Genetic Characterization of Resistance to Wheat Stem Rust Race TTKSK in Landrace and Wild Barley Accessions Identifies the *rpg4/Rpg5* Locus

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Accepted for publication 21 July 2014.

ABSTRACT

Mamo, B. E., Smith, K. P., Brueggeman, R. S., and Steffenson, B. J. 2015. Genetic characterization of resistance to wheat stem rust race TTKSK in landrace and wild barley accessions identifies the *rpg4/Rpg5* locus. *Phytopathology* 105:99-109.

Race TTKSK of the wheat stem rust pathogen (*Puccinia graminis* f. sp. *tritici*) threatens the production of wheat and barley worldwide because of its broad-spectrum virulence on many widely grown cultivars. Sources of resistance against race TTKSK were recently identified in several barley landraces (*Hordeum vulgare* subsp. *vulgare*) and wild barley accessions (*H. vulgare* subsp. *spontaneum*). The objectives of this study were to characterize the inheritance of resistance to wheat stem rust race TTKSK in four barley landraces (Hv501, Hv545, Hv602, and Hv612) and two wild barley (WBDC213 and WBDC345) accessions, map the resistance genes, and determine the allelic relationships among the genes in these accessions and the previously described *rpg4/Rpg5* locus. Resistant accessions were crossed with the susceptible cv. Steptoe and resulting F₃ populations were evaluated for resistance to race TTKSK at the seedling stage. Segregation of F₃ families in populations involving the resistance sources of Hv501, Hv545, Hv612, WBDC213, and WBDC345 fit a 1:2:1 ratio for homozygous resistant (HR)/segregating (SEG)/homozygous susceptible (HS) progenies (with $\chi^2 = 2.27$ to 5.87 and $P = 0.053$ to 0.321), indicating that a single gene confers resistance to

wheat stem rust race TTKSK. Segregation of F₃ families in cross Steptoe/Hv602 did not fit a 1:2:1 ratio (HR/SEG/HS of 20:47:43 with $\chi^2 = 11.95$ and $P = 0.003$), indicating that more than one gene is involved in imparting resistance to race TTKSK. Bulk segregant analysis using >1,500 single-nucleotide polymorphism markers positioned a resistance locus in all six populations on chromosome 5HL in very close proximity to the known location of the *rpg4/Rpg5* complex locus. Allelism tests were conducted by making crosses among resistant accessions Hv501, Hv545, and Hv612 and also Q21861 with the *rpg4/Rpg5* complex. No segregation was observed in F₂ families inoculated with race TTKSK, demonstrating that all Hv lines carry the same allele for resistance and that it resides at or very near the *rpg4/Rpg5* locus. Phenotype evaluations of the six barley accessions with wheat stem rust race QCCJ revealed resistant infection types (ITs) at a low incubation temperature and susceptible ITs at a high incubation temperature, similar to Q21861, which carries the temperature-sensitive gene *rpg4*. The accessions also exhibited low ITs against the rye stem rust isolate 92-MN-90, suggesting that they also carry *Rpg5*. This result was confirmed through molecular analysis, which revealed that all six barley accessions contain the serine threonine protein kinase domain that confers *Rpg5* resistance. These results indicate that cultivated barley is extremely vulnerable to African stem rust races such as TTKSK because even these diverse selections of landrace and wild barley accessions carry only one locus for resistance.

Stem rust, caused by *Puccinia graminis* Pers., is one of the most important diseases of wheat, barley, oat, and rye, owing to its ability to completely destroy crops in a short period of time over a large scale. Wheat stem rust, caused by *P. graminis* f. sp. *tritici* Erikss. & Henning, attacks both wheat and barley in many regions of the world. Stem rust has caused multiple widespread epidemics in the northern Great Plains region of the United States and Canada, with the most recent occurring in the 1930s and 1950s (36). Losses due to stem rust have been greatly reduced in the Great Plains region of the United States since the late 1950s due to the wide-scale deployment of resistant, early maturing cultivars, and also eradication of barberry (*Berberis vulgaris* L.), the alternate host of the stem rust pathogen (19,35). Since then, the incidence of new *P. graminis* f. sp. *tritici* races and epidemics

have been very infrequent (35). However, in 1998, a new race of *P. graminis* f. sp. *tritici*, now designated as TTKSK (also known as isolate Ug99) was detected in Uganda (32) and later found to be virulent against a widely used resistance (*R*) gene in wheat (*Sr31*) as well as many other commonly used *R* genes (16,32). Currently, race TTKSK threatens wheat and barley production worldwide. It is capable of attacking >90% of the world's wheat cultivars (40). It is also widely virulent on barley, attacking >97% of cultivars grown worldwide (47). Since its detection in Uganda, race TTKSK and its variants have spread to a number of other countries in Africa (Kenya, Ethiopia, Sudan, Tanzania, South Africa, Zimbabwe, Mozambique, and Eritrea) and also the Middle East (Yemen and Iran) (25,27,31,33,51,53,54). Variants in the "Ug99 lineage" are expected to spread to other cereal-producing regions of the world in the near future (12,40).

Although the damaging effects of stem rust, including race TTKSK, can be mitigated by fungicide applications, the extra input costs and potential negative consequences of chemical treatments on the environment warrant the use of host *R* genes to control stem rust. To date, eight stem rust *R* genes have been identified in different accessions of barley. Gene *Rpg1* was identified from barley accessions 'Chevron' (CIho 1111) and 'Peatland'

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*The e-Xtra logo stands for "electronic extra" and indicates that the online version contains two supplemental tables and one supplemental figure.

<http://dx.doi.org/10.1094/PHYTO-12-13-0340-R>
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(CIho 5267) (30, 39), is effective against most wheat stem rust races, and has protected barley from significant stem rust losses for >70 years (44). In another genetic study conducted with Peatland, Fox and Harder (10) identified *RpgU* in addition to *Rpg1*. However, TTKSK and other related African races are highly virulent on *Rpg1*. Genes *Rpg2* and *Rpg3* were identified from the accessions 'Hietpas-5' (CIho 7124) (28) and 'PI 382313' (14,15), respectively, and, like *Rpg1*, are not completely effective against race TTKSK (47). The recessive stem rust *R* gene *rpg4* was identified in breeding line 'Q21861' ('PI 584766') and confers resistance to race QCCJ (17). More recently, a recessive stem rust *R* gene (*rpg6*) was identified in '212Y1', a barley line with an introgression of *Hordeum bulbosum* L. chromatin (9).

Other *R* genes were described based on their reaction to the rye stem rust pathogen (*P. graminis* f. sp. *secalis*). The dominant *R* gene *Rpg5* (initially designated *RpgQ*) was discovered in Q21861 (4,50). Gene *Rpg5* is tightly linked to *rpg4* and is located on the long arm of barley chromosome 5H (4,6). A recessive gene designated as *rpgBH* also confers resistance to rye stem rust and was described from 'Black Hullless' (CIho 666) (43).

