Resistance to *Puccinia graminis* f. sp. *avenae* in Barley Is Associated with the *Rpg5* Locus

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ABSTRACT

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In barley, gene *Rpg5* was first identified for providing resistance to the rye stem rust pathogen (*Puccinia graminis* f. sp. *secalis*). A subsequent study determined that *Rpg5* is required for *rpg4*-mediated resistance to the wheat stem rust pathogen (*P. graminis* f. sp. *tritici*) including pathotype TTKSK (“Ug99”), which poses a major threat to global wheat and barley production. Based on the effectiveness of *Rpg5* against *P. graminis* f. sp. *tritici* and *P. graminis* f. sp. *secalis*, we assessed whether it also conferred resistance to the oat stem rust pathogen (*P. graminis* f. sp. *avenae*). A barley F₈ recombinant inbred line (RIL) population was produced by crossing ‘Q21861’ (*Rpg1* and *Rpg5*) with ‘73-G1’ (*Rpg1*), which is susceptible to *P. graminis* f. sp. *avenae*, *P. graminis* f. sp. *secalis*,

and some pathotypes of *P. graminis* f. sp. *tritici*. Seedling tests were performed on the F₈ RIL population using Australian pathotypes of *P. graminis* f. sp. *tritici*, *P. graminis* f. sp. *secalis*, *P. graminis* f. sp. *avenae*, and a putative somatic hybrid between *P. graminis* f. sp. *tritici* and *P. graminis* f. sp. *secalis* known as the ‘Scabrum’ rust. Segregation in the responses to all rust isolates for the RILs was identical (50 resistant: 52 susceptible), and fitted a 1:1 ratio ($X^2 = 0.039$, $P = 0.843$), indicating that resistance to all isolates was monogenetically inherited. Screening of the RILs and the parental lines with perfect markers for the functional *Rpg1* and *Rpg5* resistance alleles indicated that *Rpg1* was fixed, while *Rpg5* was positive in all resistant lines and negative in all susceptible lines. This suggests that different formae speciales of *P. graminis* may share common effectors, and that the *Rpg5* locus confers resistance to both *P. graminis* f. sp. *tritici* and *P. graminis* f. sp. *secalis* and the heterologous formae speciales of *P. graminis*, *P. graminis* f. sp. *avenae*.

Stem rust, caused by *Puccinia graminis*, is a major disease of cereals and grasses worldwide, and can cause complete crop failure when severe (8,25,33). The species *P. graminis* is divided into both subspecies and formae speciales, based primarily on urediniospore morphology and host range, respectively. Currently, several formae speciales of *P. graminis* are recognized including *tritici*, *secalis*, *avenae*, *agrostidis*, *poae*, *airae*, and *lolii* (1,17). The *P. graminis* f. sp. *tritici* form has a wider host range than other formae speciales (28 hosts from 8 genera) (1) and is most commonly found on wheat, but also infects barley and cereal rye, while *P. graminis* f. sp. *secalis* can infect rye and barley, but only very few wheat genotypes. The *P. graminis* f. sp. *avenae* form is not closely related to other formae speciales of *P. graminis* based on difficulty to cross hybridize (1), and it is specialized mainly to *Avena* spp. and other grasses within the Pooideae subtribe. This was further supported by DNA sequencing of the internal transcribed spacer regions of nuclear rDNA from different *P. graminis* formae speciales, which determined that *P. graminis* f. sp. *tritici* was very closely related to *P. graminis* f. sp. *dactylis*, *lolii*, and *poae* relative to f. sp. *tritici* and *secalis* (34). Evidence for both cultivated and native host grass species playing a part in the evolution and somatic hybridization of *P. graminis* also exists (12,19). A putative somatic hybrid between *P. graminis* f. sp. *tritici* and *P. graminis* f. sp. *secalis* was collected from *Elymus scaber* in a previous study and is informally referred to as the ‘Scabrum’ rust (‘Scabrum’) (19,22).

