SHORT COMMUNICATION

Multiplex PCR Assay to Detect Rust Resistance Genes *Lr26* and *Lr37* in Wheat

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Abstract: Multiplex PCR was developed and optimized for simultaneous detection of wheat leaf rust resistance genes Lr26 and Lr37. The presence of the genes was analyzed in 21 winter wheat cultivars registered in the Czech Republic. Gene Lr37 was detected in four tested cultivars (Bakfis, Biscay, Nicol, Mulan), gene Lr26 occurred only in one cultivar (Etela) and three cultivars (Clarus, Orlando and Rapsodia) were found to carry both these genes. Data obtained by PCR markers were compared with results of greenhouse and field tests. Seedling reactions of cultivars possessing Lr26 to seven different leaf rust isolates conformed to the results obtained by the marker analysis, however, there were found some discrepancies in the detections of Lr37, which could be detected in greenhouse seedling tests only with difficulties.

Keywords: leaf rust; *Lr26*; *Lr37*; multiplex PCR; wheat

Leaf rust (*Puccinia triticina* Eriks.) is one of the most prevalent diseases of wheat in the Czech Republic, causing yield losses and decreasing the grain quality. The development of genetic resistance to biotic stress is the most efficient, cost-effective and environment-friendly approach to prevent the losses caused by rust epidemics (Todorovska *et al*. 2009). At the present time more than 60 resistance genes against leaf rust are known (*Lr* genes). Some of these genes are a part of the wheat genome, some of them were introduced from the related species into it (McIntosh *et al*. 2009). A well-known fact is that resistance against leaf rust, based only on one resistance gene, can be easily overcome by new races of the pathogen. Combination of more resistance genes (gene pyramiding) can enhance the durability of resistance. To achieve a suitable combination of the genes their reliable identification is necessary. Individual resistance genes can be identified by various methods. The most conventional method is an analysis of the F_2 generation after a crossing of tested cultivars and lines with known resistance genes. Faster methods are greenhouse tests, where the reactions of the tested cultivars are compared with reactions of lines with determined genes. The latest method of verification and detection of resistance genes is the use of molecular markers that are known for most of the identified *Lr* genes (Landjeva *et al*. 2007). Molecular markers, especially PCR based markers, can be successfully used in the process of marker-assisted selection.

The aim of this study was to develop and optimize a multiplex PCR for simultaneous detection of leaf rust resistance genes *Lr26* and *Lr37* and compare the results with a greenhouse pathotype analysis.

For the presence of the genes *Lr26* and *Lr37* the following winter wheat cultivars (*Triticum*

aestivum L.) registered in the Czech Republic were analyzed: Bakfis, Baletka, Barryton, Biscay, Buteo, Clarus, Etela, Estica, Eurofit, Florett, Nicol, Helmut, Kodex, Megas, Mulan, Orlando, Pitbull, Raduza, Rapsodia, Simila, Sakura (Table 1).

The DNA for PCR analyses was extracted from leaf tissue using a DNA extraction kit (Qiagen). The quality and concentration of extracted DNA were checked by electrophoresis in 0.8% agarose gel and compared with the Lambda DNA/HindIII marker (Fermentas).

A Multiplex PCR assay, allowing a detection of more genes in one reaction, was developed and optimized to verify the presence of the genes *Lr26* and *Lr37*. Several published primers for the genes *Lr26* and *Lr37* were evaluated to choose the best mutual combination. The primers finally used were SecA2/SecA3 and Venturip/LN2 according to sequences marking the genes *Lr26* and *Lr37* published by De Froidmont (1998) and Helguera *et al*. (2003). Various annealing temperatures and concentrations of the primers and $MgCl_2$ were tested to achieve the best yield of the PCR reaction. The final PCR conditions, primers and mix composition are summarized in Table 2. The reaction was carried out in a Veriti thermal cycler (Applied Biosystems). The amplification products were separated by electrophoresis in 2% agarose gel, stained with ethidium bromide, and observed under UV light. Near isogenic *T. aestivum* lines possessing the genes *Lr26* and *Lr37* in a Thatcher background were used as a positive control.

