Research Note

RFLP Markers Linked to the Durable Stem Rust Resistance Gene *Rpg1* in Barley

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The gene, *Rpg1,* conferring stable resistance in barley to the wheat stem rust pathogen (*Puccinia graminis* f. sp. *tritici*) was mapped using two doubled haploid populations. *Rpg1* mapped to the extreme subteleomeric region of barley chromosome 1P 0.3 and 1.1 cM proximal from the molecular markers ABG704 and plastocyanin (*Plc*), respectively, and 2.2 cM distal from MWG036B. The closest marker, ABG704, was sequenced and PCR-based markers were developed.

Additional keywords: Hordeum vulgare.

Stem rust, caused by the fungus, *Puccinia graminis* Pers.:Pers f. sp. *tritici* Eriks. & E. Henn., has historically been one of the most devastating diseases of barley (*Hordeum vulgare* L.) in the northern Great Plains of the United States and Canada. This disease has been successfully controlled for over 50 yr through the widespread use of the resistance gene *Rpg1,* in nearly every commercial barley cultivar of the northern Great Plains (Steffenson 1992). The approximate location of *Rpg1* to the plus (short or β) arm of chromosome 1 has been previously reported (Jin *et al.* 1993; Kleinhofs *et al.* 1993). Here we describe detailed mapping of the *Rpg1* gene with respect to three RFLP markers, including the identification and characterization of a very closely linked marker, ABG704. These results represent an initial step toward our ultimate goal to isolate and characterize this durable stem rust resistance gene.

Two doubled haploid (DH) populations (Steptoe × Morex [S/M] and Harrington × TR306 [H/T]) were used to map *Rpg1.* One hundred fifty DH lines from each cross were developed by the *Hordeum bulbosum* technique (Chen and Hayes 1989) and provided by P. Hayes, Oregon State University, Corvallis, and an additional 101 lines were derived from the S/M cross by the anther culture technique (Dexaux 1987) and provided by P. Devaux, Florimond Desprez Co., Cappelle-En-Pevele, France. Stem rust inoculations were made with pathotype Pgt-MCC of *P. g.* f. sp. *tritici* according to the methods of Steffenson *et al.* (1993). Stem rust infection phenotypes have been described and were determined 12 days after inoculation according to the system of Stakman *et al.* (1962) as modified by Steffenson *et al.* (1993) for barley.

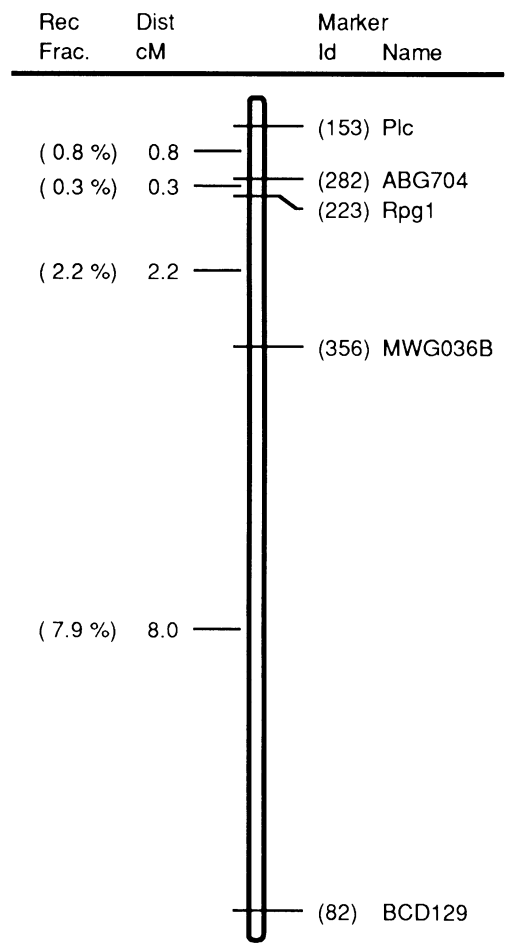

Fig. 1. A map of the telomeric region of barley chromosome 1P (β or short arm) showing the linkage relationships of the stem rust resistance gene *Rpg1* to the RFLP markers *Plc,* ABG704, MWG036, and BCD129. The *Plc* marker is the most telomeric and the BCD129 marker is closest to the centromere. Linkage analyses were made using the Macintosh II version of MAPMAKER (Lander *et al.* 1987) and G-MENDEL (v. 2) (Liu and Knapp 1990). Conversion of recombination values to centimorgans (cM) was with the Kosambi function (Kosambi 1944).