Barley (*Hordeum vulgare* L.) is considered a host to the wheat stem rust pathogen (*P. graminis* f. sp. *tritici*), the rye stem rust pathogen (*P. graminis* f. sp. *secalis*), and ‘Scabrum’ rust. Severe

epidemics of *P. graminis* f. sp. *tritici* have occurred on barley in Australia, and the disease has posed a serious threat to barley production in the United States and Canada, especially prior to the deployment of the durable resistance gene *Rpg1* in 1942 (29). In barley, several resistance genes effective to *P. graminis* f. sp. *tritici* have been described including *Rpg1* (‘Peatland’, ‘Chevron’, and ‘Kindred’) (24,27), *Rpg2* (‘Heitpas 5’) (23), *Rpg3* (‘PI 382313’) (14,15), *rpg4* (‘Q21861’) (16), and *Rpg6* in the line ‘212Y1’ that carries an introgression from *H. bulbosum* (10). *Rpg1*-mediated resistance proved durable based on the protection it has provided in North America for over 65 years; however, two pathotypes (QCCJ and TTKSK) of *P. graminis* f. sp. *tritici* were identified with virulence for *Rpg1* (28).

Three additional genes have been identified for resistance to *P. graminis* f. sp. *secalis* in barley. The partially dominant gene in ‘Q21861’ effective against North American pathotypes of *P. graminis* f. sp. *secalis* as originally designated as *RpgQ*, but more recently was designated as *Rpg5* when high resolution mapping distinguished this gene from *rpg4* (5,6,30). Numerous studies involving high resolution genetic analysis and posttranscriptional silencing of candidate genes at the putative *rpg4/Rpg5* locus on chromosome 5HL led to the hypothesis that four genes at two tightly linked loci were required in combination for resistance to *P. graminis* f. sp. *tritici* (32). Further examination of the alleles at the RMRL1 locus across both diverse domesticated and wild barley accessions determined that *HvRga1* did not contain polymorphisms that determine the compatible reactions, and that *HvAdf3* was identical at the amino acid level among all 22 accessions tested (2). This study demonstrated that these two candidates were expressed in both resistant and susceptible lines at the transcriptional level (2). *Rpg5/rpg5* allele sequence analysis determined that single mutations accounted for amino acid substitutions or truncated proteins only found in barley accessions susceptible to *Puccinia*

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graminis f. sp. *tritici*. Taken together, it can be concluded that *Rpg5* is a functional, polymorphic gene required for *rpg4/Rpg5* resistance to *P. graminis* f. sp. *tritici* and *P. graminis* f. sp. *secalis* (2). The *Rpg5* gene encodes a protein with a unique combination of three disease resistance motifs including a nucleotide binding site-leucine rich repeat (NBS-LRR) and serine threonine protein kinase (S/TPK) domain (4,18). This along with current evidence for broad resistance specificity to two different formae speciales of *P. graminis* may suggest *Rpg5* confers resistance to numerous formae speciales of *P. graminis*.

Previous studies have determined that some barley accessions showed moderate susceptibility to heterologous rust pathogen species and all known formae speciales of *P. graminis* including *P. graminis* f. sp. *avenae* (3,9,20). Barley has been classified as a near nonhost to heterologous rust species and therefore is highly suitable for genetic analysis of resistance to different formae speciales within the *P. graminis* complex. An early study identified a Canadian barley selection ('73-G1') that was moderately susceptible to *P. graminis* including *P. graminis* f. sp. *avenae*. In this study, we intercrossed '73-G1' with 'Q21861' to determine whether *Rpg5* alone conferred resistance to multiple diverse *P. graminis* cultures. Diagnostic markers for both *Rpg1* and *Rpg5* were then screened on the recombinant inbred line (RIL) population to test for cosegregation of *Rpg5* with resistant phenotypes in response to *P. graminis*. Given the unique gene structure of *Rpg5* as an NBS-STK-LRR and the broad range of resistance to multiple North American pathotypes of *P. graminis* f. sp. *tritici* and the closely related formae speciales *P. graminis* f. sp. *secalis*, we tested the hypothesis that *Rpg5* also confers resistance to Australian pathotypes of *P. graminis* f. sp. *tritici*, *P. graminis* f. sp. *secalis*, 'Scabrum', and the heterologous rust pathogen *P. graminis* f. sp. *avenae*.

