# Identification and mapping of a leaf rust resistance gene in barley line Q21861

**I.G. Borovkova, Y. Jin, B.J. Steffenson, A. Kilian, T.K. Blake, and A. Kleinhofs**

**Abstract**: Barley line Q21861 possesses an incompletely dominant gene (*RphQ*) for resistance to leaf rust caused by *Puccinia hordei*. To investigate the allelic and linkage relations between *RphQ* and other known *Rph* genes, $F_2$ populations from crosses between Q21861 and donors of *Rph1* to *Rph14* (except for *Rph8*) were evaluated for leaf rust reaction at the seedling stage. Results indicate that *RphQ* is either allelic with or closely linked to the *Rph2* locus. A doubled haploid population derived from a cross between Q21861 and SM89010 (a leaf rust susceptible line) was used for molecular mapping of the resistance locus. Bulked segregant analysis was used to identify markers linked to *RphQ*, using random amplified polymorphic DNAs (RAPDs), restriction fragment length polymorphisms (RFLPs), and sequence tagged sites (STSs). Of 600 decamer primers screened, amplified fragments generated by 9 primers were found to be linked to the *RphQ* locus; however, only 4 of them were within 10 cM of the target. The *RphQ* locus was mapped to the centromeric region of chromosome 7, with a linkage distance of 3.5 cM from the RFLP marker *CDO749*. Rrn2, an RFLP clone from the ribosomal RNA intergenic spacer region, was found to be very closely linked with *RphQ*, based on bulked segregant analysis. An STS marker, ITS1, derived from Rrn2, was also closely linked (1.6 cM) to *RphQ*.

*Key words*: *Hordeum vulgare*, *Puccinia hordei*, allelism testing, linkage, molecular markers.

**Résumé** : La lignée d'orge Q21861 possède un gène à dominance incomplète (*RphQ*) qui lui confère la résistance à la rouille naine causée par le *Puccinia hordei*. Afin de déterminer la position de ce gène et les relations alléliques entre *RphQ* et d'autres gènes *Rph* connus, des populations $F_2$ issues de croisements entre Q21861 et les lignées portant les gènes *Rph1* à *Rph14* (excluant *Rph8*) ont été évaluées quant à leur résistance à la rouille naine au stade de plantule. Les résultats indiquent que *RphQ* est soit allélique soit situé très près du locus *Rph2*. Une population de plantes dihaploïdes dérivées du croisement entre Q21861 et SM89010 (une lignée sensible à la rouille) a été employée pour réaliser une cartographie génétique de ce locus à l'aide de marqueurs moléculaires. Une analyse de ségrégants regroupés a été utilisée pour identifier des marqueurs RAPD (ADN polymorphe amplifié au hasard), RFLP (polymorphisme de longueur des fragments de restriction) et STS («sequence tagged site») liés au locus de résistance. Parmi les 600 amorces décanucléotidiques employées, 9 ont montré des fragments amplifiés qui étaient liés au locus *RphQ*; cependant seuls quatre de ceux-ci étaient à moins de 10 cM du locus. L'analyse a montré que ce locus était localisé dans la région centromérique du chromosome 7 à une distance de 3.5 cM du marqueur RFLP *CDO749*. Rrn2, un clone RFLP provenant de l'espaceur intergénique de l'ADN ribosomique, s'est avéré fortement lié au locus *RphQ* sur la base de l'analyse de ségrégants regroupés. Un marqueur STS dérivé du marqueur Rrn2, ITS1, était également fortement lié (1.6 cM) à *RphQ*.

*Mots clés* : *Hordeum vulgare*, *Puccinia hordei*, test d'allélisme, liaison génétique, marqueurs moléculaires.