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The DH lines segregated into two distinct phenotypic groups in response to infection. One group exhibited low (resistant) infection types (0;, 1 1,0; 1,2 and 2,1 like the parents Morex and TR306) and the other high (susceptible) infection types ($3,3^-$,$3,3^+$ and 3^-,2 like the parents Steptoe and Harrington). The ratio of resistant to susceptible lines was 1:1, $\chi^2 = 0.6$ ($0.25 < P < 0.5$).

The RFLP techniques have been previously described (Kleinhofs *et al.* 1993). The probes *Plc,* ABG704, MWG036, and BCD129 were used in this study because they mapped to

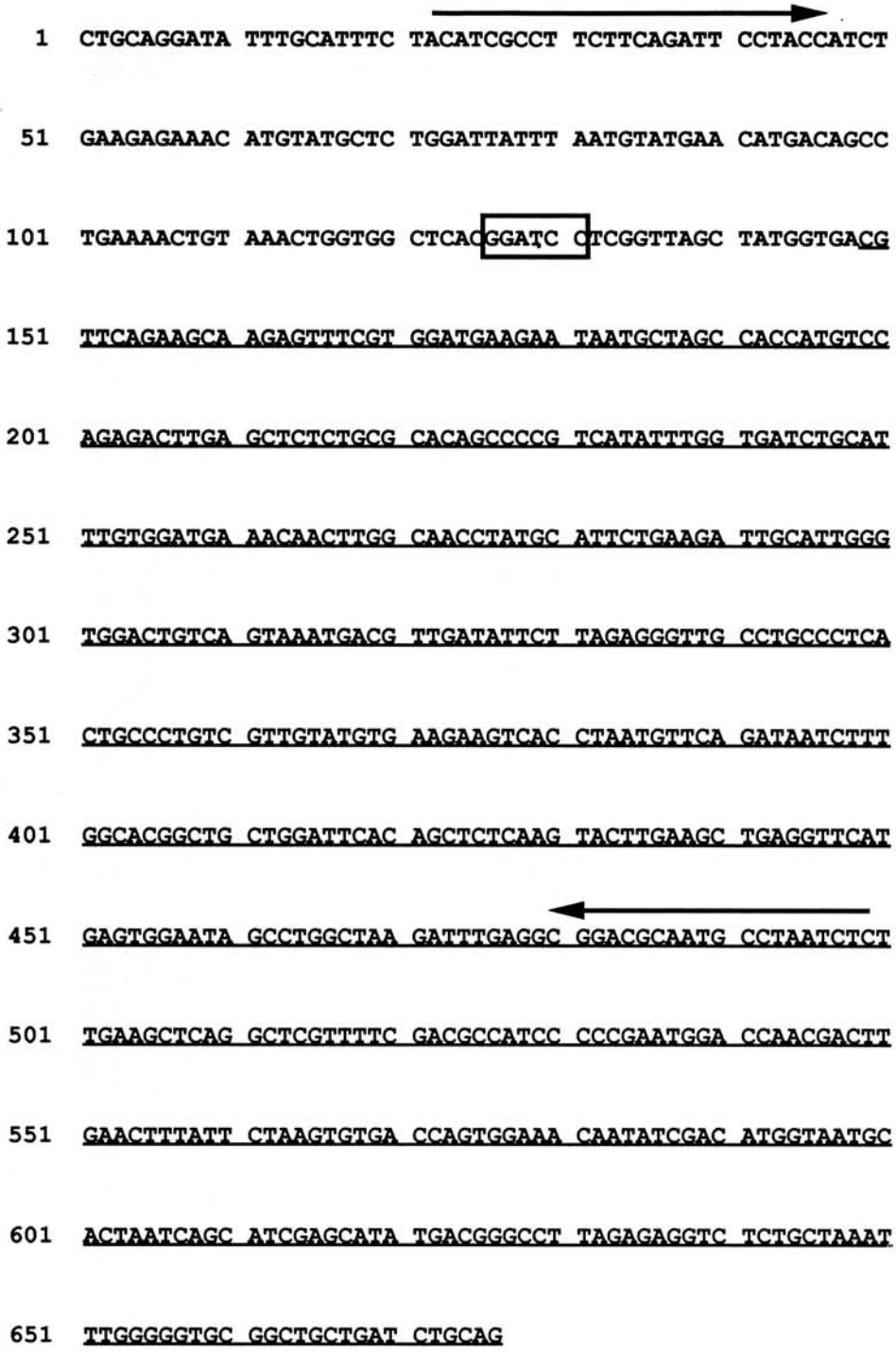

Fig. 2. Sequence of the ABG704 probe. An open reading frame is underlined. The primers used for PCR reactions are shown with an arrow above the sequence. L primer 5′ - ACATCGCCTTCTTCAGATTCCTACC - 3′ R primer 5′ - CTCTAATCCGTAACGCAGGCG -3′. The *Bam*HI site is boxed. Genomic clone ABG704 (*Pst*I insert in pGEM4Z) was sequenced using the ds DNA Cycle Sequencing System from Bethesda Research Laboratories and ^{32}P gamma-ATP labeled primers:SP6 and T7 from New England Biolabs. One microliter of plasmid miniprep was used as a template for the sequencing reaction. Amplification conditions were according to the "dirty DNA" method of the manufacturer instructions with annealing temperature at 55° C. All sequence data manipulations were done using the GCG package (Devereaux *et al.* 1984.).

the telomeric region of chromosome 1P and were polymorphic in both crosses. *Plc* is a cDNA clone encoding the precursor of barley plastocyanin (Nielsen and Gausing 1987). ABG704 and MWG036 are barley *Pst*I library genomic clones isolated by M. Saghai Maroof and Graner *et al.* (1991), respectively. BCD129 is a barley cDNA clone described by Heun *et al.