In an expression quantitative trait loci (eQTL) study of the barley population Q21861/SM89010 (Q/SM) infected with *P. graminis* f. sp. *tritici* race TTKSK at the adult plant stage in the field, Moscou et al. (24) identified a chromosome 2H *trans*-eQTL that enhances resistance through transcriptional suppression of many genes. At seedling stage, the major effect locus identified was *rpg4/Rpg5* (*Rpg-TTKSK*) on chromosome 5H. Zhou et al. (55) conducted an association mapping study of U.S. barley breeding germplasm to race TTKSK at the adult plant stage in the field and identified two QTL: one on chromosome 7H and the other on chromosome 5H, distantly proximal to *rpg4/Rpg5*. Given that *rpg4/Rpg5* is the only effective locus against race TTKSK (47), it is important to identify and genetically characterize new sources of resistance and transfer their genes into commercial cultivars.

The evaluation of a worldwide collection of barley germplasm identified a number of sources of seedling and adult plant resistance to race TTKSK in the *Hordeum* gene pool, comprising cultivars, landraces, and wild barley accessions (47) (B. Steffenson, unpublished data). Some of the most resistant accessions included landraces (*H. vulgare* subsp. *vulgare*) from Switzerland and also accessions of wild barley (*H. vulgare* subsp. *spontaneum*) from the Wild Barley Diversity Collection (WBDC) (46). Six of these resistant barley accessions were chosen for detailed study to elucidate the genetic basis of race TTKSK resistance so as to enable more efficient use in breeding. Thus, the specific objectives of this study were to (i) characterize the inheritance of

resistance to race TTKSK in landrace and wild barley accessions at the seedling stage through biparental mapping, (ii) determine the chromosomal locations of the *R* genes, and (iii) elucidate the allelic relationships among the *R* genes in these accessions and the previously described *rpg4/Rpg5* complex locus.

MATERIALS AND METHODS

Plant materials. Six *Hordeum* accessions exhibiting seedling or adult resistance to race TTKSK were crossed with susceptible 'Steptoe' (CIho 15229) barley to develop mapping populations for genetic analysis (Table 1). Four of the accessions (Hv501, Hv545, Hv602, and Hv612) were landraces originally collected from the alpine regions of eastern Switzerland (Canton Graubünden), a country previously known to be a source of stem-rust-resistant barley germplasm (44). Seed was donated by the Station federale de recherches en production vegetale de Changins in Nyon, Switzerland, courtesy of Geert Kleijer. Selection of these four accessions from a total collection of 74 landraces was based on their genetic diversity, as revealed by 12 simple sequence repeat (SSR) markers (P. Olivera, unpublished data); geographic location within Graubünden; and resistant stem rust phenotype. The two other accessions (WBDC213 and WBDC345) investigated were wild barley collected from Samarkand and Kashkadarya provinces of Uzbekistan, respectively (Table 1). These accessions are part of the WBDC (46), provided by the International Center for Agricultural Research in the Dry Areas in Aleppo, Syria, courtesy of Jan Valkoun.

Planting, inoculation, and incubation of plants. F₂ plants from the crosses were not evaluated for race TTKSK because of space limitations for increasing to the next generation within the Biosafety Level-3 (BSL-3) Containment Facility at the University of Minnesota in St. Paul. Thus, 25 to 35 plants of each F₃ family were evaluated at the seedling stage for response to race TTKSK. The tests were conducted inside the BSL-3 facility during the winter months. F₃ families and parents were planted in plastic pots (7.6 by 7.6 by 10.8 cm [length by width by height]) filled with a 50:50 mix of steam-sterilized native soil and Metro-Mix 200 (Sun Gro Horticulture, Quincy, MI) (vermiculite, sphagnum peat moss, perlite, dolomitic limestone, and a wetting agent). After planting, all pots were watered and fertilized with Osmocote controlled-release fertilizer 14-14-14 (Scott's Company, Marysville, OH) (1.4 g/pot) and Peters Dark Weather fertilizer 15-0-15 (Scott's Company) (≈40 g/liter at 1/16 dilution). Populations derived from wild barley accessions were kept at 4°C for 2 weeks to break dormancy and facilitate uniform emergence and growth prior to inoculation. All populations were grown in a

TABLE 1. Reaction of Swiss barley landraces, wild barley accessions, and susceptible control Steptoe to stem rust race *Puccinia graminis* f. sp. *tritici* TTKSK at the seedling and adult plant stages

Accession ^a	Origin	Location	Seedling reaction ^b		Sev.%/IR ^c		
			IT mode	IT range	Kenya 2008	Kenya 2009	Kenya 2010
Hv501	Switzerland	Near Bonaduz	0;1	0; to 0;1	2 MR	...	0 R
Hv545	Switzerland	Near Disentis	0;1	0; to 0;1	5 MS	...	0.5 R/5MS
Hv602	Switzerland	Unknown	0;	0; to 10;	1 R	...	0 R
Hv612	Switzerland	Near Laret	0;	0; to 0;1	20 MS-MR	...	0 R
WBDC213	Uzbekistan	Samarkand	0;	0; to 0;1	...	10 MR/20 MS-S	...
WBDC345	Uzbekistan	Kashkadarya	0;1	0; to 210;	...	Trace MS/Trace R	...
Steptoe	United States	Washington	3 ⁺	3-2 to 3 ⁺	40 S	...	20 S-MS

^a *Hordeum vulgare* (Hv) number assigned by the Station federale de recherches en production vegetale de Changins in Nyon, Switzerland; WBDC = Wild Barley (*H. vulgare* subsp. *spontaneum*) Diversity Collection accession described by Steffenson et al. (46).

^b Infection type (IT) mode represents the one or two most commonly observed ITs on plants, listed in order of their relative prevalence. IT range represents the lowest and highest ITs observed on plants. Plants were evaluated for their ITs based on the 0-to-4 scale originally developed for wheat (41) and modified for barley (45). ITs 0, 0-, 1, 2, and 23- were considered indicative of host resistance, whereas types 3-, 3, and 3+ were considered indicative of susceptibility.

^c Terminal rust severity (0 to 100%) at the adult plant stage was based on the modified Cobb scale (29,48). The infection responses (IRs) were based on the size and type of uredinia observed, where R = resistant; MR = moderately resistant; MS = moderately susceptible, and S = susceptible (21,37). Severity readings of "Trace" denote very low rust infection (<0.5%) in the field. The slash symbol (/) indicates the variation in stem rust severity at different times of disease rating during the growing season; ... indicates not tested.

greenhouse at 19 to 22°C with a 14- to 16-h photoperiod supplemented by 400-W high-pressure sodium lamps emitting a minimum of 300 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. When the plants began to emerge from the soil, they were brought into the BSL-3 facility for the remainder of the experiment. Stocks of rust isolate 04KEN156/04 of race TTKSK were prepared and utilized for inoculation following the protocols described by Steffenson et al. (42), with minor modifications. A rust spore suspension (14 mg of urediniospores per 0.7 ml of oil) was applied to 8- to 9-day-old plants with fully expanded primary leaves at a rate of ≈ 0.09 mg/plant with an atomizer pressured at 25 to 30 kPa (49). After inoculation, plants were placed in chambers misted with ultrasonic humidifiers, initially for 30 to 40 min of continuous misting and thereafter for 4 to 8 min every hour for 16 to 18 h in the dark. After the wetness period, light was provided by 400-W sodium vapor lamps (150 to 250 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$), and the mist chamber doors were slightly opened to dissipate the heat. At this time, the humidifiers were set to run for 4 to 8 min of misting every 15 min for the next 2 h. After an additional 2 h, the misters were turned off and the chamber doors were opened halfway to facilitate slow drying of the plant surfaces under continuous light for the next 3 to 4 h. Finally, when the leaf surfaces were completely dry, plants were returned to the greenhouse under the conditions previously described. The parental accessions also were evaluated in limited field trials at the Kenya Agricultural Research Institute in Njoro, Kenya according to the methods described by Zhou et al. (55).

