Allele Characterization of Genes Required for *rpg4*-Mediated Wheat Stem Rust Resistance Identifies *Rpg5* as the *R* Gene

D. Arora, T. Gross, and R. Brueggeman

Department of Plant Pathology, North Dakota State University, Fargo 58108-6050.
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ABSTRACT

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A highly virulent form of the wheat stem rust pathogen *Puccinia graminis* f. sp. *tritici* race TTKSK is virulent on both wheat and barley, presenting a major threat to world food security. The recessive and temperature-sensitive *rpg4* gene is the only effective source of resistance identified in barley (*Hordeum vulgare*) against *P. graminis* f. sp. *tritici* race TTKSK. Efforts to position clone *rpg4* localized resistance to a small interval on barley chromosome 5HL, tightly linked to the rye stem rust (*P. graminis* f. sp. *secalis*) resistance (*R*) gene *Rpg5*. High-resolution genetic analysis and post-transcriptional gene silencing of the genes at the *rpg4/Rpg5* locus determined that three tightly linked genes (*Rpg5*, *HvRga1*, and *HvAdf3*) are required together for *rpg4*-mediated wheat stem rust resistance. Alleles of the three genes were analyzed from a diverse set of 14 domesticated barley lines (*H. vulgare*) and 8 wild barley accessions (*H. vulgare* subsp. *spontaneum*) to characterize diversity that may determine incompatibility (resistance). The analysis determined that *HvAdf3* and *HvRga1* code for predicted functional proteins that do not appear to contain polymorphisms determining the compatible (susceptible) interactions with the wheat stem rust pathogen and were expressed at the transcriptional level from both resistant and susceptible barley lines. The *HvAdf3* alleles shared 100% amino acid identity among all 22 geno-

types examined. The *P. graminis* f. sp. *tritici* race QCCJ-susceptible barley lines with *HvRga1* alleles containing the limited amino acid substitutions unique to the susceptible varieties also contained predicted nonfunctional *rpg5* alleles. Thus, susceptibility in these lines is likely due to the nonfunctional *RPG5* proteins. The *Rpg5* allele analysis determined that 9 of the 13 *P. graminis* f. sp. *tritici* race QCCJ-susceptible barley lines contain alleles that either code for predicted truncated proteins as the result of a single nucleotide substitution, resulting in a stop codon at amino acid 161, a single cytosine indel causing a frame shift, and a stop codon at amino acid 217, or an indel that deleted the entire STPK domain. The three *P. graminis* f. sp. *tritici* race QCCJ-susceptible lines (Swiss landraces *Hv489*, *Hv492*, and *Hv611*) and the wild barley accession WBDC160 contain *rpg5* alleles predicted to encode full-length proteins containing a nonsynonymous nucleotide substitution that results in the amino acid substitution E1287A. This amino acid substitution present in the uncharacterized C-terminal domain is not found in any resistant line and may be important to elicit the resistance reaction. These data suggest that *rpg4*-mediated resistance against many wheat stem rust pathogen races, including *P. graminis* f. sp. *tritici* race TTKSK, rely on the *Rpg5* *R* gene; thus, *rpg4*- and *Rpg5*-mediated resistance rely on a common *R* gene and should not be considered completely distinct. The data also determined that *Rpg5* gene-specific molecular markers could be used to detect *rpg4*-mediated wheat stem rust resistance for marker-assisted selection.

Wheat stem rust caused by the biotrophic fungal pathogen *Puccinia graminis* f. sp. *tritici* was a devastating disease of wheat and barley grown in the northern Great Plains of the United States

Corresponding author: R. Brueggeman;
E-mail address: Robert.Brueggeman@ndsu.edu

Sequences reported in this article have been deposited in the GenBank database under the following accession numbers. *Rpg5* genomic sequences deposited include the following: KC495438–KC495440, KC495442, KC495443, and KC529339 (barley lines WBDC040, WBDC214, WBDC225, Swiss *Hv492*, Swiss *Hv611*, and WBDC160); *HvRga1* genomic sequences deposited include the following: KC433575–KC433575 and KC433589–KC433592 (WBDC 019, WBDC 040, WBDC 160, WBDC 214, WBDC 220, WBDC 225, Swiss *Hv492*, Swiss *Hv584*, Swiss *Hv587*, Swiss *Hv611*, Swiss *Hv619*, Swiss *Hv645*, Damon11-11, Harrington, Golden Promise, WBDC 269, and WBDC 323); *HvAdf3* genomic sequences deposited include the following: KC335499–KC335515 and KC335517–KC335518 (WBDC 019, WBDC 040, WBDC 160, WBDC 214, WBDC 220, WBDC 225, WBDC 269, WBDC 323, Swiss *Hv489*, Swiss *Hv489*, Swiss *Hv587*, Swiss *Hv611*, Swiss *Hv619*, Swiss *Hv645*, Swiss *Hv672*, Damon11-11, MD2, and Golden Promise). The following sequences were previously deposited by Brueggeman et al. (4): accession numbers EU812563 (genomic sequence from barley 'Morex'), EU878778 (genomic sequence from barley line Q21861), EU883581–EU883583 (*HvRga1* genomic sequence from barley lines Harrington, MD2, and Steptoe, respectively), EU883788–EU883790 (*rpg5* allele [*HvRga2*] genomic sequence from barley lines Harrington, MD2, and Steptoe, respectively), and EU883791 (*HvAdf3* genomic sequence from barley line Steptoe).

and Canada prior to successful management through resistance (*R*) gene deployment (23). The use of genetic resistance in both crops has been a major success, keeping stem rust in check for the past 70 years; however, the emergence of the highly virulent race of the wheat stem rust pathogen *P. graminis* f. sp. *tritici* race TTKSK in Africa and its spread has warranted concern for future production security (27). Barley production is particularly vulnerable because *P. graminis* f. sp. *tritici* race TTKSK and its variant forms have been shown to be virulent on ≈97% of barley varieties from an extensive world barley collection of cultivated and wild barley tested at the seedling stage under controlled conditions (26). *P. graminis* f. sp. *tritici* race TTKSK is also virulent on U.S. barley varieties carrying the *Rpg1* gene, the only major stem rust *R* gene deployed in commercial barley cultivars of the northern Great Plains (23). Barley is also vulnerable because the only effective *P. graminis* f. sp. *tritici* race TTKSK resistance known is the *rpg4/Rpg5* locus (24).