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## Introduction

Barley leaf rust is caused by the fungus *Puccinia hordei* Otth. It is an important disease of barley (*Hordeum vulgare* L.) in many parts of the world, including Australia, Europe, North America, and South America. In the United States, leaf rust has been particularly damaging to barley grown in the southeast region, where resistant cultivars have been used to control the disease since the early 1960s (Steffenson et al. 1993). The barley line Q21861 (PI 584766) possesses resistance to a number of pathogens (Steffenson et al. 1992; Sun et al. 1996), including *P. hordei*. The inheritance of leaf rust resistance in Q21861 was initially investigated using $F_2$ populations (Jin and Steffenson 1992; Steffenson et al. 1995). These studies identified an incompletely dominant gene, tentatively designated *RphQ*, conferring resistance to several *P. hordei*

**Table 1.** Reaction (infection types (ITs)) of parental and $F_1$ lines to *Puccinia hordei* and segregation for low and high ITs in the $F_2$ populations in crosses between Q21861 (parent A) and donors (parent B) of *Rph* genes.

| Parent B | Recognized *Rph* gene | *P. hordei* isolate[a] | IT of parent B | IT of $F_1$ | No. of $F_2$ plants with: low IT | high IT | Ratio fit | $\chi^2$ | Probability ($>\chi^2$) |
|---|---|---|---|---|---|---|---|---|---|
| Sudan (CI 6489) | *Rph1* | Aust220 | 0; | 0;,1 | 309 | 20 | 15:1 | 0.016 | 0.8981 |
| Peruvian (CI 935) | *Rph2* | ND8702 | 0;,1 | 0;,1 | 665 | 0 | No segregation | | |
| PI 531840 | *Rph2* | ND8702 | 0;,1 | 0;,1 | 312 | 0 | No segregation | | |
| PI 531841 | *Rph2* | ND8702 | 0;,1 | 0;,1 | 657 | 0 | No segregation | | |
| Estate (CI 3410) | *Rph3* | ND8702 | 0; | 0; | 644 | 49 | 15:1 | 0.797 | 0.3721 |
| Gold (CI 1145) | *Rph4* | Aust220 | 0;,1 | 1,0; | 373 | 22 | 15:1 | 0.312 | 0.5764 |
| Magnif (CI 13860) | *Rph5* | ND8702 | 0; | 0;,1 | 293 | 9 | 15:1 | 5.511 | 0.0189 |
| Bolivia (CI 1257) | *Rph6*+2 | ND8702 | 1,0; | — | 1112 | 0 | No segregation | | |
| Cebada Capa (CI 6193) | *Rph7* | ND8702 | 0; | 0; | 295 | 20 | 15:1 | 0.005 | 0.9420 |
| Hor 2596 (CI 1243) | *Rph9* | ND8702 | 0;,1 | 0; | 292 | 17 | 15:1 | 0.295 | 0.5868 |
| Clipper BC8 | *Rph10* | Aust220 | 0;,1 | — | 287 | 19 | 15:1 | 0.001 | 0.9764 |
| Clipper BC67 | *Rph11* | Aust220 | 0;,1 | 0;,1 | 290 | 19 | 15:1 | 0.005 | 0.9415 |
| Triumph (PI 290195) | *Rph12* | ND8702 | 0; | 0;,1 | 350 | 14 | 15:1 | 3.590 | 0.0581 |
| PI 531849 | *Rph13* | ND8702 | 0;,1 | 0;,1 | 448 | 21 | 15:1 | 0.491 | 0.4834 |
| PI 584760 | *Rph14* | ND8702 | 0;,1 | 0;,1 | 279 | 17 | 15:1 | 0.130 | 0.7187 |

**Note:** "—," missing data.

[a]The avirulence/virulence formula for the two *P. hordei* isolates is as follows: *Rph1, Rph2, Rph3, Rph4, Rph6, Rph7, Rph9, Rph10, Rph11, Rph12, Rph13, Rph14, / Rph5, Rph8* for Aust220 and *Rph2, Rph3, Rph5, Rph6, Rph7, Rph9, Rph12, Rph13, Rph14, / Rph1, Rph4, Rph8, Rph10, Rph11* for ND8702. Isolate Aust220 was provided by Dr. R.G. Rees (Queensland Wheat Research Institute, Toowoomba, Australia). The infection types of Q21861 to isolates ND8702 and Aust220 were 0; and 0;1, respectively.

isolates. Leaf rust resistance in Q21861 was also investigated by Poulsen et al. (1995). They found a single resistance gene in Q21861 and identified a random amplified polymorphic DNA (RAPD) marker ($OU02_{2700}$) linked to this gene at a distance of 12 cM. The purpose of this investigation was to determine the relation between *RphQ* and other described *Rph* genes by allelism testing and to map it to a specific chromosome region using molecular markers.