Rust infection phenotyping. At 12 to 14 days after inoculation, stem rust infection types (ITs) were assessed on the first

leaves of plants based on the 0-to-4 scale originally developed for wheat by Stakman et al. (41) and modified for barley (45). The IT scale used for barley is based primarily on uredinial size, as described by Miller and Lambert (23). Plants with ITs ranging from 0 to 23⁻ were classified as resistant and those from 3⁻ to 3⁺ as susceptible. Individual F₃ families were grouped into three classes of homozygous resistant (HR), segregating (SEG), or homozygous susceptible (HS) based on the reactions of individual plants.

Statistical test. Pearson's χ^2 test was used to evaluate independence of segregation for genetic ratios in the F₃ generation. The χ^2 statistic and associated *P* values were calculated using the chisq.test function in Microsoft Excel.

Sample preparation for bulked segregant analysis. HR and HS F₃ families were used for bulked segregant analysis (BSA), an efficient method for tagging and mapping disease *R* genes (13,22,34). One arbitrarily selected plant from each HR and HS family was grown in the greenhouse and the leaf tissue harvested for DNA extraction. Leaf tissue from plants representing eight F₃ families was bulked to create sets of HR and HS bulks for BSA. Three independent sets of both the HR and HS bulks were used in each population. For the Steptoe/Hv602 population, only 20 HR families were used to create the HR bulks because of the limited number of such families identified. Additionally, five seeds each of the resistant parents and Steptoe also were grown and leaf tissue was harvested 2 weeks after sowing for DNA extraction. Samples were freeze-dried using a general-purpose freeze dryer (Model 24DX48; Virtis Company, Gardiner, NY) according to the specifications of the manufacturer.

Fig. 1. Disease phenotypes of barley landraces in response to wheat stem rust race TTKSK. **A**, Highly resistant reaction of Hv545; **B**, susceptible reaction of F₁ plant from the Steptoe/Hv545 cross; **C**, susceptible reaction of Steptoe parent; **D**, susceptible plant from a homozygous susceptible F₃ family of the Steptoe/Hv612 cross; **E**, moderately susceptible plant from a segregating F₃ family of the Steptoe/Hv612 cross; **F**, resistant plant from a segregating F₃ family of the Steptoe/Hv612 cross; **G**, resistant plant from a homozygous resistant F₃ family of the Steptoe/Hv612 cross; and **H**, highly resistant reaction of Hv612.

DNA extraction and genotyping. The freeze-dried tissue was used for total genomic DNA extraction using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. DNA sample quality was checked by separating and visualizing on a 1% agarose gel. The amount of DNA in each sample was quantified by measuring absorbance at 260 nm with a spectrophotometer (Labomed, Inc., Culver City, CA). The DNA concentration of each sample was then normalized to 200 ng/μl, and 5 μl of each normalized sample was submitted for genotyping with 1,536 single-nucleotide polymorphism (SNP) markers of the Barley Oligonucleotide Pooled Assay 1 (BOPA1) (5,38). The 1,536 SNP markers were tested on the resistant parents, Steptoe, and the three independent HR and HS bulks per population using Illumina Bead Array Technology with the GoldenGate assay (7,8). The BOPA1 SNP markers were previously mapped onto the integrated molecular genetic linkage map of barley (5,26).

Genotype data analysis. Data generated by the GoldenGate assay were visualized and analyzed with the Genotyping Module of the GenomeStudio data analysis software (GSGT version 1.8.4; Illumina, San Diego, CA). SNPs between the respective resistant parents and Steptoe were identified. All of the SNP call data were manually checked, and positive hits for BSA were noted when alleles of at least two of the resistant or susceptible bulks clustered close with alleles of the resistant parent or Steptoe, respectively, in the GenCall output.

Allelism tests. To determine the allelic relationships among stem rust *R* genes in the different resistant accessions and line Q21861, a half diallel was attempted. Successful crosses were increased to the F_2 generation. F_2 seed were sown and plants were inoculated with race TTKSK according to the protocol described above.

Resistance spectrum of parents to other stem rust races. To further characterize the resistance spectrum of TTKSK-resistant accessions and also help resolve whether they may carry the *rpg4/Rpg5* *R* gene complex, three additional races or isolates of the stem rust pathogen were used. Race QCCJ was used because *rpg4* (and other genes in concert) (52) is specifically effective against it at low incubation temperatures (18 to 20°C) (17). Because *rpg4* is temperature sensitive, assays also were made with race QCCJ at 27 to 28°C, where the gene is rendered completely ineffective (17). To assay for the presence of the closely linked gene *Rpg5*, isolate 92-MN-90 of *P. graminis* f. sp. *secalis*, was used. In previous studies, *Rpg5* was found to confer a clear low reaction type to this pathogen isolate (42,50). Finally, race HKHJ of *P. graminis* f. sp. *tritici* was used because it is capable of identifying *Rpg1* in the presence of other *R* genes (49). Two replications of the parental accessions and control lines were evaluated in experiments that were repeated at least twice in time against each race or isolate. The conditions for plant growth and procedures for inoculation and IT assessment were made according to the methods described previously. The only exception was for the inoculation with race HKHJ, where a concentration of 35 mg of urediniospores per 0.7 ml of oil was used due to a lower than normal germination rate.