The recessive and temperature-sensitive *rpg4* stem rust *R* gene, presumed to control a gene-for-gene interaction between barley and several wheat stem rust races, including MCCF, QCCJ, and TTKSK, was mapped to a subtelomeric region of barley chromosome 5HL, cosegregating with the rye stem rust *R* gene *Rpg5* (4). High-resolution mapping and positional cloning identified the *Rpg5* gene and determined that *rpg4* and *Rpg5* were distinct yet tightly linked (4). The *Rpg5* gene was shown to encode a novel resistance protein containing three typical resistance domains: the

nucleotide-binding site (NBS), leucine rich repeat (LRR), and a serine threonine protein kinase domain (S/TPK). Subsequent efforts at positional cloning of the *rpg4* gene have not yet identified the gene because of the complex genetic interactions underlying the locus and resistance mechanisms (6,31). However, the recent genetic analysis of three high-resolution populations determined that the *rpg4*-mediated resistance locus (RMRL) is complex and requires at least four genes located at two tightly linked subloci (31). The two subloci have been given the designations RMRL1 and RMRL2 (Fig. 1A).

The *Rpg5*-mediated *P. graminis* f. sp. *secalis* and *rpg4*-mediated *P. graminis* f. sp. *tritici* resistances co-segregated at RMRL1 in two high-resolution mapping populations, Harrington × Q21861 and MD2 × Q21861, but segregate away from each other in the Steptoe × Q21861 population. The genetic data indicated that a second gene given the designation *rpg4*-modifier element 1 (*Rme1*) was functionally polymorphic in the Steptoe × Q21861 population and was delimited to the tightly linked RMRL2 locus (31). The previously identified *Rpg5* gene that confers dominant resistance to isolates of the rye stem rust pathogen was shown by post-transcriptional gene silencing to be required in addition to two other genes at RMRL1 for resistance against *P. graminis* f. sp. *tritici* race QCCJ (31). The other genes at RMRL1 required for *P. graminis* f. sp. *tritici* race QCCJ resistance are a second unrelated NBS-LRR gene (*HvRga1*) and the actin depolymer-

ization factor-like gene *HvAdf3* (Fig. 1). Mapping of *P. graminis* f. sp. *tritici* race TTKSK resistance in barley line Q21861 determined that resistance cosegregated with the *rpg4/Rpg5* locus in a small double-haploid population of 119 individuals from the cross Q21861 × Sm89010 (24). All the high-resolution recombinants examined also behave similarly to *P. graminis* f. sp. *tritici* race QCCJ and TTKSK, suggesting that resistance against the different wheat stem rust races is conferred by the same mechanisms at the *rpg4/Rpg5* locus (31).

The requirement of two closely linked yet unrelated NBS-LRR *R* genes is becoming a common theme to resistance mechanisms (2,3,12,13,16,17,19,21,31,32). Most of these mechanisms requiring dual NBS-LRR genes contain a genetically identifiable *R* gene with functional polymorphism and a second, closely linked nonpolymorphic NBS-LRR gene, which has been typically identified through mutagenesis or post-transcriptional gene silencing resulting in compatibility (3,13,16,17,19,21,32). Alternatively, positional cloning of the rice blast *R* genes *Pikm* and *Pi-5* identified regions containing two NBS-LRR genes: *Pikm1-TS/Pikm2-TS* and *Pi5-1/Pi5-2*, respectively. Complementation via transformation showed that both genes are required for resistance (2,12).

Similar to the *rpg4*-mediated resistance in barley, it has been shown in *Arabidopsis* that an actin depolymerization factor, *AtAdf4*, is required for specific resistance against *Pseudomonas syringae* carrying the *AvrPphB* avirulence factor mediated by the

Fig. 1. Physical map of the *rpg4/Rpg5*-mediated resistance locus and characterization of genes required for resistance. **A**, White horizontal bar represents the physical region containing the *rpg4*-mediated resistance locus (RMRL) and the two genetically defined subloci, RMRL1 and RMRL2, described by Wang et al. (31). White boxes indicate genetic markers delimiting the region and the black bars above indicate the RMRL, RMRL1, and RMRL2 delimited regions. Black arrows along the physical map indicate characterized genes in the RMRL1 region and *rpg4* modifier element 1 (*Rme1*) candidate genes (*Rme1* cg1–cg4) in the RMRL2 region. **B**, Gene structures of the genes at RMRL1 required for *rpg4*-mediated resistance. Horizontal bars represent the genomic DNA structures for *Rpg5*, *HvRga1*, and *HvAdf3*. Black indicates exons, and gray introns and dashed lines represent the 5' and 3' untranslated regions, respectively. Numbered black bars above indicate the positions of the amplicons used for allele sequencing. **C**, Agarose gel showing the amplicons used for direct sequencing of the *Rpg5*, *HvRga1*, and *HvAdf3* alleles amplified from the barley line Q21861. Genes are indicated below and the numbers above correspond to the fragments shown in B.

RPS5/PBS1-resistance mechanism (30). Based on genome sequence analysis, it appears that there are limited *Adf* genes in plants; 12 in *Arabidopsis* and rice (10). This limited number of highly conserved genes appears to play a major role in many developmental (7,29), abiotic (18), and biotic stress responses (14,30), with the concise function determined by the developmental or environmental stimuli. The minimal redundancy within the plant genomes suggests that individual *Adf* genes are differentially modulated by these diverse stimuli involving phosphorylation- and dephosphorylation-dependent signaling. The identification of highly conserved ADF proteins via mutant analysis or by post-transcriptional gene silencing required for specific resistance responses suggests that, although they are required for a specific resistance pathway, they are certainly utilized for other resistance pathways and cellular functions as well. When pathogen challenge is recognized by a cognate resistance receptor, the ADFs must be precisely regulated to mount the defense response, probably via phosphorylation or dephosphorylation mechanisms. *R* gene dogma has historically stated that resistance loci contain single distinct *R* genes, yet the recent scrutiny of many *R* gene loci has led to the paradigm shift that many resistance loci are complexes containing several genes required for resistance; however, the only component identified by genetic analysis is the one that is functionally polymorphic.

