NOVIT. BOT. UNIV. CAROL.
Praha 18/2007: 63–70

The study of the relation between wheat leaf rust *(Puccinia persistens subsp. triticina)* and winter wheat *(Triticum aestivum)* from aspect of it's genetic of resistance

TAŤÁNA SUMÍKOVÁ

ABSTRACT

The presence of the resistance genes *Lr37* and *Lr10* was verified in chosen cultivars by using infection tests of leaf rust races with known virulence genes and by using the molecular markers. The gene Lr37 was detected using published DNA SCAR marker SC-Y15 (Robert & al., 1999) in these cultivars: Apache, Banquet, Bill, Brigadier, Clever, Corsaire, Rapor and Terza. The presence of the gene *Lr10* was proved by the molecular marker *STSLrk10-6* (Schachermayr & al., 1997) in cultivars Alka, Alana, Bill, Siria, Consort, Galahad, Haven, Mercia, Rapor, Terza, Torfrida and Trémie.

Key words: molecular markers for the *Lr37* and *Lr10* genes, leaf rust resistance genes, *Puccinia persistens* subsp. *triticina* (Eriks.), Urban & Marková), winter wheat (*Triticum eastivum* L.).

INTRODUCTION

The resistance genes for wheat leaf rust in wheat are frequently studied by approximate analysis. Cultivars are infected with leaf rust isolates with defined virulence genes. The reactions of these cultivars are compared with the reactions of lines possessing known resistance genes. This analysis is very time-consuming. For quick and reliable detection of specific *Lr* genes molecular markers for these genes can be used. Molecular markers allow the detection of these genes independently of the phenotype. Nowadays molecular markers for many *Lr* genes have been developed (e.g. Feuillet & al., 1995, Schachermayr & al., 1994, Schachermayr & al., 1997, Nelson & al., 1997, Sacca & al., 1998, Robert & al., 1999, Cherukuri & al., 2003).

The *Lr37* gene which is present in many European wheat cultivars displays leaf rust resistance at the adult stage. This gene introduced into wheat chromosome *2A* from *Aegilops ventricosa* L. was found to be closely linked to yellow and stem rust resistance genes *Yr17* and *Sr38*. In the Czech Republic the *Lr37* gene is one of the most effective genes in the field conditions (Bartoš & al., 2001).

The *Lr10* gene originates from the wheat cultivars Lee and Timstein. In the Czech Republic this gene is not effective by itself but it provides a good protection in combination with other *Lr* genes for example *Lr13* (Bartoš & al., 1992, Bartoš & al., 2000).

MATERIAL AND METHODS

The seed of the unregistered cultivars originated from the Gene Bank Prague--Ruzyně and the seed of registered cultivars from the Central Institute for Supervision and Testing in Agriculture of the Czech Republic were used. Cultivars tested to detect the *Lr37* gene were Apache, Banquet, Batis, Bill, Clever, Complet, Corsaire, Svitava, Windsor, Brigadier, Rapor and Terza. Cultivars tested to detect the *Lr10* gene were Alka, Alana, Bill, Boka, Siria, Consort, Galahad, Haven, Mercia, Rapor, Terza, Torfrida and Trémie.

Greenhouse infection tests for detection the *Lr37* and *Lr10* genes were carried out by leaf rust isolates with defined virulence genes. Inoculation was carried out by rubbing the first leaf with suspensions of urediospores. Inoculated plants were kept in closed glass cylinders to keep high air humidity for 48h at 18 °C. Then the glass cylinders were opened and plants were grown 7–10 days at 18–20 °C. Infection types were evaluated 10–14 days after inoculation according to Stakman & al. (1962).

Cultivars were grown 10 days in the greenhouse and the second leaf was used for the molecular analysis. For DNA isolation from leaf tissue the DNeasy Plant Mini Kit (Qiagen) was used. Leaf tissue was mechanically disrupted in liquid nitrogen before the extraction. The approximate DNA concentration was assessed by comparison with marker Lambda DNA/*Hin*dIII.

For detection the *Lr37* gene molecular marker SCAR *SC-Y15 was* applied following the protocol by Robert & *al*. (1999) with small changes. For detection the gene *Lr10* molecular marker *STSLrk10-6* was applied following Schachermayr & al. (1997) with small changes.