Molecular characterization of the *rpg4/Rpg5* region. Genotyping for the presence or absence of *rpg4*-mediated resistance was determined using polymerase chain reaction (PCR) sequence tag site (STS) markers specific to the *Rpg5* gene. Two pairs of PCR primers were designed to specifically amplify functional or nonfunctional *Rpg5* alleles based on the presence of the serine threonine protein kinase domain (STPK; *Rpg5*+) or the protein phosphatase 2C domain (PP2C; *rpg5*-). The sequences of STS markers were designed based on the allele sequence data generated for the three genes at the *rpg4/Rpg5* locus from multiple resistant and susceptible accessions (2,4,52). For each barley line, two separate PCRs were performed using LRK-F1/LRK-R1 and RpgQ-F6/PP2C-R2 primer combinations. The 20-μl PCRs consisted of ≈100 ng of gDNA, 20 pmol of each forward and reverse

primers, 1× REDTaq PCR Reaction Buffer (Sigma-Aldrich, St. Louis, MO), 1 U of REDTaq DNA polymerase, and 0.2 mM dNTPs. Amplification was performed in a Mastercycler pro thermocycler (Eppendorf, Hauppauge, NY) 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. Primer sequences for the STS markers are given in Supplemental Table 1 along with the expected size of the PCR products. The *Rpg5* gene has three main domains (Supplemental Figure 1) (4). The *Rpg5* STS1 marker targets the leucine-rich repeat (LRR) to the STPK region and is specific to the majority of resistant lines (e.g., line Q21861 as a reference genotype). The PP2C STS1 marker amplifies the LRR to the PP2C region and is specific to susceptible lines (e.g., Steptoe). Lines that gave an *Rpg5*+ result with the *Rpg5* STS1 marker were further analyzed for an infrequent allele that gives false-positive results due to the presence of an intact protein kinase domain but a nonfunctional *rpg5* allele that contains a single C insertion causing a frame shift mutation and a predicted truncated nonfunctional Rpg5 protein (2,4). To test for this rare nonfunctional allele, a sequence was generated across the region by directly sequencing the amplicon produced using the *Rpg5*-F1 and *Rpg5*-R1 primer combination (2). The PCR conditions were the same as described above. The PCRs were purified with cycle pure spin columns (Omega Bio-Tek, Norcross, GA) and sequenced with the *Rpg5*-R1 primer. Sequencing was performed by GenScript on an ABI 3730xl (Applied Biosystems, Carlsbad, CA).

RESULTS

Genetics of resistance to race TTKSK of *P. graminis* f. sp. *tritici*. The landrace (Hv501, Hv545, Hv602, and Hv612) and wild barley (WBDC213 and WBDC345) accessions exhibited highly resistant ITs (modes of 0; to 0;1) in response to race TTKSK at the seedling stage (Table 1). In contrast, Steptoe was susceptible, exhibiting an IT mode of 3+. The resistant accessions also showed much lower rust severities and infection responses than Steptoe in the limited Kenyan field trials where they were included (Table 1). In total, 110 to 187 F_3 families from crosses between the resistant barley accessions and Steptoe were evaluated for stem rust reaction in this study (Table 2). Resistant F_3 plants exhibited ITs ranging from 0; to 12 (rarely 210; or 23-) and could be easily differentiated from susceptible plants giving ITs of 3-2 to 3+ (Fig. 1). Thus, individual families of all populations could be confidently grouped into HR, SEG, or HS categories. Segregation data for F_3 families of five populations fit a 1:2:1 ratio for HR/SEG/HS (χ^2 of 2.27 to 5.87 with *P* values of 0.321 to 0.053, respectively) (Table 2). These data indicate that a single gene confers TTKSK resistance in Hv501, Hv545, Hv612, WBDC213, and WBDC345. Segregation data for F_3 families in the Steptoe/Hv602 population did not fit a 1:2:1 ratio (χ^2 = 11.95 and *P* = 0.003), indicating that more than one TTKSK *R* gene in Hv602 confers resistance to TTKSK.

To determine whether resistance was dominant or recessive, several different aspects of the segregating populations were investigated. First, a limited number of F_1 plants were phenotyped. The IT mode of F_1 plants from the Steptoe/Hv545 population was similar to that of the susceptible parent Steptoe (3-2 versus 33+) (Fig. 1A to C), suggesting a recessive or at least a partially recessive gene. Unfortunately, no other F_1 seed were available from the other crosses. Second, the composition of individual plant reactions within each segregating F_3 family was tallied to assess possible gene action (20). At least half of the segregating families evaluated in each population (except WBDC213/Steptoe and WBDC345/Steptoe) fit a single gene ratio (20). Some of the segregating F_3 families in the six populations did not follow a clear Mendelian ratio for an expected single recessive gene as found for the Steptoe/Hv545 F_1 plant. For the Steptoe/Hv501,

Step toe/Hv602, and WBDC345/Step toe populations, 56% (27/48), 57% (27/47), and 48% (29/60) of the segregating F_3 families, respectively, fit a 1:3 ratio for resistant/susceptible plants, suggesting recessive gene action in the respective resistant parents (20). Unexpectedly, however, 23% (11/48), 32% (15/47), and 22% (13/60) of the segregating F_3 families of these three respective populations showed dominant gene action (i.e., 3:1 ratio of resistant/susceptible plants) (20). Only one segregating F_3 family among the Step toe/Hv545, Step toe/Hv612, and WBDC213/Step toe populations followed a recessive gene action ratio of 1:3 (20). The corresponding number of segregating F_3 families following a 3:1 ratio for resistant/susceptible plants for these populations was 54% (13/24), 50% (15/30), and 45% (22/49), respectively (20). For the Step toe/Hv545 population, the dominant gene action discerned from the plants within the segregating families contradicts the putative partially recessive R gene action in Hv545 deduced from the IT of the single Step toe/Hv545 F_1 plant (see below).

Genotyping of parents and bulks, and BSA. In order to determine the chromosomal location of the R genes, genetic analysis was conducted using BSA. In total, 525, 552, 564, 588, 568, and 561 SNPs were identified between the resistant parents

Hv501, Hv545, Hv602, Hv612, WBDC213, and WBDC345, respectively, and susceptible parent Step toe after screening with 1,536 BOPA1 SNPs. Markers with possible linkage to the resistance locus were determined after establishing two broad criteria. First, SNPs differentiating at least two of the HR bulks (plus the resistant parent) and two of the HS bulks (plus Step toe) were considered to be putatively linked SNP markers to the resistance loci in the TTKSK-resistant barley accessions (Table 3). Accordingly, alleles of 18 SNPs in total (1 in Step toe/Hv501, 3 in Step toe/Hv545, 4 in Step toe/Hv602, and 5 each in Step toe/Hv612 and WBDC345/Step toe F_3 families) differed between two or more of the HR and HS bulks. Of these, one SNP each in all of the three resistant and susceptible bulks of Step toe/Hv545 and Step toe/Hv612 and two SNPs in all of the three resistant and susceptible bulks of WBDC345/Step toe clustered together with the respective resistant parent (Table 3, SNPs in bold). Second, in cases where alleles of all the HR bulks were similar to the allele clusters of the resistant parent and alleles of the susceptible bulks had a different cluster from the susceptible parent, the SNP was considered to be a putative “candidate” marker linked to the identified R gene in the populations (Table 3, SNPs in italic). This second criterion was considered because a mutation or some other

Fig. 2. Consensus genetic map of the long arm of chromosome 5H of barley. The consensus map was constructed from the Step toe/Morex (SM) doubled haploid line population (18) and modified from a presentation by R. Brueggeman (*unpublished data*). Single-nucleotide polymorphism (SNP) markers detected in the subtelomeric region of the long arm of chromosome 5H through bulked segregant analysis are indicated with the thinner and longer line. SNP marker names begin with numbers according to the BOPA_C nomenclature used by Close et al. (5) followed by the name of the cross in which they were detected. Cumulative centimorgan (cM) distances are in parenthesis next to the marker name as given by Muñoz-Amatriaín et al. (26). The stem rust resistance gene complex *rpg4/Rpg5*, given in bold italics on the left-hand side of the subtelomeric region of the hypothetical chromosome arm, was previously mapped to this region (3,42). The cM position of the *rpg4/Rpg5* locus and the linked restriction fragment length polymorphism marker ABG390 is approximate based on the map position of the latter in recent barley consensus maps (11). SNP markers that were positive in each of the three resistant and susceptible bulks are indicated in bold. SNPs detected in more than one population are followed by an asterisk. The line scale on the left side of the figure gives approximate map distances in Kosambi cM at 10-cM intervals.

genetic change might contribute to the shift of the allele cluster of the susceptible bulks away from the allele cluster of the susceptible parent. Based on this criterion, alleles of 12 SNP markers in total (2 each in Steptoe/Hv501 and Steptoe/Hv545, 3 in Steptoe/Hv602, and 5 in WBDC213/Steptoe F₃ families) clustered with the resistant parent in all three resistant bulks (Table 3, SNPs in *italic*).