* (1991). The map (Fig. 1) shows that the marker ABG704 is most closely linked (0.3 cM) to *Rpg1*. After a close examination of the cross-overs in the *Rpg1* region and rechecking of all suspect data, only a single recombinant was confirmed between ABG704 and *Rpg1* in a population of 401 DH lines. The plastocyanin (*Plc*) locus is also very closely linked (1.1 cM) to *Rpg1*. Both probes hybridize strongly, are highly polymorphic, and make excellent RFLP markers. The ABG704 probe yields a small number of bands that map to the same location, and the *Plc* probe yields a single band on barley genomic DNA blots. Either probe would be suitable as an RFLP marker for *Rpg1* in a breeding situation. The closest proximal marker to *Rpg1* was generated by the probe MWG036. This probe yields a small number of bands, one of which identifies a locus, designated MWG036B, mapping 2.2 cM proximal to *Rpg1*. A different locus identified with the same probe, designated MWG036A, maps to chromosome 5 near the Mla locus (Schuller et al. 1992). Because of the difficulties in predicting in new crosses which band corresponds to which locus, MWG036 may be somewhat more difficult to use than ABG704 or Plc. The possibility of generating a locus-specific probe or primers for MWG036B exists. Additional markers include ABG312, which cosegregates with Plc in all populations and ABA301, which cosegregates with Plc in the S/M population, but is not polymorphic in the H/T population (data not shown) (Kleinhofs *et al.* 1993). Another probe, ABG399, yields several bands, one of which is polymorphic only in the H/T population and cosegregates with Plc. This locus was designated ABG399B to differentiate it from another locus, ABG399A mapped with the same probe to chromosome 3 in the S/M population (Kleinhofs *et al.* 1993). The marker BCD129 maps 10.2 cM proximal to Rpg1 and was used orient the map.