## Materials and methods

### Plant materials

To develop populations for testing allelic and linkage relations between *RphQ* and known *Rph* genes, barley line Q21861 was crossed to the donors of *Rph1* to *Rph14* (Table 1), except for Egypt 4 (CI 6461, donor of *Rph8*). $F_1$ plants of crosses were grown in the greenhouse to produce $F_2$ seed. Each individual $F_2$ population was derived from a single $F_1$ plant and ranged in size from 279 to 1112 progeny. An anther culture derived doubled haploid (DH) population (125 lines), developed from a cross between Q21861 and SM89010 (a breeding line susceptible to leaf rust) (Steffenson et al. 1995), was used for molecular mapping of the leaf rust resistance gene. These DH lines are designated the "Q/SM" population.

### Leaf rust evaluations

Parental, $F_1$, and DH lines and $F_2$ populations were grown in greenhouses at 22 ± 3°C with 12–13 h of supplemental lighting (photon flux density = 230–270 $\mu mol \cdot m^{-2} \cdot s^{-1}$, supplied by sodium vapor bulbs). Seedlings were inoculated with leaf rust isolates 7 days after planting, when the primary leaves were fully expanded. Detailed inoculation and incubation procedures have been described in previous reports (Jin and Steffenson 1994; Jin et al. 1995). After an incubation period of 12 days, infection types (ITs) were scored using the scale of Levine and Cherewick (1952). Seedlings with infection types of 3 or 4, or combinations thereof, were classified as susceptible (high IT), and those with infection types of 0, 0;, 1, or 2, or combinations thereof, were classified as resistant (low IT).

The selection of *P. hordei* isolates for evaluating progeny in the allelism tests was based on parental reactions, i.e., isolates exhibiting low ITs on both parents were used to evaluate the segregating populations. Isolate ND8702 was used to evaluate most populations because it gives low ITs in response to most of the *Rph* genes. This isolate has an avirulence/virulence formula of *Rph2, Rph3, Rph5, Rph6, Rph7, Rph9, Rph12 / Rph1, Rph4, Rph8, Rph10, Rph11* on the differential barley lines of Steffenson and Jin (1992). It is also avirulent on lines carrying two recently identified leaf rust genes, *Rph13* and *Rph14* (Jin et al. 1996). Isolate Aust220 was used to evaluate progeny in crosses involving donors of *Rph1, Rph4, Rph10,* and *Rph11* because it is one of the few known *P. hordei* isolates that is avirulent for these genes. The complete avirulence/virulence formula for this isolate is *Rph2, Rph3, Rph5, Rph6, Rph7, Rph9, Rph12, Rph13, Rph14 / Rph1, Rph4, Rph8, Rph10, Rph11.* Isolates of *P. hordei* with avirulence for *Rph8* were not available; therefore, the allelic relationship between *Rph8* and *RphQ* was not investigated in this study.

### RAPD, STS, and RFLP analyses

Total genomic DNA was isolated from individual Q/SM DH lines and combined to form resistant and susceptible bulks as described by Michelmore et al. (1991). Each bulk consisted of DNA from 10 resistant or 10 susceptible lines. Detailed procedures for DNA extraction, digestion, electrophoresis, Southern blot transfer, probe preparation, and hybridization have been reported previously (Kleinhofs et al. 1993). A total of 600 decamer primers (Operon Technologies Inc., Alameda, Calif.) were evaluated for polymorphisms between the bulks, as well as between the parents. One primer (SC10-65

**Table 2.** Centimorgan distances and summary statistics of molecular markers linked with *RphQ* on barley chromosome 7.

