance breeding also in the future.

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