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Leaf rust resistance genes of wheat: identification in cultivars and resistance sources

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Abstract. Thirty-seven wheat cultivars originating from seven European countries were examined by using sequence tagged site (STS) markers for seven *Lr* (leaf rust = brown rust) resistance genes against the fungal pathogen of wheat *Puccinia recondita* f. sp. *tritici* (*Lr9, Lr10, Lr19, Lr24, Lr26* and *Lr37*). Additionally, 22 accessions with various *Lr* genes from two germplasm collections were tested. A SCAR (sequence-characterized amplified region) marker for *Lr24* and a CAPS (Cleaved Amplified Polymorphic Sequence) marker for *Lr47* were also used to identify those genes in the wheat accessions. Each marker amplified one specific DNA fragment. Three *Lr* gene markers were identified in wheat cultivars (*Lr10*, *Lr26* and *Lr37*). Another four markers (*Lr9*, *Lr19*, *Lr24* and *Lr47*) were found in breeding lines carrying leaf rust resistance genes. The results were compared with leaf rust resistance gene postulations made in previous studies, based on multipathotype testing. Markers for *Lr10*, *Lr26* and *Lr37* may be useful in marker-assisted breeding.

Key words: leaf rust, resistance genes, STS markers, wheat.

Introduction

Leaf rust of wheat (brown rust), caused by *Puccinia recondita* f. sp. *tritici*, was observed at high incidence in three consecutive years 2000-2002 in southern regions of Poland, where it is frequently recorded. In 2001 and 2002, epidemics occurred in the central part of Poland both in wheat and in some cultivars of triticale (personal observations).

Many genes of resistance to leaf rust originate from *Triticum aestivum,* but some single leaf rust resistance genes have been introgressed into common wheat

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from wild species. These include *Lr19*, *Lr24* and *Lr29* from *Agropyron elongatum*, *Lr28*, *Lr35*, *Lr36* and *Lr47* from *Aegilops speltoides*, *Lr21*, *Lr22*, *Lr32*, *Lr39*, *Lr40*, *Lr42* and *Lr43* from *Aegilops tauschii*, *Lr9* from *Aegilops umbellulata*, and *Lr37* from *Aegilops ventricosa* (FRIEBE et al. 1996, CHEŁKOWSKI, STĘPIEŃ 2001). The resistance gene *Lr13*, originating from cv. Maris Huntsman, was present in 58% of European wheat genotypes examined by WINZELER et al. (2000), and the resistance gene *Lr26*, originating from *Secale cereale*, was present in 40% cultivars examined by the same authors. Both resistance sources were used widely in breeding programmes (WINZELER et al. 2000).

A significant number of *Lr* resistance genes have been identified by using genetic methods or by multipathotype testing (MCINTOSH et al. 1998, WINZELER et al. 2000, PARK et al. 2001). Strategies using restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) have been used successfully to develop markers for *Lr* resistance genes including: *Lr1*, *Lr9*, *Lr10*, *Lr13*, *Lr19*, *Lr23*, *Lr24*, *Lr25*, *Lr28*, *Lr29*, *Lr34*, *Lr35*, *Lr37* and *Lr47* (HELGUERA et al. 2000, CHEŁKOWSKI, STĘPIEŃ 2001). For more efficient identification and more practical usage, RFLP and RAPD markers have been converted into sequence tagged sites (STS – AUTRIQUE et al.1995, FEUILLET et al. 1995, 1997, PRINS et al.1996). The following *Lr* resistance genes originating from wild relatives can now be identified by STS specific markers or sequence-characterized amplified region (SCAR) markers: *Lr9*, *Lr19*, *Lr24*, *Lr26*, *Lr28*, *Lr35* and *Lr37* (FEUILLET et al. 1995, SCHACHERMAYR et al. 1994, 1997, DEDRYVER et al. 1996, NAIK et al. 1998, SEYFARTH et al. 1999, ROBERT et al. 1999). Recently, an STS marker identifying *Lr19* and a CAPS (cleaved amplified polymorphic sequence) marker identifying *Lr47* were characterized (HELGUERA et al. 2000, PRINS et al. 2001). Three STS markers were developed to identify the resistance genes *Lr26*, *Yr9* and *Pm8/Pm17* present in translocation from rye (IQBAL, RAYBURN 1995, MOHLER et al. 2001). The marker for *Lr37* can also be used to identify the linked genes *Yr17* and *Sr38*, all of which are present on a translocation from *Ae. ventricosa*.

