Short Communication

Diagnostic of Wheat Leaf Rust Resistance Genes by DNA Markers and their Application in MAS

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Abstract: Leaf rust caused by *Puccinia triticina* belongs to the most important wheat diseases in Europe, including Slovakia. Winter wheat varieties registered in Slovakia have been tested with already developed STS (*Lr9, Lr24)*, SCAR (*Lr35*) and SSR (*Lr13*) markers which are linked to the mentioned leaf rust resistance genes. Each of the four DNA markers linked to the individual resistance gene can be detected safely, easily and relatively fast by using the PCR reaction. This is very important for marker assisted selection (MAS) for the incorporation of the *Lr9, Lr24* a *Lr35* genes into chosen wheat genotypes with good bread-making quality or for the characterisation of wheat genetic resources (*Lr13* gene).

Keywords: *Lr* genes; PCR; wheat; *Puccinia triticina;* MAS

Leaf rust (*Puccinia triticina)* is an important pathogen of wheat. The use of resistant wheat cultivars is the most economic and environmentally safe way to reduce losses caused by leaf rust. The accumulation of several resistance genes is needed to achieve more durable resistance against this disease (Bartoš *et al*. 2002).

Some registered Slovak cultivars have the resistance genes *Lr3* (Astella, Hana, Samanta, Viginta), *Lr13* (Alka, Bruta, Estica, Lívia, Samanta, Vlada) and *Lr26* (Lívia). These genes are only partially effective or ineffective against the leaf rust races prevailing in the Slovak Republic (Bartoš *et al*. 2002). In recent European race surveys the genes *Lr9, Lr19, Lr24, Lr28, Lr35* belong to the most effective resistance genes against the prevailing leaf rust races (Bartoš *et al*. 2002; Mesterházy *et al*. 2000) and may condition durable resistance, especially their combination. It is therefore important to develop linked DNA markers, to enable the detection of the resistance genes during breeding.

The detection of resistance genes occurring in the registered cultivars (*Lr3, Lr13, Lr26*), though ineffective against leaf rust, is important for the completion of information about plant genetic resources of wheat in the Genebank.

The aim of our work was to test the suitability of developed DNA markers for the determination of linked resistance genes (*Lr9, Lr13, Lr24, Lr35*) and also the reliability of the detection.

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MATERIALS AND METHOD

Plant materials. Wheat cultivars from the Genbank at Piešťany were used for the analysis. For the detection of the *Lr9, Lr24* and *Lr35* genes a set of thirty wheat cultivars (Table 1) was used. The presence of the *Lr13* gene was studied in eight wheat cultivars.

DNA extraction. DNA of all analysed wheats was isolated according to Dellaporta *et al*. (1983).

The presence of the *Lr* genes was tested with following markers: *Lr9* with J13-STS (Schachermayr *et al*. 1994), *Lr24* with J09-STS (Schachermayr *et al*. 1995), *Lr35* with SCAR (Gold *et al*. 1999) and *Lr13* with the microsatellite marker of Röder *et al*. (1998). The amplifications were performed in PTC-200 thermocycler (MJ-Research) in 10 µl, 20 µl and 25 µl mixture volumes. As positive controls the near-isogenic lines (NIL) of the spring wheat cultivar Thatcher possessing *Lr9, Lr13, Lr24, Lr35* genes, respectively, were used.

The PCR products of STS and SCAR markers have been loaded in 1.5% agarose gels stained with ethydium bromide, followed by visualisation under UV light. The amplification products of the SSR marker were detected in 6% polyacrylamide gels stained with silver according to Bassam *et al*. (1991).

Table 1. List of wheat cultivars and the year of registration

Cultivar	Year of registration	Cultivar	Year of registration
Agra	1985	Malvina	1998
Alka	1997	Samanta	1993
Astella	1995	Sandra	1984
Balada	1999	Saxana	1990
Barbara	1993	Selekta	1985
Blava	1992	Senta	1991
Brea	1998	Sida	1993
Bruta	1994	Simona	1991
Butin	1988	Sofia	1990
Danubia	1984	Solara	1998
Hana	1985	Torysa	1992
Ilona	1989	Vega	1992
Klea	1998	Viginta	1984
Košútka	1981	Vlada	1990
Lívia	1991	Zerda	1999

RESULTS AND DISCUSSION

The amplification of *Lr9* gene was performed with the specific primer pairs J13/1 and J13/2. The STS marker allows reliable testing of this gene. The *Lr9* gene was not found in any studied Slovak bread wheat cultivars. As a positive control was used the near-isogenic line (NIL) Thatcher/*Lr9* (Th/*Lr9*). The negative control was the cultivar Thatcher. Our analysis confirmed that the *Lr9* gene is not present in these wheat cultivars (Mesterházy *et al*. 2000; Bartoš *et al*. 2002).

