# Marker-Assisted Selection for Leaf Rust Resistance in Wheat by Transfer of Gene *Lr19*

*Svetlana ŠLIKOVÁ[1], Edita GREGOVÁ[1], Pavel BARTOŠ[2] and Ján KRAIC[1]*

*[1]Division of Applied Genetics and Breeding, Research Institute of Plant Production, Piešťany, Slovak Republic; [2]Division of Genetics and Plant Breeding, Research Institute of Crop Production, Prague-Ruzyně, Czech Republic*

**Abstract**

Šliková S., Gregová E., Bartoš P., Kraic J. (2003): **Marker-assisted selection for leaf rust resistance in wheat by transfer of gene *Lr19***. Plant Protect. Sci., **39**: 13–17.

Cultivar Agrus, possessing a chromosomal substitution, and cultivar Sunnan, possessing a translocation from *Thinopyrum ponticum* (= *Agropyron elongatum*, 2*n* = 10*x*) with leaf rust resistance gene *Lr19* against *Puccinia triticina*, were crossed with the susceptible winter wheat cultivars Sofia, Simona and Lívia to transfer *Lr19* into agronomically better genotypes by marker-assisted selection. Altogether 304 individuals of the $F_2$ progeny were screened for endopeptidase phenotypes. We found null endopeptidase allele *Ep-D1c* (marker tightly liked with resistance gene *Lr19*) in 49 plants. The progenies of 40 plants of the $F_2$ generation (with *Ep-D1c*) were reselected with the same marker and tested for leaf rust reaction. Results achieved with the isozyme marker corresponded with those of the resistance tests. We obtained 28 $F_3$ families with resistance gene *Lr19* confirmed by presence of the null endopeptidase allele and by tests for leaf rust reaction. Field tests showed that Agrus increased the height of plants in the progenies, and the smallest negative effect on yield components was observed in both crosses with cultivar Sunnan.

**Keywords**: *Puccinia triticina*; leaf rust; *Lr19*; *Triticum aestivum* L.; endopeptidase; marker-assisted selection

Marker-assisted selection is an efficient tool to speed up plant breeding. It helps also in pyramiding of resistance genes. Markers linked to resistance genes have been applied for several decades. At present, the main attention is paid to DNA markers.

Wheat leaf rust (*Puccinia triticina*) belongs to the important pathogens of wheat, causes 10–15% yield losses and decreases grain quality. Breeding for resistance is the most effective way to control this pathogen. McIntosh *et al*. (1995) listed over 45 different *Lr* genes. Several of them, genes *Lr1*, *Lr3a*, *Lr10*, *Lr13*, *Lr14a*, *Lr17b*, *Lr20*, *Lr26* and *Lr37*, have been described in European wheat cultivars (Park *et al*. 2001). Slovak wheat cultivars possess usually only gene *Lr3* or *Lr26* or both (Huszár *et al*. 2000). Neither gene *Lr3* nor *Lr26* are effective against all pathotypes of leaf rust identified in 1999 and 2000 in Slovakia (Bartoš *et al*. 2001). Another *Lr* gene – *Lr19* – is known as highly effective against leaf rust (Bartoš & Stuchlíková 1999; Bartoš *et al*. 2001) both here and all over Europe (Mesterházy *et al*. 2000). *Lr19* has been introduced into wheat from the wild grass species *Thinopyrum ponticum* (syn. *Agropyrum elongatum*, 2*n* = 10*x*) and is located on chromosome 7DL of the wheat genome (McIntosh *et al*. 1995). A codominant protein marker – endopeptidase allele *Ep-D1c* – has been identified as a valuable marker linked with this gene (Winzeler *et al*. 1995).

The objective of this work was to apply this molecular marker in the selection of wheat individuals possessing leaf rust resistance gene *Lr19* and to generate resistant plants for further breeding.

Supported by the Ministry of Education, Youth and Sports of the Czech Republic, Kontakt 207/2002–3.

## MATERIALS AND METHODS

Winter wheat cultivar Agrus was used as a donor of gene *Lr19*. Agrus possesses chromosome 7Ag substituted from the wild species *T. ponticum* (syn. *A. elongatum*, 2*n* = 10*x*) (McIntosh *et al*. 1995). Gene *Lr19* had been located on chromosome 7Ag (McIntosh *et al*. 1995; Sharma & Knott 1966). Another donor of gene *Lr19* used was the Swedish spring wheat cultivar Sunnan. It possesses a translocation designated as T4 (later known as "Agatha") carrying a chromosome segment on wheat chromosome 7DL that originated from *T. ponticum*. As acceptor genotypes for gene *Lr19* the cultivars Sofia, Simona and Lívia were used. Lívia possesses gene *Lr26*, Sofia *Lr26* + *Lr3*, while Simona has no *Lr* genes. $F_2$ progenies were created from all donor × acceptor combinations.

