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ORIGINAL PAPER

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Conversion of an amplified fragment length polymorphism marker into a co-dominant marker in the mapping of the *Rph15* gene conferring resistance to barley leaf rust, *Puccinia hordei* Otth.

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Abstract Leaf rust, caused by *Puccinia hordei*, is an important disease afflicting barley (*Hordeum vulgare*) in many production regions of the world. The leaf rust resistance gene *Rph15* was identified in an accession of wild barley (*Hordeum vulgare* subsp. *spontaneum*) and is one of the most broadly effective resistance genes known. Using amplified fragment length polymorphism (AFLP) and simple sequence repeat markers, *Rph15* was mapped to chromosome 2HS in an F_2 population derived from a cross between Bowman (*Rph15*), a Bowman backcross-derived line carrying *Rph15,* and the susceptible cultivar Bowman. AFLP marker P13M40 co-segregated with *Rph15* in this mapping population and two others involving Bowman (*Rph15*) and cultivars Proctor and Nudinka. The dominant AFLP marker P13M40 was converted to a co-dominant PCR-based marker that may be useful in breeding programs employing marker-assisted selection. The allelic relationship between *Rph15* and the gene *Rph16*, also mapping to chromosome 2HS, was studied. The lack of segregation in F_2 progeny derived from the two resistance sources indicates that *Rph15* and *Rph16* are alleles of the same locus.

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Introduction

The fungal pathogen *Puccinia hordei* Otth. is the causal agent of barley leaf rust, an economically important disease afflicting barley (*Hordeum vulgare*) in many parts of the world (Clifford 1985). Under severe epidemic conditions, leaf rust can reduce the yield of susceptible cultivars by up to 62% (Cotterill et al. 1992a). Deployment of resistant cultivars is the best means of control for this disease. Evaluations of the *Hordeum* gene pool (*H. vulgare*, its wild progenitor *H. vulgare* subsp. *spontaneum*, and *H. bulbosum*) have resulted in the identification of 19 major race-specific resistance genes named *Rph1–Rph19*. However, only a few have been deployed in commercial cultivars. *Rph2*, *Rph3*, *Rph4*, *Rph7*, and *Rph9.z* (formerly *Rph12*) have been used in Europe (Dreiseitl and Steffenson 2000); *Rph2*, *Rph6*, and *Rph7* in the United States (Steffenson et al. 1993); *Rph2*, *Rph4*, *Rph7*, *Rph9.z, and Rph19* in Australia (Cotterill et al. 1994; R. Park, personal communication); and *Rph3* and *Rph9.z* in New Zealand (Cromey and Viljanen-Rollinson 1995).

Most of the reported leaf rust resistance genes have been assigned to a chromosome or specific chromosome region. In earlier studies, this was achieved by trisomic analysis or linkage of the resistance gene with previously positioned loci. *Rph1*, *Rph4* and *Rph5* were first positioned to chromosomes 2H, 1H and 3H, respectively, by trisomic analysis (Tuleen and McDaniel 1971). *Rph3* and *Rph9.z* were positioned on chromosomes 7H and 5H, respectively, through their linkage with previously mapped morphological markers (Jin et al. 1993). *Rph10* and *Rph11* were positioned on chromosomes 3H and 6H, respectively, through their linkage with isozyme markers (Feuerstein et al. 1990). The development of various molecular markers for barley has greatly facilitated the precise mapping of leaf rust resistance genes. Using molecular markers, *Rph2* was mapped to chromosome 5H

(Borovkova et al. 1997), *Rph5, Rph6* (now designated *Rph5.f*) and *Rph7* to the telomeric region of chromosome 3H (Brunner et al. 2000; Graner et al. 2000; Mammadov et al. 2003; Zhong et al. 2003), *Rph9.i* and *Rph9.z* to chromosome 5H (Borovkova et al. 1998), *Rph16* to chromosome 2H (Ivandic et al. 1998) and *Rph19* to chromosome 7H (Park and Karakousis 2002).