| Marker | Marker type | Centimorgans | θ[a] | LOD |
|---|---|---|---|---|
| ITS1 | STS | 1.6 | 0.02 | 66.94 |
| *OPI-04(2.2)* | RAPD | 3.5 | 0.03 | 55.30 |
| *CDO 749* | RFLP | 3.6 | 0.03 | 54.15 |
| *OPP-04(1.3)* | RAPD | 8.4 | 0.08 | 45.32 |
| *OPP-04(1.2)* | RAPD | 8.4 | 0.08 | 42.35 |
| *OPE-16(0.95)* | RAPD | 9.0 | 0.08 | 42.88 |
| *OPH-16(1.8)* | RAPD | 9.4 | 0.09 | 32.30 |
| *OPAH-03(0.6)* | RAPD | 9.9 | 0.09 | 37.64 |
| *SC10-76(0.6)* | RAPD | 10.0 | 0.09 | 37.12 |
| *OPK-01(2.0)* | RAPD | 14.1 | 0.12 | 33.95 |
| BG123 | STS | 17.5 | 0.15 | 29.11 |
| *OPE-02(0.6)* | RAPD | 20.9 | 0.17 | 25.22 |
| *OPP-06(0.4)* | RAPD | 23.2 | 0.19 | 20.91 |

[a]θ, recombination frequency.

(Chalmers et al. 1992)) was synthesized by National Biosciences, Inc. (NBI, Plymouth, Minn.). Protocols for PCR amplification were as previously described (Borovkova et al. 1995). Primers generating products that exhibited polymorphism between the DNA bulks were subsequently used to evaluate all DH lines.

Six sequence tagged site (STS) primer sets (BG123, ABA1, CDO749, ABC302, ABC483, and ABC168) from the Barley Genetics Project at Montana State University were used in this study. These primers are 20 bp in length and were designed from sequenced restriction fragment length polymorphism (RFLP) clones mapped to barley chromosome 7, the putative location of *RphQ* (see Results). Sequences of the STS primers are available from the Graingenes database or on the World Wide Web (http://probe.nalusda.gov/). Primer concentrations were adjusted to 100 ng/μL and PCR conditions were as reported in Tragoonrung et al. (1992). Nonpolymorphic reaction products were digested with approximately 5 U of *Hha*I, *Hae*III, *Hin*fI, *Taq*I, or *Rsa*I (Promega, Madison, Wis.) per reaction mixture, according to the manufacturer's instructions. Products were separated on 1.5% agarose gels in 1× Tris borate buffer. Gels were stained with ethidium bromide, and DNA was visualized with UV light.

Primers used to amplify the internal spacer region of the ribosomal gene (ITS2 and ITS5) were synthesized by National Biosciences, Inc. The sequences of the primers and PCR conditions were as described by Chatterton et al. (1992). Primers ITS5F (5′-GGA AGT TAA AAG TCG TAA CAA GG-3′) and ITS2R (5′-GCA TCA ATG AAG AAC GCA GC-3′) amplify the region between the 18S and 5.8S rRNA genes (ITS1).

The chromosome 7 RFLP markers *CDO749* and *Rrn2* were also evaluated in the Q/SM DH population. The CDO749 probe was obtained from M. Sorrells (Cornell University) and Rrn2 (plasmid pHv249T; Gerlach and Bedbrook 1979) was obtained from M. O'Dell. These markers were previously mapped in the Steptoe/Morex (S/M) DH population by the North American Barley Genome Mapping Project (Kleinhofs et al. 1993).

In addition to using STS and RFLP markers to position *RphQ*, we evaluated linked RAPD markers for polymorphisms in the well characterized S/M DH population (Kleinhofs et al. 1993). These RAPD markers were evaluated in 150 lines of the S/M population to ascertain a possible chromosomal location by means of their linkage with already established RFLP markers.

### Linkage analysis

The $\chi^2$ method was used to test the hypothesis of independent segregation between *RphQ* and other *Rph* genes. When a linkage relation was detected, the recombination fraction was calculated using the maximum likelihood method. Linkage analyses and map construction for molecular markers and the resistance locus were performed with the MAPMAKER computer program, version 3.0 (Lander et al. 1987). A logarithm of the odds ratio (LOD) score of 3.0 or greater was established for linkage. The Kosambi mapping function was used to convert recombination frequencies to map distances in centimorgans (cM).