MATERIALS AND METHODS

Plant materials. The parental barley lines used in this study were 'Q21861' (PI 584766; *Rpg1*, *rpg4*, *Rpg5*) and '73-G1' (*Rpg1*). In previous genetic studies the infection types (IT) produced at the seedling stage in barley line '73-G1' to Canadian pathotypes of *P. graminis* f. sp. *avenae* were consistently more compatible than those observed on other barleys. Although not fully susceptible, '73-G1' produced mesothetic reactions to *P. graminis* f. sp. *avenae* and was more resistant to *P. graminis* f. sp. *tritici* (intermediate IT) (20). An F₈ RIL population was developed and 102 progeny were used for genetic analysis of resistance to *P. graminis* in this study. Wheat ('Morocco') and oat ('Swan') cultivars were used as positive control checks for inoculation in all experiments. Seedlings were grown in plastic pots (90 mm diameter) containing a potting mix comprising composted pine bark and coarse sand in a ratio of 4:1. Pots were fertilized with a soluble nitrogenous fertilizer (Aquadol, Hortico Pty Ltd., Australia) at the rate of 30 g in 10 liter of water for 200 pots prior to sowing. The RIL progeny were sown as clumps (four lines/pot) of approximately 10 seeds each. Postinoculation, the seedlings were fertilized with Aquasol: ammonium nitrate (1:1) at a rate of 25 g in 10 liter of water per 100 pots. Plants were maintained in a temperature-controlled greenhouse at 17°C prior to inoculation and 25°C after inoculation under natural lighting.

Inoculation procedures. Inoculations were performed on 10-day-old seedlings. Urediniospores (150 mg) were suspended in approximately 5 ml of light mineral oil (Isopar L) and sprayed with a mist atomizer over the top of the seedlings. The RIL population was phenotyped with three pathogenically diverse Australian *P. graminis* f. sp. *tritici* pathotypes (34-1,2,3,4,5,6,7; 343-1,2(3), (5),6,9; and 98-1,2,3,5,6,7), two *P. graminis* f. sp. *avenae* pathotypes (41 + *Pg9*, *Pg13*, *PgA*; and 94 + *Pg13*), and single isolates of

Fig. 1. Seedling reactions of lines from left to right when incubated at 25°C with **A**, *Puccinia graminis* f. sp. *tritici* pathotype 98-1,2,3,5,6,7 on lines 'Q21861' (infection type = 0;), '73-G1' (2C), 'RIL12' (12 = C), 'RIL28' (2+C), 'Swan' oat (resistant control), and 'Morocco' wheat (susceptible control); and **B**, *P. graminis* f. sp. *avenae* pathotype 41 + *Pg9*, *Pg13*, *PgA* on lines 'Q21861' (00;), '73-G1' (X+), 'RIL12' (00;), 'RIL28' (X+), 'Morocco' wheat (resistant control), and 'Swan' oat (susceptible control). Pathotype nomenclature is essentially as described by Stakman et al. (26).

P. graminis f. sp. *secalis* (BWR '2') and 'Scabrum' ('Scabrum' + *Sr21*) (26) (Table 1). To prevent cross-contamination, the spray equipment was washed in 70% ethanol and rinsed in running tap water between successive inoculations. Seedlings inoculated with urediniospores were incubated for approximately 48 h over trays of water covered by sealed polythene hoods, under which sufficient dew (humidity) was generated. Incubation enabled the urediniospores to germinate and allowed rust infection to take place. After incubation, the plants were moved to greenhouse microclimate rooms for rust development.