The development of PCR specific markers in polyploid species with large genomes, such as hexaploid common wheat, is more complex than in diploid species, because PCR can result in the amplification of multiple fragments of the same size from more than one genome (HELGUERA et al. 2000). For this reason, STS markers described in the literature may give false-positive answers concerning the presence of the marker in different lines and cultivars with different genetic backgrounds. The aim of this paper was to use the existing STS markers to identify leaf rust resistance genes in winter wheat cultivars from European countries.

Material and methods

Plant material

Thirty-seven European wheat cultivars were tested with three PCR-based DNA markers for leaf rust resistance genes (*Lr10*, *Lr26* and *Lr37*). Swiss cultivars were obtained from RAC – Station Federale de Recherches en Production Vegetale de Changins, Nyon, Switzerland. Czech and German cultivars originated from the Gene Bank of VURV – Research Institute of Crop Production, Prague–Ruzyně, Czech Republic. British cultivars were kindly supplied by the University of Reading, Reading, United Kingdom. Polish cultivars were supplied by breeding companies in Szelejewo and Polanowice. Seeds of Thatcher isogenic accessions conferring resistance genes *Lr1–Lr45* were kindly supplied by Prof. R.A. McIntosh and Dr. R.F. Park, University of Sydney, Plant Breeding Institute Cobbitty, Australia, and by Dr. M. Csösz, Cereal Research Institute, Szeged, Hungary. Seeds of accessions conferring gene *Lr47* were kindly supplied by Prof. A. Lukaszewski, Department of Botany and Plant Sciences, University of California, Riverside, USA. The cultivars Agent and Agatha 235 and translocation lines with *Lr9* were supplied by Dr. W.J. Raupp, Department of Plant Pathology, Kansas State University, USA, and winter wheat acessions with genes *Lr9*, *Lr19* and *Lr24* were supplied by Dr. P. Bartoš, Research Institute of Crop Production, Prague-Ruzyně, Czech Republic. These resistance genes were transferred from CIMMYT spring germplasms to the Czech wheat cultivars Regina, Viginta and Zdar by STUCHLIKOVA (1993).

DNA isolation

DNA was extracted from 7-day-old seedling leaves by a modified CTAB method (according to DOOHAN et al. 1998). Briefly, 0.15 g of fresh leaves (five plants of each accession, containing approximately 2-5 µg of DNA) were harvested to an Eppendorf tube, frozen at –70°C and then freeze-dried. After grinding the material with a pestle, 1 ml of CTAB buffer with 0.4% 2-mercaptoethanol was added, followed by 100 µl of a chloroform-octanol (24:1/vol.) mixture. Samples were then incubated at 65°C for 25 minutes. After the addition of 400 µl of a chloroform-octanol mixture, samples were shaken vigorously and left at room temperature for 10 minutes.

After centrifugation (10 minutes at 11,000 rpm), the aqueous upper phase was transferred to a new Eppendorf tube. 50 µl of 3M sodium acetate were then added and DNA was precipitated with 800 µl of ice-cold ethanol (96%) and left in the refrigerator for 2 h. The precipitate was centrifuged at 12,000 rpm for 20 minutes, the supernatant was removed, and DNA was washed carefully with 1 ml of cold

ethanol (75%) and redissolved in 200 µl of TE buffer, pH 8.0. The extracts were stored at –20°C until used.

