# Determination of the Cluster of Wheat Rust Resistance Genes *Yr17*, *Lr37* and *Sr38* by a Molecular Marker

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**Abstract**

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.

A translocation from *Aegilops ventricosa* carrying genes *Yr17*, *Lr37* and *Sr38* was verified in cultivars Hussar, Eureka, Torfrida, Renan, Rendezvous, Rapier and Brigadier by the molecular marker SCAR *SC-Y15*. Of the cultivars recently registered in the Czech Republic, only the western European cultivars Corsaire, Apache, Complet and Bill possessed the translocation carrying *Yr17*, *Lr37* and *Sr38*. Cultivars Corsaire, Apache, Bill and Complet were highly or medium resistant to yellow rust and leaf rust in 1999–2001 field trials at Prague-Ruzyně, although virulence to *Yr17* was found in the 2001 virulence survey also in the Czech Republic. Cultivars Corsaire, Apache and Bill displayed an above average resistance to stem rust, whereas Complet was susceptible.

**Keywords**: rusts; wheat; resistance genes; *Yr17*; *Lr37*; *Sr38*; molecular marker

Knowledge of genes for resistance to diseases is important to estimate the risk of the disease according to the virulence in the population of the pathogen. It is also essential for plant breeders for the choice of the most suitable resistance genes and their combinations for the breeding process. The most common procedure for the postulation of resistance genes is based on Flor's gene-for-gene hypothesis. Reactions of the studied cultivars to a set of pathotypes are compared with reactions of lines with determined resistance genes. However, crosses and analysis of the $F_2$ generation are necessary for genetic proof of the presence of a certain gene. The development of molecular markers of resistance genes offers new possibilities for a fast and relatively reliable determination of resistance genes.

Reviews on molecular markers of wheat leaf rust resistance genes have been published by several authors (GUPTA *et al*. 1999; LANGRIDGE & CHALMERS 1998) and examples of molecular markers of *Lr* genes were also given by ROBERT *et al*. (1999, 2000a, b).

The cluster of genes *Yr17*, *Lr37* and *Sr38* was transferred to wheat in a translocation from *Aegilops ventricosa* (2*n* = 28, $D^v$ $D^v$ $M^v$ $M^v$). It is located on the short arm of chromosome 2A (BARIANA & MCINTOSH 1993, 1994). Originally it was transferred to line VPM1 (a cross of *Aegilops ventricosa*, *Triticum persicum* and cv. Marne Desprez) and is carried by many cultivars derived from this line.

The line VPM1 had been first described as an important source of resistance to eyespot (MAIA 1967). Later, resistance to wheat rusts was revealed in cultivars derived from line VPM1. However, there is no linkage between the gene for eyespot resistance and those for resistance to rusts. Gene *Pch2* for eyespot resistance has been located on chromosome 7D (WORLAND *et al*. 1988). Other data on the location of eyespot resistance also exist (MCINTOSH *et al*. 1998), however, chromosome 2A, on which the cluster of rust resistance genes is located, has not been mentioned. On the other hand it has been found that the *A. ventricosa* segment on chromosome 2AS of line VPM1 carries also the cereal cyst nematode resis-

Supported by the Ministry of Agriculture of the Czech Republic, Project No. EP 1311, and by the Ministry of Education, Youth and Sports of the Czech Republic, Project Barrande 2000-007-1.

tance gene Cre5 (JAHIER *et al*. 2001). BONHOMME *et al*. (1995) found a gene for leaf rust resistance on a line (L22) carrying a translocation between an *A. ventricosa* segment and the chromosome arm 2AS of wheat. They provisionally designated the gene *Lr* (*L22*) and identified a RFLP marker closely linked to it. ROBERT *et al*. (1999) proved that *Lr* (*L22*) and *Lr37* were identical. Recently SEAH *et al*. (2001) described a cloned disease resistance gene-like sequence by which the gene cluster *Yr17*, *Lr37* and *Sr38* can be assayed.

The objective of our work was to verify the application of the molecular marker for the determination of the gene cluster *Yr17*, *Lr37* and *Sr38* in the Institute of Crop Production in Prague-Ruzyně before it will be used for genetic analysis of resistance within the framework of a joint French-Czech Barrande project. This project enabled us to apply the sequence of the primers after ROBERT *et al*. (1999) that are otherwise confidential.