At present, many of the leaf rust resistance genes derived from cultivated barley have limited value for plant breeding because *P. hordei* pathotypes with virulence for them have appeared (Cotterill et al. 1992b; Steffenson et al. 1993; Dreiseitl and Steffenson 2000). In Europe, *Rph7* still gives an effective level of leaf rust resistance; however, virulence for this gene has been reported in Israel (Golan et al. 1978), Morocco (Parlevliet et al. 1981), and the United States (Steffenson et al. 1993). Wild barley (*H. vulgare* subsp. *spontaneum*) collections have been evaluated by several research groups in an effort to identify leaf rust resistance with potentially greater durability (Manisterski et al. 1986; Feuerstein et al. 1990; Moseman et al. 1990; Jin et al. 1995). Several new resistance genes were identified from this research and designated *Rph10*, *Rph11*, *Rph15* and *Rph16* (Feuerstein et al. 1990; Chicaiza 1996; Franckowiak et al. 1997; Ivandic et al. 1998). Unfortunately, leaf rust pathotypes with virulence for some of these recently identified *Rph* genes (e.g., *Rph10* and *Rph11*) are fairly common (Fetch et al. 1998; B. Steffenson, unpublished). *Rph15* was derived from PI 355447, a *H. vulgare* subsp. *spontaneum* accession collected in Israel (Jin et al. 1995; Chicaiza 1996; Franckowiak et al. 1997). This gene was evaluated for its reaction to a diverse collection of over 350 *P. hordei* isolates from around the world and conferred resistance to all but one of the isolates (90–3 from Israel) (Fetch et al. 1998; B. Steffenson, unpublished). Thus, *Rph15* represents one of the most widely effective leaf rust resistance genes reported from *Hordeum* and may therefore be useful in barley-improvement programs.

Information on the chromosomal location of resistance genes can be useful in breeding for resistance. Moreover, the identification of closely linked markers for such genes can facilitate the selection and transfer of leaf rust resistance in programs employing marker assisted selection. Here, we report the genetic mapping of *Rph15* to chromosome 2HS of barley, and the development of a co-dominant PCR-based marker suitable for the detection of this gene in practical breeding programs. In addition, the allelic relationship between *Rph15* and another leaf rust resistance gene, *Rph16*, mapping to the same region of chromosome 2H, was resolved.

Materials and methods

Plant material

A Bowman (PI 483237) backcross-derived line carrying *Rph15* (I95–282–2) was obtained from the cross PI 355447/5×Bowman and provided by J.D. Franckowiak, North Dakota State University, Fargo, ND (Chicaiza 1996; J. D. Franckowiak and B. Steffenson, unpublished). The backcrossed-derived line I95–282–2 is hereafter referred to as Bowman (*Rph15*). Bowman (*Rph15*) was crossed again to Bowman, and the resulting F_2 progeny (in this case BC_6F_2) were used for mapping *Rph15*. F_2 progeny were scored as resistant or susceptible to leaf rust isolate ND97–21. Resistant F_2 progeny were increased and then phenotyped again in the F_3 generation to identify homozygous resistant F_2 plants. Crosses were also made between Bowman (*Rph15*) and the leaf rust-susceptible barley cultivars Proctor (PI 467822) and Nudinka (PI 428495). Homozygous resistant and susceptible segregants were also identified for these populations as described above. A set of ditelosomic wheat-barley addition lines, developed from the wheat cultivar Chinese Spring and barley cultivar Betzes, were obtained from A.K.M.R. Islam, University of Adelaide, Australia (Islam 1983). These lines were used to determine the chromosomal location of molecular markers linked to the resistance gene. Preliminary mapping information suggested that *Rph15* may reside near *Rph16* (Ivandic et al. 1998) on chromosome 2HS. To resolve the allelic relationship between the resistance genes, crosses were made between Bowman (*Rph15*) and HS084, the source of *Rph16*. Seeds from HS084 were provided by A. Graner, Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany.

