SHORT COMMUNICATION

Determination of Leaf Rust Resistance Genes *Lr10*, *Lr26* and *Lr37* by Molecular Markers in Wheat Cultivars Registered in the Czech Republic

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Abstract: Twenty-seven winter wheat cultivars registered in the Czech Republic were tested by molecular markers for the presence of *Lr26* and *Lr37*, and twenty-eight cultivars for the presence of *Lr10*. Gene *Lr37* was determined in eleven cultivars, gene *Lr10* in ten cultivars and gene *Lr26* in four cultivars. Eight cultivars had combinations of two *Lr* genes, one cultivar possessed all the three *Lr* genes. The results of marker analyses were compared with multipathotype analysis which confirmed the presence of *Lr26* but did not enable the verification of the presence of *Lr10* and *Lr37*. Seedling resistance was compared with resistance of the studied cultivars in the field.

Keywords: wheat leaf rust; *Puccinia triticina*; rust resistance; genes *Lr10*, *Lr26* and *Lr37*; molecular markers; registered wheat cultivars; Czech Republic

The knowledge of genes for resistance in grown wheat cultivars is important for wheat growers, specialists in the plant disease control as well as for wheat breeders. Wheat growers can decrease the risk of yield losses by growing cultivars differing in genes for rust resistance. The necessity of rust control by fungicides can be assessed according to resistance genes in the grown cultivars and virulence in the rust population. Wheat breeders need to know resistance genes because their suitable combinations in gene pyramiding can enhance the durability of resistance.

There are several methods for identification of resistance genes. The classical method is based on resistance analysis of the F_2 generation of the crosses between the tested cultivars and lines with known resistance genes. A faster method is based on comparison of reactions of the tested cultivar to a set of different pathotypes with reactions of lines possessing determined resistance genes (multipathotype test). The development of molecular biology has enabled the identification of resistance genes by molecular markers.

This paper presents results of the determination of leaf rust resistance genes *Lr10*, *Lr26* and *Lr37* by molecular markers in winter wheat cultivars registered in the Czech Republic.

MATERIAL AND METHODS

The majority of winter wheat cultivars registered in the Czech Republic between 2003 and 2008, cv. Estica registered in 1995 were analysed by molecu-

lar markers. The same cultivars were tested in a greenhouse at the seedling stage for reaction to seven leaf rust pathotypes with determined virulence. Reactions of the tested cultivars were compared with reactions of near isogenic lines possessing relevant *Lr* genes in Thatcher background. Inoculation was carried out by rubbing the first leaf with water suspension of urediospores. Inoculated plants were kept in a greenhouse in closed glass cylinders for 24 hours at 18°C–22°C. After incubation plants grew for 14 days at the same temperatures in a greenhouse and then infection types were recorded according to Stakman *et al.* (1962).

DNA was extracted from the second wheat leaf 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 the ladder Lambda DNA/HindIII (Fermentas, Vilnius, Lithuania). The genes *Lr10*, *Lr26* and *Lr37* were identified with the use of PCR with published primers marking these genes. The PCR conditions and primers are shown in Table 1. Near isogenic *T. aestivum* lines containing the corresponding *Lr* genes in Thatcher background were used as a positive control. The thermal cyclers Veriti (Applied Biosystems, San Francisco, USA) and UNO II Biometra (Schöeller Instruments, Prague, Czech Republic) were 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.

The primer combination Lrk10D1/Lrk10D2 was developed to detect the gene *Lrk10* which is completely linked with *Lr10* gene (Feuillet *et al*. 1997) and located at the distal region of the short arm of chromosome 1A with a distance 8 cm to the microsatellite (*Glu-3*)-*1A*. It is called sequence-tagged site marker (STS) which is specific to the *Lr10* leaf rust resistance gene *STSLrK10-6* (Schachermayr *et al.* 1997).

The pair of primers Ventriup/LN2 was designed to amplify the N-allele of the *Xcmwg682* of the chromosome 2NS translocated to the short arm of wheat chromosome 2AS.

The primer pair SecA2/SecA3 was designed to amplify a sequence ω-secalin gene (locus *SEC-1b*) located on the short arm of the rye chromosome 1R.