Disease assessment. Disease response was assessed 12 days after inoculation using a modified 0 to 4 scale based on uredinal size (21). Variations of the ITs were indicated by the use of "–" (less than average for the class), "+" (more than average for the class), "C" (chlorosis), "N" (necrosis), and X to denote a mesothetic IT with a mixture of ITs on the same leaf.

Molecular marker analysis. Molecular marker analysis was performed on the parents and the RIL population using primer pairs previously designed to both the functional (*Rpg5*-LRK) and

nonfunctional alleles (*Rpg5*-LRK/PP2C) of the *Rpg5* resistance gene, and to the functional *Rpg1* gene (*Rpg1*-N) (Table 2) (7). Brueggeman and coauthors (4) previously determined that there was a putative translocation insertion deletion (indel) event, which replaced the serine/threonine protein kinase (S/TPK) domain required for a functional *Rpg5* gene with a putative functional protein phosphatase 2C (PP2C). The *Rpg5*-LRK primers were previously designed to specifically amplify a 1,046-bp fragment of genomic DNA of the functional *Rpg5* gene spanning intron 2 (7). The *Rpg5*-LRK/PP2C primers were designed across the junction between the leucine-rich repeat domain and the PP2C domain and only amplified an 840-bp fragment from genomic DNA when the line contained the PP2C domain, which is a feature of only susceptible lines (7).

Genomic DNA was extracted from 102 RILs and the parental genotypes using the CTAB method essentially as described by Fulton et al. (11). Primer pairs previously designed to *Rpg1* and both the functional and nonfunctional alleles of *Rpg5* (Table 2) were screened essentially as described by Derevnina et al. (7). In brief, polymerase chain reaction (PCR) amplifications were performed in an automated thermocycler (MasterCycler, Eppendorf) using 96 well plates (Astral Scientific). PCR products were amplified from a 10- μ l reaction containing 40 μ M dNTP, 10 $\times$ Immolase Buffer (Bioline), 2.5 mM MgCl₂, 1 μ M of each primer, 0.2 U of Immolase Taq polymerase (Bioline), and approximately 40 ng of genomic DNA. Thirty-five cycles were conducted in each assay consisting of the following profiles. Annealing temperature and primer sequence information is provided in Table 2. For Rpg1-N: denaturation at 94°C for 30 s, anneal for 45 s, extension at 72°C for 60 s. For *Rpg5*-LRK and *Rpg5*-LRK/PP2C: denaturation at 94°C for 30 s, anneal for 60 s, extension at 72°C for 60 s. 'Chevron' and 'Q21861' were used as positive controls for the presence of *Rpg1* and *Rpg5* resistance alleles, respectively.

TABLE 1. Isolates of *Puccinia graminis* used for phenotypic analysis of the 'Q21861'/'73-G1' recombinant inbred line population

Pathogen species	Pathotype virulence	Culture accession
<i>P. graminis</i> f. sp. <i>tritici</i>	34-1,2,3,4,5,6,7 343-1,2(3),(5),6,9 98-1,2,3,5,6,7	74-L-1 010047 033537
<i>P. graminis</i> f. sp. <i>avenae</i>	41 + <i>Pg9</i> , <i>Pg13</i> , <i>PgA</i> 94 + <i>Pg13</i>	962508 923034
<i>P. graminis</i> f. sp. <i>secalis</i>	BWR '2' Rye stem rust	71327
<i>P. graminis</i> f. sp. <i>secalis</i> $\times$ <i>P. graminis</i> f. sp. <i>tritici</i>	Scabrum + <i>Sr21</i>	970220

TABLE 2. Description of markers used to assess the 'Q21861'/'73-G1' F₈ recombinant inbred line population for the presence and absence of the *Rpg1* and *Rpg5* alleles