## Results

### Allelic and linkage relations of *RphQ* with other *Rph* genes

The reaction of parental and $F_1$ lines to *P. hordei* and segregation for IT in the $F_2$ populations are given in Table 1. Segregation for resistance and susceptibility occurred in all crosses, except those involving lines Peruvian, PI 531840, PI 531841, and Bolivia. No susceptible plants were observed in crosses with these lines. Peruvian, PI 531840, PI 531841, and Bolivia share a common gene (*Rph2*) for leaf rust resistance (Jin et al. 1996). The lack of segregation in these crosses involving the *Rph2* donors indicates that *RphQ* is either allelic with or closely linked to the *Rph2* locus. $F_2$ progeny in most of the other crosses segregated approximately 15:1 for resistance/susceptibility, as expected for the independent segregation of two genes. A deficiency in the number of susceptible plants in the cross involving line Magnif (a donor of *Rph5*) suggested a linkage relation between *RphQ* and *Rph5* with a recombination fraction of 34.5 ± 5.7%.

### Mapping of *RphQ* with RAPD, STS, and RFLP markers

Of the 600 decamer primers evaluated, 9 generated products that were linked to the *RphQ* locus in the Q/SM population within the range of 3.5–23.2 cM (Table 2). Six markers (*OPI-04(2.2)*, *OPAH-03(0.6)*, *OPP-04(1.2)*, *OPH-16(1.8)*, *OPE-02(0.6)*, and *SC10-76(0.6)*) were in coupling and four markers (*OPP-06(0.4)*, *OPP-04(1.3)*, *OPK-01(2.0)*, and *OPE-16(0.95)*) were in repulsion with *RphQ*. Only one primer (OPP-04) generated a codominant marker. Five of the RAPD markers (*OPP-04(1.2)*, *OPP-04(1.3)*, *OPH-16(1.8)*, *OPE-16(0.95)*, and *OPAH-03(0.6)*) mapped within 8.4–9.9 cM from *RphQ*, forming a cluster. The RAPD marker identified as closest to *RphQ* was *OPI-04(2.2)*, with a linkage distance of 3.5 cM.

In an earlier study, the *RphQ* locus was found to be associated with the *s* locus (controlling rachilla hair length) on the long arm of chromosome 7 with a recombination fraction of 36.4% (Steffenson et al. 1995). Although the linkage was considered tenuous because of skewed segregation for *s*, this information implicated chromosome 7 as the probable location of *RphQ*. Further evidence for a chromosome 7 location of *RphQ* was obtained when linked RAPD markers were evaluated for polymorphism in the S/M population. The RAPD marker *OPAH-03(0.65)*, linked to *RphQ* at a distance of 9.9 cM in the Q/SM population, was polymorphic in the S/M population. The OPAH-03 primer generated a marker that was linked to *Ubi2* at a distance of 4.5 cM in the S/M population. *Ubi2* is an RFLP marker located near the centromeric region of chromosome 7 (Kleinhofs et al. 1993). With the information of a putative location of *RphQ* near the centromeric region, RFLP clones from this region were used to evaluate the Q/SM bulks. Two clones, CDO749 and Rrn2, which

**Fig. 1.** Linkage map of *RphQ* with associated RAPD, STS, and RFLP markers on barley chromosome 7. The order and distances of loci were derived by multiple linkage analysis using the MAPMAKER software program, version 3 (Lander et al. 1987). Centimorgan distances were calculated from recombination percentages with the Kosambi function. Recombination fraction is given as a percentage in parentheses at the left. The letter "C" indicates the approximate location of the centromere of chromosome 7.

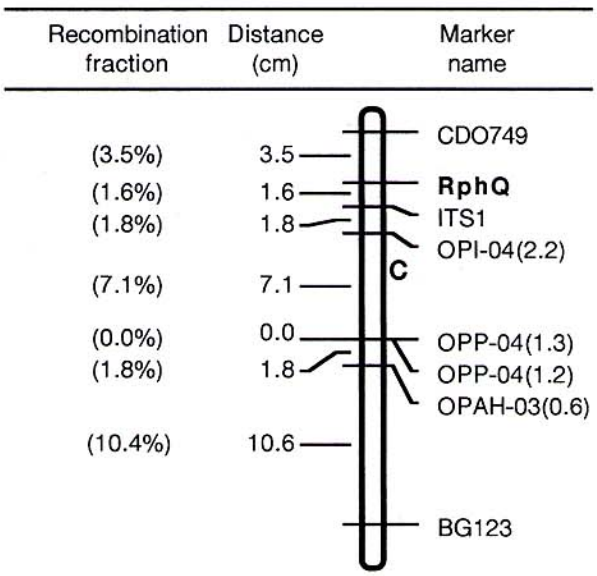
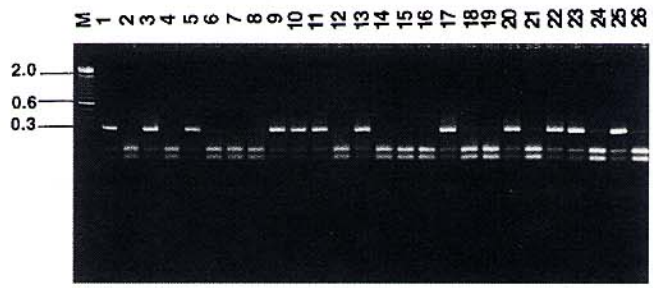



