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Genetics and Resistance

e-Xtra*

Vulnerability of Barley to African Pathotypes of *Puccinia graminis* f. sp. *tritici* and Sources of Resistance

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ABSTRACT

The emergence of widely virulent pathotypes (e.g., TTKSK in the Ug99 race group) of the stem rust pathogen (*Puccinia graminis* f. sp. *tritici*) in Africa threatens wheat production on a global scale. Although intensive research efforts have been advanced to address this threat in wheat, few studies have been conducted on barley, even though pathotypes such as TTKSK are known to attack the crop. The main objectives of this study were to assess the vulnerability of barley to pathotype TTKSK and identify possible sources of resistance. From seedling evaluations of more than 1,924 diverse cultivated barley accessions to pathotype TTKSK, more than 95% (1,844) were found susceptible. A similar high frequency (910 of 934 = 97.4%) of susceptibility was found for the wild progenitor (*Hordeum vulgare* subsp. *spontaneum*) of cultivated barley. Additionally, 55 barley lines with characterized or putative introgressions from various wild *Hordeum* spp. were also tested against pathotype TTKSK but none was found resistant. In total, more than 96% of the 2,913 *Hordeum* accessions tested were susceptible as seedlings, indicating the extreme vulnerability of the crop to the African pathotypes of *P. graminis* f. sp. *tritici*. In total, 32 (1.7% of accessions evaluated) and 13 (1.4%) cultivated and wild barley accessions, respectively, exhibited consistently highly resistant to moderately resistant reactions across all experiments. Molecular assays were conducted on these resistant accessions to determine whether they carried *rpg4*/*Rpg5*, the only gene complex known to be highly effective against pathotype TTKSK in barley. Twelve of the 32 (37.5%) resistant cultivated accessions and 11 of the 13 (84.6%) resistant wild barley accessions tested positive for a functional *Rpg5* gene, highlighting the narrow genetic base of resistance in *Hordeum* spp. Other resistant accessions lacking the *rpg4*/*Rpg5* complex were discovered in the evaluated germplasm and may possess useful resistance genes. Combining *rpg4*/*Rpg5* with resistance genes from these other sources should provide more durable resistance against the array of different virulence types in the Ug99 race group.

Stem rust is considered one of the most important plant diseases because it can cause complete destruction of one of mankind's most important food crops, wheat (*Triticum aestivum* L.), over a wide area and in a very short period of time. Due to its worldwide importance on a major world food crop, the wheat stem rust fungus (*Puccinia graminis* f. sp. *tritici* Erikss. & Henning) is one of the most intensively studied plant pathogens, particularly with regard to its biology, physiology, genetics, epidemiology, and, more recently, genomics (Duplessis et al. 2011; Leonard and Szabo 2005). On the host side, the body of literature on the resistance of wheat to stem rust is voluminous and has contributed greatly to our evolving knowledge of breeding plants for disease resistance.

Barley (*Hordeum vulgare* L.) also can be attacked by stem rust and, in fact, is host to two such pathogens: the wheat stem rust fungus (*P. graminis* f. sp. *tritici*) and the rye stem rust fungus (*P. graminis* f. sp. *secalis* Erikss. & Henning). The former is the most important and widespread stem rust pathogen on barley (Steffenson 1992), except in some areas of northern and central Russia (Gorbunova 1979). Stem rust can be a serious problem of barley in the major production areas of the Upper Midwest (especially North Dakota and Minnesota) and Pacific Northwest (Washington) in the United States, the Prairie Provinces of Canada (Cereal Rust Bulletin 2012; Steffenson 1992), and also in northeastern Australia (Dill-Macky et al. 1991). It also was reported infecting barley crops in East Africa (Eritrea, Ethiopia, Kenya, and Uganda), the Middle East (Yemen, Iran, and Iraq), Central Asia (Kazakhstan and Tajikistan), South Asia (Bhutan, Nepal, and Pakistan), the Caucasus region (Georgia), and South America (Uruguay) (D. Hodson, K. Nazari, M. Rahmatov, and S. German, personal communication). In the early part of the 20th century, barley grown in the Upper Midwest region of the United States and adjacent provinces of Canada suffered several epidemics during the same years (especially 1935 and 1937) as wheat (Roelfs 1978; Steffenson 1992). Since the mid-1940s, stem rust of barley has been kept under control through the widespread use of cultivars carrying the resistance gene *Rpg1* (Steffenson 1992). Other factors, however, have contributed to this long-lasting disease control such as (i) a now largely resistant wheat crop that has kept the pathogen population small; (ii) barley's mostly northern cultivation area and shorter maturation period contributing, in most years, to a delayed onset of rust infection on an already ripening crop; (iii) a basal level of resistance underlying that conferred by *Rpg1*; and (iv) removal of the pathogen's alternate host (various barberry species in the genus *Berberis*) from production regions, thereby eliminating an early and local source of inoculum as well as new virulence types of the pathogen generated through sexual hybridization (Roelfs 1982; Steffenson 1992). The barley *Rpg1* monoculture was established over several million hectares in North America and, therefore, was extremely vulnerable to epidemics should a virulent pathotype of *P. graminis* f. sp. *tritici* ever arise. Indeed, in 1988, a pathotype (QCC, now designated QCCJB) of *P. graminis* f. sp. *tritici* with virulence for