The amplified fragments 580bp (for the *Lr37* gene) and 282bp (for the *Lr10* gene) were separated on 2% agarose gel (Sigma) and visualized under UV light after staining with ethidium bromide. Ladder Lambda DNA/*Hin*dIII was loaded on the gel together with the amplified fragments.

The methods are described in detail in my Diploma thesis (Sumíková, 2004).

RESULTS

All cultivars were tested in greenhouse by above mentioned leaf rusts isolates. The resistant reaction of leaf rust pathotypes 333 and 600 avirulent to the *Lr37* gene showed the presence of the *Lr37* gene in cultivars Apache, Banquet, Bill, Clever, Corsaire, Rapor, Svitava and Terza (Table 1).

The presence of the *Lr10* gene was shown in all tested cultivars except for cultivar Boka according to the resistant reaction of leaf rust pathotypes 1947 and 4332 avirulent to the *Lr10* gene (Table 2).

Tab. 1: Greenhouse test to detect the *Lr37* gene

l. r. pathotyp / *cultivar*	*virulent to the Lr37 gene*					*avirulent to the Lr37 gene*	
	4332	9017	1887	52A	1947	**333**	**600**
Apache	2–3	3	3	3	3	0;	2–3
Banquet	2–3	3	3	3	3	0	2
Batis	2–3	3	3	3	2	2–3	3
Bill	1	3	2	2 > 3	2	0	2
Brigadier	3	–	–	0	–	3	–
Clever	3	3	2	3	2	0;	2
Complet	3	–	–	3	–	3	3
Corsaire	3	–	–	3	–	; 1	2–3
Rapor	; 1	3	2	3	2	2	1–2
Svitava	2–3	3	3	3	2	0;	2
Terza	0	3	0	2–3	1	0	1–2
Windsor	2–3	3	3	3	2	1	3

Tab. 2: Greenhouse test to detect the *Lr10* gene

l. r. pathotyp / *cultivar*	*virulent to the Lr10 gene*				*avirulent to the Lr10 gene*	
	333	1887	600	9017	**1947**	**4332**
Alana	1	3	3	3	2	1–2
Alka	–	2	–	–	0	–
Consort	2–3	–	–	–	0	–
Galahad	2	3	3	3	0	1–2
Haven	0	;	; 1	3	2–3	1–2
Lr 10	3	3	3	3	2	2
Mercia	3	3	3	3	1	1–2
Siria	0	–	–	–	–	–
Torfrida	;	2	3	3	1–2	1
l. r. pathotyp / *cultivar*	347	628	600	9018	**1947**	**4332**
Boka	3	3	3	3	3	3
Trémie	2	1	3	2	2>3	1

0; 1 2 – resistant; 3 – susceptible

Molecular markers were applied to verify the results obtained in infection tests.

Specific PCR product 580bp which proved the presence of the *Lr37* gene was produced in cultivars Apache, Banquet, Bill, Brigadier, Clever, Corsaire, Rapor, Terza and NIL Thatcher *Lr37* after application the molecular marker SCAR

SC-Y15. Sterile water was used as a negative control. Near isogenic line Thatcher *Lr37* was used as a positive control. The application of the marker gave distinct results (Fig. 1a, 1b).

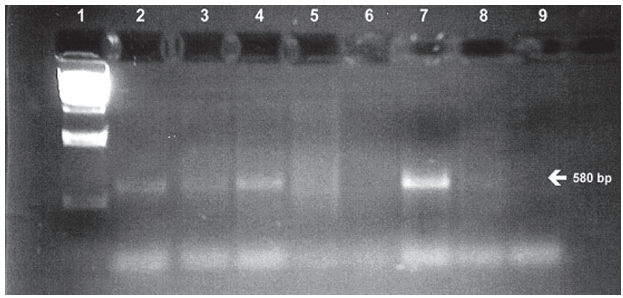

Fig. 1a: Samples scanned with the marker SCAR *SC-Y15*
1 – Marker Lambda DNA/*Hin*DIII, 2 – Apache, 3 – Bill, 4 – Clever, 5 – Corsaire, 6 – Complet, 7 – NIL cv. Thatcher *Lr*37, 8 – Terza, 9 – Sterile water