RESULTS

Molecular marker analyses

The results of analyses of wheat cultivars by molecular markers for the genes *Lr37, Lr26* and *Lr10* are summarized in Table 2 and Figures 1–3. Out of the 27 tested cultivars DNA from 11 cultivars gave a positive reaction with the marker for *Lr37* and DNA from 4 cultivars with the marker for *Lr26*. Twenty-eight cultivars were tested for *Lr10*; the additonal cultivar was Boka. DNA from 10 of them showed a positive reaction with the marker. Marker analyses showed that most cultivars had combinations of the tested genes.

Greenhouse tests

Reactions of the near isogenic lines possessing *Lr10, Lr26* and *Lr37* to seven leaf rust pathotypes

Table 1. PCR conditions and primers

Gene	Chromosome location	Nucleotide sequence	Amplification conditions	PCR product
Lr10	1 AS	Lrk10D1: GAA GCC CTT CGT CTC ATC TG Lrk10D2: TTG ATT CAT TGC AGA TGA GAT CAC G	94°C for 3 min; 35 cycles of 94°C for 30 s, 58°C for 30s, 72°C for 45 s; 72°C for 10 min	286 bp*
Lr26	1 BS	SecA2: GTT TGC TGG GGA ATT ATT TG SecA3: TCC TCA TCT TTG TCC TCG CC	94°C for 5 min; 30 cycles of 94°C for 30 s, 60°C for 30 s, 72°for 60 s; 72°C for 5 min	412 bp**
Lr37	2 AS	Ventriup: AGG GGC TAC TGA CCA AGG CT LN2: TGC AGC TAC AGC AGT ATG TAC ACA AAA	94°C for 5 min; 30 cycles of 94°C for 30 s, 58°C for 30 s, 72°C for 60 s; 72°C for 5 min	262 bp***

*by Schachermayr *et al.* (1997), **by De Froidmont (1998), ***by Helguera *et al.* (2003)

Table 2. Reaction of the cultivars in the greenhouse and in the field

Cultivar	*Lr* genes*	Registered	Greenhouse classification*** – isolate							Field classification**
			ASU	4332	333	347/6	4332/3	4003/4	628	
Anduril		2006	3	3	3	3	3	3	2+	7.5
Bakfis	*Lr10, Lr37*	2008	3	2+	1–2	;1	;1	2+	;1	5
Baletka	*Lr10*	2008	3-	1–2	2+	2	;1	3–	;1–2	8
Barryton	*Lr10, Lr37*	2007	;	3	3	3	;	3–	1–2	5
Biscay	*Lr10, Lr37*	2005	;1	2	;1–2	2+	1–2	;1	;1	8
Bohemia		2007	;1	3	3	3	3	3	3	6
Buteo		2006	1–2	3	3	3	3	3	3	7
Caphorn	*Lr10, Lr37*	2004	;1	;	;	3-	;1	;1–2+	;1	8
Clarus	*Lr26, Lr37*	2003	;	;1	;	;	3	3	3	8
Dromos		2006	1–2	3	3	3	3	3	3	6
Estica		1995	3	3	3	3	3	3	3	-
Etela	*Lr10, Lr26*	2006	;	;	;	;	3	3	3	6
Eurofit		2006	3	3	3	3	3	3	3	7
Florett		2006	3	3	3	3	;	3	;1	5
Nikol	*Lr37*	2008	3	3	3	3	3	3	3	–
Helmut		2008	1–2	3	3	3	1–2	3	1–2	7
Kerubino		2007	3	3	3	3	3	3	3	5
Kodex	*Lr37*	2008	;(3)	3	3	2–3	3–	3–	2–3	6
Megas	*Lr10*	2008	3	3	3	3–	;	3	;	6
Mulan	*Lr10, Lr37*	2007	;1–2	;1–2	;1–2	;1–2	;	;	;	8
Orlando	*Lr26, Lr37*	2008	;	;	;	;	;1–2	;	;	9
Pitbull	*Lr10*	2008	3	;1–2	;1–2+	2-3	;	3–	;1–2+	6
Raduza		2006	1–2	3	3	3	3	3	3	6
Rapsodia	*Lr10, Lr26, Lr37*	2003	;	;	;	;	;	1–2	;	8
Sakura		2007	;1	3	3	3	3	3	;1	6
Simila		2006	3	3	3	3	3	3	3	7
Sultan	*Lr37*	2008	2–3	3	3	3	3	3	2–3	7
NIL *Lr26*			;	;	;	;	3	3	3	
NIL *Lr37*			3	3	3	3	3	3	3	
NIL *Lr10*			3	3-	3-	3	3-	3	3	