Analyses of endopeptidases were carried out according to Koebner *et al*. (1988) and Winzeler *et al*. (1995). Protein extracts for isoelectrofocusing were prepared either from leaves or from embryos. Extracts were loaded into prefocused polyacrylamide gel containing ampholyte (Pharmalyte pH 4.2–4.9). Fast Black K salt and $N_\alpha$-benzoyl-DL-arginine-2-napthylamide hydrochloride were used for specific endopeptidase staining. Genetic interpretation of alleles at the *Ep-D1* locus followed the study of Koebner *et al*. (1988). Segregation of alleles at the *Ep-D1* locus in the $F_2$ generation was evaluated by $\chi^2$ test.

Leaf rust isolate 4332 SaBa was used for phytopathological testing. This isolate overcomes resistance of genes *Lr26*, *Lr3* and *Lr3*+*Lr26*. Infection tests were carried out at the first leaf stage in greenhouse conditions at a temperature of 18–22°C with supplemental illumination with fluorescent tubes. Plants were inoculated by rubbing of first leaves with a urediospore/water suspension and kept 24 h at high humidity in closed glass cylinders. Infection types were scored 14 d after inoculation, using the scale developed by Stakman *et al*. (1962).

Seeds of $F_2$ individuals and their parents were sown in field plots of the RIPP Piešťany. The progenies of $F_2$ generations were sown in three rows 1 m long. Plant height was measured from soil surface to the tip, excluding awns. Thirty ears from the central row were harvested and evaluated. The *t*-test was used to compare the $F_3$ generation and their parents.

## RESULTS AND DISCUSSION

### Screening of hybrids for the presence of *Ep-D1c* allele

Segregation of the endopeptidase null allele linked with gene *Lr19* in the $F_2$ generation is shown in Table 1 and Figure 1. Altogether 304 plants of the five parent combinations were analysed. The null allele *Ep-D1c* was found in 49 plants and these were cultivated until harvest.

Segregation in the $F_2$ generation of Sofia × Sunnan and Lívia × Agrus fitted the expected ratio of 3:1, whereas the $F_2$ generations of Sofia × Agrus, Simona × Agrus and Simona × Sunnan did not fit the expected ratio. Our results agree with those of Winzeler *et al*. (1995) who found a fit with the 3:1 ratio ($P = 0.0554$) only if the donor of *Ep-D1c* was used as the maternal component in a cross. The transfer of allele *Ep-D1c* by pollen was reduced, the *P* value for a 3:1 ratio being 0.0004 in their study. A reduced transfer of genes located on segments of alien chromosomes has been described also for the transfer of gene *Lr3*8 (Bartoš *et al*. 1998). Similarly, Marais *et al*. (2001) showed that the translocation with gene *Lr19* from *Thinopyrum ponticum* gener-

Table 1. Segregation of the isozyme marker for *Lr19* in $F_2$ populations of wheat crosses

| Cross | Plants possessing marker | | Plants without the marker | | Total number of plants | $\chi^2$ 3:1 | *P* |
|---|---|---|---|---|---|---|---|
| | number | (%) | number | (%) | | | |
| Sofia × Agrus | 7 | 13.5 | 45 | 86.5 | 52 | 3.694 | 0.054 |
| Sofia × Sunnan | 5 | 29.4 | 12 | 70.4 | 17 | 0.177* | 0.674 |
| Simona × Agrus | 13 | 12.8 | 88 | 87.1 | 101 | 7.879 | 0.005 |
| Simona × Sunnan | 9 | 14.1 | 55 | 85.9 | 64 | 4.095 | 0.043 |
| Lívia × Agrus | 15 | 21.4 | 55 | 78.6 | 70 | 0.477* | 0.490 |

*not statistically significant difference at the level $P > 0.05$

ally showed reduced pollen transmission from the $BCF_1$ population.