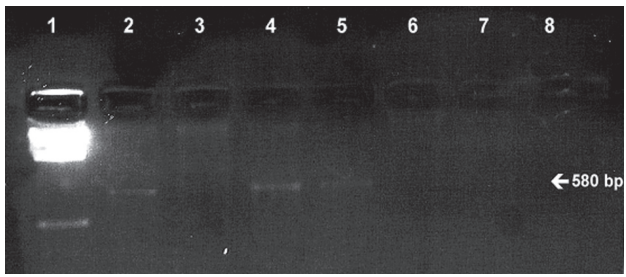

Fig. 1b: Samples scanned with the marker SCAR *SC-Y15*
1 – Marker Lambda DNA/*Hin*DIII, 2 – Banquet, 3 – Batis, 4 – Brigadier, 5 – Rapor, 6 – Svitava, 7 – Windsor, 8 – Sterile water

The results obtained with both methods shown that cultivars Apache, Bill, Clever, Corsaire, Terza, Banquet, Brigadier and Rapor might possess the *Lr37* gene.

The presence of the gene *Lr10* was proved by the molecular marker *STSLrk10-6* in all tested cultivars and NIL Thatcher *Lr10*, except for cv. Boka which was susceptible also in infection test. Near isogenic line Thatcher *Lr37* and sterile water were used as a negative control. Near isogenic line Thatcher *Lr10* was used as a positive control. The application of the marker gave distinct results (Fig. 2a, 2b).

The results obtained with both methods showed that all tested cultivars, except for cv. Boka, might possess the *Lr10* gene.

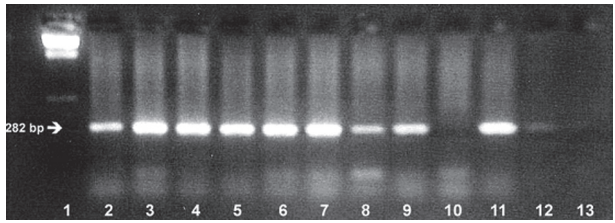

Fig. 2a: Samples scanned with the STS marker *Lrk10-6*.
1 – Marker Lambda DNA/*Hin*DIII, 2 – NIL cv. Thatcher Lr10, 3 – Galahad, 4 – Mercia, 5 – Haven, 6 – Torfrida, 7 – Consort, 8 – Alka, 9 – Alana, 10 – Boka, 11 – Trémie, 12 – Trémie, 13 – Sterile water

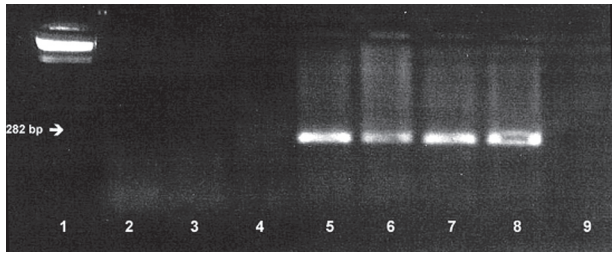

Fig. 2b: Samples scanned with the STS marker *Lrk10-6*.
1 – Marker Lambda DNA/*Hin*DIII, 2 – Boka, 3 – Boka, 4 – NIL cv. Thatcher *Lr*37, 5 – NIL cv. Thatcher *Lr*10, 6 – Terza, 7 –Bill, 8 – Rapor, 9 – Sterile water

DISCUSSION AND CONCLUSIONS

The presence of the *Lr*37 gene was previously supposed according to the tests with a set of leaf rust pathotypes in cultivars Brigadier, Terza, Rapor (Park & al., 2001; Singh & al., 2001; Winzeler & al., 2000) and in cultivars Apache, Bill, Clever, Corsaire (Bartoš & al., 2003b).

By molecular marker SCAR *SC-Y15* the presence of this gene was previously confirmed in cultivars Apache, Bill, Corsaire (Ambrozková & al., 2002, Bartoš & al., 2003a) and Clever (Ambrozková & al., 2002; Bartoš & al., 2003a; Stępień & al., 2003). The gene was detected also in cultivars Brigadier (Robert & al., 1999, 2000; Ambrozková & al., 2002; Stępień & al., 2003), Rapor and Terza (Stępień & al., 2003).