Table 1. Presence of the examined rust resistance genes and data on field resistance

Cultivar	Origin	Registered in the Czech Republic	Presence of *Lr26* and/or *Lr37**	Field resistance**
Bakfis	BE	2008	*Lr37*	5
Baletka	BE	2008		8
Barryton	FR	2007	(*Lr37*)	5
Biscay	DEU	2005	*Lr37*	8
Buteo	DEU	2006		7
Clarus	FR	2003	*Lr26*, *Lr37*	8
Estica	NLD	1995		–
Etela	CZE	2006	*Lr26*	6
Eurofit	AU	2006		7
Florett	FR	2006		5
Helmut	DEU	2008		7
Kodex	DEU	2008	(*Lr37*)	6
Megas	DEU	2008		6
Mulan	DEU	2007	*Lr37*	8
Orlando	DK	2008	*Lr26*, *Lr37*	9
Nikol	CZE	2009	*Lr37*	–
Pitbull	DEU	2008		6
Raduza	CZE	2006		6
Rapsodia	FR	2003	*Lr26*, *Lr37*	8
Sakura	CZE	2007		6
Simila	CZE	2006		7

*According to Hanzalová *et al.* (2009); in brackets: data not confirmed by our results; **according to data from the Central Institute for Supervising and Testing in Agriculture of the Czech Republic; scale 1–9, 1 – susceptible, 9 – resistant

Table 2. Reaction conditions and mix composition of multiplex PCR

Reaction mix (total volume 25µl)	Amplification conditions	PCR product (bp)	Primers/references
2.5µl 10× PCR buffer Mg free (Biotools)	94°C – 3 min	412 (*Lr26*)	SecA2, SecA3
1.5µl 50mM MgCl2 (Biotools)	94°C – 30s		(De Froidmont 1998)
0.5µl 2.5mM dNTPs (Promega)	58°C – 30s		
(SecA2, SecA3, Venturip, LN2)	35 ×	262 (*Lr37*)	Venturip, LN2 (Helguera *et al.* 2003)
0.1µl Taq DNA polymerase (Biotools)	72°C – 45s		
1µl DNA (20ng/µl)	72°C – 10 min		

Methodology and results of greenhouse tests were described in detail by Hanzalová *et al*. (2009).

To compare seedling reactions of the winter wheat cultivars to seven leaf rust isolates with the field leaf rust reactions, data of the Central Institute for Supervising and Testing in Agriculture from the years 2006–2009 and our records were used. The disease severity was evaluated in a 1–9 point assessment scale, where a rating of 9 was without symptoms (resistance) and a rating of 1 means maximum attack (susceptibility).

Molecular markers, especially those based on PCR assay, have been successfully used many times to detect various *Lr* genes in marker-assisted selection (Urbanovic *et al.* 2006; Gajnullin *et al.* 2007; Vida *et al.* 2009). The use of a multiplex PCR assay allows the detection of more than one gene in a single reaction. In the present study we optimized the multiplex PCR assay to detect resistance genes *Lr26* and *Lr37*. The results of the analysis are shown in Figure 1. Cultivars Bakfis, Biscay, Nicol and Mulan gave positive reactions to the gene *Lr37.* The single gene *Lr26* was found only in the cultivar Etela. DNA from cultivars Clarus, Orlando and Rapsodia amplified two specific products showing the presence of both tested genes.

Genes *Lr26* and *Lr37* do not originate from the wheat genome. Resistance gene *Lr37* was introduced from *Aegilops ventricosa* together with resistance genes to stripe rust (*Yr17*), stem rust (*Sr38*) and *Heterodera avenae* resistance gene (*Cre5*). It is located on the chromosome 2A, closely linked with *Yr17* and *Sr38* in coupling and with *Lr17* in repulsion (McIntosh *et al*.1995). Although rust races with virulence to these genes have been recorded (Bayles *et al*. 2000; Robert *et al.* 2000), this gene cluster is still useful in combination with other rust resistance genes and therefore various molecular markers have been developed to identify these genes. Robert *et al.* (1999) described a SCAR marker, closely but not completely linked (0.8 +/– 0.7 cM) to *Yr17* and tentatively to *Lr37* and *Sr38*. A PCR marker from the sequence of a nucleotide-binding-site of leucine-rich repeat disease resistance gene located on the 2NS chromosome segment from *Ae. ventricosa*, completely