The *Lr24* gene was detected with a specific STS marker (Schachermayr *et al*. 1995), completely linked with this gene. Two specific primers amplify the 350 bp long DNA fragment in cultivars with this gene. Using this marker, we did not find the *Lr24* gene in Slovak bread wheat cultivars. Virulence for the *Lr24* gene occurred in some European countries like Germany, Bulgaria and Romania (Mesterházy *et al*. 2000) but has not been found in the rest of Europe.

The *Lr35* gene was tested by the specific SCAR marker (Gold *et a*l. 1999) generated by the primer pair SR39F2 and SR39R3, that identify a 900 bp DNA band in resistant plants. We did not find the *Lr35* gene in any tested wheat cultivar. The used SCAR marker identifies this gene reliably. Since *Lr35* confers adult plant resistance, the SCAR marker can be used in MAS for introgression of *Lr35*.

The *Lr13* gene was tested using the microsatellite marker Xgwm630 of Röder *et al*. (1998) which is located at 10.7 cM distance from this resistance gene (Seyfarth *et al*. 2000). The presence of the *Lr13* gene was studied in a set of eight wheats. The DNA marker was present in the cultivars Samanta, Vlada, Alka and Blava and absent in the cultivars Agra, Astella, Balada and Barbara. The results obtained with the microsatellite marker were compared with determinations of *Lr13* by seedling infections by Bartoš *et al*. (2002). The only difference was observed in the cultivar Blava, in which *Lr13* was not found by Bartoš *et al*. (2002). The difference might be caused by the difficult determination of adult plant resistance by seedling reactions in the above mentioned paper. The difference could be also due to the distance of 10.7 cM (Seyfarth *et al*. 2000) between the *Lr13* gene and the DNA marker. The *Lr13* gene is present in several Slovak wheat cultivars and lacks effectivity if used single. However, ineffective or partially effective genes could be used in combination with other *Lr* genes. For

example, combinations of *Lr13* with *Lr34* resulted in very high levels of resistance due to synergism between both genes (Sawney *et al*. 1992). The SSR marker used for the detection of *Lr13* gene is not optimal for further application in MAS because the rather large genetic distance. According to Mohan *et al*. (1997) molecular markers should cosegregate or should have a genetic distance of less than 1 cM from the resistance gene for a successful application in MAS. But the mentioned DNA marker could be used for screening of wheat genetic resources of the Genbank.

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Abstrakt

Gregáňová Ž., Kraic J., Gálová Z. (2003): **Diagnostika génov rezistencie proti hrdzi pšeničnej pomocou DNA markerov a ich využitie v MAS**. Czech J. Genet. Plant Breed., **39**: 127–129.

Hrdza pšeničná *(Puccinia triticina)* patrí medzi významné choroby pšenice v celej Európe. V slovenských odrodách pšenice boli testované, inými autormi už skôr vyvinuté, STS (*Lr9, Lr24* gény), SCAR (*Lr35* gén) a SSR (*Lr13* gén) markery, ktoré sú vo väzbe so spomínanými génmi rezistencie (*Lr* gény) proti hrdzi pšeničnej. Všetky štyri DNA markery viazané k jednotlivým génom rezistencie je možné spoľahlivo, jednoducho a pomerne rýchlo detegovať pomocou PCR reakcie, čo je veľmi dôležité pre ich následné využitie v MAS pri zabudovávaní do vybraných genotypov s dobrou pekárskou kvalitou (*Lr9, Lr24* a *Lr35* gény) alebo pri charakterizovaní genotypov genetických zdrojov pšenice v kolekcii Génovej banky (*Lr13* gén).

Kľúčové slová: *Lr* gény; PCR; pšenica; *Puccinia triticina;* MAS

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