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Rpg1 was first discovered in the northwestern Great Plains (Martens et al. 1989) and, a few years later (1990 to 1991), caused scattered losses on barley in both the Upper Midwest region of United States and eastern Prairie Provinces of Canada (Harder and Dunsmore 1991; Roelfs et al. 1993a,b). During this time, the frequency of pathotype QCCJB increased rapidly (reaching >90% of all races identified from commercial fields in the United States in 1990) due to its ability to attack not only barley cultivars with *Rpg1* but also a few wheat cultivars in the central Great Plains (Roelfs et al. 1993a). Removal of these stem-rust-susceptible wheat lines from cultivation led to a concomitant decrease in pathotype QCCJB so that, by 1997, it was not detected on the crop in the annual rust surveys (McVey et al. 2002). Since the early 1990s, no significant losses have been reported due to stem rust on barley in the Upper Midwest region (USDA-ARS 2016). This example highlights the importance of removing susceptible hosts, whatever they may be, from the Great Plains epidemiological region.

Success also has been attained in controlling stem rust of wheat in the northern Great Plains of North America. Through the deployment of cultivars with multiple resistance genes, losses to stem rust in wheat have been minimal since the mid-1950s (Leonard and Szabo 2005; USDA-ARS 2016). Similar successes have been reported in Australia and other major wheat production areas through this same gene-pyramiding scheme (Bariana et al. 2007; Park 2007; Singh et al. 2004). However, the world is now faced with one of the greatest biotic threats to stable wheat production in more than 50 years: widely virulent *P. graminis* f. sp. *tritici* pathotypes from Africa (Singh et al. 2008, 2015). In 1998, heavy stem rust infections were observed on wheat lines carrying the widely deployed resistance gene *Sr31* in a field nursery in southwest Uganda. The stem rust isolate collected from this nursery was designated "Ug99" (an abbreviation for the country of origin and year it was received and processed for virulence analysis) (Pretorius et al. 2000) and was subsequently assayed for its virulence phenotype on 16 differential lines of wheat (Jin et al. 2008; Roelfs and Martens 1988). Isolate Ug99 was initially keyed to pathotype TTKS (Wanyera et al. 2006). Subsequent virulence typing on an additional set of four wheat differential lines (with *Sr* genes *Sr24, Sr31, Sr38,* and *SrMcN*) led to the expanded and current pathotype designation of TTKSK (Jin et al. 2008).

Since it was first discovered in Uganda in 1998 (Pretorius et al. 2000), pathotype TTKSK has been detected across many countries in Africa (Egypt, Eritrea, Ethiopia, Kenya, Rwanda, Sudan, and Tanzania) and also in the Middle East (Iran and Yemen) (Nazari et al. 2009; RustTracker.org 2016; Singh et al. 2008). It is certain to spread to other countries given the ease by which rust urediniospores can be disseminated over long distances by wind and also international travelers. In addition to pathotype TTKSK, a number of other variants (i.e., TTKST, TTTSK, TTKSP, PTKSK, PTKST, TTKSF, TTKSF+*Sr9h*, TTKTK, TTKTT, TTHST, TTHSK, and PTKTK) in the "Ug99 race group" have been described (Patpour et al. 2016; Pretorius et al. 2012; RustTracker.org 2016; Singh et al. 2015), thereby complicating the process of screening and breeding for disease resistance. These widely virulent pathotypes are a major threat to food security because 90 to 95% of the world wheat acreage and 85 to 95% of breeding materials for various countries are susceptible (Singh et al. 2006; 2011).

