Evaluation and Mapping of a Leaf Rust Resistance Gene Derived from *Hordeum vulgare* subsp. *spontaneum*

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Abstract: Studies of marker development were performed on a doubled haploid population derived from the cross of a highly resistant line *H. spontaneum* 677 × Krona (susceptible). Previous segregation studies on F_2 and F_3 populations revealed that the resistance of *H. spontaneum* 677 was likely due to a single dominant gene. Bulked segregant analysis using AFLPs and SSRs was conducted to identify markers linked to this leaf rust resistance gene. By this approach the resistance gene was located on barley chromosome 2H with the closest markers linked at 6.1 cM (E35M54b) and 13.6 cM (Bmac0218) based on the analysis of 83 DH-lines. In order to get first hints whether this gene may be allelic to *rph16* located on chromosome 2H STS marker MWG 2133 co-segregating with *rph16* was tested but it turned out to be monomorphic. However, in a resistance test with a set of four different isolates of *Puccinia hordei, H. spontaneum* 677 showed a different reaction pattern from that of *H. spontaneum* 680, the source of *rph16*. Tests of allelism to confirm these results are in progress.

Keywords: *Hordeum vulgare*; barley; *Puccinia hordei*; leaf rust; resistance; SSRs; AFLPs; genetic mapping

Leaf rust caused by *Puccinia hordei* Otth is an important disease of barley in Central Europe. New virulent isolates as well as combinations of virulent genes have overcome most of the resistance genes known so far and only the leaf rust resistance gene *Rph7* is still effective in Europe. For this reason it is necessary to identify new sources of resistance. Resistance in *Hordeum vulgare* subsp. *vulgare* was shown to be very limited but a high variability was found in the wild progenitor *Hordeum vulgare* subsp. *spontaneum* (= *Hordeum spontaneum* = *Hvs*) (Jin *et al*. 1996; Weibull *et al*. 2003), which is a valuable source for broadening the genetic base of resistance to *P. hordei*, therefore 500 *H. vulgare* subsp. *spontaneum* accessions were screened for resistance and out of these, 38 lines with complete resistance to a set of known isolates of *P. hordei* including *Rph7* virulence were identified.

In this respect *rph16* derived from *Hordeum spontaneum* 680 was already mapped on chromosome 2H (Ivandic *et al.* 1998). The present study aims at the mapping of leaf rust resistance of *H. spontaneum* 677, which is likely due to a dominant gene (Walther *et al*. 1999).

Respective markers will be useful tools for marker-assisted selection and gene pyramiding in breeding programs for leaf rust resistance.

MATERIAL AND METHODS

Plant material. Genetic mapping was performed in a population of 83 doubled haploid lines (DH) which was produced by anther culture from F_1 plants derived from a cross between *H. spontaneum* 677 (resistant) × Krona (susceptible).

Resistance tests. The standard leaf rust isolate I-80 virulent to *Rph1*, *2*, *3*, *4*, *8*, *9*, *10*, *11* and *12*, but avirulent to the resistance gene in *H. spontaneum* 677 was used for phenotyping the *H. spontaneum* 677 × Krona mapping population.

The determination of qualitative resistance to leaf rust is carried out by means of a seedling test in the greenhouse. Seedlings were incubated with urediniospores for 24 h at 18°C and 100% humidity in a growth chamber. Plants were scored 8–10 days after inoculation according to the scale of Levine and Cherewick (1952). Infection types 0, 1 and 2 indicate host resistance and types 2-3, 3 and 4 host susceptibility. The χ^2 test was used to assess segregation ratios.

All isolates found in Europe are avirulent for *Rph7*. The standard isolate I-80 of *P. hordei* possesses a wide range of virulence (Table 1). For further differentiation of the resistant *H. spontaneum* accessions resistance tests were carried out in the U.S. Isolates (90-3, 92-7, 90-5) with virulence/avirulence patterns, that have not been observed in the *P. hordei* population present in Europe, were used (Table 1).

DNA isolation and linkage analysis. DNA samples were prepared from the fresh leaf tissue of green-house-grown barley plants. Standard procedures like CTAB-based DNA isolation were carried out as described by Saghai Maroof *et al*. (1984). Besides this, a fast small-scale DNA isolation according to Dorokhov *et al*. (1997) was applied. DNA concentration was measured on the fluorometer DyNA Quant 200 (Hoefer/Amersham Biosciences).

For marker identification bulked segregant analysis (BSA) was carried out using equal amounts of DNA from 10 resistant and 10 susceptible DH lines (Michelmore *et al*. 1991). For marker development SSRs (Ramsay *et al*. 2000) and AFLPs were used (Vos *et al*. 1995). In order to get information about the chromosomal location of the gene, 5 SSRs per chromosome were analysed in the first step.

The PCR reactions for SSRs were performed in a total volume of 20 µl in a thermal cycler PTC 200 (Biozym Diagnostics GmbH) and consisted of 50 ng template DNA, 1 × PCR buffer, 1.5mM $MgCl_2$, 0.3µM of forward and reverse primer, 200µM dNTPs, 1 unit Taq polymerase (Roche Diagnostics GmbH). The amplification products were separated in a denaturing polyacrylamide gel in a Sequi-Gen Cell (BioRad Laboratories Inc.). The DNA fragments were detected using the silver-staining method.