**Reselection of $F_3$ generation**

Embryos from selected plants were used for the isolation and analysis of endopeptidases to confirm the presence of null allele *Ep-D1c* (Figure 2). The presence of *Ep-D1c* was confirmed in 35 progenies of 40 plants selected from the five $F_2$ generations. In the Sofia × Agrus progenies, *Ep-D1c* was found in six of the seven tested, while *Ep-D1a* was determined in one progeny. All analysed Sofia × Sunnan and Simona × Sunnan $F_2$ progenies possessed marker allele *Ep-D1c*. Of the 10 tested progenies of the $F_2$ generation from combination Simona × Agrus, seven possessed allele *Ep-D1c* and three allele *Ep-D1a*. Of the 12 progenies of the $F_2$ generation from Lívia × Agrus, 11 progenies carried *Ep-D1c*.

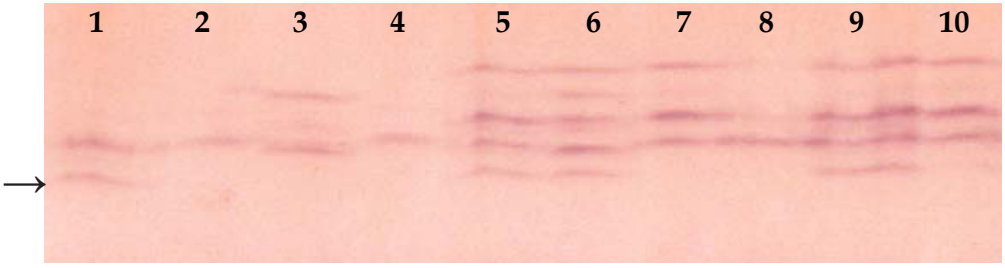
**Reaction to leaf rust in the greenhouse**

Reaction to leaf rust was tested in 32 progenies of the $F_2$ generation. A resistant reaction was characterised by chlorotic flecks without sporulation of the fungus (IT0), a susceptible reaction by abundant sporulation without chlorosis formation (IT3-4). Three of the 32 progenies segregated for resistant and susceptible plants, one progeny was susceptible, while 28 were resistant. In 87.5% of the progenies the results of the isozyme analysis coincided with those of the infection test. The discrepancy between the results has not been further studied to reveal reasons for the differences. As Winzeler *et al*. (1995) calculated the genetic distance between gene *Lr19* and allele *Ep-D1c* to be 0.33 ± 0.33 cM, we presume that such a discrepancy may be caused by some ambiguity in the evaluation of leaf endopeptidase patterns.

**Field plots**

Preliminary results from field plot tests showed an influence by cultivar Agrus on the progenies; it increased plant height, and lowered the weight of kernels per ear and weight of thousand kernels. The *t*-test showed statistically significant differences in these traits between the crosses Sofia × Agrus and Sofia, Simona × Agrus and Simona, Lívia × Agrus and Lívia (Table 2). Yield components were least negatively affected in both crosses where Sunnan was used as a donor parent. The *t*-test showed statistically significant differences in ear length between Sofia × Sunnan and Sofia, Simona × Sunnan and Simona (Table 2). Cultivar Simona showed the highest level of the yield components compared to crosses Sofia × Agrus, Sofia × Sunnan, Simona × Agrus, Simona × Sunnan, and Lívia × Agrus (Table 2).

Our data indicate that the donor genotype with a chromosomal substitution (Agrus) had a more negative effect on yield components than the donor with a chromosomal translocation (Sunnan). Similarly, Ortelli *et al*. (1996) have shown that the presence of *Lr9* (introduced from *Aegilops umbellulata*) reduced grain yield in wheat. Knott (1989) studied the effect of the transfer of genes for leaf rust resistance from nine alien sources (incl. *Agropyron elongatum*, *Triticum speltoides* and *Secale cereale*) into commercial cultivars. The results varied greatly for different transfers, some lines were undesirable in agronomical characteristics but with good traits of quality. Reynolds *et al*. (2001) found a significant increase in yield (13%), final




1. Sofia – allele *Ep-D1a*; 2. (Sofia × Agrus) – *Ep-D1c;* 3. Agrus – *Ep-D1c;* 4. (Sofia × Agrus) – *Ep-D1c;* 5. Simona – *Ep-D1a;* 6. (Simona × Agrus) – *Ep-D1a;* 7. (Simona × Agrus) – *Ep-D1c;* 8. Sunnan – *Ep-D1c*; 9. Lívia – *Ep-D1a;* 10. (Lívia × Agrus) – *Ep-D1c*

Figure 1. Endopeptidase zymograms of parents and $F_2$ progenies – leaf extracts (arrow indicates allele *Ep-D1a* or null allele *Ep-D1c*)