Characterization of >8,000 recombinant gametes in three separate barley populations with the same resistance source (Q21861) and three different susceptible parents (Harrington, MD2, and Steptoe) could not separate the three candidate genes required for *rpg4*-mediated resistance present at the RMRL1 locus, indicating that recombination in the region was rare and the genes are typically transferred as a single genetic unit (4,31). However, three recombination events in the Steptoe × Q21861 population determined that a second locus, designated RMRL2, contains a gene or genes required for *rpg4*-mediated resistance against *Puccinia graminis* f. sp. *tritici* races QCCJ and TTKSK. The RMRL2 segregates away from RMRL1 by 0.01 centimorgans in

the Steptoe × Q21861 high-resolution mapping population and, thus, is tightly linked yet genetically distinct from RMRL1. The gene required for *rpg4*-mediated resistance at RMRL2, given the designation *Rme1*, has yet to be identified, although sequencing of the ≈200-kb genetically delimited RMRL2 region is completed and candidate *Rme1* genes have been identified (R. Brueggeman and X. Wang, unpublished data). These data suggest that *rpg4*-mediated resistance, previously considered the single *rpg4* *R* gene, is actually a resistance complex requiring the concerted action of at least four genes at the RMRL (31). This research helped to elucidate the underlying molecular mechanisms determining compatibility and incompatibility and has helped lead to the understanding of important questions concerning rust resistance mechanisms in barley. However, we are still left to answer the fundamental questions of what genes are required for *rpg4*-mediated resistance against the wheat stem rust pathogens and what molecular markers can be used to select for *rpg4*-mediated resistance.

Previous research analyzing *Rpg5* alleles from the single unimproved resistant barley line Q21861 and a limited number of susceptible genotypes suggested that a nonfunctional *rpg5* gene could be used to identify resistant or susceptible barley genotypes containing what has been considered *rpg4*-mediated resistance against the wheat stem rust pathogen (4,31). Here, we tested this hypothesis by analyzing alleles of the three genes recently identified as required for functional *rpg4*-mediated resistance from a total of 9 *P. graminis* f. sp. *tritici* race QCCJ-resistant (*rpg4+*) and 13 *P. graminis* f. sp. *tritici* race QCCJ-susceptible (*Rpg4-*) barley genotypes representing a diverse group of cultivated and wild barley accessions from around the world (Table 1). The allele analysis confirmed that *Rpg5* is the polymorphic *R* gene at the *rpg4/Rpg5* locus and predicted that functional Rpg5 proteins corresponded with *rpg4*-mediated wheat stem rust resistance. Thus, molecular markers developed based on polymorphisms in *rpg5* alleles predicted to encode nonfunctional proteins can be used to select for *rpg4/Rpg5*-mediated wheat stem rust resistance.

TABLE 1. Reaction of barley lines to *Puccinia graminis* f. sp. *secalis* isolate 92-MN-90 and *P. graminis* f. sp. *tritici* pathotypes QCCJ, HKHJ and MCCF

Line	Origin	R5 group ^a	Genotype	IT-M (general reaction) ^b			
				<i>P. graminis</i> f. s. <i>secalis</i> isolate		<i>P. graminis</i> f. sp. <i>tritici</i> pathotype	
				92-MN-90	QCCJ	HKHJ	MCCF
				<i>Rpg5+</i>	<i>rpg4+</i>	<i>Rpg1+</i>	<i>Rpg1 a/o rpg4+</i>
				IT-M (GR)	IT-M (GR)	IT-M (GR)	IT-M (GR)
Q21861	Mexico	1 R	<i>Rpg1+</i> , <i>rpg4+/Rpg5+</i>	0;1 (R)	0;1 (R)	0;1 (R)	0;1 (R)
G.P.	Chester, England	1 S	<i>rpg1-</i> , <i>Rpg4-/rpg5-</i>	3 (S)	3 (S)	3 (S)	3 (S)
MD2	Manitoba, Canada	1 S	<i>rpg1-</i> , <i>Rpg4-/rpg5-</i>	3 (S)	3 (S)	3 (S)	3 (S)
Steptoe	Washington, United States	2 S	<i>rpg1-</i> , <i>Rpg4-/rpg5-</i>	3 (S)	3 (S)	3 (S)	3 (S)
Morex	Minnesota, United States	2 S	<i>Rpg1+</i> , <i>Rpg4-/rpg5-</i>	3 (S)	3 (S)	21 (MR)	12 (R)
Harrington	Saskatchewan, Canada	3 S	<i>rpg1-</i> , <i>Rpg4-/rpg5-</i>	3-2 (MS)	3-2 (MS)	3 (S)	3-2 (MS)
Hv489	Trans, Switzerland	4 S	<i>rpg1-</i> , <i>Rpg4-/rpg5-</i>	3 (S)	3 (S)	3 (S)	3 (S)
Hv492	Scheid, Switzerland	4 S	<i>rpg1-</i> , <i>Rpg4-/rpg5-</i>	3 (S)	3 (S)	3 (S)	3 (S)
Hv584	Fuornis, Switzerland	3 R	<i>rpg1-</i> , <i>rpg4+/Rpg5+</i>	0;1 (R)	0;1 (R)	3 (S)	0;1 (R)
Hv587	Platta, Switzerland	3 R	<i>rpg1-</i> , <i>rpg4+/Rpg5+</i>	0;1 (R)	12 (R)	3 (S)	12 (R)
Hv611	Plan, Switzerland	4 S	<i>rpg1-</i> , <i>Rpg4-/rpg5-</i>	3 (S)	3 (S)	3 (S)	3 (S)
Hv619	Fetan, Switzerland	3 R	<i>rpg1-</i> , <i>rpg4+/Rpg5+</i>	10; (R)	12 (R)	3 (S)	10; (R)
Hv645	Scuol, Switzerland	1 S	<i>rpg1-</i> , <i>Rpg4-/rpg5-</i>	3 (S)	3 (S)	3 (S)	3 (S)
Hv672	Surava, Switzerland	3 S	<i>Rpg1+</i> , <i>Rpg4-/rpg5-</i>	3 (S)	3 (S)	12 (R)	12 (R)
Damon 11-11	Israel	2 R	<i>rpg1-</i> , <i>rpg4+/Rpg5+</i>	0;1 (R)	12 (R)	3 (S)	0;1 (R)
WBDC 019	West Azerbaijan, Iran	3 S	<i>rpg1-</i> , <i>Rpg4-/rpg5-</i>	3 (S)	3 (S)	3 (S)	3 (S)
WBDC 040	Haifa, Israel	4 R	<i>rpg1-</i> , <i>rpg4+/Rpg5+</i>	0;1 (R)	0;1 (R)	3 (S)	12 (R)
WBDC 160	Sweida, Syria	4 S	<i>rpg1-</i> , <i>Rpg4-/rpg5-</i>	3 (S)	3 (S)	3 (S)	3 (S)
WBDC 214	Samarkand, Uzbekistan	4 R	<i>rpg1-</i> , <i>rpg4+/Rpg5+</i>	0;1 (R)	10; (R)	3 (S)	21 (MR)
WBDC 220	Chimkent, Kazakhstan	4 R	<i>rpg1-</i> , <i>rpg4+/Rpg5+</i>	10; (R)	10; (R)	3 (S)	12 (R)
WBDC 225	Dushanbe, Tajikistan	4 R	<i>rpg1-</i> , <i>rpg4+/Rpg5+</i>	0;1 (R)	12 (R)	3 (S)	12 (R)
WBDC 269	Lebanon	3 S	<i>rpg1-</i> , <i>Rpg4-/rpg5-</i>	3 (S)	3 (S)	3 (S)	3 (S)

^a R5 groups were assigned based on the genotype of the *Rpg5* alleles.

