Presence of a Translocation from *Aegilops ventricosa* in Wheat Cultivars Registered in the Czech Republic

Pavel BARTOŠ, Jaroslava OVESNÁ, Alena HANZALOVÁ, Jana CHRPOVÁ, Veronika DUMALASOVÁ, Miroslav ŠKORPÍK and Václav ŠÍP

Division of Genetics and Plant Breeding and Division of Molecular Genetics, Department of Applied Genetics, Research Institute of Crop Production, Prague-Ruzyně, Czech Republic

Abstract: The presence of a translocation from *Aegilops ventricosa* carrying the genes for rust resistance *Yr17, Lr37* and *Sr38* was analysed in recently registered, mostly western European wheat cultivars in the Czech Republic. By means of a PCR marker the presence of the translocation was determined in cvs. Bill, Clarus, Clever, Corsaire, Rapsodia, and in the Czech cv. Rheia. Novel are the data for cvs. Rapsodia, Clarus and Rheia. Infection tests indicated the presence of additional leaf rust resistance genes in cultivars with the translocation, except in cv. Rheia. Segregating progenies of six crosses between cv. Renan possessing *Lr37*and different cultivars susceptible to leaf rust were tested for the presence of the translocation with *Yr17*, *Lr37* and *Sr38* by an infection test as well as by a molecular marker. High coincidence between the results from infection tests and those by the marker has been proved.

Keywords: winter wheat; *Aegilops ventricosa*; translocation; registered cultivars; *Lr37*; leaf rust resistance; molecular marker

Knowledge of genes for disease resistance in registered cultivars of wheat (*Triticum aestivum* L.) is important not only for farmers but also for wheat breeders who use commercial cultivars in crosses. In western European wheat breeding programs, resistance genes from the line VPM 1 possessing translocations from *Aegilops ventricosa* Tausch (syn. *Triticum ventricosum* Ces.) are important sources of resistance to rusts, eyespot and nematodes. Line VPM 1 was developed from the cross *Aegilops ventricosa/Triticum persicum* (*T. turgidum* var. *carthlicum*)// 3* Marne (*T. aestivum*) with the aim to transfer the eyespot resistance from *Ae. ventricosa* to wheat (Maia 1967). In addition to gene *Pch1* for eyespot resistance, further genes for resistance to yellow rust (*Yr17*), leaf rust (*Lr37*), stem rust (*Sr38*), powdery mildew (*Pm4b*) and cereal cyst nematode (*Cre5*) have been found in VPM 1. The genes for rust resistance and that for cereal cyst nematode resistance were located in wheat chromosome 2AS (Bariana & McIntosh 1993; Jahier *et al*. 2001), the gene for eyespot resistance in chromosome 7D (Worland *et al*. 1988), while the powdery mildew resistance gene probably originated from *Triticum persicum* (Bariana & McIntosh 1994).

Since 1994 many western European wheat cultivars have been registered in the Czech Republic. Among them the presence of resistance genes from VPM 1 can be expected because of their specific reactions and field behaviour. Molecular markers can be applied to verify the presence of the translocation from *Ae. ventricosa* carrying rust resistance. The main objective of our study was to determine which of the recently registered cultivars possess the translocation with rust resistance genes. Another aim was to compare the postulated presence

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of gene *Lr37* in progenies of crosses with cv. Renan based on greenhouse reactions of leaf rust (*Puccinia triticina* Eriks.) with results obtained by means of a molecular marker.

MATERIAL AND METHODS

Seed of the tested registered cultivars was identical with the seed from the Czech Official Trials and was supplied by the Central Institute for Supervising and Testing in Agriculture of the Czech Republic, except cvs. Renan and Arina that originated from the Gene Bank, Prague-Ruzyně, whereas Thatcher* 8/VPM1 (NIL *Lr37*) and Democrat/6* Thatcher (NIL *Lr3a*) were obtained from the Non-Profit Cereal Research Organization, Szeged, Hungary. Progenies of the crosses between cv. Renan and susceptible cultivars not possessing the cluster of rust resistance genes from *Ae. ventricosa* originated from a research breeding program of the Department of Genetics and Plant Breeding. Rust isolates originated from the Rust and Powdery Mildew Collection, Prague-Ruzyně. Cultivars were grown in the greenhouse and the first or second leaf was used for the tests. Greenhouse infection tests were carried out with 12 leaf rust isolates to determine a spectrum of rust reactions characteristic for *Lr37*. Plants were inoculated by rubbing the leaves with a water/urediospore suspension. After inoculation, plants were kept in closed glass cylinders for 24 h at 20 ± 2°C to achieve a high air humidity. Then they were kept in the greenhouse at similar temperatures and evaluated after 14 d according to Stakman *et al*. (1962).