*according to Figures 1, 2, 3; **scale 1–9, 1 – susceptible, 9 – resistant; *** – chlorosis; 1, 2 – resistant; 3, 4 – susceptible

revealed that only reactions of the line with *Lr26* can be useful for postulation of the genes analyzed by the markers (Table 2). Cvs. Etela and Clarus had identical reactions with the *Lr26* near isogenic line. Other cultivars that also have *Lr26*, Orlando and Rapsodia, were resistant to all tested pathotypes. Obviously, they possess other resistance gene(s) in addition to *Lr26.* None of the pathotypes used was avirulent to *Lr37* or *Lr10* near isogenic lines. Gene *Lr37* is only rarely effective at the seedling stage. Pedigrees of the tested cultivars (Table 3) supported some results of the marker analyses. The overwhelming majority of the tested cultivars were resistant to one rust pathotype at least, which indicates that they have other genes for specific resistance alone or in addition to the genes determined by us.

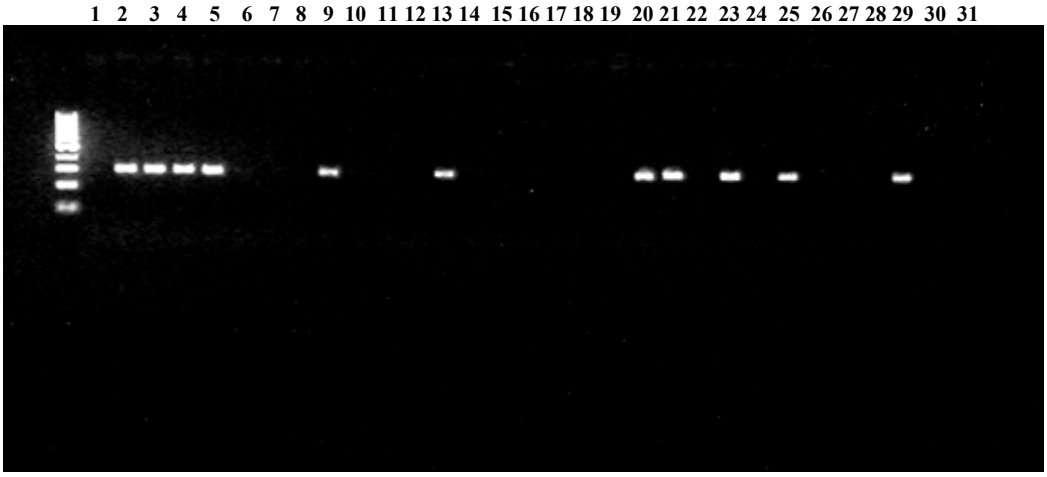

Figure 1. Detection of the *Lr10* resistance gene in 28 wheat cultivars

GeneRuler 100bp DNA ladder (Fermentas), 1 – Anduril, 2 – Baletka, 3 – Bakfis, 4 – Barryton, 5 – Biscay, 6 – Bohemia, 7 – Boka, 8 – Buteo, 9 – Caphorn, 10 – Clarus. 11 – Dromos, 12 – Estica, 13 – Etela, 14 – Eurofit, 15 – Florett, 16 – Nikol, 17 – Helmut, 18 – Kerubino, 19 – Kodex, 20 – Megas, 21 – Mulan, 22 – Orlando, 23 – Pitbull, 24 – Raduza, 25 – Rapsodia, 26 – Sakura, 27 – Simila, 28 – Sultan, 29 – *Lr10*, 30- *Lr34*, 31- *Lr37*