^b Mode infection type (IT-M) observed based on a 0-to-4 scale of Stakman et al. (22). GR is the general reaction: R = resistant, MR = moderately resistant, MS = moderately susceptible, and S = susceptible.

MATERIALS AND METHODS

Barley germplasm. A collection of six cultivated barley lines (*Hordeum vulgare*), eight Swiss landraces (*H. vulgare*), and eight wild barley accessions (*H. vulgare* subsp. *spontaneum*) were phenotyped and alleles of *Rpg5*, *HvRga1*, and *HvAdf3* were analyzed. All of the barley cultivars utilized in this study, with the exception of 'Golden Promise', were previously selected for the positional cloning of the *Rpg5* gene and in the effort to identify the *rpg4* gene (4,31). The *P. graminis* f. sp. *tritici* race QCCJ-susceptible Golden Promise was included because it is the most efficiently transformed barley genotype (11). The eight Swiss landraces and seven of the wild barley accessions were previously utilized to determine allele diversity of the stem rust *R* gene *Rpg1* (15). The eight wild barley accessions evaluated in this study have differential reactions to the rust isolates used and were collected from diverse regions of the Fertile Crescent, the center of barley origin (Table 1).

Stem rust isolates and phenotyping. The barley lines used in this study were phenotyped for their infection types (ITs) to single-pustule isolates (QCC-2, A5-MCCF, and R29M) of three wheat stem rust races (*P. graminis* f. sp. *tritici* races QCCJ, MCCF, and HKHJ, respectively) and the rye stem rust isolate 92-MN-90. The race designations given to the *P. graminis* f. sp. *tritici* isolates used were designated by wheat differentials, as described by Roelfs et al. (20). The *rpg4/Rpg5* resistance complex confers recessive and temperature-dependent resistance to *P. graminis* f. sp. *tritici* race QCCJ and TTKSK. The *Rpg1* gene is ineffective against both *P. graminis* f. sp. *tritici* races QCCJ and TTKSK. The *Rpg1* gene and the *rpg4/Rpg5* complex confer dominant and recessive or temperature-sensitive resistance to *P. graminis* f. sp. *tritici* race MCCF, respectively. The *Rpg1* gene confers specific resistance to the isolate *P. graminis* f. sp. *tritici* race HKHJ; thus, HKHJ is virulent on lines containing the *rpg4/Rpg5* locus. The *Rpg5* gene alone confers dominant resistance to the rye stem rust isolate 92-MN-90. The stem rust evaluations were done at North Dakota State University. Fresh urediniospores, increased on the universal susceptible barley 'Steptoe', were applied to 7- to 9-day-old seedlings after primary leaves were fully expanded using a spore-oil suspension atomizer. The inoculum, 10 mg of urediniospores per 1 ml of Solitol oil, was applied to the seedlings at a rate of ≈ 0.1 mg/seedling. The seedlings were incubated as previously described by Steffenson et al. (24), except that, after incubation in a mist chamber and

drying, the plants were returned to a growth chamber set to a 14-h photoperiod and day and night temperatures of 22.5 ± 1 and $19 \pm 1^\circ\text{C}$, respectively. Fourteen days after inoculation, the ITs were assessed using the 0-to-4 scale modified for barley from the scale developed for wheat by Stakman et al. (22), as previously described by Sun and Steffenson (28).

Allele sequencing and analysis. Genomic DNA was isolated from young barley leaves (7- to 10-day-old seedlings) from a single plant of each of the 22 selected barley lines. The plants were grown in 6-in. plastic pots containing Sunshine brand potting mix 1 supplemented with Osmocote 14-14-14 at 14.3 g/pot in a growth chamber under cool fluorescent lights with a 14-h photoperiod and day and night temperatures of 22.5 ± 1 and $19 \pm 1^\circ\text{C}$, respectively. All DNA was isolated according to Edwards et al. (9). Pairs of overlapping primers specific to each gene required for *rpg4*-mediated resistance (*Rpg5*, *HvRga1*, and *HvAdf3*) were designed to produce ≈ 1 -kb overlapping amplicons. The primer sequences, sizes of amplicons produced, and relative positions of the *Rpg5*, *HvRga1*, and *HvAdf3* primers are given in Table 2 and Figure 1B and C. The polymerase chain reaction (PCR) reactions were carried out on all 22 barley genotypes and analyzed using all 17 primer combinations (Tables 1 and 2). The 25- μl PCR reactions consisted of ≈ 100 ng of gDNA, 20 pmol of each forward and reverse primers, $1\times$ Go Taq Flexi Buffer (Promega Corp., Madison, WI), 1 U of Go Taq Flexi DNA polymerase, and 0.2 mM dNTPs. Amplification was performed in a Mastercycler pro (Eppendorf, Hauppauge, NY) thermocycler using the following parameters: 95°C for 4 min; 35 cycles of 95°C for 30 s, 62°C for 1 min, and 72°C for 1 min; followed by 72°C for 5 min. Amplification of each reaction was confirmed by loading 10 μl of each PCR reactions onto 1% agarose gels (Fig. 1C). The remaining 20 μl of each reaction was purified using cycle pure spin columns (Omega Bio-Tek, Norcross, GA). Direct sequencing was performed by Genscript (Piscataway, NJ) on an ABI 3730xl (Applied Biosystems, Carlsbad, CA) using the respective forward and reverse primers. Contigs were generated for each allele and allele alignment was performed using Vecor NTI software (Life Technologies, Grand Island, NY). The deduced amino acid sequences for each group of alleles identified are given in Figure 2.