PCR amplification and gel electrophoresis

Eight different markers for leaf rust resistance genes were used: STS markers for the genes *Lr10*, *Lr19* and *Lr24*, SCAR markers for the genes *Lr9*, *Lr24*, *Lr26* and *Lr37*, and a CAPS marker for gene *Lr47*. Each sample contained 0.75 U of Taq DNA polymerase (Finnzymes Oy, Espoo, Finnland), 2.5 µl of PCR buffer, 12.5 pmol of forward/reverse primers (Table 1), 2.5 mM of each dNTP, and about 50 ng of plant DNA. PCR conditions (Perkin Elmer DNA Thermal Cycler) for all primer sets were optimised in initial studies (Table 1). Amplification products were electrophoresed at 5 V/cm for about 3 h in 1.5% agarose gel (Amersham Biotech), stained with ethidium bromide, visualised under UV light and photographed (Syngen UV visualiser). The amplification product for the *Lr47* marker was digested with *Sac*I restriction enzyme prior to electrophoresis.

Table 1. Sequences of primers, PCR conditions and references for markers used to identify resistance genes in wheat cultivars and lines

Marker	Sequence of primers 5'-3'	PCR programme	Reference
Lr9	fwd: TCC TTT TAT TCC GCA CGC CGG rev: CCA CAC TAC CCC AAA GAG ACG	94°C 6 min., 45 cycles (92°C 1 min., 62°C 1 min., 72°C 2 min.), 72°C 4 min.	SCHACHERMAYR et al. (1994)
Lr10	fwd: GTG TAA TGC ATG CAG GTT CC rev: AGG TGT GAG TGA GTT ATG TT	94°C 3 min., 35 cycles (94°C 45 s, 57°C 45 s, 72°C 30 s), 72°C 3 min.	SCHACHERMAYR et al. (1997)
Lr19	CAT CCT TGG GGA CCT C CCA GCT CGC ATA CAT CCA	94°C 5 min., 30 cycles (94°C 1.30 min., 55°C 2 min., 72°C 1.30 min.), 72°C 5 min.	PRINS et al. (2001)
Lr24	fwd: TCT AGT CTG TAC ATG GGG GC rev: TGG CAC ATG AAC TCC ATA CG	94°C 4 min., 40 cycles (92°C 1 min., 60°C 1 min., 72°C 2 min.), 72°C 5 min.	SCHACHERMAYR et al. (1995)
Lr26	fwd: CAT CCT TGG GGA CCT C rev: CCA GCT CGC ATA CAT CCA	94°C 2 min., 35 cycles (94°C 30 s, 63°C 1 min., 72°C 2 min.), 72°C 5 min.	MOHLER et al. (2001)
Lr37	confidential data	94°C 4 min., 35 cycles (94°C 1 min., 65°C 2 min., 72°C 1 min.), 72°C 5 min.	ROBERT et al. (1999)
Lr47	GCT GAT GAC CCT GAC CGG T GGG CAG GCG TTT ATT CCA G	94°C 4 min., 40cycles (94°C 30 s, 55°C 30 s, 72°C 30 s), 72°C 5 min.	HELGUERA et al. (2000)

Table 2. Identification of three STS markers for leaf rust resistance genes in European winter wheat cultivars