Marker name	Sequence (5'–3')	Size (bp)	Annealing temperature (°C)
RPG1-N ^a	F:CGGCTAATCACATCAAGTAA R:AGCCCATCATCAATAGACAA	669	61
<i>Rpg5</i> -LRK ^a	F:CTGCTGGCACAGAGTCTGCCTTGAG R:ACTCTCGGGTCTGAAGTTCGTGTG	1,046	62
<i>Rpg5</i> -LRK/PP2C ^a	F:CTGCTGGCACAGAGTCTGCCTTGAG R:CCCAGGTTTGCGATGAAGAGAGTC	840	62

^a All primer pairs used for molecular marker analysis were previously published in Derevnina et al. (7).

TABLE 3. Summary of the infection types (ITs) and marker analysis of representative recombinant inbred line (RIL) and parental genotypes of the F₈ RIL population of 'Q21861'/'73-G1' inoculated with isolates of *Puccinia graminis* f. sp. *avenae*, *P. graminis* f. sp. *tritici*, *P. graminis* f. sp. *tritici* $\times$ *P. graminis* f. sp. *secalis*, and *P. graminis* f. sp. *secalis*

Plant identifier	Plant genotype (<i>Rpg5/rpg5</i>)	<i>P. graminis</i> f. sp. <i>avenae</i>		<i>P. graminis</i> f. sp. <i>tritici</i>			<i>P. graminis</i> f. sp. <i>tritici</i> × <i>secalis</i>	<i>P. graminis</i> f. sp. <i>secalis</i>	<i>Rpg5</i>	<i>Rpg5</i>	
RIL/parent	Allele	94 + <i>Pg13</i>	41 + <i>Pg9</i> , 13,a	343-1,2(3), (5),6,9	98-1,2,3, 5,6,7	34-1,2,3, 4,5,6,7	Scabrum + <i>Sr21</i>	BWR ‘2’	<i>Rpg5</i> LRK	<i>Rpg5</i> LRK/PP2C	<i>Rpg1-N</i>
1	<i>rpg5</i>	X ^a	X–	X– ^b	2+C ^c	2C	X–	X+3 ^b	–	+	+
9	<i>Rpg5</i>	0;	0;	;12–	1 =	0;	;12+C	nd ^d	+	–	+
64	<i>rpg5</i>	X	X	2+C ^c	2+C	2C	X	X+3	–	+	+
72	<i>Rpg5</i>	0;	0;	;1 =	0;	0;	;12–C	0;	+	–	+
87	<i>Rpg5</i>	0;	0;	;1–C	;12–C	0;	;12–C	0;	+	–	+
99	<i>rpg5</i>	X+	X+	2+C	2+C	2++C	X-	X+3	–	+	+
Q21861	<i>Rpg5</i>	00;	00;	;12–C	;1=C	0;	0;	0;	+	–	+
73-G1	<i>rpg5</i>	X+	X+	2+C	2+C	2+C	2+C	X+3	–	+	+
Chevron	<i>rpg5</i>	nd	nd	nd	nd	nd	nd	nd	–	+	+

^a X denotes a mixed reaction type including pustules of three different sizes (1, 2, and 3), the – and + following the X refers to either a higher or lower proportion of pustules with lower IT, respectively.

^b X+3 denotes a large proportion of IT 3 pustules compared with others and suggests that the reaction is almost a 3 but also has some mixing of ITs on the same leaf.

^c The symbols C and ; denote the presence of chlorosis and flecking, respectively.

^d nd denotes no data.