Although not a major world food crop, barley is nonetheless an important staple for some of the most impoverished people living in the highlands of Ethiopia, Eritrea, Yemen, Tibet, Nepal, Ecuador, and Peru (Grando and Gomez Macpherson 2005). It is also a major food staple in some countries of North Africa, Central Asia, and the Baltic region (Grando and Gomez Macpherson 2005). In many Western countries, barley is an important component of the agricultural economy for its use as malt in brewing, animal feed, and also specialty foods (Ullrich 2011). Because stem rust can markedly decrease both the yield and quality of barley (Dill-Macky et al. 1991; Harder and Dunsmore 1991; Mwando et al. 2012), it is important that an investigation be made to assess the potential vulnerability of the crop to *P. graminis* f. sp. *tritici* pathotypes such as TTKSK and also identify possible resistance sources. Thus, the main objective of this study was to evaluate the reaction of a large and diverse collection of *Hordeum* germplasm to pathotype TTKSK at the seedling stage and assess the vulnerability of the crop. During the course of these evaluations, sources of resistance were discovered. A preliminary report of this work was previously published (Steffenson et al. 2012).

MATERIALS AND METHODS

Plant materials. For the stem rust evaluations, a large collection of barley cultivars, breeding lines, and landraces were obtained from various gene banks, research institutions, universities, private companies, and individual collaborators around the world. This germplasm (1,924 accessions in total) represents a broad sampling of the genetic diversity in cultivated barley. Data on the type of germplasm, originating institution, region or country where developed or cultivated, seed supplier or organization, seedling infection types (ITs) to pathotype TTKSK, corresponding adult plant reactions of selected seedling resistant accessions in the field, and results of molecular assays for functional resistance genes are given in Supplementary Table S1. In addition to cultivated barley, accessions of wild barley (*H. vulgare* subsp. *spontaneum* C. Koch.) Thell. (Supplementary Table S2) and barley lines with confirmed or suspected chromosomal introgressions from wild *Hordeum* spp. (i.e., *H. vulgare* subsp. *spontaneum*, *H. bulbosum* L., *H. depressum* (Scribn. & Sm.) Rydb., *H. compressum* Griseb., *H. brachyantherum* Nevski, and *H. bogdanii* Wil.) (Supplementary Table S3) also were evaluated for their stem rust phenotype. The *H. vulgare* subsp. *spontaneum* germplasm (934 accessions in total) included 300 accessions of the Wild Barley Diversity Collection (WBDC), previously used in other disease resistance studies (Ames et al. 2015; Roy et al. 2010; Steffenson et al. 2007), as well as many other accessions across the geographic range of the subspecies. The vast majority of wild barley accessions were from the Fertile Crescent but a broad representation also was obtained from Central Asia, North Africa, and the Caucasus region. Most of the introgression lines in cultivated barley were developed from crosses involving *H. bulbosum* and *H. vulgare* subsp. *spontaneum* (Wendler et al. 2015) but a few were made using other *Hordeum* spp. (Schooler 1974; Schooler and Franckowiak 1981). To assess whether previously identified resistance genes in barley are effective against pathotype TTKSK, we tested the *Rpg1* source 'Chevron' (CIho 1111) (Powers and Hines 1933; Shands 1939), the *Rpg2* source 'Hietpas-5' (CIho 7124) (Patterson et al. 1957), the *Rpg3* source 'GAW-79' (plant introduction [PI] 382313) (Jedel 1990; Jedel et al. 1989), the *rpg4/Rpg5* sources 'Q21861' (PI 584766) (carrying in addition *Rpg1*) and 'Q/SM20' (a Q21861/'SM89010' doubled-haploid progeny carrying only *rpg4/Rpg5*) (Brueggeman et al. 2008; Jin et al. 1994; Sun et al. 1996; Sun and Steffenson 1997) (B. Steffenson, unpublished), the *rpg6* source '212Y1' (Fetch et al. 2009), and the *rpgBH* (formerly designated *S* gene) source 'Black Hulless' (PI 24849) (Steffenson et al. 1984; Sun and Steffenson 2005). Susceptible controls included 'Hiproly' barley (PI 60693) and the barley landrace PI 532013, which is extremely susceptible to stem rust at both the seedling and adult plant stages (B. Steffenson, unpublished). In addition, 'Line E' (PI 357308) and 'McNair 701' (CItr 15288) were included as susceptible wheat controls. Previous research revealed that accessions carrying genes at the complex *rpg4*/*Rpg5* locus are resistant to pathotype TTKSK (Steffenson et al. 2009); thus, Q21861 and Q/SM20 also were considered to be resistant controls in the experiments.