Resistance tests

Leaf rust isolate ND97–21 (virulence/avirulence formula of *Rph1*, *Rph4*, *Rph8*/*Rph2*, *Rph3*, *Rph5*, *Rph6*, *Rph7*, *Rph9*, *Rph10*, *Rph11*, *Rph12*, *Rph13*, *Rph14*, *Rph15*) was used for phenotyping the Bowman (*Rph15*)×Bowman mapping population. In preliminary experiments, this isolate elicited clear resistant and susceptible infection types (ITs) on Bowman (*Rph15*) (ITs of 0;) and Bowman (ITs of 3–4), respectively. Eight-day-old seedlings were inoculated with urediniospores from isolate ND97–21 and then incubated in the dark for 18 h at 20°C in growth chambers where the relative humidity (RH) was maintained near 100%. Plants were then incubated in a growth chamber at 20–22°C with 70–80% RH and a 16-h photoperiod. ITs were scored 10 days after inoculation using the rating scale of Levine and Cherewick (1952). ITs of 0, 0;, 1 and 2 were considered indicative of resistance, and ITs of 3 and 4 were considered indicative of susceptibility. The chi-square test was used to assess segregation ratios for goodness of fit to expected ratios. A test of allelism between *Rph15* and *Rph16* was performed with *P. hordei* isolate ND8702 (virulence/avirulence formula for *Rph1*, *Rph4*, *Rph8*, *Rph10*, *Rph11*/*Rph2*, *Rph3*, *Rph5*, *Rph6*, *Rph7*, *Rph9*, *Rph12*, *Rph13*, *Rph14*, *Rph15*) according to the methods of Zhong et al. (2003).

Linkage and molecular marker techniques

Linkage was expressed in centimorgans (cM) and was calculated using the Kosambi mapping function (Kosambi 1944). Linkage was initially identified by a bulked segregant procedure (Michelmore et al. 1991), and further mapping was done on the resistant and susceptible F2 segregants. Amplified fragment length polymorphism (AFLP) markers were screened essentially as described (Vos et al. 1995), except that ^{32}P-ATP was used instead of ^{33}P-ATP for the labeling of *Pst*I primers. *Pst*I primers used for AFLP were P11–P26 and *Mse*I primers were M31–M78. Relevant primers were as follows:

P12 P12 5′-GACTGCGTACATGCAGAC
P13 P13 5′-GACTGCGTACATGCAGAG
P20 P20 5′-GACTGCGTACATGCAGGC
P22 P22 5′-GACTGCGTACATGCAGGT
M40 M40 5′-GATGAGTCCTGAGTAAAGC
M49 M49 5′-GATGAGTCCTGAGTAACAG
M63 M63 5′-GATGAGTCCTGAGTAAGAA
M71 M71 5′-GATGAGTCCTGAGTAAGGA.

The MWG2133 sequence was amplified using primers 5′-GCTGGAAAGTATGTGTATGAG and 5′-GATGCTGTACTTACAGATCC. Microsatellite markers developed by Liu et al. (1996) and Ramsay et al. (2000) were selected and used according to their published protocols.

Conversion of AFLP into cleaved amplified polymorphic sequence (CAPS) markers

Polymorphic AFLP bands were prepared from polyacrylamide gels by cutting out the band with a scalpel. A piece of the band was then subjected to re-amplification using the same primer combinations as in the selective amplification. Re-amplified AFLP bands were either sequenced directly or cloned using a TOPO-TA cloning kit (Invitrogen, Carlsbad, Calif.). Sequencing was performed using a Perkin-Elmer 377 DNA sequencer (Applied Biosystems, Foster City, Calif.). Based on the internal sequence of the polymorphic AFLP bands, PCR primers were designed for genomic amplification of the internal sequence. Primer sequences used for the P13M40CAPS marker were 5′-ATTCTCGAAGCTCCGCC and 5′-GCACCAAGAAGGGAGCG. Amplified bands from both parents were sequenced in this manner and internal single nucleotide polymorphisms were identified. For *Nhe*I CAPS, cleavage was performed directly in the PCR mixture without any fragment purification, and cleavages were analyzed on agarose gels. The PCR mixture contained 1×PCR Buffer II supplemented with 1.5 mM $MgCl_2$ and 5 U AmpliTAQ Gold (all obtained from Applied Biosystems), 0.2 mM of each dNTP, 0.2 μM of each primer and 40 ng DNA in a volume of 20 μl. The reaction was first heated at 94°C for 10 min and then the cycling parameters were 94°C for 30 s, 58°C for 30 s and 72°C for 40 s, repeated 40 times.