RNA extraction. RNA was extracted from leaf tissue collected at the second-leaf stage from three plants for each of the 22 selected barley lines. The plants were grown in 6-in. plastic pots containing Sunshine brand potting mix 1 supplemented with Osmocote 14-14-14 at 14.3 g/pot in a temperature-controlled green-

TABLE 2. Primers used for *Rpg5*, *HvRga1*, and *HvAdf3* allele analysis

Fragment	Primers	Primer sequences	Size	Location ^a
<i>Rpg5</i> -1	<i>Rpg5</i> -F1/ <i>Rpg5</i> -R1	CCGCCTACCACACCTCCGATTCCAC/TCAGGTTTGATGGCTGTCTCTGGAG	1,087	-303/785
<i>Rpg5</i> -2	<i>Rpg5</i> -F2/ <i>Rpg5</i> -R2	TGTGCCGGATGTCAGATCTGGACCTC/GAAGTACATTGTTGCTCTCTCCTCAGA	1,048	492/1,539
<i>Rpg5</i> -3	<i>Rpg5</i> -F3/ <i>Rpg5</i> -R3	CATCAATGTTCTAACATGGCGTATC/GGGACATTCTGATGCTTTCCGGATC	1,143	1,396/2,538
<i>Rpg5</i> -4	<i>Rpg5</i> -F4/ <i>Rpg5</i> -R4	GGTGTGTCATGTACAGTTGATCAC/CTATCTGAGATCTAACCGTGTATTG	1,258	2,351/3,584
<i>Rpg5</i> -5	<i>Rpg5</i> -F5/ <i>Rpg5</i> -R5	AGATGCACCTATCTGCATCGAGCAC/ATGTCGAGCCTGAGACTACTGACAC	1,016	3,422/4,438
<i>Rpg5</i> -6	<i>Rpg5</i> -F6/ <i>Rpg5</i> -R6	CCCCATACTTCAAATTACACTTCGTGCC/GCAACCTTCATTCTGACAGACCATG	1,019	4,234/5,252
<i>Rpg5</i> -7	<i>Rpg5</i> -F7/ <i>Rpg5</i> -R7	CTATTGAGAAGTATGCACTGACCAG/GCACCAGCACTCCTGTGTGTTTCGAC	1,136	5,023/6,182
<i>Rpg5</i> -8	<i>Rpg5</i> -F8/ <i>Rpg5</i> -R8	ACGCTCCGAGGAGGAGGTTTACAG/GCTTTGGTTTCAGCCGAACAGACGAG	985	5,993/6,977
<i>Rpg5</i> -9	<i>Rpg5</i> -F9/ <i>Rpg5</i> -R9	GCTTGGGGATGCAAGCATAGAGCTG/GAGTGATGTGTATAGCTTTGGCGTG	1,001	6,775/7,775
<i>Rpg5</i> -10	<i>Rpg5</i> -F10/ <i>Rpg5</i> -R10	TGCATCTATCTGCTCATGCAAGGAG/AACAATATTACCTGCGGCACCAAC	1,082	7,302/8,383
<i>HvRga1</i> -1	<i>HvRga1</i> -F1/ <i>HvRga1</i> -R1	TCAACCGCTTTTCGCTCATATGCATG/GAAGTTGCTCTTCCGGATCCTGATC	740	-323/418
<i>HvRga1</i> -2	<i>HvRga1</i> -F2/ <i>HvRga1</i> -R2	TTGGCCGCTCGAATGGCAGCAGATC/CCCCTGAATGAATGTGCGCAAATAG	1,063	146/1,208
<i>HvRga1</i> -3	<i>HvRga1</i> -F3/ <i>HvRga1</i> -R3	GTAGTCATGATAAGATGGTGC/TTGCAATCTACTAGTCCATACGA	939	949/1,887
<i>HvRga1</i> -4	<i>HvRga1</i> -F4/ <i>HvRga1</i> -R4	GATGGAATGTGGTCTAGGACTGTAG/TCTCTTTGCTGCGCAGATTCTTCTC	1,086	1,715/2,800
<i>HvRga1</i> -5	<i>HvRga1</i> -F5/ <i>HvRga1</i> -R5	GAATCCCCTGATAAGGCGATGGCTG/CTGCGTCAGCTTGCTCTCAACTTG	1,027	2,526/3,552
<i>HvRga1</i> -6	<i>HvRga1</i> -F6/ <i>HvRga1</i> -R6	GCTACTTGAAGGTGACTGCACACAG/AACAGGTTTGCACTGCAGACAGTAG	1,061	3,189/4,249
<i>HvAdf3</i> -Q1	<i>HvAdf3</i> -F1/ <i>HvAdf3</i> -QR1	ATCATACGAGCAGCTCCTGTC/CGATCCCATGCGCAGGTATGCCAAG	660	-80/581
<i>HvAdf3</i> -S1	<i>HvAdf3</i> -F1/ <i>HvAdf3</i> -MR1	ATCATACGAGCAGCTCCTGTC/AACCAAGCACTCAATTCTCGAAGAG	718	-80/639 ^b

^a Location of amplicons is given as position of the 5' terminal nucleotide from the forward and reverse primers in relation to the ATG start codon, with A being position 1 from the Q21861 sequence.

^b Location of amplicons is given as position of the 5' terminal nucleotide from the forward and reverse primers in relation to the ATG start codon, with A being position 1 from the Morex sequence.

house with a 14-h photoperiod and day and night temperatures of 22 and 18°C, respectively. The RNA was extracted using a Trizol mini method modified from Chomczynski and Sacchi (8). Secondary seedling leaf tissue (≈4 cm) was ground in 1.5-ml microcentrifuge tubes with 1 ml of Trizol reagent (prewarmed at 65°C) using disposable microfuge pestles. After complete maceration of leaf tissue, the sample was placed in a 65°C water bath for 10 min. The homogenized mixture was centrifuged at 9,000 rpm for 10 min to pellet the cellular debris and ≈1 ml of the supernatant was transferred to a new 1.5-ml microcentrifuge tube. Chloroform (400 µl) was added, the solution was vortexed for 5 s, and the samples were left at room temperature for 5 min prior to 15 min of centrifugation at 9,000 rpm in a 4°C bench-top centrifuge. Supernatant (≈400 µl) was transferred to a new 1.5-ml microcentrifuge tube and 100 µl of isopropanol and 100 µl of a 0.8 M sodium citrate + 1.2 M sodium chloride solution was added. The mixture was homogenized by gentle inversion and centrifuged at 13,000 rpm for 15 min at 4°C. The pelleted RNA was gently washed with ice-cold 75% ethanol and left to dry for 10 min after pipetting out the excess ethanol. The RNA was then resuspended in 30 µl of ultrapure water containing 0.003% RNasin. The integrity of the RNA was determined by the visualization of four ribosomal RNA bands after separation on a 0.5× Tris-borate-EDTA, 1% agarose gel.