Results of greenhouse leaf rust inoculation tests on progenies of several crosses in F_{3-4} generations (Table 3) involving Renan (a cultivar possessing *Lr37*) and leaf rust susceptible cultivars were compared with results of tests with the molecular marker. For the greenhouse tests 15 seeds were used and isolate 333, avirulent on *Lr37* at the seedling stage, was used to inoculate the progenies. In the test with the marker, mixed DNA from two plants was analysed.

A PCR (Vlr2) marker was applied following the modified protocol by Seah *et al.* (2001) to determine presence of the translocation carrying rust resistance genes. The reaction mixture consisted of 100 ng of genomic DNA 5 µl 10 × PCR buffer (Promega), 4 µl 25mM $MgCl_2$, 5 µl 2mM dNTPs, 5 µl of each 10 pmol/µl primer, 22.5 µl H_2O and 1U Taq polymerase. The PCR amplifications were done in a cycler Techne Flexigene under the following conditions: 2 min denaturation at 94°C followed by 35 cycles of 1 min denaturation at 94°C, 2 min annealing at 61°C and 2 min extension at 72°C followed by final extension 3 min at 72°C. The amplified fragments were separated on 2% agarose gel (Serva) and visualised under UV light after staining with ethidium bromide. A 100bp DNA Ladder (Fermentas) was loaded onto the gel together with the amplified fragments.

RESULTS AND DISCUSSION

As shown in Figure 1, cvs. Renan, Rapsodia, Clarus, Rheia, Bill, Clever, Corsaire and the Thatcher 8/VPM 1 line (NIL Lr37) showed a positive reaction with the PCR marker, whereas no amplification was recorded for cvs. Boka, Ilias, Versailles and Svitava. The data are supported by the results obtained by Ambrozková *et al.* (2002), and Bartoš *et al.* (2003) who used the molecular marker SCAR *SC Y-15* (Roberts *et al.* 1999). They found the presence of the VPM 1 translocation carrying rust resistance genes in cvs. Apache, Bill, Corsaire, Clever and Bill. Novel are the data for cvs. Rapsodia, Clarus and Rheia. However, the sequences of the SC-*Y15*

1 – NIL *Lr37*; 2 – Boka; 3 – Renan; 4 – Complet; 5 – Rapsodia; 6 – Clarus; 7 – Rheia; 8 – Bill; 9 – Clever; 10 – Corsaire; 11 – Ilias; 12 – Versailles; 13 – Svitava

Figure 1. Samples scanned with PCR (Vlr2)

primer have been made available to us by INRA and GIE CLUB 5 only for research purposes. The Vlr2 primer gave identical results as the SC-*Y15* (Ambrozková *et al*. 2002; Bartoš *et al*. 2003). Its application is not limited only for research purposes and can be therefore used for marker assisted selection more easily.

Infection tests (Table 1) did not reveal any spectrum of reactions characteristic only for *Lr37*. The resistant reaction to isolate 333 was characteristic not only for *Lr37* but e.g., also for *Lr3a*. Differences in the reactions of cultivars possessing *Lr37* to the applied rust isolates, and resistance to some rust isolates that are virulent to *Lr37*, indicate that probably all tested cultivars also possess other leaf rust resistance genes in addition to *Lr37*, except cv. Rheia. Stepień *et al*. (2004) state *Lr13* for cv. Apache and *Lr10* and *Lr13* for cv. Clever in addition to *Lr37*.

Registered cultivars possessing the cluster of rust resistance genes *Yr17*, *Lr37* and *Sr38* contribute to the diversity of resistance genes in the Czech Republic. Leaf rust resistance gene *Lr37* (in combination with other genes) conditions a very good level of field resistance. According to data from the Central Institute for Supervising and Testing in Agriculture of the Czech Republic (Přehled odrůd obilnin 2003/Survey of Cereal Cultivars 2003) the leaf rust resistance of cv. Corsaire was classified by the degree 8, of cvs. Bill and Apache by 7, Rheia by 6.5. The stem rust resistance in infection field trials in Prague-Ruzyně was also classified relatively high (Corsaire and Bill 7, Apache and Rheia 6) using the scale: 1 susceptible, 9 resistant. Yellow rust resistance gene *Yr17* is no longer effective in western Europe and virulence to it was found also in Central Europe.