Results

AFLP marker screen on bulked segregants

Bulked segregant analysis with AFLPs was applied to the Bowman (*Rph15*)×Bowman F_2 population segregating for *Rph15*. Four homozygous resistant F_2 progeny formed the resistant bulk, and nine homozygous susceptible F_2 progeny formed the susceptible bulk. In total, 576 *Pst*I+2 and *Mse*I+3 primer combinations were screened for detection of polymorphic bands between the resistant and susceptible bulks. Nine AFLP bands were identified as polymorphic between the two bulks. Of these, four bands were detected in the resistant bulk, and five were detected in the susceptible bulk. Only the four bands in the resistant bulk, i.e. those linked in coupling with *Rph15*, were used for mapping. These four AFLP fragments were cut from the polyacrylamide gel, re-amplified using the same primers as in the AFLP reaction and sequenced. Polymorphic AFLP fragments detected with primer combinations P20M49, P12M71, and P22M63 were relatively short, approximately 100 bp. One band detected with the primer combination P13M40 was significantly longer at 276 bp (Fig. 1A).

Conversion of the P13M40 AFLP marker to a co-dominant marker

The sequences of the AFLP markers P20M49, P12M71 and P22M63 were considered to be too short for reliable conversion to co-dominant markers. The P13M40 band was sequenced both before and after cloning. No ambiguities were found that could otherwise have indicated the presence of multiple bands. Primer sequences were obtained from the ends of the P13M40 AFLP fragment and used for the amplification of the corresponding DNA from Bowman, the susceptible parent that lacks the polymorphic P13M40 AFLP band. A DNA sequence linked to *Rph15* was obtained in this manner from both parents used in the mapping population (Fig. 2); the NCBI database accession number for P13M40 from Bowman (*Rph15*) is AY360395 and for P13M40 from Bowman is AY360396. A polymorphism found in the middle of this sequence is located in a recognition site for restriction enzyme *Nhe*I and could therefore be used to convert the AFLP marker into a CAPS marker (Konieczny and Ausubel 1993). The segregation of the CAPS marker obtained, P13M40 CAPS, in a set of F2 segregants is

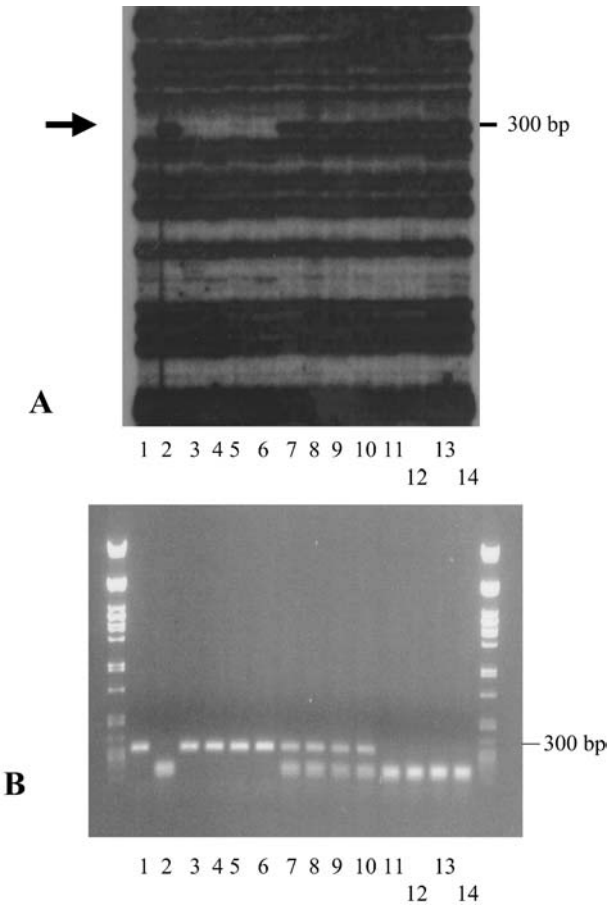

Fig. 1 **A** Segregation of the amplified fragment length polymorphism (AFLP) marker P13M40 in a set of F2 segregants derived form the cross Bowman (*Rph15*)×Bowman. *1* Bowman, *2* Bowman (*Rph15*), *3–6* susceptible F2 segregants, *7–10* heterozygous resistant F2 segreants, *11–14* homozygous resistant F2 segregants. The location of the relevant AFLP band is indicated by the *arrow*. **B** The AFLP marker P13M40 was converted to the CAPS marker P13M40 CAPS, and tested on the same DNA samples as in **A**. Amplified DNA was digested with *Nhe*I and separated on an agarose gel. Size marker is lambda-*Pst*I