For AFLP analysis, template DNA (300 ng) from the parents and bulks was digested with 5 units of the restriction enzymes *Eco*RI and *Mse*I. For template preparation the selection of biotinylated DNA restriction fragments was omitted. The adapter ligation, pre-amplification and selective amplification with Cy5-labelled *Eco*RI +3 primers were carried out according to the AFLP protocol suggested by GibcoBRL. The detection of the amplified DNA fragments was performed on an automatic laser fluorescence sequencing machine (ALFexpress, Amersham Biosciences).

Linkage analysis was performed with the MAPMAKER software program, version 3.0 (Lander *et al*. 1987). The Kosambi function was used to convert recombination frequencies to map distances in centimorgans (Kosambi 1944).

RESULTS AND DISCUSSION

Reactions of the host differential lines and of the *Hvs* accessions to 4 isolates differentiating resistant lines are presented in Table 1. *H. spontaneum* 677 turned out to be resistant to isolates 92-7, 90-3 and I-80 but susceptible to isolate 90-5. In contrast to this, *H. spontaneum* 680 (*rph16*) is exclusively susceptible to isolate 90-3 and shows the same reaction as the line I 95-282-2 (*Rph15*). *H. spontaneum* 677 shows the same reaction to these 4 isolates as the line PI 531849 with the known *Rph13* gene, but in another resistance test *H. spontaneum* 677 shows to the isolates I 8-2 and I 30-1+4280 resistant reactions and PI 531849 susceptibility.

By analysing the progeny of the cross L 94 × *H. spontaneum* 677 in F_2 and F_3 a good fit to a segregation ratio of 3r:1s was observed, giving a hint to a single dominant gene encoding resistance to *P. hordei* in this line (Walther *et al*. 1999).

The results of the disease scoring of DH-lines of the *H. spontaneum* 677 × Krona cross using rust isolate I-80 are shown in Figure 1.

A segregation ratio of 50r:33s was determined. χ^2 value for a 1r:1s segregation is 3.48 and for 3r:1s segregation indicative of the presence of two resistance genes is 9.60 suggesting that one gene is involved in resistance to isolate I-80 in *H. spontaneum* 677. However, it has to be noticed that *H. spontaneum* 677 is scored 0 while Krona is scored 4. In the DH population lines showing a reduced level of resistance (infection type 0-2) or susceptibility (infection type 2-3) were also

Table 1. Reactions of a set of barley differentials and of *Hordeum spontaneum* accessions to inoculation with four isolates of *P. hordei*

Differential lines	Isolate 90-3	Isolate 92-7	Isolate 90-5	Isolate I-80
Sudan (*Rph1*)	S	S	S	S
Peruvian (*Rph2*)	S	S	S	S
Estata (*Rph3*)	R	S	R	S
Gold (*Rph4*)	S	S	S	S
Magnificent (*Rph5*)	S	S	R	R
Bowman/Bol (*Rph6*)	S	S	S	R
Cebada capa (*Rph7*)	R	S	R	R
Egypt 4 (*Rph8*)	S	S	S	S
Hor 2596 (*Rph9*)	S	S	S	S
Clipper BC8 (*Rph10*)	S	S	S	S
Clipper BC67 (*Rph11*)	S	S	S	R
Triumph (*Rph12*)	S	S	S	S
PI 531849 (*Rph13*)	R	R	S	R
PI 584760 (*Rph14*)	S	S	S	R
I 95-282-2 (*Rph15*)	S	R	R	R
***Hvs* accessions**				
H. sp. 680 (*rph16*)	S	R	R	R
H. sp. 677	R	R	S	R

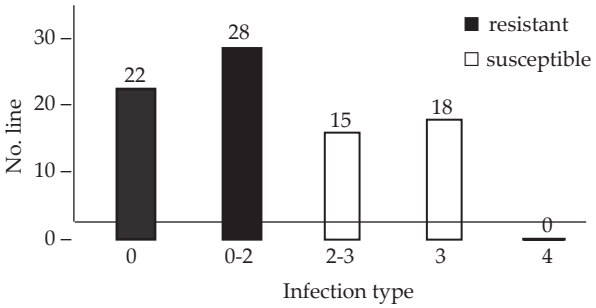

Figure 1. Reaction of 83 doubled-haploid lines derived from the cross *H. spontaneum* 677 × Krona to leaf rust isolate I-80

observed. Therefore, additional genes influencing the level of resistance may be involved.