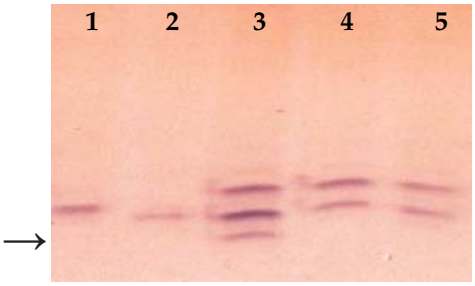



Figure 2. Endopeptidase zymograms of lines of the $F_3$ generations with *Ep-D1c* allele and the susceptible line with *Ep-D1a* allele – embryo extracts (arrow indicates allele *Ep-D1a* or null allele *Ep-D1c*)

1. (Sofia × Agrus) – (*Ep-D1c*); 2. (Sofia × Agrus) – (*Ep-D1c*); 3. (Simona × Agrus) – *Ep-D1a;* 4. (Simona × Agrus) – (*Ep-D1c*); 5. (Lívia × Agrus) – (*Ep-D1c*)

Table 2. Small scale field test of $F_3$ generations possessing *Lr19* (average value of traits)

| Cross | Plant height (cm) | Ear length (cm) | No. of kernels per ear | Weight of kernels per ear (g) | Thousand kernel weight (g) |
|---|---|---|---|---|---|
| Sofia × Agrus | 102.62 | 8.17 | 35.21 | 1.19* | 33.65* |
| Sofia × Sunnan | 99.40 | 7.25* | 41.16* | 1.67* | 40.22 |
| Simona × Agrus | 108.57* | 7.83* | 35.45* | 1.19* | 33.48* |
| Simona × Sunnan | 97.16 | 8.17* | 45.40 | 1.54* | 38.00 |
| Lívia × Agrus | 86.06 | 7.33* | 30.01* | 1.06* | 35.06* |
| Sofia | 81.00 | 8.61 | 48.80 | 2.07 | 43.32 |
| Simona | 93.33 | 9.83 | 55.27 | 2.44 | 47.01 |
| Lívia | 57.66 | 6.67 | 37.65 | 1.71 | 45.52 |

*statistically significant difference at the level $P \leq 0.05$

biomass (10%) and grain number (15%) associated with introgression of alien chromatin carrying *Lr19* in all backgrounds studied.

This study presents an unconventional approach to create 28 wheat progenies of the $F_2$ generation resistant to the important pathogen *Puccinia triticina* by use of a molecular marker and marker-assisted selection. The success was assisted by good coincidence between the marker-based selection and infection tests that identified resistant plants. Preliminary field tests also indicate that some resistant progenies of the $F_2$ generation resemble the acceptor parents.

## References

Bartoš P., Hanušová R., Blažková V., Škorpík M. (1998): Wheat cultivar Amigo and line W49 as sources of disease resistance. Czech J. Genet. Plant Breed., **34**: 49–54.

Bartoš P., Stuchlíková E. (1999): Wheat leaf rust races/pathotypes in the Czech Republic in 1997–1998. Plant Protect. Sci., **35**: 51–56.

Bartoš P., Huszár J., Hanzalová A., Herzová E. (2001): Wheat leaf rust races/pathotypes in Slovakia in 1999–2000. Plant Protect. Sci., **37**: 85–90.

Huszár J., Cagáň Ľ., Vančo B. (2000): Biologická regulácia vybraných chorôb a škodcov pšenice letnej. SPÚ, Nitra, 4–39.

Knott D.R. (1989): The effect of transfers of alien genes for leaf rust resistance on the agronomic and quality characteristics of wheat. Euphytica, **44**: 65–72.

Koebner R.M.D., Miller T.E., Snape J.W., Law C.N. (1988): Wheat endopeptidase: genetic control, polymorphism, intrachromosomal gene location, and alien variation. Genome, **30**: 186–192.

Marais G.F., Marais A.S., Groenewald J.Z. (2001): Evaluation and reduction of *Lr19-149*, a recombined form of the *Lr19* translocation of wheat. Euphytica, **121**: 289–295.

Mesterházy Á., Bartoš P., Goyeau H., Niks R.E., Csösz M., Andersen O., Casulli F., Ittu M., Jones E., Manisterski J., Manninger K., Pasquini M., Rubiales D., Schachermayr G., Strzembicka A., Szunics L., Todorova M., Unger O., Vančo B., Vida G., Walther

U. (2000): European virulence survey for leaf rust in wheat. Agronomie, **20**: 793–804.

McIntosh R.A., Wellings C.R., Park R.F. (1995): Wheat Rusts: An Atlas of Resistance Genes. CSIRO Australia/Kluwer Acad. Publ., Dordrecht, 29–82.