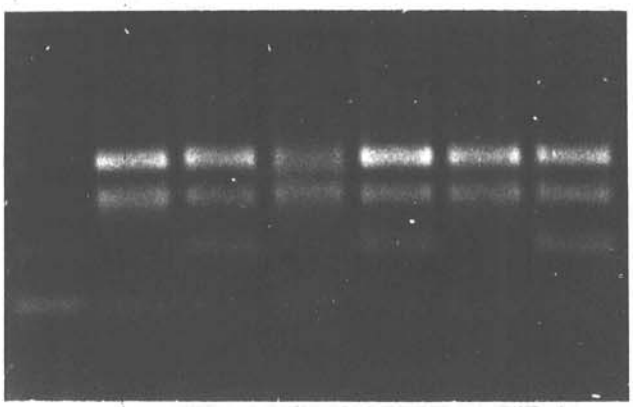

Fig. 3. A 250-bp restriction fragment of the PCR amplified DNA product is present in barley lines carrying the *Rpg1* allele and absent in those with the *rpg1* allele. From left, lane 1, Bethesda Research Laboratories 123-bp molecular mass markers; lanes 2, 4, and 6, Steptoe, Harrington, and 80-tt-30 DNA carrying the stem rust susceptible allele *rpg1*; lanes 3, 5, and 7, Morx, TR306, and 80-TT-29 DNA carrying the stem rust resistance allele *Rpg1*. Three different amplification products of approximately the same size are indicated by the results, i.e., those without a *Bam*HI restriction site resulting in an approximately 500-bp band identical with the undigested product, those with a single *Bam*HI restriction site resulting in approximately 400- and 100-bp nonpolymorphic bands and those with a single *Bam*HI restriction site resulting in two approximately equal about 250-bp fragments yielding the polymorphic band. PCR primers were designed based on the sequence obtained for ABG704 using the OLIGO program (Rychlik and Rhoads 1989). Primers were custom made at the Laboratory of Biotechnology and Bioanalysis at Washington State University. PCR reactions (25 µl) contained 1× buffer (Promega), 1.5 mM $MgCl_2$, 0.1 mM dNTPs, 10 pmoles of each primer, and 1 unit of Taq polymerase (Promega). The reaction conditions were: denaturation for 4 min (94° C), 35 cycles of 94° C for 1 min, 58° C for 1 min and 2 min at 72° C, final extension at 72° C for 10 min and storage at 10° C. To detect polymorphisms, 10% of the reaction was digested with the appropriate restriction enzymes in a 30-µl reaction. Restriction digests were resolved on 2% agarose gels.

To design primers, we sequenced the ABG704 probe (Fig. 2). The sequence has a long open reading frame (underlined in Fig. 2). Primers were designed based on the sequence and used to amplify target sequences from total genomic DNA of the four parents and two nearly isogenic lines 80-TT-29 (*Rpg1)* (CI 16129) and 80-tt-30 (*rpg1*) (CI 16130). The isogenic lines were developed by G. Wiebe from the cross (Wisconsin Barbless × Chevron) × Composite Cross XI and are 29 and 30 backcross generations selections, respectively (Steffenson *et al.* 1993). These primers yielded a single band of the expected size (478 bp). Polymorphism between the barley lines carrying the resistant allele *Rpg1* and those carrying the susceptible allele *rpg1* was observed after *Bam*HI (Fig. 3) and *Rsa*I digestion (not shown). Digestion with the restriction enzyme *Bam*HI revealed a 250-bp fragment that was present only in the three genotypes possessing *Rpg1*, but not in those possessing *rpg1* (Fig. 3). The use of *Bam*HI was suggested by the presence of a restriction site for this enzyme in the sequenced fragment and this enzyme is compatible with the PCR buffer. Among other retriction enzymes (*Hae*III, *Hin*fl, *Hpa*II, *Mbo*I, *Rsa*I, and *Taq*I) tested, *Rsa*I showed excellent polymorphism with the ABG704 primers. This enzyme, however, is not compatible with the PCR buffer and thus is not as convenient to use. The probe ABG704 was selected for PCR primer development due to its very close linkage with *Rpg1* and highly polymorphic nature. PCR-based markers may be more suitable than RFLP markers in breeding laboratories where Southern hybridization is not practical.

Our long-term goal is to clone the *Rpg1* gene. The North American Barley Genome Mapping Project (NABGMP) has generated nearly 1,000 lines from three crosses polymorphic for the *Rpg1* gene. The availability of the DH and near-isogenic lines make this genome suitable for fine structure mapping and the eventual cloning of the *Rpg1* gene. By virtue of the telomeric location of *Rpg1,* additional markers specific to this region can also be readily generated. We have previously cloned and characterized barley teleomere associated repeated sequences (Kilian and Kleinhofs 1992). These sequences are dispersed throughout the barley chromosome telomeric regions and are interspersed with low copy sequences. Combined with the availability of telosomic barley-wheat addition lines (Islam 1983) and the lack of cross-hybridization between the barley telomere associated repeats and wheat DNA, all telomeric low copy sequences are theoretically available for cloning. We hope to exploit this knowledge for the cloning of the *Rpg1* gene. Additional efforts toward this goal include attempts to generate *rpg1* deletion mutants using fast neutron irradiation. Such mutants would

open the possibilities to exploit genome subtraction and related techniques for gene isolation (Sun *et al.* 1992).

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