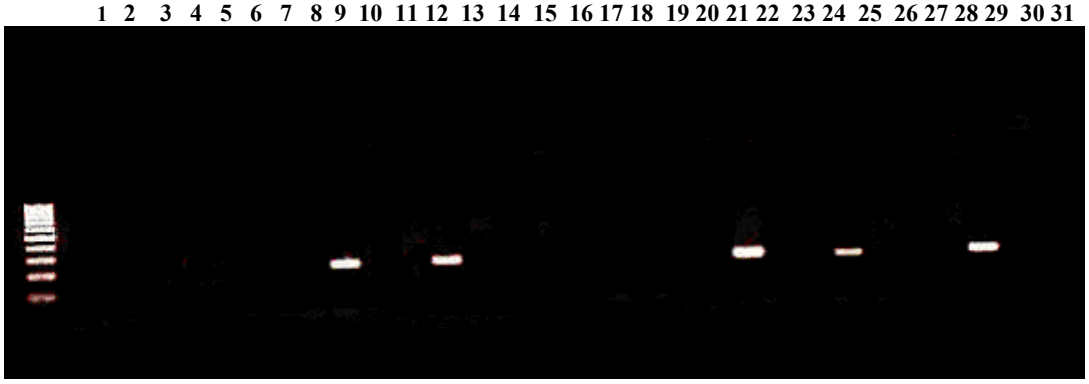

Figure 2. Detection of the *Lr26* resistance gene in 27 wheat cultivars

GeneRuler 100bp DNA ladder (Fermentas), 1 – Anduril, 2 – Bakfis, 3 – Baletka, 4 – Barryton, 5 – Biscay, 6 – Bohemia, 7 – Buteo, 8 – Caphorn, 9 – Clarus, 10 – Dromos, 11 – Estica, 12 – Etela, 13 – Eurofit, 14 – Florett, 15 – Nikol, 16 – Helmut, 17 – Kerubino, 18 – Kodex, 19 – Megas, 20 – Mulan, 21 – Orlando, 22 – Pitbull, 23 – Raduza, 24 – Rapsodia, 25 – Sakura, 26 – Simila, 27 – Sultan, 28 – *Lr26*, 29 – *Lr37*, 30 – water

Figure 3. Detection of the *Lr37* resistance gene in 27 wheat cultivars

GeneRuler 100bp DNA ladder (Fermentas), 1 – Anduril, 2 – Bakfis, 3 – Baletka, 4 – Barryton, 5 – Biscay, 6 – Bohemia, 7 – Buteo, 8 – Caphorn, 9 – Clarus, 10 – Dromos, 11 – Estica, 12 – Etela, 13 – Eurofit, 14 – Florett, 15 – Nikol, 16 – Helmut, 17 – Kerubino, 18 – Kodex, 19 – Megas, 20 – Mulan, 21 – Orlando, 22 – Pitbull, 23 – Raduza, 24 – Rapsodia, 25 – Sakura, 26 – Simila, 27 – Sultan, 28 – *Lr26*, 29 – *Lr37*, 30 – water

Table 3. Pedigree of the tested wheat cultivars

Cultivar	Pedigree
Anduril	Residence × Eiffel
Bakfis	Pegassos × Vlasta
Baletka	Alka × Astella
Barryton	Reaper × Asketis
Biscay	CPB 79 × Hussar
Bohemia	(540i-92 × 6192a-92) × (540i-92 × Kontrast)
Buteo	(LP 4285.90 × LP 3273.87) × Pegassos
Caphorn	(S14579/454 × Rialto) × Beaufort
Clarus	[(Norman × D 84/412) × Haven] × Naseman
Dromos	(ZE 8710 × Batis) × Kimon
Estica	Arminda × Virtue
Etela	HE 3691 × Apollo
Eurofit	Pegassos × Kontrast
Florett	PBIS 95-82 × G 31
Nikol	HE 6118 × Apache
Helmut	Complet × (SL 63/87-9 × Darwin)
Kerubino	WW 1972 × IG 31
Kodex	(SB 8512 × 2.192) × LP 780.3.92
Megas	(Agent × Pico) × Caesar
Mulan	(Ronos × Estica) × Maverick
Orlando	Savannah × Sokrates
Pitbull	Florida × Estica
Raduza	RU 23 × Alveor
Rapsodia	[(Hornet × Haven) × Haven] × Haven
Sakura	Hana × Estica
Simila	Samanta × Estica
Sultan	Ebi × CWW95/26

Field tests

Table 2 presents also data from field tests. Seven cultivars out of eleven cultivars possessing *Lr37* alone or in combination with other genes were classified by the values 7–9 (i.e. resistant) in the field and three cultivars by 5–6; one cultivar with *Lr37* was not classified in the field. Five cultivars lacking *Lr37* were classified by the note 7 and one cultivar possessing only *Lr10* by the note 8.