Reverse transcription and reverse-transcription PCR. RNA obtained from seedling tissue of each line was converted into cDNA using the Promega GoScript reverse-transcription (RT) system following the manufacturer's standard procedure. RNA (≈0.5 µg) was added to RT mix for each genotype, normalized

based on the intensity of the bands visualized on the agarose gel, and checked using a Qubit Fluorometer. The 20-µl RT reactions were diluted 1:1 with ultrapure H₂O and RT-PCR was set up using exon-specific primer pairs that amplified across at least 1 intron from each of the genes required for *rpg4*-mediated resistance (*Rpg5*, *HvRga1*, and *HvAdf3*). The 25-µl RT-PCR reaction mix was the same as described for genomic DNA, except that 2 µl of the diluted cDNA was used as the template. Amplification was performed in a Mastercycler pro (Eppendorf) thermocycler using the following parameters: 95°C for 1 min; 35 cycles of 95°C for 30 s, 62°C for 30 s, and 72°C for 30 s; followed by 72°C for 5 min for GAPDH, *Rpg5*, and *HvAdf3* amplification. To amplify the *HvRga1*-specific cDNA fragment, 33 cycles of 95°C for 1 min, 62°C for 1 min, and 72°C for 1 min were used, keeping the other conditions the same. The resistant barley line Q21861 was used as the genomic control for each amplicon. The RT reactions were visually normalized using primers specific to the GAPDH housekeeping gene. The *HvGAPDH* primers used were previously reported by Horvath et al. (11). The primer sequences, amplicon sizes, and positions of the genes on the Q21861 genomic template are given in Table 3 and Figure 3.

RESULTS

Stem rust phenotyping. The *P. graminis* f. sp. *tritici* race QCCJ susceptible and resistant control genotypes utilized in these experiments reacted as expected, with the resistant line Q21861 (*rpg4*+, *Rpg5*+) exhibiting resistant ITs and the susceptible controls Steptoe, 'Morex', 'Harrington', Golden Promise, and line

Fig. 2. Protein domain structures and amino acid alignment for the *Rpg5*, *HvRga1*, and *HvAdf3* alleles. Boxes represent the protein domains with amino acid positions and domain labels shown above. Barley lines were grouped based on their *RPG5* amino acid sequences and general reaction types with R = resistant and S = susceptible. Differences in amino acid sequences compared with group 1 resistant (G1R) are highlighted with light gray vertical bars. Barley lines contained in each group are as follows: GR1 (Q21861); GR2 (Damon 11-11, WBDC040, WBDC214, WBDC220, and WBDC225); GR3 (*Hv584*, *Hv587*, and *Hv619*); G1S (Golden Promise, MD2, and *Hv645*); G2S (Steptoe and Morex); G3S (Harrington, *Hv672*, WBDC019, and WBDC269); and G4S (*Hv489*, *Hv492*, *Hv611*, and WBDC160).

MD2 (*Rpg4*–, *rpg5*–) exhibiting susceptible ITs (Table 1). The Swiss landraces *Hv584*, *Hv587*, and *Hv619* showed *P. graminis* f. sp. *tritici* race QCCJ-resistant ITs, with medians ranging from 0;1 to 12. The Swiss landraces *Hv489*, *Hv492*, *Hv611*, *Hv645*, and *Hv672* exhibited susceptible *P. graminis* f. sp. *tritici* race QCCJ phenotypes, with the median IT for all being 3. The wild barley accessions Damon 11-11, WBDC040, WBDC214, WBDC220, and WBDC225 showed *P. graminis* f. sp. *tritici* race QCCJ resistance, with the median ITs ranging from 0;1 to 12. The wild barley accessions WBDC019, WBDC160, and WBDC269 showed *P. graminis* f. sp. *tritici* race QCCJ susceptibility, with median ITs of 3. The *P. graminis* f. sp. *tritici* race QCCJ-resistant Swiss landraces and wild barley accessions were previously identified as resistant to *P. graminis* f. sp. *tritici* race MCCF, with the exception of Damon 11-11, which was not included in the previous study. However, only one genotype contained an intact *Rpg1* gene and was resistant to the *Rpg1*-avirulent *P. graminis* f. sp. *tritici* race HKHJ, suggesting that the race MCCF resistance in the majority of the resistant accessions was conferred by a second source of wheat stem rust resistance, presumed to be the *rpg4* gene (15). The wheat stem rust *P. graminis* f. sp. *tritici* race

MCCF phenotyping data (Table 1) were consistent with the general reaction designations of lines utilized in previous studies (5,15).
The *P. graminis* f. sp. *secalis* isolate 92-MN-90 stem rust phenotyping data on all the susceptible and resistant control lines reacted as expected, with the resistant line Q21861 (*Rpg5*+), exhibiting the resistant IT 0;1 and the susceptible Steptoe, Morex, Harrington, Golden Promise, and experimental line MD2 (all *rpg5*–) exhibiting susceptible ITs, with the median IT ranging from 3-2 to 3 (Table 1). These data were similar to the phenotyping data previously reported for the these lines (4). The general reactions for *P. graminis* f. sp. *secalis* isolate 92-MN-90 were very similar to those of *P. graminis* f. sp. *tritici* race QCCJ, suggesting that resistance to both wheat and rye stem rust pathogens is conferred by a functional *rpg4/Rpg5* resistance locus. The *H. spontaneum* line Damon 11-11 exhibited a resistant IT, suggesting that Damon 11-11 contains a functional *rpg4/Rpg5* resistance complex.
Allele sequencing and analysis. Allele analysis of the *HvAdf3* gene showed a very high level of gene conservation, with only two synonymous nucleotide substitutions identified in the 22

TABLE 3. Primers used for expression analysis of the *Rpg5*, *HvRga1*, and *HvAdf3* alleles

Gene	Primers	Primer sequences	Amplicon size	Location ^a
<i>Rpg5</i>	QST Kin F1/ <i>Rpg5</i> R7.3	AAAATGCGCCACACGGAACCTTCA/GCAACCTTCATTCTGACAGACCATG	354/748	4,505/5,252
<i>HvRga1</i>	R4-F2/R4-R4	TTGGCCGCTCGAATGGCAGCAGATC/TCTCTTTGCCTGGCAGATTCTTCTC	1,227/2,655	146/2,800
<i>HvAdf3</i>	ADF3 F2/ADF3 R3	CCAACCTCCGGATCCATCAACAATG/GTCGTCCATCTTGTACACCACGAAC	117/356	–22/334

^a Amplicon sizes are given for cDNA/gDNA from the Q21861 sequence. The location of amplicons is given as position of the 5' terminal nucleotide from the forward and reverse primers in relation to the ATG start codon, with A being position 1 from the Q21861 gDNA sequence.