Plant growth environment, inoculation protocol, and infection/incubation conditions for seedling evaluations. Seedling evaluations of all germplasm to stem rust pathotype TTKSK were done at the Minnesota Agricultural Experiment Station/Minnesota Department of Agriculture Plant Growth Biosafety Level-3 (BSL-3) Containment Facility on the St. Paul campus of the University of

Minnesota. Five seeds of each accession were planted into individual peat pots (7.0 in diameter by 9.0 cm in height) supported within plastic flats, each holding 16 pots. The growth media used was a 50:50 mixture of steam-sterilized native soil and Sunshine MVP mix (Sungro Horticulture Distributors, Quincy, MI), a growing medium containing vermiculite, Canadian sphagnum peat moss, coarse perlite, starter nutrient charge, gypsum, and dolomitic limestone. Plants were fertilized at planting with Osmocote 14-14-14 (0.3 g/pot; Scott's Company, Marysville, OH) and Peters Dark Weather 15-0-15 (40 g/liter at 1/16 dilution; Scott's Company) formulations. To break possible seed dormancy in the accessions and achieve more uniform plant growth for infection, the planted seed were initially incubated at 4°C for 2 to 5 days. Then, they were brought into the BSL-3 greenhouse and grown at 19 to 22°C with supplemental lighting provided by 400-W high-pressure sodium lamps emitting photons at a minimum of 300 μmol s^{-1} m^{-2} for 14 h/day.

A single pustule isolate (04KEN156/04) of pathotype TTKSK (Jin and Singh 2006; Steffenson et al. 2009) was used in all experiments and increased on susceptible McNair 701 wheat. Urediniospores were collected with a cyclone spore collector (G-R Manufacturing Co., Manhattan, KS) (Browder 1971), desiccated over a saturated salt solution at approximately 20% relative humidity (RH), and stored in size 00 gelatin capsules (Gallipot Inc., St. Paul, MN) enclosed within cryovials (Corning Life Sciences, Tewksbury, MA) in an ultralow-temperature freezer (–80°C) until needed. In the morning before inoculation, the rust was removed from the freezer and immediately heat shocked in a water bath at 45°C for 15 min (Rowell 1985). Then, the rust was rehumidified for at least 3 h by incubating the opened gelatin capsules over a saturated salt solution at approximately 80% RH. Germination tests for the rust were performed by spraying a suspension of urediniospores dispersed in a lightweight mineral oil (Soltrol 170; Phillips Petroleum, Bartlesville, OK) onto Petri plates containing 2% water agar, incubating the plates at 22°C for at least 3 h in the dark, and then examining 100 to 200 arbitrarily selected urediniospores for extended germtubes with a compound microscope at ×40. Occasionally, fresh rust, newly collected from infected plants, was used for inoculation. In either case, the germination rate of urediniospores was 90% or higher.

For inoculation, urediniospores were placed into new gelatin capsules containing the oil carrier (concentration of 15 mg of urediniospores per 0.7 ml of oil) and then applied to 9-day-old plants (fully expanded primary leaves) in each flat using special atomizers (G-R Manufacturing Co.) (Browder 1971) pressured by a pump set at 25 to 30 kPa. Urediniospores were applied at approximately 0.175 mg/plant. The concentration of inoculum was approximately four times higher than that used in previous studies (Steffenson et al. 2009; Sun and Steffenson 2005) but was necessary to ensure good infection on barley within the drier, high-air-flow environment of the BSL-3. Immediately after inoculation, the plants were placed in front of a small electric fan for 3 to 5 min to hasten the evaporation of the oil carrier from leaf surfaces. Thereafter, an additional 90 min of evaporation time was provided to reduce the phytotoxicity of the oil carrier. Then, plants were placed in mist chambers and exposed to approximately 30 min of continuous misting from ultrasonic humidifiers, followed by 16 h of periodic misting (2 min of misting every 15 min) in the dark. After the dark period, lights (400-W high-pressure sodium vapor lamps emitting photons at 300 μmol s^{-1} m^{-2}) were turned on to complete the final stages of the infection process while the misters continued to run under the same regime. After a minimum of 5 h of light exposure, the misters were turned off and chamber doors were opened, facilitating the slow drying of plant surfaces over the next several hours. When the plants were completely dry, they were moved back to the greenhouse under the conditions previously described. The general conditions used for the stem rust infection period were based on the methods of Rowell (1985).