Most of the 1,536 SNP markers used in this study were previously mapped onto the integrated consensus map of barley (5,18,26). Almost all SNPs that had their alleles clustered with alleles of the respective parent in all three resistant and susceptible bulks were located within a 10-centimorgan (cM) region of the long arm of chromosome 5H between SNP markers 11_10528 and 11_10869 (Table 3; Figs. 2 and 3). Likewise, most SNPs that had their alleles clustered with the resistant parent in all three resistant bulks (positive SNPs)—regardless of their pattern in the HS bulks of all six populations—were located within the same region of chromosome 5HL. Other SNPs where two of the resistant bulks clustered with the resistant parent or where two or more of the susceptible bulks clustered with the susceptible parent also were located in the same interval of chromosome 5H in all populations (Table 3; Figs. 2 and 3). This result suggests that all six barley accessions contain an *R* gene mapping to the same

region of chromosome 5H. The *rpg4/Rpg5* complex locus in Q21861 also maps to this same region of chromosome 5HL (42). One exception to note is that, in the Steptoe/Hv602 population, an SNP (marker 11_21061) that maps at 99.39 cM on the same arm of chromosome 5H had all three resistant bulks clustered with the resistant parent (Table 3; Fig. 2). This region is interesting because Zhou et al. (55) identified a novel adult plant resistance locus against race TTKSK in the 69.3- to 103.9-cM interval of 5HL. An SNP marker (11_21472) mapping to the long arm of chromosome 3H at 66.62 cM had three of the resistant bulks clustered with the resistant parent in the Steptoe/Hv612 population.

Allelism tests. Crosses for the half diallel among the resistance sources and to Q21861 were only obtained for 6 of the 21 possible combinations: Hv545/Hv602 (300 progeny), Hv545/Hv612 (140 progeny), Hv602/Hv612 (280 progeny), Q21861/Hv501 (680 progeny), Q21861/Hv545 (500 progeny), and Q21861/Hv612 (760 progeny) (Supplemental Table 2). Successful crosses were not obtained for the other combinations, due mostly to flowering time differences and poor pollen production. All progeny from crosses Hv545/Hv602, Hv545/Hv612, and Hv602/Hv612 exhibited resistant ITs to race TTKSK, indicating that the same

Fig. 3. Clustering of sample GoldenGate assay results from GenomeStudio that were considered a positive hit for single-nucleotide polymorphism markers linked with TTKSK resistance in landrace and wild barley accessions using bulked segregant analysis. **A**, The three susceptible bulks clustered with the susceptible genotype Steptoe and the three resistant bulks clustered with the resistant genotype Hv612; **B**, the three resistant bulks clustered with the resistant genotype Hv545 but the three susceptible bulks did not cluster with Steptoe; **C**, the three susceptible bulks clustered with Steptoe and two of the three resistant bulks clustered with the resistant genotype WBDC345; and **D**, two of the susceptible bulks clustered with Steptoe and the two of the resistant bulks clustered with Hv545. The cluster position of the bulks (resistant versus susceptible) and the parents (resistant versus Steptoe) are indicated with arrows (HR = homozygous resistant and HS = homozygous susceptible). The X axis is normalized θ . A normalized θ value nearest 0 is homozygous for allele A and a θ value nearest 1 is homozygous for allele B. The Y axis is normalized R (7).

allele imparts resistance in the three Swiss landraces. Similar results were obtained in populations involving landraces Hv501, Hv545, and Hv612 crossed to Q21861, indicating that the *R* genes were allelic to the *rpg4/Rpg5* complex. One F₂ plant derived from Q21861/Hv501 and three F₂ plants derived from Q21861/Hv612 showed an intermediate IT but these were retested and later confirmed to be resistant to race TTKSK. Attempts at other crosses for the half diallel were not successful; however, some deductions may be made based on the current results. Although Hv602 was not successfully crossed to Q21861, it likely carries an *R* gene allelic to the *rpg4/Rpg5* complex based on the allelism of Hv602 to Hv612 and Hv612 to Q21861.

Resistance spectrum of parents to other stem rust races. Landrace and wild barley accessions were inoculated with three

additional races or isolates of *P. graminis* to help resolve whether they contain the same *R* gene complex of *rpg4/Rpg5* and also to profile their resistance spectrum. Steptoe, the susceptible control, gave high ITs to all races tested (Table 4). The landrace and wild barley accessions reacted the same as the resistant controls of Q21861 (with *rpg4/Rpg5* and *Rpg1*) and QSM20 (with *rpg4/Rpg5*) to race QCCJ at low-temperature (ITs of 0; to 0;1) and high-temperature (3-2) incubation. The TTKSK-resistant accessions also were evaluated against *P. graminis* f. sp. *secalis* isolate 92-MN-90 to determine whether they carry *Rpg5*. All accessions exhibited low ITs (0; to 0;1), similar to those of Q21861 and QSM20, suggesting that they also carry *Rpg5*. Finally, the six TTKSK-resistant accessions were evaluated to race HKHJ to assess whether they might carry *Rpg1*. Accessions Hv501, Hv612,

TABLE 2. Segregation for resistance in F₃ families from crosses of landrace and wild barley accessions with Steptoe to race TTKSK of *Puccinia graminis* f. sp. *tritici* at the seedling stage

Cross	Number of F ₃ families ^a			Expected ratio	χ^2 value (2 df)	Probability (> χ^2) ^b
	HR	SEG	HS			
Steptoe/Hv501	32	48	40	1:2:1	5.87	0.053 ns
Steptoe/Hv545	48	98	35	1:2:1	3.11	0.211 ns
Steptoe/Hv602	20	47	43	1:2:1	11.95	0.003 **
Steptoe/Hv612	39	88	60	1:2:1	5.36	0.068 ns
WBDC213/Steptoe	30	52	36	1:2:1	2.27	0.321 ns
WBDC345/Steptoe	32	76	52	1:2:1	5.40	0.067 ns

^a Homozygous resistant (HR), segregating (SEG), or homozygous susceptible (HS).

^b Symbols: ns = not significant at 0.05 and ** = significant at 0.01.