Fig. 3. Expression analysis of the *Rpg5*, *HvRga1*, and *HvAdf3* alleles. Lines analyzed are labeled at the top. All lanes used cDNA template, except the last lane is Q21861 genomic DNA and the first lane is a 100-bp molecular marker. Horizontal bars below the gel images represent the gene structure for each gene analyzed; black represents exons, and gray introns and dashed lines are the 5' and 3' prime untranslated regions, respectively. Numbered arrows to the left indicate the approximate size of cDNA and genomic DNA amplicons. **A**, *Rpg5* analysis; **B**, *HvRga1* analysis; **C**, *HvAdf3* analysis; and **D**, *HvGAPDH* housekeeping gene.

barley genotypes examined. The barley landrace *Hv672* had a single-nucleotide substitution, G233C, that resulted in no change to the amino acid sequence. The *H. spontaneum* line WBDC019 also contained a single synonymous nucleotide substitution, C245T, that resulted in no change in the amino acid sequence. The 100% conservation of the *HvAdf3* protein suggests that no functional polymorphism exists between the genotypes examined; thus, differences at *HvAdf3* did not contribute to the compatible or incompatible interaction with the wheat stem rust pathogen in the genotypes tested.

The allele analysis of the *HvRga1* gene determined that this NBS-LRR domain gene was predicted to encode a full-length 859-amino-acid resistance-like protein from all the alleles examined. There were eight amino acid sequence differences among the nine *P. graminis* f. sp. *tritici* race QCCJ-resistant alleles examined. Compared with the Q21861-resistant allele, considered a group 1 resistant genotype, the S146N, E149Q, A190S, S290A, L325I, A445G, N474D, and R843G amino acid substitutions (Fig. 2) were present in *P. graminis* f. sp. *tritici* race QCCJ-resistant genotypes and, therefore, considered not to effect the incompatible (resistant) interaction with the pathogen. The group 1 susceptible lines MD2 and Golden Promise contained one amino acid substitution, Q624K, that was unique to these susceptible genotypes. The group 3 susceptible Harrington contained the substitutions S156Y and C324R that were unique to this susceptible cultivar. The group 3 susceptible accession WBDC019 contained the two substitutions C772S and V846I, unique to this wild barley accession. Although these differences could result in nonfunctional *HvRGA1* proteins, these alleles were present with predicted nonfunctional *rpg5* alleles containing mutations that resulted in truncated proteins. Thus, the compatible interaction with *P. graminis* f. sp. *tritici* race QCCJ in these susceptible barley genotypes was probably due to the nonfunctional *rpg5* alleles.

The *Rpg5* allele analysis determined that all of the resistant genotypes contained predicted functional RPG5 proteins; however, there was significant allele diversity that provides some hint to regions important for stem rust resistance function. The barley line Q21861, the original source of *rpg4* and the variety utilized in the cloning of the *Rpg5* gene, was given the designation group 1 resistant (Fig. 2; Table 1). The *Rpg5* allele analysis determined that the four *H. spontaneum* wild barley accessions WBDC040, WBDC214, WBDC225, and Damon 11-11 contained *Rpg5* alleles that only differed from the Q21861 allele by the single amino acid substitution R205G (Fig. 2) and were given the designation group 2 resistant (Table 1). The genotypes containing the group 3 resistant alleles, including *Hv584*, *Hv587*, and *Hv619*, had the greatest *Rpg5* diversity for an allele encoding a functional *P. graminis* f. sp. *tritici* race QCCJ and *P. graminis* f. sp. *secalis* isolate 92-MN-90 stem rust *R* gene. The diversity of the group 3 allele compared with the Q21861 allele includes a 5-amino-acid deletion at the N terminus (amino acids 59 to 63) and a 10-amino-acid insertion at the C terminus (amino acids 1,367 to 1,376). The group 3 resistant alleles also contained single-nucleotide substitutions that introduced 15 amino acid substitutions (L64M, R205G, S272P, S507N, D619N, S820C, A926T, S930A, N1138D, L1284V, H1288R, A1293E, I1297F, F1303S, and Q1366A) (Fig. 2).

The group 1 susceptible genotypes (MD2, Golden Promise, and *Hv645*) contain a single cytosine insertion within the first exon, resulting in a frame shift and a predicted truncated RPG5 protein that results in a stop codon at amino acid position 217 (Fig. 2).

The group 2 and group 3 susceptible genotypes (Harrington, Morex, Steptoe, *Hv672*, WBDC019, and WBDC269) code for a predicted nonfunctional RPG5 protein due to the insertion or deletion event leading to the deletion of the protein kinase domain and insertion of the *HvPP2C1* gene (Fig. 2) (4,31).

The group 4 susceptible genotypes (*Hv489*, *Hv492*, *Hv611*, and WBDC160) code for a predicted full-length RPG5 protein that

was identical to the group 3 resistance allele, except for the two amino acid differences S820C and E1287A. The Q21861 (group 1 resistant) allele contained the serine residue at position 820; thus, this amino acid substitution probably does not contribute to a nonfunctional RPG5 protein. However, the alanine at position 1,287 is unique to this susceptible allele and is considered the polymorphism that is responsible for the compatible interaction with the wheat stem rust pathogen. It must be further explained that the *HvRga1* allele in the group 4 susceptible genotypes are identical to the group 3 resistant *HvRga1* alleles, except for the aspartic acid residue at position 474. However, the WBDC040 group 2 resistant accession contains aspartic acid at position 474 (Fig. 2); thus, this difference in the *HvRga1* allele probably does not contribute to susceptibility in the group 4 susceptible genotypes.