**Fig. 2.** DNA fragments generated by primers ITS5 and ITS2 (ITS1 region), digested with the restriction enzyme *Taq*I and electrophoresed in an agarose gel. Lanes: 1, Q21861 parent; 2, SM89010 parent; 3, resistant bulk; 4, susceptible bulk; 5, 9, 10, 11, 13, 17, 20, 22, 23, and 25, resistant plants; 6, 7, 8, 12, 14, 15, 16, 18, 19, 24, and 26, susceptible plants; 21, recombinant plant (resistant phenotype). M is a 100 bp molecular weight ladder (Gibco BRL).




were previously mapped to the centromeric region of the short arm of chromosome 7 (Kleinhofs et al. 1993), were polymorphic in the Q/SM bulks and closely linked to *RphQ*. Evaluation of the Q/SM population with CDO749 showed that it was linked to *RphQ* at a distance of 3.5 cM.

Although closely linked markers were identified in the vicinity of *RphQ*, the orientation of these markers was still not clear. Several available STS primers (ABC302, BG123, ABC168, and CDO749), designed from RFLP clones located on chromosome 7, were used to amplify the DNA of Q/SM bulks. Of these markers, only BG123 was found to be polymorphic after digestion with *Hha*I. The BG123 STS primers produced a marker that was linked to *RphQ* at a distance of 17.4 cM. The reported distance between the *Rrn2* and BG123 loci in the S/M population is about 14 cM (Kleinhofs et al. 1993). Thus, our linkage data for the Q/SM population were in agreement with those found for the S/M population. The STS marker BG123, however, was not polymorphic in the S/M population. Therefore, it was necessary to use additional markers to verify that the STS marker BG123 was indeed located on the long arm of chromosome 7. To do that, we used the STS primers for ABA1, which produce a marker that cosegregates with BG123 in the S/M population (Kleinhofs et al. 1993). We found that the ABA1 STS marker also cosegregated with BG123 in the Q/SM population. This result confirmed the location of the BG123 STS marker on the long arm of chromosome 7. Having obtained the two flanking markers *CDO749* and BG123 STS, we constructed a map for the linkage relationships between *RphQ* and linked RAPD, STS, and RFLP markers (Fig. 1). The internal transcribed region between the 18S and 26S rRNA genes of barley has been sequenced (Chatterton et al. 1992). Four primers, ITS5, ITS4, ITS2, and ITS3 (White et al. 1990), were analyzed in the Q/SM population. The pair ITS5 and ITS2, which amplify spacer ITS1 between the 18S and 5.8S rRNA genes, produced a 0.3-kb fragment in the PCR reaction, which was polymorphic in the Q/SM population after restriction with *Taq*I. This marker showed close linkage (1.6 cM) with *RphQ* and was present in the resistant parent Q21861 (Fig. 2).