## MATERIAL AND METHODS

The gene cluster *Yr17*, *Lr37* and *Sr38* was investigated in a set of cultivars in which gene *Lr37* has been postulated (PARK *et al*. 2001; SINGH *et al*. 2001; WINZELER *et al*. 2000), as well as in some cultivars registered in the Czech Republic, selected mainly because of the presence of western European cultivars in their pedigrees. The seed of the unregistered cultivars Hussar, Eureka, Torfrida, Renan, Rendezvous, Rapier and Brigadier originated from the Gene Bank Prague-Ruzyně, seed of registered cultivars (Table 1) from the Central Institute for Supervision and Testing in Agriculture of the Czech Republic. Cultivars were grown in the greenhouse and the second (in few cases first) leaf was used for the test. For DNA isolation from plant tissue the DNeasy Plant Mini Kit (Qiagen) was used. Plant tissue was mechanically disrupted in liquid nitrogen before the extraction. Molecular marker for *Yr17* SCAR *SC-Y15* was applied following the protocol by ROBERT *et al*. (1999). The amplified fragments were separated on 3% high resolution agarose gel (Sigma) and visualised under UV light after staining with ethidium bromide. 50bp ladder (Sigma) was loaded on the gel together with the amplified fragments.

Rust severity on selected cultivars was assessed in field trials in which the spreader was inoculated with a mixture of rust isolates separately for leaf-, stem- and yellow rust. The highest rust severity was designated by 1, the lowest by 9.

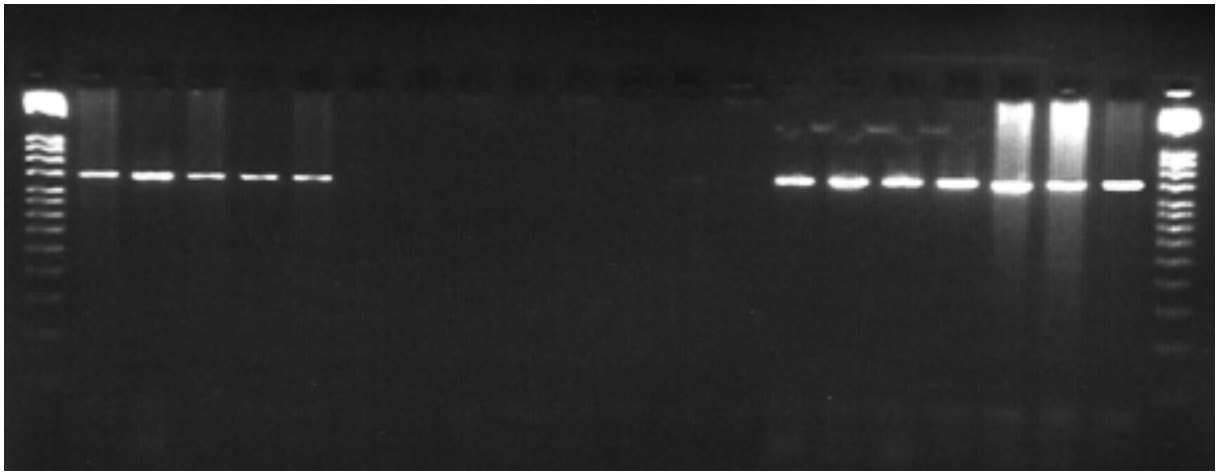
## RESULTS AND DISCUSSION

The presence of the gene cluster was proved by the molecular marker in foreign cultivars Hussar, Eureka, Torfrida, Renan, Rendezvous, Rapier and Brigadier (Fig. 1) in which it has been postulated by WINZELER *et al*. (2000), GOYEAU & PARK (1997), SINGH *et al*. (2001), PARK *et al*. (2001) and in most of them also defined by the molecular marker by ROBERT *et al*. (1999, 2000a, b). Only in cvs. Torfrida and Rapier the molecular marker was applied probably for the first time.

The application of the marker gave distinct results and strong signals (Fig. 1).