Expression analysis. RT-PCR was run for all the genotypes with primer pairs specific to each gene. The housekeeping gene GAPDH was amplified from all 22 cDNAs and Q21861 genomic DNA to determine the integrity of the cDNA samples (Fig. 3). The *HvGAPDH* primers amplified the ≈250-bp cDNA amplicon, showing that all 22 samples contained GAPDH expressed at the mRNA level. The RT-PCR reactions using the *HvAdf3*-specific and *HvRga1*-specific primers amplified a 117-bp band for the *HvAdf3* gene and a 1,227-bp band for the *HvRga1* gene. The sizes correspond to the expected amplicon sizes, showing that these are amplified from cDNA with introns spliced out. The *HvAdf3* cDNA produced a weak band the size of the genomic amplicon (356 bp) that is due to either nonspliced mRNA or genomic contamination. However, the mRNA was treated with DNAase prior to cDNA synthesis and a genomic band did not amplify from the other primer combinations, suggesting that it may be the product of an unspliced or alternate splice form. The amplification, although a bit variable, determined that both *HvAdf3* and *HvRga1* genes are expressed at the transcript level from all 22 genotypes analyzed. The *Rpg5*-specific amplification showed expression in all the resistant genotypes. Some of the susceptible genotypes (i.e., those with an intact STPK domain and a stop codon at amino acid position 217) and the group 4 susceptible lines with a full-length *rpg5* allele were expressed at the transcript level. Thus, all genotypes except the group 2 susceptible genotypes (Steptoe, Morex, Harrington, *Hv672*, and wild barley accessions WBDC019 and WBDC269) produced the expected 354-bp cDNA band corresponding to the LRR to STPK junction, with introns three and four properly spliced. The results show that the *rpg5*-susceptible alleles containing the STPK domain are expressed at the transcript level but are predicted to code for truncated proteins and the susceptible alleles missing the STPK domain do not have *Rpg5* STPK region transcripts as expected. All the RT-PCR reactions were run with a Q21861 genomic control to show the size of the amplicons without the introns spliced out. Using the Q21861 genomic DNA as the template in each reaction produced amplicon of 748, 2,655, and 356 bp for *Rpg5*, *HvRga1*, and *HvAdf3*, respectively (Fig. 3).

DISCUSSION

The Upper Midwest region of the United States and the central Prairie Provinces of Canada comprise the largest barley production region in North America. Currently, the only known stem rust *R* gene deployed in barley cultivars grown in the region is the single *Rpg1* gene. This gene has conferred remarkably durable resistance, considering the vast acreage on which varieties containing *Rpg1* have been grown and the disease pressure exerted; however it is ineffective against *P. graminis* f. sp. *tritici* race QCCJ and, more importantly, race TTKSK and its lineage. This leaves barley production in North America vulnerable to stem rust epidemics should *P. graminis* f. sp. *tritici* race TTKSK be introduced.

The only known source of resistance effective against *P. graminis* f. sp. *tritici* race TTKSK in barley is the *rpg4* gene, warranting further research to understand this complex resistance mechanism. Recent research identified three genes at the RMRL that are required for wheat stem rust race QCCJ resistance and, presumably, TTKSK resistance. This conclusion is supported by the observation that all recombinants used to genetically dissect the *rpg4/Rpg5* locus behave similarly to both races (4,31). Because resistance to *P. graminis* f. sp. *tritici* races QCCJ and TTKSK conferred at the *rpg4/Rpg5* locus has been shown to be genetically similar (31), QCCJ was used as a TTKSK surrogate in this study due to the restrictions on the use of TTKSK in North America.

The recent identification of the three genes at RMRL required for resistance (31) prompted our research to determine whether *P. graminis* f. sp. *secalis* and *P. graminis* f. sp. *tritici* resistance or susceptibility correlated with functional or nonfunctional *Rpg5*, *HvRga1*, or *HvAdf3* genes. To test the hypothesis that polymorphisms in one of these three genes should determine compatibility or incompatibility with the pathogen, we sequenced alleles from a diverse collection of cultivated and wild barley accessions. A comprehensive evaluation of a germplasm collection of 74 barley landraces from Switzerland on the reaction type to diverse pathotypes of *P. graminis* f. sp. *tritici* and isolates of *P. graminis* f. sp. *secalis* revealed that three lines designated *Hv584*, *Hv587*, and *Hv619* were resistant to *P. graminis* f. sp. *secalis* isolate 92-MN-90 and *P. graminis* f. sp. *tritici* races QCCJ and TTKSK, presumed to be conferred by functional *rpg4/Rpg5* resistance alleles (15,25,26). It was also determined that the lines were resistant to the wheat stem rust race MCCF but did not have a functional *Rpg1* gene. Thus, it was proposed that a functional *rpg4* gene conditioned the *P. graminis* f. sp. *tritici* race MCCF resistance as well (15). Recent allelism tests between Q21861 and *Hv584* determined that the two lines contain alleles of *rpg4/Rpg5* (R. Brueggeman, unpublished data). Therefore, we included the set of Swiss landraces and wild barley accessions that were previously characterized for *Rpg1* allele diversity (15) in our set of genotypes used for *Rpg5*, *HvRga1*, and *HvAdf3* allele analysis. The wild barley accession Damon 11-11 was also included because it contains resistance to *P. graminis* f. sp. *secalis* isolate 92-MN-90 and *P. graminis* f. sp. *tritici* races MCCF and QCCJ that was previously mapped to the *rpg4/Rpg5* locus (1).

All the barley genotypes containing resistance to *P. graminis* f. sp. *tritici* race QCCJ contain a predicted functional *Rpg5* allele and most of the susceptible genotypes examined contain a predicted truncated nonfunctional *rpg5* allele (Table 1; Fig. 2). The exceptions are the susceptible *H. vulgare* landraces *Hv489*, *Hv492*, and *Hv611* and the *H. spontaneum* line WBDC160, which are predicted to encode full-length RPG5 proteins. Interestingly, these alleles contain a nonsynonymous nucleotide substitution that results in the amino acid substitution E1287A that is unique to these four susceptible genotypes compared with all the resistant alleles. All resistant alleles contain glutamate at position 1,287, which is frequently an amino acid involved in protein active or binding sites. It is quite possible that the substitution with the nonreactive alanine residue, rarely involved in protein function, results in the loss of stem rust function by causing a loss of protein-protein interaction or activity. The group 4 susceptible genotypes also contain *HvRga1* and *HvAdf3* alleles with amino acid differences compared with the Q21861 allele but that are shared with other resistant genotypes, suggesting that susceptibility is not due to nonfunctional *HvRga1* or *HvAdf3* alleles. Thus, we have identified a new susceptible *Rpg5* allele that can be attributed to this single amino acid difference. It appears that this amino acid in the C-terminal region is important for stem rust resistance function and will be a target for future functional analysis of the RPG5 protein.

The data presented here suggest that the rye stem rust *R* gene *Rpg5*, previously considered distinct from *rpg4*, is the polymor-

phic *R* gene required for *rpg4*- and *Rpg5*-mediated resistance, and the polymorphisms in the *Rpg5* gene could provide perfectly linked markers to determine whether barley lines or cultivars contain *rpg4*-mediated resistance to the wheat stem rust pathogens. These markers could also be utilized to screen barley lines to determine whether newly identified sources of TTKSK resistance are novel. Although the rye and wheat stem rust resistance mechanisms rely on the same *R* gene component, the requirement of the *Rme1* gene and the recessive nature of the wheat stem rust resistance versus the dominant nature of the rye stem rust resistance suggests that the two stem rust pathogens interact differently with the *Rpg5* gene and function via different resistance mechanisms.

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