RESULTS

Reaction of barley genotypes to different *P. graminis* cultures. 'Q21861' was resistant to all *P. graminis* cultures tested in this study (Table 3; Fig. 1). ITs were lower (00;) in response to infection by *P. graminis* f. sp. *avenae*, *P. graminis* f. sp. *secalis*, 'Scabrum', and *P. graminis* f. sp. *tritici* pathotype 34-1,2,3,4,5,6,7 compared with the reactions to *P. graminis* f. sp. *tritici* pathotypes 343-1,2(3),(5),6,9 (IT ;12-C) and 98-1,2,3,5,6,7 (IT ;1=C). All resistant RIL progeny had near-immune reactions to *P. graminis* f. sp. *avenae*, *P. graminis* f. sp. *secalis*, and pathotype 34-1,2,3,4,5,6,7, and exhibited low ITs (ranging from 0; to ;12C) to pathotypes 343-1,2(3),(5),6,9, 98-1,2,3,5,6,7, and 'Scabrum'. Conversely, the '73-G1' parent and the susceptible RIL lines had higher ITs to both *P. graminis* f. sp. *avenae* and *P. graminis* f. sp. *secalis* than to both *P. graminis* f. sp. *tritici* and 'Scabrum', which gave intermediate ITs. The IT of the 'Q21861' parent was often lower (0; to ;1-C) than that seen among resistant RIL progeny (0; to ;12+C). In all cases, there was no infection of *P. graminis* f. sp. *tritici* pathotypes on oat variety 'Swan' and no infection of *P. graminis* f. sp. *avenae* on wheat variety 'Morocco', indicating that there was no cross-contamination of cultures in this experiment (Fig. 1).

Genetic analysis of resistance to the different *P. graminis* cultures in the 'Q21861'/'73-G1' RIL population. The phenotypic assessment of the F₈ RIL population derived from intercrossing 'Q21861'/'73-G1' identified two distinct phenotypic classes when assessed by all stem rust fungal cultures (Table 4). From a total of 102 RIL progeny, the segregation of 50 resistant:52 susceptible fitted a 1:1 ratio ($\chi^2 = 0.039$, $P = 0.843$) in tests with all rust isolates, indicative of single gene inheritance pattern (Table 4). Cosegregation patterns of resistant and susceptible lines across three separate pathotypes of *P. graminis* f. sp. *tritici*, two pathotypes of *P. graminis* f. sp. *avenae*, and single isolates of *P. graminis* f. sp. *secalis* and 'Scabrum' indicated that the same or very tightly linked genes are involved in resistance to all rust cultures. Resistance to all *P. graminis* f. sp. *tritici* pathotypes cosegregated with resistance to *P. graminis* f. sp. *avenae*, *P. graminis* f. sp. *secalis*, and 'Scabrum' in all cases.

Molecular marker analysis. Molecular analysis was performed on the parental and RIL lines of the 'Q21861'/'73-G1' F₈ population using previously published perfect markers for both *Rpg1* and *Rpg5* (7). Both parents, the positive-*Rpg1* control 'Chevron', and all RIL progeny were positive for the *Rpg1* marker allele, confirming that the 'Q21861'/'73-G1' population was fixed for *Rpg1* (Table 3). Further screening with two previously published primer pairs designed to both the functional (*Rpg5*-LRK) and nonfunctional (*Rpg5*-LRK/PP2C) alleles of the *Rpg5* gene demonstrated that 'Q21861' was positive for *Rpg5*-LRK and negative for *Rpg5*-LRK/PP2C allele. Conversely, 'Chevron' and '73-G1' were positive for the *Rpg5*-LRK/PP2C allele and negative for *Rpg5*-LRK, suggesting that both lines do not carry *Rpg5*. There was cosegregation of RIL lines positive for *Rpg5*-LRK allele and resistance ITs to all *P. graminis* formae speciales. In all cases, resistant RILs were positive for *Rpg5*-LRK and negative for *Rpg5*-LRK/PP2C. Conversely, susceptible RILs were negative for *Rpg5*-LRK and positive for the *Rpg5*-LRK/

PP2C allele (Tables 3 and 4). χ^2 analysis found a nearly perfect fit to a 1 *Rpg5*:1 *rpg5* segregation ratio for both marker analysis and phenotypic assessment of resistance and susceptibility (Table 4).