Disease assessment. At 12 to 14 days after inoculation, the ITs on each accession were scored using a 0-to-4 scale. The IT scale used for barley is a modification of the one developed for wheat by Stakman et al. (1962) and is based primarily on uredinial size, as described by Miller and Lambert (1955). Plus (+) and minus (–) symbols were used denote more or less sporulation of classically described uredinia, respectively. Barley frequently exhibits two or more ITs on a single leaf (i.e., a mesothetic reaction) when infected with *P. graminis* (Sun and Steffenson 2005). All of the observed ITs were recorded in order of their frequency on the leaves; however, only the two most common ones (i.e., the IT mode) are presented in the data tables because they usually comprise more than 85% of all those observed on individual accessions (Zhou et al. 2014). ITs were classified into five general categories as follows: 0 or 0; ("zero-fleck", a hypersensitive reaction) as highly resistant (HR), 1 as resistant (R), 2 as moderately resistant (MR), 3– as moderately susceptible (MS), and 3, 3+, or 4 as susceptible (S).

The experiment was conducted in a completely randomized design with one replicate and was repeated once. To obtain more robust data on germplasm likely to be used in breeding, accessions exhibiting consistently HR to MR reactions were repeated at least two additional times to confirm the phenotype. The same was done for accessions exhibiting variable reactions (i.e., reaction classes of HR, R, or MR versus MS or S) between experiments or those that showed clear segregation among individual plants. Some cultivated and wild barley accessions were only tested once due to seed quantity limitations but, in all of these cases, the plants exhibited only or predominantly S reactions to pathotype TTKSK. The resistant (Q21861 and Q/SM20) and susceptible (Hiproly, Line E, and McNair 701) controls were included approximately every 50 accessions to monitor both the infection levels and expected ITs in each experiment.

To provide a succinct summary of results from this large dataset, accessions were categorized into three groups: those exhibiting only HR, R, or MR reactions across all experiments (accessions highlighted in green in Supplementary Tables S1 and S2); those exhibiting predominantly HR, R, or MR reactions within the IT mode across experiments (accessions highlighted in light green) or in individual plants of accessions that were phenotypically mixed due to possible segregation or seed admixtures (accessions highlighted in blue); and those exhibiting only or predominantly MS to S reactions within the IT mode across experiments (accessions not highlighted). The number and percentage of accessions in each of these three groups were summarized across continents/regions and countries for cultivated barley and across countries for wild barley.

Assessment of adult plant resistance in the field. Repeated attempts were made to obtain adult plant resistance data on the entire germplasm panels at the Kenyan Agricultural and Livestock Research Organization in Njoro, Kenya and in Greytown, South Africa; however, these efforts failed due to low rust infection. From 2009 to 2015, we focused our efforts on obtaining adult plant resistance data on a subset of *Hordeum* accessions exhibiting consistently low seedling ITs because previous studies revealed that such reactions were usually indicative of all-stage resistance. Still, in a number of these stem rust nurseries, the level of infection obtained was insufficient to reliably separate known S from R accessions. Thus, only data from the 2014 Kenya and 2012 and 2015 South Africa nurseries are presented. Methodology for initiating stem rust epidemics at the different locations was similar, and a completely randomized plot design with one replicate was used.

For the 2014 off-season Kenya nursery (planting in December with scoring in April), the protocols of Njau et al. (2013) were followed, except that needle inoculations of spreader plants (i.e., injecting a urediniospore-water suspension into stems) were made in addition to the direct foliar spray inoculations. The pathotypes used in Kenya consisted of a mixture of TTKSK (avirulence/virulence formula of *Sr36, Tmp, 24/Sr5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, 31, 38, McN*) and TTKST (*Sr36, Tmp/Sr5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 30,*

17, 9a, 9d, 10, 24, 31, 38, McN). The local susceptible wheat control 'Red Bobs' was included every 100 rows in the nursery to monitor the level of infection in the nursery.