TABLE 3. Chromosomal position of markers associated with resistance against stem rust race TTKSK in landrace and wild barley accessions as determined by bulked segregant analysis

Chromosome ^a	Position (cM) ^b	SNP designation ^c	Mapping population ^d	HR ^e	HS ^f
3H L	66.62	11_21472	Steptoe/Hv612	2	3
5H L	51.51	11_20501	Steptoe/Hv602	1	3
	99.39	11_21061	Steptoe/Hv602	3	1
	152.93	11_10901	WBDC213/Steptoe	3	1
	155.45	11_10528	WBDC213/Steptoe	3	1
	155.66	11_21024	WBDC213/Steptoe	3	0
	157.61	11_10336	Steptoe/Hv545	3	2
	157.61	11_10336	Steptoe/Hv602	2	2
	157.61	11_10336	Steptoe/Hv612	3	2
	157.61	11_10336	WBDC345/Steptoe	2	3
	157.61	11_20646	Steptoe/Hv545	3	1
	157.61	11_20646	Steptoe/Hv602	2	2
	157.61	11_20646	Steptoe/Hv612	2	2
	157.61	11_20646	WBDC345/Steptoe	3	3
	157.61	11_21018	Steptoe/Hv545	3	0
	158.28	11_11464	WBDC345/Steptoe	2	3
	162.98	11_11216	Steptoe/Hv545	3	3
	162.98	11_11216	Steptoe/Hv612	3	2
	162.98	11_11216	WBDC213/Steptoe	3	1
	162.98	11_11216	WBDC345/Steptoe	3	3
	163.72	11_20546	Steptoe/Hv501	2	2
	163.72	11_20546	Steptoe/Hv602	2	2
	163.72	11_20546	Steptoe/Hv612	2	3
	163.72	11_20686	WBDC345/Steptoe	2	3
	164.15	11_20644	Steptoe/Hv602	3	1
	164.15	11_20644	WBDC213/Steptoe	3	1
	165.28	11_10869	Steptoe/Hv501	3	1
	165.28	11_10869	Steptoe/Hv545	2	2
	165.28	11_10869	Steptoe/Hv602	2	2
	165.28	11_10869	Steptoe/Hv612	3	3
	168.44	11_20536	Steptoe/Hv501	3	0
	168.44	11_20536	Steptoe/Hv602	3	0

^a Chromosome and arm.

^b Single-nucleotide polymorphism (SNP) marker position along chromosome, according to Muñoz-Amatriaín et al. (26).

^c SNP marker BOPA_C nomenclature according to Close et al. (5). SNP markers that were positive in each of the three resistant and susceptible bulks are indicated in bold; SNP markers that were positive in each of the three resistant bulks and in none or one of the susceptible bulks were considered as putative "candidate" SNPs and are indicated in italics.

^d Mapping population in which associations were identified.

^e Number of homozygous resistant (HR) bulks (out of three) which have alleles identical to the respective resistant parent.

^f Number of homozygous susceptible (HS) bulks (out of three) which have alleles identical to the respective susceptible parent.

WBDC213, and WBDC345 exhibited high ITs (3-2 to 3-3) to race HKHJ, suggesting that they lack *Rpg1*. Accessions Hv545 and Hv602 gave intermediate ITs of 210; and 213-, respectively. These two accessions exhibited similar ITs in repeated evaluations and, therefore, possess a resistance spectrum that is different from the other studied accessions. Q21861 and QSM20 gave low (0;) and high (33+) ITs to race HKHJ, confirming the presence and absence, respectively, of *Rpg1* in the accessions.

Molecular characterization of the *Rpg5* region. The parental lines were genotyped at the *rpg4/Rpg5* region using STS markers (4,52) (GenBank accession number EU812563) to assay for the presence of *Rpg5*. All accessions contained the nucleotide binding site (NBS) of the *Rpg5* gene (Table 5). Additionally, all resistant parents and Q21861 contained an intact STPK domain at the 3' end of the *Rpg5* gene, indicating that the gene is functional in all resistant accessions. Steptoe contained the nonfunctional allele of *Rpg5* because it lacks the STPK domain.

DISCUSSION

Race TTKSK is a serious threat to wheat and barley production worldwide because of its virulence on multiple *R* genes of agricultural importance. Steffenson et al. (42) previously reported that the stem rust resistance locus *rpg4/Rpg5* in Q21861 was the only one described in barley that confers resistance against race TTKSK. In an effort to identify additional genes for resistance to race TTKSK, a large and diverse collection of *Hordeum* germplasm was evaluated at the seedling stage (47). Several Swiss landrace (Hv501, Hv545, Hv602, and Hv612) and wild barley (WBDC213 and WBDC345) accessions were among the most resistant identified to race TTKSK. To fully characterize the genetics of resistance in these accessions, the following studies were conducted: (i) biparental populations were developed to determine the inheritance of resistance and define the chromosomal locations of the resistance loci using BSA, (ii) allelism tests were made to resolve the relationships of genes among some of the resistance sources, (iii) accessions were tested to other stem rust races to characterize the resistance spectrum of the genes and postulate their possible identity, and (iv) specific primers were used for detecting *Rpg5*, a gene implicated with *rpg4* in conferring resistance to race TTKSK.

TABLE 4. Seedling infection types (ITs) of parental lines and controls in response to wheat stem rust races *Puccinia graminis* f. sp. *tritici* QCCJ and HKHJ and rye stem rust isolate *P. graminis* f. sp. *secalis* 92-MN-90

Accession ^b	IT mode ^a			
	QCCJ		HKHJ	92-MN-90
	21°C	28°C	21°C	21°C
Hv501	0;1	3-2	3-2	0;
Hv545	0;1	3-2	210;	0;
Hv602	0;1	3-2	213-	0;1
Hv612	0;1	3-2	3-2	0;
WBDC213	0;	3-2	3-3	0;
WBDC345	0;1	3-2	3-2	0;1
Q21861	0;1	3-2	0;	0;
QSM20	0;1	3-2	33+	0;
Steptoe	3-2	33+	3-3	3-2

^a IT mode represents the one or two most commonly observed ITs on plants, listed in order of their relative prevalence, at the incubation temperatures under which plants were grown after stem rust inoculation. Plants were evaluated for their ITs based on the 0-to-4 scale originally developed for wheat (41) and modified for barley (45). ITs 0, 0;, 1, 2, and 23- are considered indicative of host resistance, and types 3-, 3, and 3+ are indicative of susceptibility.

^b *Hordeum vulgare* number assigned by the Station federale de recherches en production vegetale de Changins in Nyon, Switzerland; WBDC: Wild Barley (*H. vulgare* subsp. *spontaneum*) Diversity Collection accession described by Steffenson et al. (46).