Cultivar	Origin	Postulated *Lr* genes as a reaction type*	*Lr10*	*Lr37*	*Lr26*
Titlis	CH	10+37	+	+	–
Lona	CH	13+20	–	–	–
Equinox	CH	?	–	+	+
Zenith	CH	?	–	–	–
Terza	CH	10+37	–	+	–
Frisal	CH	–	–	–	–
Barra	CH	3a+13+26+?	–	–	–
Danis	CH	10+13	+	–	–
Boval	CH	10+13+14a	–	–	–
Arina	CH	13	–	–	–
Siria	CZ	10+13	+	–	–
Alka	CZ	10	+	–	–
Sparta	CZ	26	–	–	+
Livia	CZ	26	–	–	+
Piko	D	13	+	+	–
Pegassos	D	?	–	–	–
Batis	D	13	–	–	–
Kris	D	?	+	+	–
Flair	D	?	–	–	–
Cardos	D	?	–	+	–
Rapor	F	10+13+37	+	+	–
Slade	GB	?	+	+	–
Clever	GB	?	+	+	–
Rialto cz.	GB	10+13+26	+	–	+
Rialto	GB	10+13+26	+	–	+
Hereward	GB	10+13	+	–	–
Consort	GB	10+13	+	–	–
Charger	GB	10+13	+	–	–
Brigadier	GB	13+26+37	+	+	+
Apollo	GB	13+26	–	–	–
Abbot	GB	?	–	–	–
Reaper	GB	10+13+?	–	–	–
Fiocco	I	3a+13+26+?	–	–	–
Pegaso	I	10	+	–	–
Turnia	PL	26	–	–	–
Jubilatka	PL	26	–	–	+
Wilga	PL	26	–	–	+
Michigan Amber	USA	13	–	–	–

*Acc. to WINZELER et al. (2000)
– not present
? not identified

Results

Seven amplification fragments corresponding to STS and SCAR markers of resistance genes were amplified from DNA of the accessions examined (Table 2 and 3, Figures 1-4). A marker fragment of the CAPS marker for the *Lr47* gene was also amplified. The *Lr9* marker, with a DNA fragment of 1100 bp, was identified in two winter wheat breeding lines: Lr9 CIM2/3^{+}Mara and Lr9 CIM10/3^{+}Mara (Table 3). The marker for *Lr10* was identified in 16 of the 37 cultivars tested as a fragment of 310 bp. The amplification product of the *Lr19* marker was 130 bp and was present in the near-isogenic line Tc Lr19 and in three lines carrying this gene: Lr19 CIM60/3^{+}Zdar, Lr19 CIM60/3^{+}Viginta and Lr19 CIM65/3^{+}Viginta. An STS marker and a SCAR marker for *Lr24*, which produce fragments of 310 bp and 700 bp, respectively, were found in two winter wheat lines: Lr24 CIM90/3^{+}Regina and Lr24 CIM91/3^{+}Regina (Table 3). The STS marker for *Lr26* (located on the 1B/1R translocation from *Secale cereale*) yielded a 1200 bp amplification fragment, and was identified in 8 cultivars (Table 2). The molecular marker for *Lr37* was identified in 10 cultivars (Table 2) and was 580 bp. The size of the amplification products for *Lr47* were 450 bp and 380 bp. Digestion of the 450 bp product with the restriction enzyme *Sac*I produced 260 bp and 190 bp frag-

Table 3. Identification of STS markers for leaf rust resistance genes in winter wheat accessions and breeding lines

Accession	Origin	Present *Lr* gene	*Lr* genes identified by STS markers
Lr9 CIM2/3^{+}Mara	CZ	*Lr9*	*Lr9*
Lr9 CIM10/3^{+}Mara	CZ	*Lr9*	*Lr9*
Lr19 CIM60/3^{+}Zdar	CZ	*Lr19*	*Lr19*
Lr19 CIM60/3^{+}Viginta	CZ	*Lr19*	*Lr19*
Lr19 CIM65/3^{+}Viginta	CZ	*Lr19*	*Lr19*
Lr24 CIM90/3^{+}Regina	CZ	*Lr24*	*Lr24*
Lr24 CIM91/3^{+}Regina	CZ	*Lr24*	*Lr24*
Pavon *Lr47*	USA	*Lr10*	*Lr10*+*Lr47*
Agatha 235	USA	*Lr19*	*Lr19*
Agent	USA	*Lr24*	*Lr24*
Teewon	USA	*Lr24*	none
Transfer T47	USA	*Lr9*	*Lr9*
CS T40	USA	*Lr9*	*Lr9*
CS T41	USA	*Lr9*	*Lr9*
CS T44	USA	*Lr9*	*Lr9*