Probably for the first time the marker SCAR *SC-Y15* was used in cultivars Banquet and Svitava. Bartoš & al. (2003b) mentioned the presence of some resistance gene to leaf rust (the undetermined *Lr* gene) in cv. Banquet. The results of infection tests and also the application of the marker SCAR *SC-Y15* showed the

resistant reaction. It could signify that the undetermined resistance gene is the *Lr37* gene, but this supposition must be confirmed by other tests.

Cv. Svitava was resistant to the infection test but susceptible in the molecular test. The resistant reaction in the infection test was caused by the presence of the *Lr3* gene not the *Lr37* gene (Bartoš et al., 2003b). The leaf rust pathotypes 333 and 600 are avirulent to both genes *Lr3* and *Lr37* so this might misrepresent the results.

The absence of the *Lr37* gene was postulated in cultivars Batis, Complet and Windsor. It was shown by the susceptible results in infection greenhouse test and also the specific PCR product was not presented after application the marker SCAR *SC-Y15*. Previously was the absence of this gene confirmed by the molecular marker in cv. Windsor (Ambrozková & al., 2002), cv. Batis (Stępień & al., 2003) and cv. Complet (Bartoš & al., 2003a).

The presence of the *Lr10* gene was previously proved or supposed in cultivars Alka, Alana, Siria (Bartoš & al., 2003b), in cultivars Trémie, Consort, Terza (Winzeler & al., 2000; Park & al., 2001; Singh & al., 2001) and in cultivars Galahad, Haven and Torfrida (Singh & al., 2001).

The *Lr10* gene was confirmed by the molecular STS marker *Lrk10-6* in cultivars Alka, Siria, Consort, Rapor, Galahad and Trémie (Dumalasová, 2000; Blažková & al., 2002; Stępień & al., 2003).

All of tested cultivars, except for cv. Boka, were resistant in infection tests to leaf rust pathotyps avirulent to the *Lr10* gene. After application of the STS marker *Lrk10-6 the* specific PCR product was shown in all these cultivars except cv. Boka. These results confirmed the presence of the *Lr10* gene in all cultivars, except Boka which was also susceptible in the infection tests. In cultivars Haven, Mercia and Torfrida the presence of this gene was verified for the first time.

The results of infection test revealed that cultivars Bill, Rapor and Terza might have the *Lr*10 gene. After application of the STS marker *Lrk10-6* it was confirmed. The presence of this gene was previously confirmed only in cv. Rapor (Blažková & al., 2002).

Generally a big agreement between the results obtained in infection greenhouse tests and the results obtained with molecular markers was detected in all tested cultivars.

ACKNOWLEDGEMENTS

I would like to thank to the Research Institute of Crop production, Prague-Ruzyně, Department of Applied Genetics and the Department of Botany, Charles University of Prague for research facilities and support during my work.

REFERENCES

Ambrozková M., Dedryver F., Dumalasová V., Hanzalová A. & Bartoš P. (2002): Determination of the cluster of wheat rust resistance genes Yr17, Lr37 and Sr38 by a molecular marker. – Plant Protect. Sci., 2: 41–45.

Bartoš P., Bonevová D., Marešová J. & Hanzalová A. (2003a): Rezistence pšenice ke rzím přenesená z Aegilops ventricosa v odrůdách registrovaných v České Republice. – In: Zborník abstraktov – XVI. Slovenská a česká konferencia o ochraně rastlín, Nitra 2003: 68.

Bartoš P., Hanzalová A. & Blažková V. (2000): Leaf rust resistance of winter wheat cultivars registered in the Czech Republic. – Acta Phytopath. Entomol. Hung., 35 (1–4): 149–151.

Bartoš P., Hanzalová A. & Stuchlíková E. (2001): Wheat leaf rust races/pathotypes in the Czech Republic in 1999–2000. – Plant Protect. Sci., 37 (1): 10–16.

Bartoš P., Huszár J., Hanzalová A. & Marešová J. (2003b): Leaf rust resistance genes in winter wheat cultivars registered in the Czech and Slovak Republics. – In: Zborník abstraktov – XVI. Slovenská a česká konferencia o ochraně rastlín, Nitra 2003: 66.