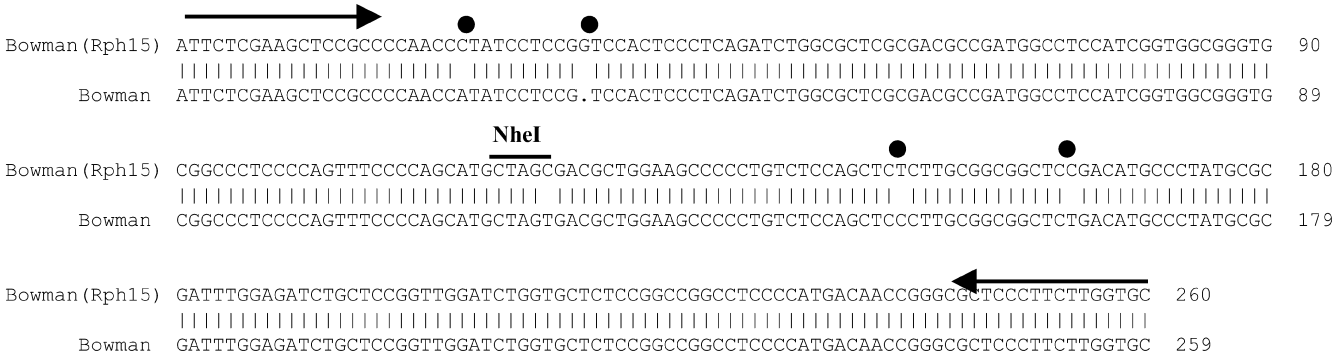

Fig. 2 Alignment of the internal sequence of the P13M40 AFLP fragment derived from the Bowman (*Rph15*) line to the corresponding sequence amplified from Bowman. Primer sites are indicated at the ends of the sequence. The polymorphism at an *Nhe*I *restriction enzyme recognition site* is indicated in the middle of the sequence. Other polymorphisms are indicated by *dots* above the sequence

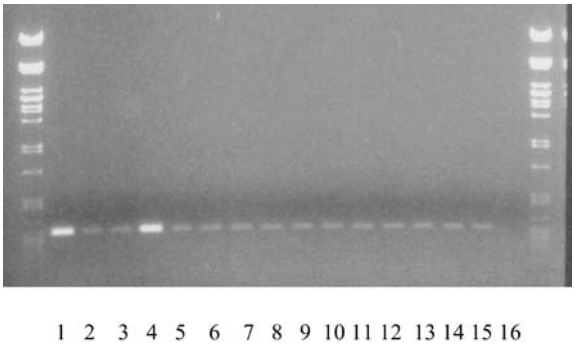

Fig. 3 Amplification of the P13M40 CAPS marker from a set of ditelosomic wheat-barley addition lines, containing chromosome arms of barley cultivar Betzes introduced into the wheat cultivar Chinese Spring. Above background amplification was detected in the addition line carrying barely chromosome 2HS. *1* Betzes, *2* Chinese Spring, *3–15* addition lines carrying barley chromosomes 1HS, 2HS, 2HL, 3HS, 3HL, 4HS, 4HL, 5HS, 5HL, 6HS, 6HL, 7Hα and 7Hβ, respectively. Sample *16* is a water control

shown in Fig. 1B. In total, five polymorphisms were detected between the two parents in the sequenced band and these have been indicated in the alignment of these sequences in Fig. 2.

Amplification from wheat-barley ditelosomic addition lines

Assignment of the P13M40 marker to the right chromosome arm of barley was performed by amplification of this marker from a set of ditelosomic wheat-barley addition lines. The P13M40 marker was thereby found to be amplified from the addition line 2HS (Fig. 3), although a slight background amplification could be seen from all wheat lines, including the parent cultivar Chinese Spring. We tentatively assigned the P13M40 marker and *Rph15* to the barley chromosome 2HS and proceeded to test this with AFLP and single sequence repeat (SSR) markers that had previously been mapped to this chromosome arm.