DISCUSSION

Many molecular markers have been described for *Lr* genes and often more markers based on different procedures have been developed for the same gene (http://maswheat.ucdavis.edu, Landjeva *et al*. 2007). In the Czech Republic Ovesná (RICP, Prague) participated in the ring test for validation of several STS markers for *Lr* genes. Markers for *Lr10* (Blažková *et al*. 2002) and *Lr37* (Ambrozková *et al*. 2002; Bartoš *et al*. 2004) were applied to test for the presence of these genes in winter wheat cultivars registered in the Czech Republic. In these tests gene *Lr10* was determined in cvs. Siria and Alka, gene *Lr37* in cvs. Apache, Bill, Clarus, Caphorn, Clever, Corsaire, Rapsodia and Rheia. The results summarized in the present paper verified its presence in cvs. Clarus and Rapsodia. Gene *Lr37* in cvs. Apache, Bill, and Caphorn was determined by two molecular markers also by Blaszczyk *et al*. (2004) and in cv. Bill by Stepień *et al.* (2003).

Resistance gene *Lr37* originates from *Aegilops ventricosa* and is located in a translocation on wheat chromosome 2AS. It is closely linked with *Yr17* and *Sr38* in coupling and with *Lr17* in repulsion (McIntosh *et al*. 1995). Gene *Lr37* is possessed by many West European wheat cultivars. Whereas yellow rust resistance conditioned by *Yr17* broke down soon after the cultivars possessing this translocation became widespread, stem rust resistance seems to be effective in Central Europe, though virulence has already been recorded. In Germany (Lind 2008, personal communication) virulence to *Lr37* in adult plants has been found and caused a loss of resistance of the cv. Tommi. In our analysis the presence of *Lr37* was determined in cvs. Bakfis, Barryton, Biscay, Caphorn, Nikol (HE 77/97), Kodex, Mulan, Orlando and Sultan in addition to cvs. Clarus and Rapsodia mentioned earlier.

Resistance gene *Lr26* originates from rye and appears in wheat in a 1BL.1RS translocation or substitution 1R(1B). It is completely linked with *Yr9, Sr31* and *Pm8*. Translocation 1BL.1RS is widespread in wheat cultivars throughout the world. In addition to resistance, translocation 1BL.1RS has a positive effect on yield, but a negative effect on quality. In the Czech Republic resistance genes on this translocation are no longer effective to the prevailing pathotypes of powdery mildew, leaf rust and yellow rust, only stem rust resistance remains effective. Only four cultivars possessed *Lr26*, namely Etela, Orlando, Clarus and Rapsodia. The last two cultivars have both translocations, from rye as well as from *Aegilops ventricosa*. Besides the above-mentioned cultivars the 1BL.1RS

translocation was determined earlier in the registered cultivars Lívia, Mona, Rialto, and Solara. Bradová and Šašek (2007) separated two lines in the cv. Karolinum by gliadin analysis. The line with Gld1B3 indicating the presence of the 1BL.1RS translocation, i.e. gene *Lr26*, prevailed. In the cv. Windsor three lines have been determined, one of them with 1BL.1RS translocation.

Resistance gene *Lr10* was derived from bread wheat and was originally reported in cvs. Lee and Timstein. It is located on chromosome 1A and distributed in many wheat cultivars. Gene *Lr10* is not widely effective on its own but may play a positive role in gene combinations (McIntosh *et al*. 1995). In addition to the above listed cultivars possessing *Lr10* in combination with *Lr37* (Bakfis, Barryton, Biscay, Caphorn Mulan and Rapsodia) or *Lr26* (Clarus, Etela and Rapsodia) gene *Lr10* was determined by the molecular marker in cvs. Baletka, Barryton, Megas and Pitbull. Cv. Rapsodia possesses *Lr10* in combination with both *Lr37* and *Lr26*. Previously analysed cultivars Alka and Siria (Blažková *et al*. 2002) possess *Lr10* in combination with *Lr13*.

It is difficult to ascribe resistance observed in the field to individual resistance genes tested in our analyses. Obviously, also other resistance genes play an important role in the field resistance of the tested cultivars than merely genes analysed by us.

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