Table 1. Tested cultivars registered in the Czech Republic

| Cultivar | Pedigree | Registered | Reaction of the marker | Company |
|---|---|---|---|---|
| Samara | WW-WN 156/Regina | 1995 | – | Selgen, a. s., CR |
| Šárka | UH677/Mironovskaya nizkoroslaya//Avalon/Mironovskaya nizkoroslaya | 1997 | – | Selgen, a. s., CR |
| Contra | Kronjuwel/M.Marksman | 1998 | – | Saatzucht J. Breun, GdbR, D |
| Corsaire | Viking/Rendezvous | 1999 | + | Florimond Desprez, F |
| Apache | Axial/NRPB 84 4233 | 1999 | + | Nickerson S.A., Station de Recherches, F |
| Semper | Obelisk//CEB 8451/Arminda | 1999 | – | Cebeco Zaden B.V., NL |
| Sepstra | Orestis/Greif | 1999 | – | F. von Lochow-Petkus GmbH, D |
| Drifter | Rones/Estica | 2000 | – | Nickerson Pflanzenzucht, GmbH, D |
| Ludwig | Ares/Farmer | 2000 | – | Probstdorfer Saatzucht, GmbH, A |
| Complet | Boxer/M.Huntsman/Monopol | 2000 | + | Saatzucht Firlbeck KG, D |
| Windsor | Apollo/Gawein | 2001 | – | Saatzucht J. Breun, GdbR, D |
| Bill | multicross dihaploid | 2002 | + | Nordsaat, Saatzuchtgesellschaft, GmbH, D |



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

1 – 50bp marker, 2 – Thatcher *Lr37* near isogenic line, 3 – Corsaire, 4 – Complet, 5 – Apache, 6 – Bill, 7 – Ludwig, 8 – Contra, 9 – Semper, 10 –Windsor, 11 – Sepstra, 12 – Drifter, 13 – Samara, 14 – Šárka, 15 – Hussar, 16 – Eureka, 17 – Torfrida, 18 – Renan, 19 – Rendezvous, 20 – Rapier, 21 – Brigadier, 22 – 50bp marker

Figure 1. Samples scanned with the SCAR *SC-Y15*

Results obtained with several cultivars registered in the Czech Republic (Table 1) show that only four of them (Corsaire, Apache, Complet and Bill), all of western European origin, possess the cluster of resistance genes *Yr17*, *Lr37* and *Sr38*. They were highly resistant to yellow rust and medium resistant to leaf rust. Stem rust resistance was above average in cvs. Corsaire, Apache and Bill but low in cv. Complet (Table 3). Tested foreign cultivars not registered in the Czech Republic gave positive reactions with the marker (Table 2).

At least three hypotheses could be given to explain the stem rust susceptibility of cv. Complet, and to gain a more detailed understanding of the genetic basis of resistance to all three rusts. Recombination is the first one. A recombination of 0.5 + 0.5% cM was estimated for *Sr38* (or *Yr17*) and *Lr37* by BARIANA and MCINTOSH (1993). Of 103 lines F3 VPM1 × Harrier, one was scored as a recombinant, but its genotype was not further examined. ROBERT *et al*. (2000a, b) also found of 141 plants $F_2$ VPM1 × Thésée one recombinant event between the SCAR marker and the *Yr17* resistance gene. Another possible explanation for the stem rust susceptibility of cv. Complet may be the presence of a suppressor of the resistance gene. KERBER and GREEN (1980) found that chromosome 7D in cv. Canthatch carried a gene that inhibited an expression of stem rust resistance gene(s) on an A or B genome chromosome. Finally, a presence of genes modifying the expression of resistance in cv. Complet cannot be exclud-

Table 2. Tested cultivars not registered in the Czech Republic

| Cultivar | Pedigree | Reaction of the marker |
|---|---|---|
| Hussar | Squadron/Rendezvous | + |
| Eureka | Mirunovskaya 808/Maris Huntsman /3/ VPM-1/Moisson//Courtot | + |
| Torfrida | Rendezvous/( Moulin/Mercia ) | + |
| Renan | Mirunovskaya 808/Maris Huntsman//VPM-1/Moisson /3/Courtot | + |
| Rendezvous | VPM-1/(SIB) Hobbit//Virtue | + |
| Rapier | Maris Ranger/Mayo 64//Chile 8293/3/Maris Ranger/4/Maris Beacon | + |
| Brigadier | Squadron/Rendezvous | + |