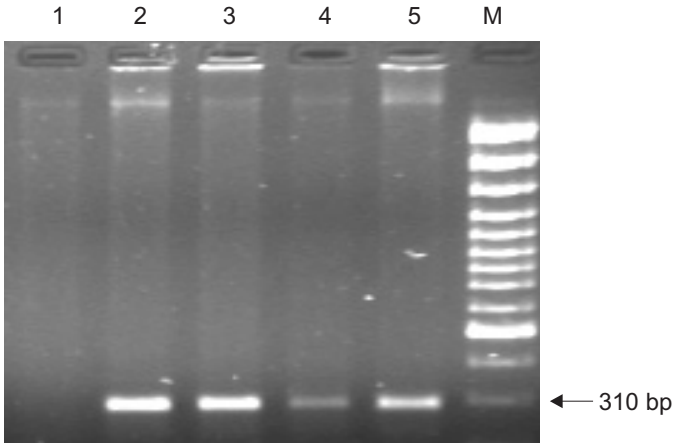

Figure 1. Amplification products of PCR using *Lr10* marker. Lanes from 1 to 5 are: control, Kris, Slade, Clever, Tc Lr10. M = 100 bp marker.

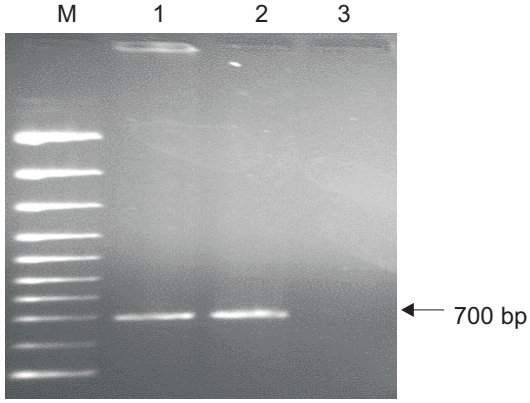

Figure 2. Amplification products of PCR using *Lr24* marker. Lanes from 1 to 3 are: Tc Lr24, Agent, control. M = 100 bp marker.

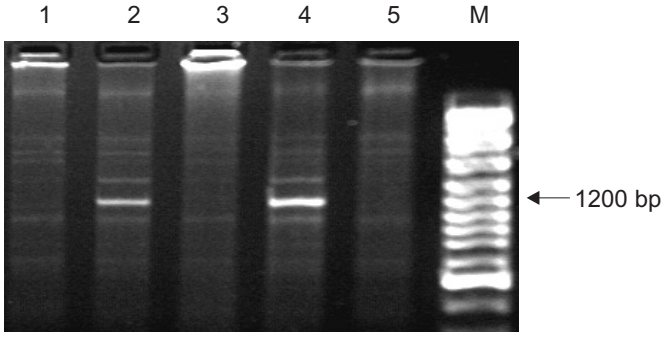

Figure 3. Amplification products of PCR using *Lr26* marker. Lanes from 1 to 5 are: control, Barra, Equinox, Piko, Rialto, Michigan Amber. M = 100 bp marker.

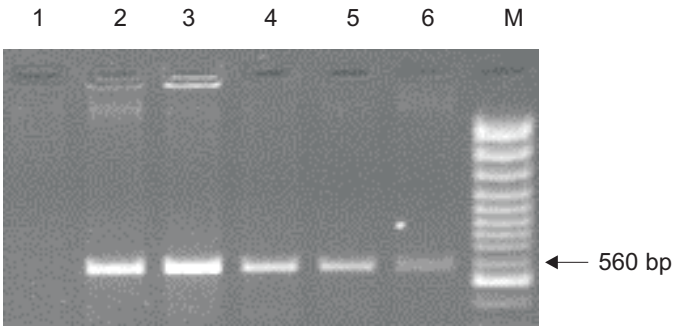

Figure 4. Amplification products of PCR using *Lr37* marker. Lanes from 1 to 6 are: control, Kris, Slade, Clever, Equinox, Tc Lr37. M = 100 bp marker.

ments and a low-intensity 450 bp fragment. The CAPS marker for resistance gene *Lr47* was identified in the line Lr47 Pavon (Table 3).