DISCUSSION

Several genes for resistance in barley to wheat stem rust caused by *P. graminis* f. sp. *tritici* have been identified and designated, two (*Rpg1* and *Rpg5*) of which have been cloned and characterized (4,8). Previous studies have determined that a single dominant gene in 'Q21861', designated as *RpgQ*, provided high levels of resistance (00;) to *P. graminis* f. sp. *secalis* (31). This gene was subsequently designated *Rpg5* (5), and was recently shown to be required for *rpg4*-mediated resistance effective to a wide range of *P. graminis* f. sp. *tritici* pathotypes, including TTKSK (2,32). The present study reports on the genetic analysis of resistance to multiple formae species of *P. graminis* including *P. graminis* f. sp. *tritici*, *P. graminis* f. sp. *secalis*, 'Scabrum', and *P. graminis* f. sp. *avenae* in an F₈ RIL population derived from an intercross between the *P. graminis* susceptible barley line '73-G1' and 'Q21861'. This population was used in conjunction with molecular markers perfectly linked to both *Rpg1* and *Rpg5* to test the hypothesis that resistance at the *Rpg5* locus is effective in response to *P. graminis* f. sp. *tritici*, *P. graminis* f. sp. *secalis*, and the heterologous rust pathogen *P. graminis* f. sp. *avenae*.

χ^2 analysis of phenotypic data indicated that the resistant responses to *P. graminis* f. sp. *tritici*, *P. graminis* f. sp. *secalis*, 'Scabrum', and *P. graminis* f. sp. *avenae* observed in the 'Q21861'/'73-G1' RIL population was monogenically inherited. Cosegregation of phenotypic data with molecular marker analysis of the RIL progeny found that resistance always cosegregated with the presence of the *Rpg5* marker allele, implying that resistance to *P. graminis* f. sp. *avenae* was associated with *Rpg5*. Martens and coworkers (20) previously performed allelism tests on two barley cultivars that were resistant to *P. graminis* f. sp. *avenae* and no segregation was observed, suggesting the presence of the same gene for resistance to *P. graminis* f. sp. *avenae* in both genotypes, which differed from the resistance gene effective against *P. graminis* f. sp. *tritici*. Phenotypic assessment of the parental genotypes of the 'Q21861'/'73-G1' RIL population in the present study determined that the 'Q21861' parent was highly resistant to all isolates of *P. graminis* tested. Conversely, '73-G1' was moderately susceptible to Australian *P. graminis* f. sp. *avenae* pathotypes but showed an intermediate level of resistance to the Australian *P. graminis* f. sp. *tritici* pathotypes used in this study. Martens et al. (20) originally determined that '73-G1' was moderately susceptible to Canadian pathotypes of *P. graminis* f. sp. *avenae* and resistant to a Canadian pathotype of *P. graminis* f. sp. *tritici*. Previous studies on resistance to different formae speciales of *P. graminis* in barley have never reported on full susceptibility in barley to *P. graminis* f. sp. *tritici* or *P. graminis* f. sp. *avenae* but rather mesothetic reactions with a mixture of ITs consistent with moderate susceptibility (7,9,20). Molecular marker analysis in this study determined that both parents and all RIL progeny were positive for the presence of *Rpg1*, indicating the gene was fixed in this population. Despite this, there was no clear indication of incompatibility associated with *Rpg1* to any of the Australian *P. graminis* cultures used in this study. Derevnina and coworkers (7) previously reported the inability to detect *Rpg1* with Australian *P. graminis* f. sp. *tritici* pathotypes, including 98-1,2,3,5,6,7 that was used in the current study (7). It is however possible that the observed resistance in '73-G1' is an uncharacterized resistance gene effective against Australian *P. graminis* f. sp. *tritici* isolates.