Mapping of *Rph15* in the Bowman (*Rph15*)×Bowman population

We identified 208 susceptible segregants out of 813 F2 lines from the Bowman (*Rph15*)×Bowman cross, representing a sixth backcross of the *Rph15* gene into the recurrent parent Bowman background. The segregation conformed well, with a 3:1 segregation, as expected for a fully dominant resistance gene. A chi-square test for a 1:1 segregation rejected this hypothesis at $P<0.005$. A chi-square test for a 3:1 segregation did not reject the test hypothesis at $P>0.5$. In addition, we included 30 resistant segregants as a reference in the mapping population. The mapping of *Rph15* was performed on this population using the P13M40 CAPS marker that had been previously developed. We also used a set of previously described AFLP and microsatellite markers from chromosome 2HS and attempted to place them on the map with *Rph15*. The closest marker identified was the P13M40 CAPS marker, which co-segregated with *Rph15* in this mapping population of 200 susceptible segregants (Fig. 4A). Additional markers identified from the AFLP screen were P20M49, P12M71 and P22M63, which mapped 10.9 cM, 2.6 cM and 1.6 cM proximal to *Rph15,* respectively. The five additional AFLP markers that were linked in repulsion were not tested on the mapping population. In addition to the above markers, the previously mapped AFLP marker E4140–8 (Castiglioni et al. 1998) was found to be linked at 2.6 cM proximal to *Rph15*. An additional marker was obtained from MWG2133, an RFLP marker that was amplified and sequenced based on primers from the MWG2133 sequence deposited in the NCBI database (Ivandic et al. 1998; accession numbers AJ234791 and AJ234792). The MWG2133 sequence from Bowman (*Rph15*), deposited in the NCBI database under accession number AY217120, contains an indel of 25 bp, making it possible to use this as a sequence tagged site (STS)

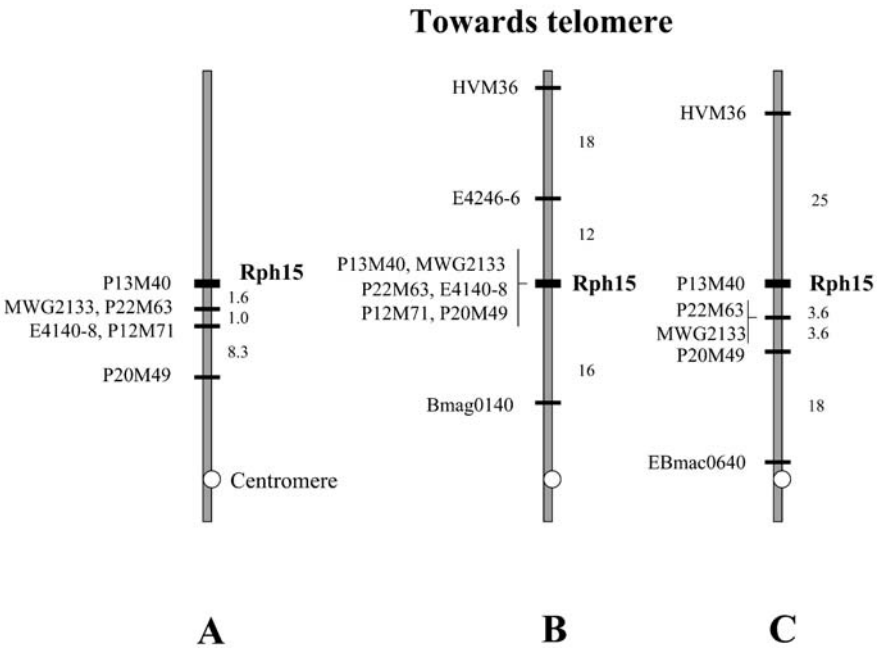

Fig. 4 Map of barley chromosome 2HS in the area surrounding *Rph15* using three mapping populations. **A** Bowman (*Rph15*)×Bowman **B** Bowman (*Rph15*)×Proctor **C** Bowman (*Rph15*)×Nudinka. Linked AFLP markers identified from the marker screen were P22M63, P12M71, P20M49, and P13M40. The approximate location of the centromere is indicated. Genetic distance is given in centimorgans (cM)

marker as was done by Ivandic et al. (1998). The MWG2133 marker mapped 1.6 cM proximal to *Rph15* and co-segregated with the P22M63 marker. With the exception of P13M40, which co-segregated with *Rph15*, all markers mapped proximal to the target gene. No SSR markers were found that detected polymorphisms between the parents of this cross. The dearth of markers identified was likely due to the highly homozygous structure of the BC_6F_2 population used in the mapping.