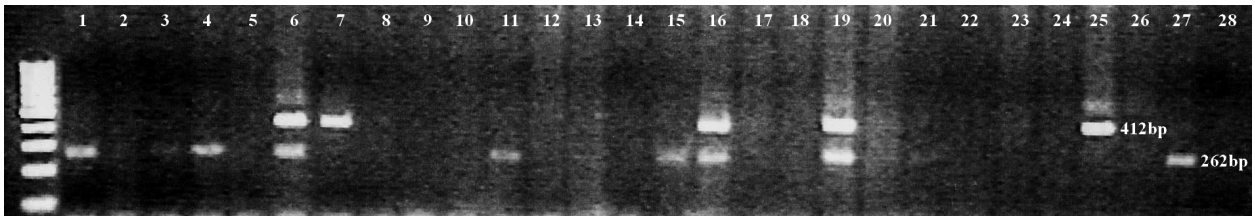

Figure 1. GeneRuler 100bp DNA ladder (Fermentas), 1 – Bakfiz, 2 – Baletka, 3 – Baryton, 4 – Biscay, 5 – Buteo, 6 – Clarus, 7 – Etela, 8 – Estica, 9 – Eurofit, 10 – Florett, 11 – Nicol, 12 – Helmut, 13 – Kodex, 14 – Megas, 15 – Mulan, 16 – Orlando, 17 – Pitbull, 18 – Raduza, 19 – Rapsodia, 20 – Simila, 21 – Sakura, 22 – *Lr1*, 23 – *Lr10*, 24 – *Lr13*, 25 – *Lr26*, 26 – *Lr34*, 27 – *Lr37*, 28 – water

linked to the rust resistance genes *Yr17*, *Lr37* and *Sr38* in 14 isogenic lines, has been reported by Seah *et al.* (2001). Helguera *et al.* (2003) used a PCR assay to amplify the N-allele of *Xcmwg682* of the translocation 2NS/2AS.

The resistance gene *Lr26* originates from rye and is located on a 1BL.1RS translocation in wheat. The translocation has been widely used in wheat breeding programs throughout the world since it carries also resistance genes to stem rust (*Sr31*), stripe rust (*Yr9*), powdery mildew (*Pm8*) and greenbug. Several markers to detect the wheat/rye translocation are known (De Froidmont 1998; Mago *et al*. 2002).

The gene *Lr37* was first identified in the French cultivar VPM1 (Bariana & McIntosh 1993) and is currently found in many West European wheat cultivars. Many of these cultivars have been registered in the Czech Republic and the presence of the gene *Lr37* was verified by field tests, greenhouse tests and by means of molecular markers (Ambrozková *et al.* 2002; Bartoš *et al*. 2004; Hanzalová *et al*. 2009). The gene *Lr37* provides resistance at the adult plant stage and for that reason it is rarely effective at the seedling stage. Consequently the gene *Lr37* can be detected in greenhouse seedling tests only with difficulties. Therefore marker-assisted selection employed in this case may be very useful, although the markers need not be effective in all cases (Robert *et al*. 2000; Helguera *et al*. 2003). The presence of *Lr37* in the cultivars Barryton and Kodex (Hanzalová *et al*. 2009) was not confirmed by our analyses. High levels of field resistance have been recorded in all tested cultivars possessing this gene (Table 1), except the cultivar Bakfis, where the level of field resistance has been classified at a rating of 5 (medium resistance). The reasons for this reaction remain unknown.

The gene *Lr26* occurs in many successful wheat cultivars containing the 1BL.1RS translocation, including the Veery lines developed at CIMMYT (Rajaram *et al*. 1983). In the Czech Republic resistance genes on this translocation are no longer effective against the prevailing pathotypes of powdery mildew, leaf rust and yellow rust and only the resistance against stem rust remains effective. Despite this fact the 1BL.1RS translocation is used in wheat breeding to enhance the yield of cultivars and in combination with other resistance genes (such as *Lr37*), the gene *Lr26* may provide a sufficient level of field resistance (Moreno-Sevilla & Baenziger 1995). Reactions of the line with *Lr26* in our greenhouse tests were found to be useful for the postulation of the gene *Lr26* analyzed also by molecular markers. The results of both analyses agreed in all cases (data not shown). The marker used in the PCR assay was found to correlate well with earlier analyses (Hanzalová *et al.* 2009).

Multiplex PCR can represent an efficient tool for testing wheat cultivars for individual resistance genes. However, in general it is desirable to combine the molecular analysis with traditional methods to achieve reliable results.

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