Genetic analysis of the segregating populations clearly indicated that a single gene confers seedling resistance to race TTKSK in accessions Hv501, Hv545, Hv612, WBDC213, and WBDC345. In accession Hv602, more than one gene was involved in conferring seedling resistance (Table 2). Monogenic inheritance for resistance to different races or isolates of the wheat and rye stem rust pathogens have been reported in a number of barley accessions in previous studies (9,10,14,17,28,39,43,50). Steffenson et al. (42) reported that resistance to race TTKSK in Q21861 segregates as a single gene that lies at the *rpg4/Rpg5* region. It appears that this same gene complex confers resistance in the accessions characterized in this study. Perhaps this complex locus and some other gene is involved in conferring resistance in Hv602. The population Steffenson et al. (42) evaluated had only 129 progeny and, therefore, segregation of the two closely linked genes was unlikely. The recessive gene *rpg4* controls resistance to race QCCJ in Q21861 at low incubation temperatures (18 to 21°C) (17). This gene also was thought to confer resistance to rye stem rust in a partially dominant fashion (50) until Brueggeman et al. (4) identified recombinants exhibiting resistance to *P. graminis* f. sp. *secalis* isolate 92-MN-90 and not race QCCJ in a single large segregating population (Steptoe/Q21861). However, the second tightly linked locus containing the *Rme1* (*rpg4*-modifier element 1) gene identified in the Steptoe/Q21861 population that is required for *rpg4*-mediated resistance is only polymorphic in this population and not in the other two large populations analyzed (MD2/Q21861 and Harrington/Q21861). These data suggested that the *Rpg5* gene conferring resistance to rye stem rust is partially dominant and lies only a few recombination units from *Rme1*, yet is the polymorphic resistance (*R*) gene at the locus and the major determinant of *rpg4*-mediated resistance (4,52).

Efforts to characterize the gene action (recessive versus dominant) of TTKSK resistance in the resistance sources did not yield clear-cut results. The IT mode of a single F₁ plant from the Steptoe/Hv545 population and the ratio of plant reaction types within 48 to 57% of segregating F₃ families of the Steptoe/Hv501, Steptoe/Hv602, and WBDC345/Steptoe populations were suggestive of a recessive or at least partially recessive-acting *R* gene. In contrast, the ratio of plant reaction types in other segregating F₃ families was suggestive of a dominant gene. One explanation for the lack of conclusive results concerning gene action based on segregating F₃ families is that multiple genes are likely involved in the phenotype, as recently found by Wang et al. (42), and, therefore, some F₃ plants might represent various recombinations of genes at other loci that interact with the *rpg4/Rpg5* complex of genes. The contention of additional genes being involved was supported by the IT data observed in the Steptoe/Hv501 popu-

TABLE 5. Genotyping and sequencing of landrace and wild barley accessions for the wheat stem rust resistance gene *Rpg5*

Accession	<i>Rpg5</i> ^a	Kinase ^b	5' end insertion ^c	Sequence ^d
Hv501	+	+	-	Unknown
Hv545	+	+	-	Unknown
Hv602	+	+	-	+
Hv612	+	+	-	Unknown
WBDC213	+	+	-	Unknown
WBDC345	+	+	-	Unknown
Q21861	+	+	-	+, D
Steptoe	+	-	Unknown	-, D

^a *Rpg5* nucleotide binding site region was present.

^b The C-terminal protein kinase is intact (+ indicates resistant genotype) or replaced by the PP2C gene (-indicates susceptible genotypes).

^c The 5' end of the allele does not contain an insertion that results in a frame shift and truncated *Rpg5* protein (-).

^d Resistant genotype (+) or susceptible genotype (-) based on the sequenced portions of the allele; D indicates that the entire allele has been sequenced (52).

lation, where some resistant families exhibited intermediate ITs instead of the typical 0; to 0;1 types shown by the resistant parent. In addition, some plants in the susceptible families of the Steptoe/Hv602 population had quite variable ITs, ranging from 3⁻2 to 3⁺. This suggests again that there might be complementary *R* genes involved in the populations. In fact, segregation data of the Steptoe/Hv602 population indicated that TTKSK resistance involves more than one gene. Indeed, the recessive nature of *rpg4* is determined at the *Rpg5* locus by the HvPP2C dominant susceptibility factor (R. Brueggeman, unpublished data). Preliminary expression analysis data suggest that the expression levels of *Rpg5* and the interaction of *Rpg5* with HvPP2C may determine the recessive versus dominant nature of *rpg4/Rpg5*-mediated resistance, suggesting that other genes segregating in these populations, possibly transcriptional regulators similar to those proposed by Moscou et al. (24), could be influencing the nature of gene action in the resistance sources (R. Brueggeman, unpublished data). Further evaluation of the *rpg4/Rpg5* locus and other loci influencing the resistance in the populations reported here may help determine the dominant or recessive nature of *rpg4/Rpg5*-mediated resistance.

The *rpg4* locus (now the *rpg4/Rpg5* complex locus) was mapped to the long arm of barley chromosome 5H using molecular markers (3,6). *Rpg5* was later isolated through positional cloning (4). Recent high-resolution recombinant analysis by Wang et al. (52) indicated that the *rpg4/Rpg5* region spans an ≈290-kbp physical region and contains several candidate genes. Wang et al. (52) have implicated *Rpg5*, along with other tightly linked genes in the region, in *rpg4*-mediated resistance against races TTKSK and QCCJ. The multiple genes in the *rpg4/Rpg5* region required for resistance to TTKSK and QCCJ often segregate as a single locus because they are very closely linked (i.e., within an ≈70-kbp genetic interval). Research is underway to characterize additional informative recombinants in the region to resolve which genes are essential for conferring TTKSK resistance (52). This information will be useful for identifying the genes needed to confer TTKSK resistance in the landrace and wild barley accessions.

After demonstrating a monogenic inheritance pattern for all but one of the resistant accessions, BSA was used to approximate the chromosomal locations of the TTKSK resistance loci. Several SNP markers identified using BSA in the six populations were previously mapped to the subtelomeric region of the long arm of chromosome 5H in the 158- to 165-cM interval between SNP markers 11_10336 and 11_10869 (26). The SNPs identified by BSA lie in a region coincident with *rpg4/Rpg5*. A previous but lower-resolution mapping study of the *R* gene *rpg4* in the Q/SM population using restriction fragment length polymorphism (RFLP) markers identified MWG740 and ABG390 as linked markers (3). That study mapped *rpg4* 5.7 cM distal from the RFLP marker ABG390. On recent consensus maps, ABG390 lies in the same genomic region with the SNP markers detecting the TTKSK resistance locus in the current study (26) (<http://wheat.pw.usda.gov/GG2/index.shtml>). Steffenson et al. (42) mapped the genes conferring TTKSK resistance in Q21861 to the *rpg4/Rpg5* complex locus based on the cosegregation of this resistance with the previously mapped *rpg4* locus conferring resistance to race QCCJ and also resistance to *P. graminis* f. sp. *secalis* isolate 92-MN-90 (50). Moscou et al. (24) mapped qualitative TTKSK seedling stage resistance in the Q/SM population to 146.78 cM on chromosome 5H using mRNA transcript abundance with the Barley1 Affymetrix array. Based on the chromosomal position in consensus maps of RFLP markers linked to the *rpg4* locus, specifically ABG390, the TTKSK resistance locus detected by BSA analysis in this study lies very near the *rpg4/Rpg5* complex locus. In BSA, SNP markers that were positive in each of the three resistant and susceptible bulks and also detected in more than one population were positioned at a more proximal location

on chromosome 5H, closer to the putative location of *rpg4/Rpg5* (Fig. 2, SNPs in bold). Other markers, not positive in all three resistant and susceptible bulks and detected in only one of the populations, were positioned at more distal locations on the chromosome. The other interesting region to note is the 69.3- to 103.9-cM interval of chromosome 5HL, where a novel adult plant TTKSK resistance locus was identified through association mapping in U.S. barley breeding germplasm (55). BSA of the Steptoe/Hv602 population identified a marker (SNP 11_21061) that maps at 99.39 cM on the same arm of chromosome 5H. This region of chromosome 5HL may contain a gene that interacts with the *rpg4/Rpg5* complex locus to impart TTKSK resistance in Hv602.