Mapping of *Rph15* in the Bowman (*Rph15*)×Proctor and Bowman (*Rph15*)×Nudinka populations

To confirm the results obtained in the Bowman (*Rph15*)×Bowman population and to exploit further AFLP markers previously mapped by Castiglioni et al. (1998), we tested AFLP and microsatellite markers on F2 populations from the crosses Bowman (*Rph15*)×Proctor (the Proctor mapping population) and Bowman (*Rph15*)×Nudinka (the Nudinka mapping population). The Proctor mapping population consisted of 28 susceptible F2 plants and 24 resistant F2 plants. The Nudinka mapping population consisted of 14 susceptible F2 plants and 24 resistant F2 plants. The resistant segregants were only included as a reference, whereas the actual mapping was based on the susceptible segregants. AFLP markers P13M40, P20M49, P22M63 and P12M71, previously identified in the initial AFLP marker screen, also detected polymorphisms between Bowman (*Rph15*) and Proctor. However, no recombinants could be detected between these markers and *Rph15* in the Proctor mapping population (Fig. 4B). Markers MWG2133 and E4140–8 were also polymorphic and co-segregated with *Rph15* in this population. AFLP marker E4246–6, previously mapped to 2HS by Castiglioni et al. (1998), was polymorphic in the Proctor mapping population and mapped 12 cM distal to *Rph15*. The SSR markers HVM36 and Bmag0140 were also polymorphic in this mapping population and were positioned 30 cM distal and 16 cM proximal to *Rph15*, respectively. In the Nudinka mapping population, the AFLP markers P13M40, P22M63 and P20M49 were polymorphic, whereas E4140–8 and P12M71 were not. The P13M40 CAPS marker also co-segregated with *Rph15* in this mapping population, whereas markers P22M63 and P20M49 detected one and two recombinants, respectively (Fig. 4C). The SSR markers HVM36 and EBmac0640 were polymorphic in the Nudinka mapping population, mapping 25 cM distal and 25 cM proximal to *Rph15*, respectively. The two recombinants between *Rph15* and P20M49 in relation to EBmac0640 allowed us to position markers P22M63, MWG2133 and P20M49 proximal to *Rph15*. This result suggests that markers P12M71 and E4140–8 should also be positioned proximal to the gene (Fig. 4A).

A test for allelism between *Rph15* and *Rph16*

Since *Rph15* was found to reside near *Rph16* (Ivandic et al. 1998) on chromosome 2HS, crosses were made between Bowman (*Rph15*) and HS084 (source of *Rph16*) to test for allelism. No susceptible plants were found in 1027 F_2 progeny tested, suggesting that *Rph15* and *Rph16* are allelic.

Discussion

Rph15 is a broadly effective leaf rust resistance gene that was originally derived from an *H. vulgare* subsp. *spontaneum* accession from Israel. Markers linked to *Rph15* were identified using bulked segregant analysis and were subsequently mapped to chromosome 2HS using wheat-barley addition lines. Bulked segregant analysis is an efficient method by which to identify markers linked to a target gene and is superior to methods involving near-isogenic lines (Giovannoni et al. 1991; Michelmore et al. 1991). The present investigation confirms the utility of bulked segregant analysis for initial marker screens, even in highly homozygous backcross populations as used here. Indeed, bulked segregant analysis, together with the use of wheat-barley addition lines, represents one of the fastest and most reliable methods for mapping an uncharacterized gene to a chromosome arm. However, one potential problem with this procedure is the presence of wheat sequences that amplify background wheat DNA, thus complicating the interpretation of the amplification pattern. Such was the case with the P13M40 marker (Fig. 3). This finding was not unexpected given the significant sequence similarity that exists between the two cereal species (Ahn et al. 1993).). A more reliable means of assigning the right chromosome position would seem to be the use of AFLP polymorphisms between Proctor and Nudinka (Castiglioni et al. 1998). This procedure can also be conveniently employed as part of an extensive AFLP marker screen.