Ortelli S., Winzeler M., Winzeler P., Fried P.M., Nösberger J. (1996): Leaf rust resistance gene *Lr9* and winter wheat yield reduction: I. Yield and yield components. Crop Sci., **36**: 1590–1595.

Park R.F., Goyeau H., Felsenstein F.G., Bartoš P., Zeller F.Z. (2001): Regional phenotypic diversity of *Puccinia triticina* and wheat host resistance in western Europe, 1995. Euphytica, **122**: 113–127.

Reynolds M.P., Calderini D.F., Condon A.G., Rajaram S. (2001): Physiological basis of yield gains in wheat associated with the *Lr19* translocation from *Agropyron elongatum*. Euphytica, **119**: 137–141.

Sharma D., Knott D.R. (1966): The transfer of leaf rust resistance from *Agropyron* to *Triticum* by irradiation. Can. J. Genet. Cytol., **8**: 137–143.

Stakman E.C., Stewart P.M., Loegering W.Q. (1962): Identification of physiologic races of *Puccinia graminis* var. *tritici*. Minn. Agr. Exp. Sci. J., Ser. Paper, 4691.

Winzeler W., Winzeler H., Keller B. (1995): Endopeptidase polymorphism and linkage of the *Ep-D1c* null allele with the *Lr19* leaf rust resistance gene in hexaploid wheat. Plant Breeding, **114**: 24–28.

Received for publication January 7, 2003
Accepted after corrections March 3, 2003

## Súhrn

Šliková S., Gregová E., Bartoš P., Kraic J. (2003): **Markerom podporovaná selekcia pri prenose génu rezistencie Lr19 proti hrdzi pšenicovej do pšenice**. Plant Protect. Sci., **39**: 13–17.

Uskutočnili sme prenos génu rezistencie *Lr19* proti hrdzi pšenicovej do genotypov pšenice, kombináciou klasickej hybridizácie s postupmi selekcie pomocou markerov. Odroda Agrus so substitúciou chromozómu 7Ag z *Thinopyrum ponticum* (= *Agropyron elongatum*, 2*n* = 10*x*) a Sunnan s chromozomálnou translokáciou z *Thinopyrum ponticum* na chromozóme 7DL, v ktorých bola prítomnosť génu rezistencie *Lr19* potvrdená, boli krížené s odrodami Sofia, Simona a Lívia. Z 5 kombinácií rodičov (Sofia × Agrus, Sofia × Sunnan, Simona × Agrus, Simona × Sunnan a Lívia × Agrus) bolo získaných 304 rastlín $F_2$ generácie. Všetky rastliny boli analyzované na prítomnosť, resp. neprítomnosť nulovej alely *Ep-D1c* pomocou izoelektrickej fokusácie endopetidáz extrahovaných z listových segmentov. Prítomnosť alely *Ep-D1c*, biochemický marker génu rezistencie *Lr19* proti hrdzi pšenicovej, bola zistená v 49 rastlinách. Segregácia alely v potomstve poukazuje na zníženie prenosu génu rezistencie *Lr19* peľom z odrôd Agrus a Sunnan do potomstiev. Potomstvo $F_2$ generácie bolo pestované v poľných podmienkach, znova selektované na detekciu prítomnosti alely *Ep-D1c* pomocou analýzy endopeptidáz izolovaných z embryí a testované proti hrdzi pšenicovej. Fytopatologické testy boli vykonané na potomstve z 32 selektovaných rastlín, v ktorých sme identifikovali prítomnosť alely *Ep-D1c*. Úspešný prenos génu *Lr19* bol potvrdený v potomstve z 28 selektovaných rastlín, ktoré boli rezistentné proti hrdzi pšenicovej a zároveň v nich bola potvrdená prítomnosť markerovacej alely *Ep-D1c*. Predbežné výsledky poľných testov naznačujú, že odroda Sunnan s translokáciou z *Thinopyrum ponticum* má nižší negatívny vplyv na úrodotvorné prvky potomstva ako odroda Agrus so substitúciou.

**Kľúčové slová**: *Puccinia triticina*; hrdza pšenicová; *Lr19*; *Triticum aestivum* L.; endopeptidázy; selekcia pomocou markerov

*Corresponding author:*

Ing. Svetlana Šliková, Výskumný ústav rastlinnej výroby, Divizia aplikovanej genetiky a šľachtenia, Bratislavská cesta 122, 92 168 Piešťany, Slovenská republika
tel.: + 421 337 722 311, fax: + 421 337 726 306, e-mail: slikova@vurv.sk