Steffenson et al. (46) identified DArT markers significantly associated with wheat stem rust (race MCCF) resistance in the *rpg4/Rpg5* gene complex region of chromosome 5H through association mapping in the WBDC. A biparental mapping study with one of the resistant wild barley accessions (WBDC348, also known as 'Damon') identified a single major gene conferring resistance to stem rust races MCCF and QCCJ in the same bin as *rpg4/Rpg5* (1). Research is underway to continue high-resolution recombinant analysis in the *rpg4/Rpg5* region to precisely map these resistant loci (52).

To provide additional data regarding the relationships among the *R* genes identified in the six accessions and also the *rpg4/Rpg5* complex in Q21861, tests of allelism were made. No segregation was observed in crosses between the Swiss landraces (Hv501, Hv545, and Hv612) and Q21861 with the *rpg4/Rpg5* complex. This indicates that the genes conferring TTKSK resistance in the landraces are either allelic with those at the *rpg4/Rpg5* locus or are closely linked to it. Further confirmation of this finding was obtained from the allelism tests among selected Swiss landraces. No segregation was observed in any of these populations, demonstrating that Hv501, Hv545, Hv612, and Hv602 all carry the same allele for resistance to race TTKSK and that it resides at *rpg4/Rpg5* locus.

To obtain more data as to whether the resistant accessions contain the same *R* gene complex of *rpg4/Rpg5*, additional phenotype evaluations were made with *P. graminis* f. sp. *tritici* races QCCJ and HKHJ and *P. graminis* f. sp. *secalis* isolate 92-MN-90. These tests were critical because the genes have unique hallmarks: *rpg4* is temperature sensitive (17) and *Rpg5* specifically confers resistance to rye stem rust without the need for other genes (50). The Swiss landraces and wild barley lines exhibited resistant ITs against race QCCJ at low temperature and susceptible ITs at high temperature, similar to those exhibited by Q21861 with the *rpg4/Rpg5* complex (4,42). The resistant accessions also gave low ITs against *P. graminis* f. sp. *secalis* isolate 92-MN-90, similar to those exhibited by Q21861, suggesting that they also carry *Rpg5* (Table 4). These results strongly support our hypothesis that the six resistance sources contain the *rpg4/Rpg5* locus. Tests with race HKHJ indicated that Hv501, Hv612, WBDC213, and WBDC345 likely lack *Rpg1*. This result is in agreement with *Rpg1*-specific marker analysis that revealed the absence of this gene in the Swiss landraces (B. Steffenson and R. Brueggeman, personal communication). Two accessions (Hv545 and Hv602) exhibited unexpected ITs in response to race HKHJ over multiple evaluations. Known controls with *Rpg1* gave classical ITs of 0; to 10; when tested with race HKHJ. In contrast, Hv545 and Hv602 exhibited low to intermediate ITs of 210; and 213⁻, respectively, to this race. *Rpg1*-specific marker analysis indicated that these two landraces lack *Rpg1* (B. Steffenson and R. Brueggeman, personal communication). However, they may carry a partially effective gene against race HKHJ. This result should be verified further, including testing the two accessions with stem rust races under different temperature conditions.

Molecular haplotyping provided another strong piece of evidence concerning the presence of a functional *Rpg5* gene in the

six resistant accessions. *Rpg5* encodes a protein with NBS, leucine-rich, and protein kinase domains (4). Molecular characterization of the *Rpg5* region with STS markers indicated that all six resistant accessions contain a functional *Rpg5* gene (Table 5). A sequenced portion of the allele also revealed that the STPK domain at the C-terminus end of the *Rpg5* gene is intact. The STPK domain is a functionally crucial unit of *Rpg5* for conferring resistance. These data demonstrate that TTKSK resistance in landrace and wild barley accessions likely involves *Rpg5*. By lieu of its close linkage to other genes, the presence of *Rpg5* in these sources also strongly suggests the presence of *rpg4* and other nearby genes needed for conferring TTKSK resistance. The latest research on stem rust resistance mediated by the *rpg4/Rpg5* region suggests that *Rpg5* is the *R* gene that is responsible for the gene-for-gene interaction determining *rpg4*-mediated resistance and is the only reliable gene with polymorphism that can be used to determine the presence of *rpg4*-mediated resistance (2). In the future, any newly discovered barley accessions with TTKSK resistance should be initially screened for the presence of the functional *Rpg5* gene to determine whether or not the resistance might be novel. This test will likely serve to detect other genes at the locus because *rpg4* and *Rpg5* are likely conserved, as revealed by their discovery both in landrace and also wild barley accessions.

In summary, segregation data from F₃ families developed from crosses of landrace and wild barley accessions with susceptible Steptoe indicated that a single locus confers resistance to race TTKSK in five of the six populations and that more than one locus governs resistance in Hv602. Molecular genetic mapping by BSA, together with molecular haplotyping for a functional *Rpg5* gene and screening with rye stem rust, demonstrate that the TTKSK *R* genes in the landrace and wild barley accessions map to the *rpg4/Rpg5* region. Taken together, these data indicate that the TTKSK *R* genes in the barley accessions are simply alleles of the *rpg4/Rpg5* gene complex. Q21861 is the original source of the *rpg4/Rpg5* gene complex and is one of the best-known accessions possessing a high level of adult plant resistance against race TTKSK. Several barley breeding programs in North America are introgressing *rpg4/Rpg5* into elite breeding lines for resistance to race TTKSK. However, future work should be done to identify additional sources of resistance so that barley cultivars can be developed with a broad spectrum of resistance to *P. graminis* f. sp. *tritici* races, including race TTKSK and its variants.

ACKNOWLEDGMENTS

This study was supported, in part, by funds provided through a grant from the Bill & Melinda Gates Foundation and the UK Department for International Development to Cornell University for the Borlaug Global Rust Initiative Durable Rust Resistance in Wheat Project; the Lieberman-Okinow Endowment at the University of Minnesota; American Malting Barley Association; and United States Department of Agriculture–Agricultural Research Service Specific Cooperative Agreement: Development of Stem Rust Resistant Barley for the Upper Midwest, Agreement Number 58-3640-0-648. We thank the Minnesota Supercomputing Institute for hosting a software utilized in analysis of data included in this publication.

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