Table 3. Rust severity in field trials (classification 1–9, 1 highly susceptible, 9 highly resistant)

| Cultivar | Yellow rust | | | Stem rust | | | Leaf rust | |
|---|---|---|---|---|---|---|---|---|
| | 1999 | 2000 | 2001 | 1999 | 2000 | 2001 | 2000 | 2001 |
| Complet | 9 | 9 | 9 | 3 | 2 | 2-3 | 5 | 5 |
| Apache | 9 | 9 | 9 | 5 | 4 | 7 | 5 | 4 |
| Bill | 9 | 9 | 9 | 6 | 6 | 8 | 5 | 6 |

ed either. BARIANA and MCIINTOSH (1994) described an effect of the cytoplasm on the expression of *Sr38*.

Yellow rust resistance governed by gene *Yr17* was overcome in Europe successively. Virulence to *Yr17* was found in the UK in 1994 (BAYLES & STIGWOOD 1994) and in France in 1998 (ROBERT *et al*. 2000a, b). After HOVMØLLER (2001) in 1997–1999 the two most common pathotypes of yellow rust in Denmark possessed virulence to *Yr17*. Virulence to *Yr19* was also recorded in Hungary (MANNINGER – pers. commun.). In the Czech Republic it has been determined in field samples of yellow rust from 2001, although cultivars possessing *Yr17* are not grown on a large scale (BARTOŠ – unpublished).

Leaf rust resistance governed by gene *Lr37* is adult plant resistance, but by certain leaf rust isolates it can be revealed already at the seedling stage. It has probably remained effective till now in all of Central Europe. Virulence at the seedling stage is common in Germany, Spain and Poland. Field resistance has been reported from Hungary, Germany and the UK in the summary of experiments carried out in the framework of COST817 (MESTERHÁZY *et al*. 2000). The level of field resistance varied considerably. Susceptibility in the field was reported from Roumania (MESTERHÁZY *et al*. 2000).

Stem rust resistance gene *Sr38* governed medium resistance in our field trials but was not effective to all stem rust isolates tested in the greenhouse (BARTOŠ – unpublished).

The foreign cultivars Apache, Bill, Complet and Corsaire with the cluster of rust resistance genes *Yr17*, *Lr37* and *Sr38* broaden the spectrum of resistance genes present in cultivars registered in the Czech Republic. Unfortunately, this happens rather late after the introduction of this resistance to western Europe where cultivars with *Yr17*, *Lr37* and *Sr38* cover a large area. For this reason the effectiveness of these resistance genes may be limited and break down soon, as has already happened with *Yr17*.

***Acknowledgement***: We thank INRA and GIE CLUB 5 for making the sequences of the *SC-Y15* primers available to us.

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Received for publication February 20, 2002
Accepted after corrections June 11, 2002

**Souhrn**

AMBROZKOVÁ M., DEDRYVER F., DUMALASOVÁ V., HANZALOVÁ A., BARTOŠ P. (2002): **Stanovení translokace genů rezistence ke rzím *Yr17*, *Lr37* a *Sr38* na pšenici molekulární sondou**. Plant Protect. Sci., **38**: 41–45.

Marker SCAR *SC-Y15* (ROBERT *et al*. 1999) jsme použili pro stanovení genů ve vazbě *Yr17*, *Lr37* a *Sr38*. Tímto markerem byla ověřena přítomnost translokace z *Aegilops ventricosa* nesoucí zmíněné geny v odrůdách Hussar, Eureka, Torfrida, Renan, Rendezvous, Rapier a Brigadier. Z odrůd nedávno registrovaných v České republice byla prokázána přítomnost uvedené translokace v odrůdách Corsaire, Apache, Complet a Bill. Odrůdy Corsaire, Apache, Complet a Bill byly vysoce či středně rezistentní ke rzi plevové a rzi pšeničné v polních pokusech hodnocených v Praze-Ruzyni v letech 1999–2001, přestože v roce 2001 byla zjištěna virulence ke genu *Yr17* při regionálním průzkumu patotypů rzi plevové. Odrůdy Corsaire, Apache a Bill měly nadprůměrnou odolnost ke rzi travní, kdežto odrůda Complet byla náchylná.

**Klíčová slova**: rzi; pšenice; geny rezistence; *Yr17*; *Lr37*; *Sr38*; molekulární marker

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