A set of 35 previously described SSR markers was screened for detection of polymorphic bands between the resistant and susceptible bulks (Ramsey *et al*. 2000). As a result of the bulked segregant analysis polymorphisms were detected on chromosome 2H. In the next step additional SSR markers for chromosome 2H were analysed and mapped. Linkage was detected between the

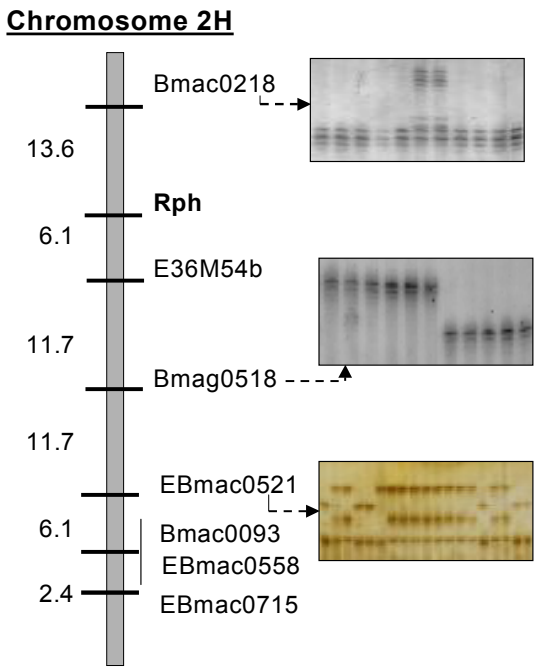

Figure 2. Partial map of barley chromosome 2H using the mapping population *H. spontaneum* 677 × Krona. Genetic distance is given in centimorgans (cM)

resistance locus and the SSR markers Bmac0218, Bmag0518, EBmac0521, EBmac0558, Bmac0093 and EBmac0715 (Figure 2). SSRs EBmac0415, Bmac0134, HVM26, HVM63, EBmac0557 and Bmag0003 were monomorphic. The closest linked SSR markers are Bmac0218 and Bmag0518, which are flanking the gene at a distance of 13.6 cM and 17.8 cM, respectively.

Because SSRs are quite distantly linked to the resistance derived from *H. spontaneum* 677, AFLP marker saturation was conducted using about 200 AFLP primer combinations.

Polymorphic DNA fragments between the parents and the bulks were amplified by using the AFLP primer combinations E39M58, E42M48, E37M33 and E36M54. Out of them the AFLP marker E36M54b was mapped at a distance of 6.1 cM to the resistance gene. The total map including 6 SSR markers and one AFLP marker constitutes 51.4 cM. The map position of the leaf rust resistance gene is shown in Figure 2.

Unfortunately, linkage detected up to now is quite loose. Therefore, additional AFLPs will be screened and besides this phenotypic analysis will be repeated on those genotypes showing no unequivocal reactions, i.e. 2–3 scores. Analysing 42 DH lines of this population which were scored resistant (infection type 0) or susceptible (infection type 3) closer linkage was observed, i.e. 4.8 cM for Bmac0218 and 9.7 cM for Bmag0518.

As the gene of *H. spontaneum* 677 like *rph16* is located on chromosome 2H, the STS marker MWG 2133 developed by Ivandic *et al*. (1998) and co-segregating with *rph16* was analysed but it turned out to be monomorphic in our DH-population. Therefore, no information could be obtained whether resistance of *H. spontaneum* 677 was located at exactly the same chromosomal region. As it has been found recently that *Rph15* and *rph16* are allelic (Weerasena *et al*. 2004), extensive tests for allelism will be carried out in future.

Without respect to chromosomal location resistance to leaf rust derived from *H. spontaneum* 677 is likely to be prospective for future breeding programmes as it shows a different resistance spectrum in comparison with the *Rph15*/*rph16* locus.

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Abstrakt

Kopahnke D., Nachtigal M., Ordon, F., Steffenson B. J. (2004): **Zhodnocení a zmapování genu rezistence ke rzi ječné odvozeného z** *Hordeum vulgare* subsp**.** *spontaneum*. Czech J. Genet. Plant Breed., **40**: 86–90.

Testy nutné pro získání markeru byly prováděny na populaci dihaploidů odvozených z křížení vysoce rezistentní linie *H. spontaneum* 677 × Krona (náchylná odrůda). Předcházející studie štěpení F_2 a F_3 populací ukázaly, že rezistenci *H. spontaneum* pravděpodobně řídí jeden dominantní gen. Byla provedena analýza segregantů (BSA) s použitím AFLP a SSR s cílem identifikovat markery, které jsou ve vazbě s tímto genem rezistence ke rzi ječné. Na základě analýzy 83 dihaploidních linií byl gen rezistence lokalizován na chromozomu 2H ječmene s nejbližším markerem ve vazbě 6.1 cM (E35M54b) a 13.6 cM (Bmac0218). Aby byly získány předběžné údaje o tom, zda tento gen může být alelický s genem *rph16*, lokalizovaným na chromozomu 2H, byl k testům použit STS marker MWG 2133, kosegregující s *rph16*; ukázalo se však, že je monomorfní. V testu rezistence čtyřmi různými izoláty *Puccinia hordei* jevilo *H. spontaneum* 677 odlišné spektrum reakcí od *H. spontaneum* 680, zdroje *rph16*. Probíhají testy alelismu k ověření získaných výsledků.

Klíčová slova: *Hordeum vulgare*; ječmen; *Puccinia hordei*; rez ječná; rezistence; SSR; AFLP; genetické mapování

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