In the South Africa rust nurseries, barley accessions were sown in 0.5-m rows spaced 75 cm apart. Each barley row was flanked on one end by the stem-rust-susceptible bread wheat line L37-07. The barley screening trial formed part of a larger wheat stem rust screening nursery of approximately 10,000 lines, specifically designed to provide abundant *P. graminis* f. sp. *tritici* inoculum from numerous spreader rows and blocks. Beginning in early August of each year, stem rust epidemics were created by repeatedly spray inoculating susceptible spreader plants in the surrounding wheat nursery. For the inoculations, urediniospores suspended in Soltrol oil were applied onto plants with an ultra-low-volume sprayer (Micron Group, Bromyard, England) in the late afternoon. Then, strategically positioned spreader rows were covered with plastic sheeting to facilitate dew formation and, hence, infection by the pathogen. Pathotype PTKST (*Sr9h*, *21*, *27*, and *36*; *Tmp*/*5*, *6*, *7b*, *8a*, *8b*, *9a*, *9b*, *9d*, *9e*, *9g*, *10*, *11*, *17*, *24*, *30*, *31*, *38*; and *McN*) was used in all experiments and bulk harvested on the day of inoculation from infected wheat seedlings (carrying *Sr31*) grown in a greenhouse. The local susceptible wheat control L37-07 was included every 100 rows in the nursery to monitor the level of infection in the nursery.

Adult plant resistance was assessed based on the severity of rust occurring on the stems and leaf sheaths of plants. Rust severity was visually estimated using the modified Cobb Scale (0 to 100%) (Peterson et al. 1948) when plants were at the mid- to hard-dough stage of development (Zadoks et al. 1974). In addition to severity, accessions also were assessed for their adult plant infection response (IR) according to the scale of R = minute to small uredinia surrounded by chlorosis or necrosis, MR = medium-sized uredinia often surrounded by chlorosis, MS = medium to large erumpent uredinia with little or no chlorosis, and S = very large erumpent uredinia with little or no chlorosis (Roelfs et al. 1992). IRs were recorded in order of their relative frequency (e.g., MR-MS) when more than one was observed on an accession.

Molecular assays for the *Rpg5* and *Rpg1* stem rust resistance genes. The *rpg4*/*Rpg5* gene complex (Brueggeman et al. 2008) is the only one known to confer a high level of all-stage resistance to pathotype TTKSK in barley (Steffenson et al. 2009). To determine whether the *Hordeum* accessions found to be resistant carried this gene complex, molecular assays were conducted for the functional *Rpg5* resistance allele and its described susceptibility alleles. Genomic DNA from select resistant *Hordeum* accessions was extracted by the cetyltrimethylammonium bromide method and used for polymerase chain reaction (PCR) and sequencing applications (Murray and Thompson 1980). The primer pairs LRK-F1 (CTGCTGGCACAGAGTCTGCCTTGAG) versus LRK-R1 (ACT

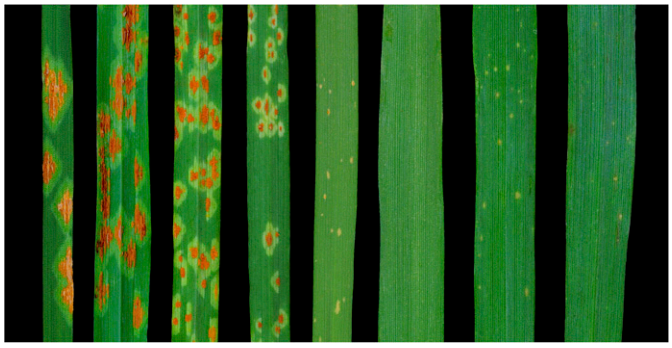

Fig. 1. Seedling infection types of wheat control McNair 701 and select cultivated and wild barley accessions to *Puccinia graminis* f. sp. *tritici* pathotype TTKSK in the greenhouse. Left to right: McNair 701 wheat and barley accessions PI 532013, Hiproly, WBDC123, WBDC119, Q21861, Brandham II, and SH98073.