The comparison of the results of greeenhouse and marker tests is given in Table 2. Whereas homozygous and heterozygous progenies could be distinguished by testing 15 plants in greenhouse infection tests, the analyses with mixed DNA from two plants selected by chance could not distinguish homozygous from heterozygous progenies. A positive reaction with the PCR marker could indicate homozygous resistant or heterozygous progenies, a negative reaction homozygous susceptible or heterozygous progenies. Therefore, the results obtained by the two different methods can be compared only if the lines are homozygous, with all plants being either resistant or susceptible. Identical results of the infection tests and analyses with the marker were found in the progenies of three crosses, whereas in the progenies of the other three crosses one or two differences were revealed. Yet if all progenies from all crosses are considered, differences were found in 4 out of 98 progenies.

As isolate 333 is avirulent also to some other leaf rust resistance genes it can be applied only

Table 1. Reactions of cultivars possessing *Lr 37* and of NIL *Lr37,* as well as of cv. Svitava and NIL *Lr3* to leaf rust isolates

Cultivar – NIL line	Postulated gene	Leaf rust isolate						
		333	600	628	1887	7087	347	1947
Bill	*Lr 37+*	0 ;	2–-3	; N	; 1–2	3	2 - 3	; 1–2 N
Clever	*Lr 37+*	0 ;	; 1	; 1	3	3	3	2–3
Rheia	*Lr 37*	0 ;	2–3	3	3	3	3	3
Svitava	*Lr 3a*	0 ;	; 1	3	3	3	3	3
NIL *Lr 3*	*Lr 3a*	0 ;	; 1	3	3	3	3	3
		333	9071	9072	9095	9077	Ch-b	1947
Clarus	*Lr 37 +*	0 ;	3	3	1–2	3	; 1	3
Rapsodia	*Lr 37 +*	0 ;	3	3	; 1	; 1–2	; 0	; 0
Corsaire	*Lr 37 +*	0 ;	3	3	2 +	3	0 ; 1	3
NIL *Lr 37*	*Lr 37*	; 1–2	3	3	3	3	3 –	3 –

Table 2. Comparison of seedling infection test and marker analysis

Cross (Progeny)		Number of homozygous progenies		
		infection test	marker	total
Renan/Arina (F_4)	R	4	5	
	S	19	18	23
Renan/Arina//Šárka (F_4)	R	10	10	
	S	10	10	20
Renan/Vlasta (F_3)	R	6	6	
	S	5	5	11
Renan/Šárka (F_3)	R	4	5	
	S	7	6	11
Renan/Arina//RU 703 (F_3)	R	4	6	
	S	6	4	10
Renan/Rheia (F_3)	R	23	23	
	S	–	–	23
Σ				98

R – resistant; S – susceptible

to test progenies of crosses in which none of such resistance genes is present and only *Lr37* is involved. This restriction does not apply to tests by molecular marker. Therefore, use of molecular markers is advantageous when resistance genes are pyramided, i.e. cumulated in one genotype. Markers are available to identify other *Lr* genes (Blasczyk *et al*. 2004), but a laboratory equipped for molecular work is needed for the analyses. On the other hand, infection tests do not require any laboratory equipment and can be carried out in greenhouses commonly available at plant breeding stations.

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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., **38**: 41–45.

Bariana H.S., McIntosh R.A. (1993): Cytogenetic studies in wheat. XV. Location of rust resistance genes in VPM 1 and their genetic linkage with other disease resistance genes in chromosome 2A. Genome, **36**: 476–482.

Bariana H.S., McIntosh R.A. (1994): Characterization and origin of rust and powdery mildew resistance genes in VPM1 wheat. Euphytica, **76**: 53–61.

Bartoš P., Bonevová D., Marešová J., Hanzalová A. (2003): Rust resistance derived from *Aegilops ventricosa* in wheat cultivars registered in the Czech Republic. In: XVI. Slovak and Czech Plant Protect. Conf., 16.–17. September 2003, Nitra, Slovakia, Abstr. Suppl.: 68.