Previous studies suggest that genetic resistance in barley to heterologous rust pathogens is highly diverse and largely dependent on plant genotype (9,13). The literature reports on a large reservoir of genes for nonhost resistance that often overlap with partial resistance mechanisms to adapted pathogens. A recent study

TABLE 4. Joint genotypic and phenotypic segregation analysis of the recombinant inbred line population when tested with three pathotypes of *Puccinia graminis* f. sp. *tritici*, two pathotypes of *P. graminis* f. sp. *avenae*, a single pathotype of *P. graminis* f. sp. *secalis* and single isolate of *P. graminis* f. sp. *tritici* × *P. graminis* f. sp. *secalis* ('Scabrum' rust)

Genotype/phenotype	Number of lines			χ^2 (1R:1S)
	Resistant (R)	Susceptible (S)	Total	
<i>Rpg5</i>	50	0	50	No seg.
<i>rpg5</i>	0	52	52	No seg.
χ^2 (1 <i>Rpg5</i> :1 <i>rpg5</i>)	50	52	102	0.039 ^a

^a $P = 0.843$ ($P = 3.84$ and 6.64 at 1 df for 5 and 1%, respectively).

identified four to five quantitative trait locus (QTL) for prehaustorial resistance to Canadian and Australian pathotypes of *P. graminis* f. sp. *avenae* in a barley doubled haploid population (9). Similar findings were also observed in response to numerous heterologous rusts in three separate mapping populations, where each of the resistant parents contributed different QTL for resistance (13). In the present study, a single gene, either *Rpg5* or a gene very closely linked to *Rpg5* and showing a similar incompatible phenotype, was effective against two Australian pathotypes of *P. graminis* f. sp. *avenae*, however genetic analysis with a larger population size may aid in the further identification of additional minor resistance QTL.

Although all resistant lines were found to carry the *Rpg5*-LRK marker, and all susceptible lines carried the *Rpg5*-LRK/PP2C marker, it is unknown if the resistance to *P. graminis* f. sp. *avenae* is due to *Rpg5* or to one or more closely linked genes at the *Rpg5* locus. In an RIL population size of 102 lines, the estimated linkage distance is 5 to 6 cM, thus a larger population size is needed to determine conclusively if the resistance to *P. graminis* f. sp. *avenae* (and other formae speciales) is due to *Rpg5*. The *Rpg5* locus is complex, with at least four genes at two loci (RMRL1 and RMRL2) needed to confer high level of *rpg4* resistance to *P. graminis* f. sp. *tritici* pathotypes QCCJ and TTKSK (32). Further research using high resolution analyses and gene silencing found that of three tightly linked genes (*HvAdf3*, *HvRgal*, *Rpg5*), the *Rpg5* gene was needed to confer *rpg4* and *Rpg5* resistance to *P. graminis* f. sp. *tritici* and *P. graminis* f. sp. *secalis* (2). Similar research is needed to elucidate the gene conferring resistance to 'Scabrum' and *P. graminis* f. sp. *avenae*, but it appears that this gene is near the *Rpg5* locus and may in fact be the *Rpg5* gene that confers resistance to *P. graminis* f. sp. *tritici* and *P. graminis* f. sp. *secalis*.

In summary, we have determined that a single gene at or near the *Rpg5* locus (contributed by 'Q21861') is effective against multiple Australian pathotypes of *P. graminis* f. sp. *tritici*, *P. graminis* f. sp. *secalis*, and 'Scabrum' as well as the heterologous rust *P. graminis* f. sp. *avenae*. Analysis using diagnostic markers for both resistant and susceptible alleles of the *Rpg5* gene found that only resistant lines were positive for the marker, but further testing with a larger population size is needed to perform fine-mapping and confirm if it is indeed *Rpg5* that confers resistance to *P. graminis* f. sp. *avenae*. Additional testing with other formae speciales of *P. graminis* including f. spp. *lolii* and *dactylis* is planned to determine the role of *Rpg5* in these pathosystems and whether *Rpg5* is involved in PAMP-triggered immunity in barley.

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