TABLE 1. Summary of the infection type (IT) mode, range, and general reaction (GR) of controls and accessions with recognized stem rust resistance genes to *Puccinia graminis* f. sp. *tritici* pathotype TTKSK at the seedling stage, their corresponding adult plant reactions in the field, and results of molecular assays for functional resistance genes

			Seedling reaction			Adult plant reaction[a]					
			TTKSK			TTKSK/TTKST Kenya[b]		PTKST South Africa		Assays[c]	
Accession	Other[d]	Description	IT[e]	Range[f]	GR[g]	Sev (%)	IR	Sev (%)	IR	*Rpg5*	*Rpg1*
Chevron	PI 38061	Source of *Rpg1*	3–, 2	2, 3– to 3	MS-MR	15 (3–50)	MS-S	17 (0.5–60)	MS-S	–	+
Hietpas 5	CIho 7124	Source of *Rpg2*	3	3	S	16 (1–30)	MS-S	10 (0–15)	MR-MS	–	–
GAW 79-3	PI 382313	Source of *Rpg3*	3–, 2	2, 1 to 3–, 2	MS-MR	22 (10–40)	MS-S	31 (5–50)	S	–	–
Q21861	PI 584766	Source of *rpg4/Rpg5* plus *Rpg1*	0;, 1	0; to 2,1	HR-R	2 (0–5)	R-MR	4 (0–30)	MR-R	+	+
Q/SM20	–	Source of *rpg4/Rpg5*	1, 0;	0;, 1 to 1, 2	R-HR	1 (1–10)	MR-R	14 (5–20)	MR-MS	+	–
212Y1	–	Source of *rpg6*	3–, 2	2 to 3–, 2	MS-MR	…	…	…	…	–	–
Black Hulless	PI 24849	Source of *rpgBH*	3	3– to 3	S	15 (–)	S-MS	66 (5–95)	S	–	–
Hiproly	PI 60693	Susceptible barley control	3	3– to 3	S	23 (5–70)	S-MS	36 (3–90)	S-MS	–	–
PI 532013	2794	Susceptible barley control	3	3 to 3+	S	53 (25–80)	S	71 (12–100)	S	–	–
McNair 701	CItr 15288	Susceptible wheat control	4	3+ to 4	S	…	…	…	…	–	–
Line E	CItr 357308	Susceptible wheat control	4	3 to 4	S	…	…	…	…	–	–

[a] Adult plant reaction was assessed at the mid- to hard-dough stage of development as the percentage of stem and leaf sheath tissue infected by stem rust, estimated using the modified Cobb scale (0 to 100%) (Peterson et al. 1948), and also as the type of uredinia (i.e., infection response [IR]) observed, where R = resistant, MR = moderately resistant, MS = moderately susceptible, and S = susceptible (Roelfs et al. 1992). Adult plant severity and IR data are based on experiments conducted in Kenya from 2012 to 2015 and in South Africa from 2013 to 2015. Sev = average severity, with range in parentheses, and … indicates data missing or not applicable.

[b] The 2014 Kenya nursery had a mixture of pathotypes TTKSK and TTKST.

[c] Molecular assays to determine a functional or nonfunctional *Rpg5* gene were conducted according to the methods of Arora et al. (2013) and Mamo et al. (2015), and those for *Rpg1* were done according to Eckstein et al. (2003), as modified by Derevnina et al. (2014). Detailed descriptions of the assays are given in the Materials and Methods section. Symbols: + indicates the presence of the functional gene and a – indicates the lack of the functional gene.

[d] Other designator.

[e] ITs for wheat were scored based on the 0-to-4 scale of Stakman et al. (1962) and those for barley were scored based on the system of Stakman et al. (1962), as modified by Miller and Lambert (1955). The IT mode represents the one or two most common ITs observed in order of frequency on accessions over all experiments. Symbols + and – denote more or less sporulation of classically described uredinia, respectively. Seedling IT data are based on experiments conducted in 2014 and 2015.

[f] IT range is the lowest and highest types observed on accessions over all experiments.

[g] GR was assigned for the IT mode where 0 or 0; is highly resistant (HR); 1 is R; 2 is MR; 3– is MS; and 3, 3+, or 4 is S.