Blasczyk L., Chelkowski J., Korzun V., Kraic J., Ordon F., Ovesná J., Purnhauser M., Tar M., Vida G. (2004): Ring test results of STS markers for leaf rust resistance genes in wheat. In: Chelkowski J., Stepień L. (eds): Microscopic Fungi – Host Resistance Genes, Genetics and Molecular Research. Inst. Plant Genet. Pol. Acad. Sci., Poznań, 53–58.

Jahier J., Abelard P., Tanguy A.M., Dedryver F., Rivoal R., Khatkar S., Bariana H.S. (2001): The *Aegilops ventricosa* segment on chromosome 2AS of the wheat cultivar "VPM 1" carries the cereal cyst nematode resistance gene *Cre5.* Plant Breed., **120**: 125–128.

Maia N. (1967): Obtention de blés tendres résistants au piétin-verse (*Cercosporella herpotrichoides*) par croisements interspécifiques. C.R. Acad. Sci. Fr., **53**: 149–154.

Roberts O., Abelard C., Dedryver F. (1999): Identification of molecular markers for the detection of the yellow rust resistance gene *Yr17* in wheat. Mol. Breed., **5**: 167–175.

Seah S., Bariana H., Jahier J., Sivasithamparam K., Lagudah E.S. (2001): The introgressed segment carrying rust resistance genes *Yr17, Lr37 and Sr38* in wheat can be assayed by a cloned disease resistance gene-like sequence. Theor. Appl. Genet., **102**: 600–605.

Stepień L., Blasczyk L., Wiśniewska H., Chelkowski J. (2004): Spring wheat resistance against powdery mildew, leaf rust and Fusarium blight and identification of resistance genes using STS and SSR markers. In: Chelkowski J., Stepień L. (eds): Microscopic Fungi – Host Resistance Genes, Genetics and Molecular Research. Institute of Plant Genetics PAS, Poznań, Poland, 77–86.

Stakman E.C., Stewart D.M., Loegering W.Q. (1962): Identification of physiological races of *Puccinia graminis* var. *tritici.* US Dept. Agric. ARS E617.

Worland A.J., Law C.N., Hollins T.W., Koebner R.M.D., Guira A. (1988): Location of a gene for resistance to eyespot (*Pseudocercosporella herpotrichoides*) on chromosome 7D of bread wheat. Plant Breed., **101**: 43–51.

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Souhrn

Bartoš P., Ovesná J., Hanzalová A., Chrpová J., Dumalasová V., Škorpík M., Šíp V. (2004): **Translokace z** *Aegilops ventricosa* **v odrůdách pšenice registrovaných v České republice**. Czech J. Genet. Plant Breed., **40***:* 31–35.

Odrůdy pšenice převážně západoevropského původu registrované v nedávné době v České republice byly analyzovány na přítomnost translokace z *Aegilops ventricosa* nesoucí geny rezistence ke rzím *Yr17*, *Lr37* a *Sr38*. Pomocí PCR markeru byla zjištěna přítomnost studované translokace v odrůdách Bill, Clarus, Clever, Corsaire, Rapsodia a v české odrůdě Rheia. Nové je zjištění této translokace v odrůdách Rapsodia, Clarus a Rheia. Infekční testy prokázaly přítomnost dalšího nebo dalších genů rezistence ke rzi pšeničné v uvedených odrůdách kromě odrůdy Rheia. Ve štěpících potomstvech křížení odrůdy Renan, která má gen *Lr37*, s různými odrůdami náchylnými ke rzi pšeničné byla zjištěna vysoká shoda mezi výsledky získanými molekulárním markerem a infekčními testy o přítomnosti genu *Lr37*.

Klíčová slova: pšenice ozimá; *Aegilops ventricosa*; translokace; registrované odrůdy; *Lr37*; rezistence ke rzi pšeničné; molekulární marker

Corresponding author:

Ing. Pavel Bartoš, DrSc., Výzkumný ústav rostlinné výroby, odbor genetiky a šlechtění, 161 06 Praha 6-Ruzyně, Česká republika
tel.: + 420 233 022 243, fax: + 420 233 022 286, e-mail: bartos@vurv.cz