The P13M40 AFLP marker co-segregated with *Rph15* in all three populations. Since this marker has potential utility in marker-assisted selection, it was converted to the co-dominant CAPS marker P13M40 CAPS. The P13M40 marker comprises 276 bp, which in this case was large enough to detect polymorphisms via restriction enzymes. In most AFLP profiles, the mean AFLP band length is around 150 bp, with few bands larger than 300 bp. A substantial portion of the polymorphic bands may, therefore, be too small for efficient conversion to a co-dominant marker. For example, of the six linked AFLP bands detected in this study, only P13M40 was considered long enough for direct conversion to a CAPS marker. A cut-off limit of 200 bp for AFLP to CAPS conversion was also used by De Jong et al. (1997). Similar considerations led Caranta et al. (1999) and Ballvora et al. (2001) to develop CAPS markers from AFLP markers of sizes 645 bp, 200 bp and 700 bp, respectively, however, Dussle et al. (2002) converted an AFLP marker to a 152 bp CAPS marker. This is probably close to the lower size limit for this marker type. Sometimes, polymorphic AFLP bands may arise due to indels within the AFLP fragment. In such cases, co-dominant STS markers can be developed even from short AFLP fragments (Paltridge et al. 1998; Prins et al. 2001; Dussle et al. 2002). To convert short AFLP bands to CAPS markers, several investigators have extended AFLP sequences using inverse PCR. This approach was successfully applied by Bradeen and Simon (1998), who managed to convert an AFLP marker linked to the carrot Y2 locus to a co-dominant marker based on the identification of an indel in the extended sequence. Similar approaches were also adopted by Bendahmane et al. (1997), De Jong et al. (1997), Bensch et al. (2002) and Wen et al. (2002). Alternatively, an AFLP fragment could be used as a hybridization probe to detect polymorphisms using Southern blot analysis. This approach was successfully performed by Mienie et al. (2002). An AFLP fragment can also be used to obtain genomic clones spanning the AFLP sequence. This procedure does, however, rely on the AFLP fragment being derived from a single-copy sequence in the genome. Most polymorphic AFLP fragments do indeed correspond to single-copy sequences in the genome. This seems to be especially true when methylation-sensitive enzymes such as *Pst*I are used.

Rph15 is one of the most widely effective leaf rust resistance genes reported from *Hordeum*. From over 350 different isolates of *P. hordei* evaluated, only one demonstrated virulence for *Rph15*. This virulent isolate (90–3) was collected in Israel, where the wild barley host and pathogen have co-evolved for thousands of years (Anikster and Wahl 1979). *Rph* genes derived from *H. vulgare* subsp. *spontaneum* may not confer any greater durability than those derived from cultivated barley. For example, *Rph10* and *Rph11* were derived from *H. vulgare* subsp. *spontaneum*, yet over 35% of the *P. hordei* isolates tested carried virulence for these genes (B. Steffenson, unpublished). Nevertheless, the broad resistance spectrum provided solely by *Rph15* may be useful in some barley production areas of the world. Alternatively, *Rph15* may be combined or "pyramided" with other genes as a means to expand the resistance spectrum against *P. hordei* and increase durability. Since the phenotype conferred by different resistance genes may be similar, their presence in a cultivar cannot be easily verified when several of them occur together as in the gene-pyramiding approach. Molecular markers linked to the resistance genes are therefore a prerequisite for efficient gene pyramiding. The PM13M40 CAPS marker identified in this study co-segregated with *Rph15* in three different populations and will be useful in breeding programs utilizing marker-assisted selection.

No segregation was observed among 1027 F_2 progeny derived from the cross of Bowman (*Rph15*)×HS084 (the source of *Rph16*). This strongly suggests that *Rph15* and *Rph16* are alleles of the same locus. HS084 was reported to express resistance to all leaf rust isolates in the collection at Aschersleben, Germany, including those with wide virulence from Israel and Morocco (Ivandic et al. 1998). Only a few isolates of *P. hordei* have been tested on Bowman (*Rph15*) and HS084 together in comparative studies. Both sources were resistant to the widely virulent cultures 89–3, 90–5, Neth28, 92–6 and 92–7, but susceptible to 90–3 (B. Steffenson, unpublished). Distinct IT differences were detected between the resistance sources to isolates 89–3 and Neth28: HS084 exhibited ITs of 0;N, whereas Bowman (*Rph15*) exhibited ITs of 1,2c or 2,1c. Based on the data from this study and

the locus/allele nomenclature system of Franckowiak et al. (1997), *Rph16.ae* (i.e. the original locus and allele designations of the gene) should be renamed as *Rph15.ae*.

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