Discussion

The results obtained for *Lr9*, *Lr19* and *Lr24* markers, were in agreement with their identification by STUCHLIKOVA (1993) and showed that markers for these genes should be useful in marker-assisted breeding. These genes have not been deployed in Europe to date. Similarly, a CAPS marker for *Lr47* was specific to a line containing this resistance gene, but the CAPS procedure is more complicated and problems with restriction enzyme digestion efficiency may occur.

A marker for *Lr10* was identified in 16 of the 37 cultivars tested. This was generally in agreement with previous postulations (WINZELER et al. 2000) except that Boval and Terza, regarded previously as possessing *Lr10*, did not possess the *Lr10* marker, and that the cultivars Piko and Brigadier that were supposed to lack *Lr10*, were positive for the marker. Those mismatches can be explained by incomplete linkage between the marker and the gene, or by incorrect phenotyping during multipathotype testing. It is also possible that grain samples of these cultivars, or the pathogen races used as inoculum, were not homogeneous. The marker for *Lr10* was also found in the line Lr47 Pavon, a translocation line derived from cv. Pavon, the original source of *Lr10* (MCINTOSH et al. 1998).

The *Lr26* marker was identified in seven cultivars (Equinox, Sparta, Livia, Rialto, Brigadier, Jubilatka and Wilga), and with the exception of Equinox, all were previously postulated to carry *Lr26*. In contrast, four cultivars (Barra, Apollo, Fiocco, Turnia) known to carry the T1B/1R translocation on which *Lr26* is located (WINZELER et al. 2000), gave negative results (Table 2). Further examination is necessary to explain the disagreement. It may be possible that the cultivars tested could not be homogeneous.

The molecular marker for *Lr37* was identified in 10 cultivars (Titlis, Equinox, Terza, Piko, Kris, Cardos, Rapor, Slade, Clever and Brigadier). The cultivars Titlis, Terza, Rapor and Brigadier were previously described as *Lr37*–positive (WINZELER et al. 2000, PARK et al. 2001) and these results confirmed the earlier results. This marker was also identified in six additional cultivars: Equinox, Piko, Kris, Clever, Slade, Cardos, three of which (Kris, Clever, Slade) were resistant to leaf rust under field conditions in 2001 and 2002 (Chełkowski, unpublished). Resistance gene postulations have not been conducted for these cultivars to date. The cultivar Rendezvous, a source of rust resistance (MCINTOSH et al. 1998), was a common parent of cvs. Kris, Clever and Slade, and so it is probable that these cultivars possess this gene.

Seven cultivars (Batis, Capo, Terza, Toronit, Titlis, Barra, Beaufort) were the most resistant among 72 wheat cultivars tested in ten European countries, and may be considered as sources of resistance (WINZELER et al. 2000). The presence of unidentified resistance genes in several cultivars exhibiting resistance at all locations was suggested. WINZELER et al. (2000) concluded that not all gene combinations are effective in Europe and care is needed if attempts are made to pyramid resistance genes in new cultivars. Gene combinations present in Kris, Clever, Terza and Titlis are in particular contributing to leaf rust resistance and may be used in breeding programmes.

Conclusions

The results of our experiments show the usefulness of the markers tested in identifying some resistance genes in various cultivars, especially when used in conjunction with multipathotype testing with leaf rust isolates at the pre-breeding stage. However, exceptions to previous results of multipathotype tests were obtained in the present study, demonstrating the need for caution when interpreting the results of both approaches.

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