Bartoš P., Stuchlíková E. & Hanušová R. (1992): Fyziologická specializace rzi pšeničné [*Puccinia persistens* Plow. var. *triticina* (Eriks.), Urban & Marková] v Československu v letech 1987 až 1990. – Genet. a Šlecht., 28 (1): 103–119.

Blažková V., Bartoš P., Park R. F. & Goyeau H. (2002): Verifying the presence of leaf rust resistance gene Lr10 in sixteen wheat cultivars by use of a PCR – based STS marker. – Cereal Research Communications, 30 (1–2): 9–16.

Cherukuri D. P., Gupta S. K., Charpe A., Koul S., Prabhu K. V., Singh R. B., Haq Q. M. R. et Chauhan S. V. S. (2003): Identification of a molecular marker linked to an *Agropyron elongatum*-derived gene *Lr19* for leaf rust resistance in wheat. – Plant Breed., 122 (3): 204–208.

Dumalasová V. (2000): Study of selected cereal pathogens and their interactions with host plant. – 61 p. – MS. [Ph. D. Thesis, depon. in dept. of Botany, Charles Univ. Praha]

Feuillet C., Messmer M., Schachermayr G. & Keller B. (1995): Genetic and physical characterization of the Lr1 leaf rust resistance locus in wheat (*Triticum aestivum* L.). – Mol. Gen. Genet., 248 (5): 553–562.

Nelson J. C., Singh R. P., Autrique J. E. & Sorrells M. E. (1997): Mapping genes conferring and suppressing leaf rust resistance in wheat. – Crop. Sci., 37: 1928–1935.

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

Robert O., Abelard A. & Dedryver F. (1999): Identification of molecular markers for the detections of the yellow rust resistance gene Yr17 in wheat. – Molecular Breeding, 5: 167–175.

Robert O., Dedryver F., Rolland B., Abelard C. & Jaudeau B. (2000): Relationships between molecular identification of the gene Yr17 and adult plant resistance against stripe and leaf rusts in bread wheat varieties. – Acta Phytopath. Entomol. Hung., 35 (1–4): 59–63.

Sacco F., Suárez E. Y. & Naranjo T. (1998): Mapping of the leaf rust resistance gene *Lr3* on chromosome 6B of Sinvalocho MA wheat. – Genome, 41 (5): 686–690.

Schachermayr G., Siedler H., Gale M. D., Winzeller H., Winzeler M. & Keller B. (1994): Identification and localisation of molecular markers linked to the Lr9 leaf rust resistance gene of wheat. – Theor. Appl. Genet., 88: 110–115.

Schachermayr G., Feuillet C. & Keller B. (1997): Molecular markers for the detection of the wheat leaf rust resistance gene Lr10 in diverse genetic backgrounds. – Molecular Breeding, 3: 65–74.

Singh D., Park R. F. & McIntosh R. A. (2001): Postulation of leaf (brown) rust resistance genes in 70 wheat cultivars grown in the United Kingdom. – Euphytica, 120: 205–218.

Stakman E. C., Stewart D. M. & Loegering W. Q. (1962): Identification of physiologic races of Puccinia graminis var. tritici. – U. S. Dept. Agr., Agr. Res. Service, E 617.

Stępień Ł., Golka L. & Chełkowski J. (2003): Leaf rust resistance genes of wheat: identification in cultivars and resistance sources. – J. Appl. Genet., 44 (2): 139–149.

Sumíková T. (2004): Studium vztahu rzi pšeničné (*Puccinia persistens subsp. triticina*) a ozimé pšenice (*Triticum aestivum*) z hlediska genetiky rezistence. – 100 p. – MS. [Diploma Thesis, depon. in dept. of Botany, Charles Univ. Praha]

Winzeler M., Mesterházy Á., Park R. F., Bartoš P., Csösz M., Goyeau H., Ittu M., Jones E., Löschenberger F., Manninger K., Pasquini M., Richter K., Rubiales D., Schachermayr G., Strzembicka A., Trottet M., Unger O., Vida G. & Walther U. (2000): Resistance of European winter wheat germplast to leaf rust. – Agronomie, 20: 783–792.

Mgr. Taťána Sumíková
Department of Breeding Methods
Research Institute of Crop production
Drnovská 507
CZ-161 06 Prague 6
Czech Republic