## Discussion

The allelism tests performed between Q21861 and other donors of *Rph* genes clearly indicate that *RphQ* is either an allele of *Rph2* or a locus closely linked to it. *RphQ* can be differentiated from *Rph2* in various donors based on its infection response to several *P. hordei* isolates (Y. Jin and B.J. Steffenson, unpublished results). A new allele was recently identified at the *Rph2* locus in PI 531841 (Jin et al. 1996). These data strongly suggest that *Rph2* is a complex locus. The results of this study also clearly indicate that *Rph2* is located just distal to the chromosome 7 secondary constriction (site of the ribosomal RNA locus). Since there is very little recombination between the ribosomal RNA locus and the centromere on chromosome 7, the linkage map suggests a close association between the centromere and *Rph2*. At the physical map level, however, *Rph2* seems to reside in the chromosome 7 satellite. An unknown leaf rust resistance gene from the barley line TR306 was recently mapped to the same region of chromosome 7 flanked by the RFLP markers *ABG497* and *MWG635A* (B.J. Steffenson, unpublished results). Our results from an allelism test (718 progeny tested) between Q21861 and TR306 indicate that both carry the same gene. This result is further evidence for the chromosome 7 location of *Rph2*. The linkage relationship between *Rph2* and *Rph5*, observed from this and a previous study (Jin et al. 1996), place both resistance loci on chromosome 7. Several other leaf rust resistance genes have been positioned on the barley chromosomes. Based on linkage to previously mapped loci, *Rph4* was mapped to chromosome 5 (McDaniel and Hathcock 1969), *Rph10* to chromosome 3 (Feuerstein et al. 1990), *Rph11* to chromosome 6 (Feuerstein et al. 1990), and *Rph12* to

chromosome 7 (Jin et al. 1993*a*, 1993*b*). *Rph1* and *Rph7* were associated with chromosomes 2 and 3, respectively, using primary trisomics (Tuleen and McDaniel 1971). *Rph3* was thought to be on chromosome 1, based on its linkage with $X_a$ (Jin et al. 1993*a*); however, we have not been able to corroborate the chromosome 1 location of $X_a$ using either molecular or morphological markers (Y. Jin et al., unpublished results).

Among the nine RAPD markers found linked to the *RphQ* locus in the Q/SM population, only *OPAH-03(0.6)* was polymorphic in the S/M population. This result underscores a limitation of RAPD markers for mapping loci, as they may be present only in specific crosses and may not be applicable to others. Five RAPD markers were mapped at 8–10 cM from the *RphQ* locus in this study. The presence of such a cluster may be associated with the location of these markers in the centromeric region. Clusters of RAPD markers were also found in other barley crosses (Giese et al. 1994) and in lettuce (Kesseli et al. 1991).

A map was constructed for *RphQ* and linked RAPD, STS, and RFLP markers (Fig. 1). Some of the identified RAPD markers could not be assigned to a map position with confidence and were not included in the final map. Similar problems with positioning RAPD markers have been reported in barley, lettuce, and sorghum (Kesseli et al. 1991; Kleinhofs et al. 1993; Pammi et al. 1994).

Morphological markers were useful for locating disease resistance genes on specific barley chromosomes prior to the development of molecular markers. Using crosses between various resistance donors and morphological marker stocks, several rust resistance loci were mapped to a chromosome by their association with known morphological markers (Jin et al. 1993*a*, 1993*b*; McDaniel and Hathcock 1969). Even with all the advances in molecular genome mapping, morphological markers may still provide important information for mapping. For example, the association of the chromosome 7 locus *r* (controlling the degree of awn barbing) with *rpg4*, a gene conferring resistance to *Puccinia graminis* f.sp. *tritici* (Jin et al. 1994), provided a critical clue for the putative location of this stem rust resistance locus. With this information, we were able to efficiently map this gene on the long arm of chromosome 7 (Borovkova et al. 1995). A similar approach was followed in mapping *RphQ* in this study. The *RphQ* locus was initially found to be associated with the *s* locus on the long arm of chromosome 7 with a recombination fraction of 36.4% (Steffenson et al. 1995). Although the linkage was considered tenuous because of skewed segregation for *s*, it provided us with information on a possible chromosome region, which we pursued in our molecular mapping efforts.

Molecular markers have significantly enhanced our understanding of complex loci for disease resistance in several host–pathogen systems (Dickinson et al. 1993; Hulbert and Michelmore 1985; Hulbert and Bennetzen 1991; Jones et al. 1993). The closely linked RAPD, STS, and RFLP markers identified in this study will be useful for resolving the *Rph2* complex further. Additional markers identified in this region of chromosome 7 may hasten the mapping of other loci linked to *Rph2*.

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