CTCGGGTCTGAAGTTCCGTGTG) or PP2C-R2 (CCCGAGG TTTGCGATGAAGAGAGTC) were used to distinguish functional *Rpg5* alleles from the most common nonfunctional *Rpg5* allele, which contains an insertion that replaces the protein kinase domain with a protein phosphatase domain (Brueggeman et al. 2008). The diagnostic primer combinations produce dominant markers consisting of amplicons across the LRR to STPK or LRR to PP2C junction and were previously described (Mamo et al. 2015). Accessions showing a predicted functional STPK domain were sequenced across the *Rpg5* 5′ region that contains a single cytosine insertion, resulting in a rare nonfunctional *rpg5* allele as the result of a frame shift and nonfunctional truncated RPG5 protein (Arora et al. 2013; Brueggeman et al. 2008). To accomplish this, an amplicon was generated from the primer pair R5-F1 (CCGCCTACCACACCTCCGATTCCAC) versus R5-R1 (TCAGGTTTGATGGCTGTCTCTGGAG) and then directly sequenced with the R5-F1 primer. Functional *Rpg5* alleles contain the sequence GCAGGATCCCCCCATCACGG, whereas nonfunctional *rpg5* alleles, such as 'Golden Promise', contain the sequence GCAGGATCCCCCCCCATCACGG. Accessions were also sequenced across the 3′ STPK domain region that contains a second rare susceptible *rpg5* allele, predicted to encode full-length proteins with a nonsynonymous nucleotide substitution that results in the amino acid substitution E1287A, as reported by Arora et al. (2013). For this protocol, an amplicon was generated using the primers RPG5-F10 (TGCATCTATCTGCTCATGCAAGGAG) versus RPG5-R10 (AACAATATTCACCTGCGGCACCAAC). This product was then directly sequenced using the RPG5-SEQ-R1 (AGTGGCTTGA GAGCTTCAAC) primer (suggested by R. Brueggeman, personal communication). The sequencing results were translated and aligned using Vector-NTI software to determine whether the accessions contained predicted functional or nonfunctional RPG5 proteins. Accessions with functional *Rpg5* alleles have the sequence TCCTTCCCCGCGAGGG or TCCTTCCCCACGAGGG, corresponding to groups 3R and 1R, respectively, whereas accessions with nonfunctional *rpg5* alleles have the sequence TCCTTCCCCGCG CGGG, corresponding to group 4S, as described by Arora et al. (2013). Because genes at the *rpg4*/*Rpg5* locus are tightly linked with each other, a positive assay for a functional *Rpg5* gene was presumed to be indicative for the presence of a functional *rpg4*/*Rpg5* gene complex (Arora et al. 2013). In summary, genotypes with a functional *Rpg5* gene (i) amplify a product from the LRK-F1 versus LRK-R1 PCR, (ii) do not amplify a product from the LRK-F1 versus PP2C reaction, (iii) do not contain the Golden Promise-like sequence within the amplicon generated by R5-F1 versus R5-R1, and (iv) possess either the group 3R- or 1R-like sequence within the amplicon of RPG5-F10 versus RPG5-R10.

Accessions resistant to pathotype TTKSK at the seedling stage were also assayed for the presence of *Rpg1* because it may confer a low level of residual resistance against virulent pathotypes as compared with accessions lacking any major resistance genes. Assays for a functional or nonfunctional *Rpg1* gene were performed as described by Eckstein et al. (2003) using the revised primer sequence for the

TABLE 2. Summary of general reactions of cultivated barley accessions from different countries to *Puccinia graminis* f. sp. *tritici* pathotype TTKSK at the seedling stage

Continent, region, country	Number (%) exhibiting reactions[a]			Total[b]
	Only HR to MR	PD R to MR or Seg	Only or PD MS to S	
North America				
Canada	9 (6.0)	2 (1.4)	138 (92.6)	149
Mexico	0 (0.0)	2 (22.2)	7 (77.8)	9
United States	2 (0.4)	19 (3.6)	502 (96.0)	523
Continent total	11 (1.6)	23 (3.4)	647 (95.0)	681
South America				
Argentina	0 (0.0)	0 (0.0)	10 (100.0)	10
Bolivia	0 (0.0)	0 (0.0)	10 (100.0)	10
Brazil	0 (0.0)	1 (3.2)	30 (96.8)	31
Chile	0 (0.0)	0 (0.0)	12 (100.0)	12
Ecuador	0 (0.0)	0 (0.0)	10 (100.0)	10
Peru	0 (0.0)	0 (0.0)	17 (100.0)	17
Uruguay	2 (14.3)	0 (0.0)	12 (85.7)	14
Continent total	2 (1.9)	1 (1.0)	101 (97.1)	104
Africa				
Algeria	0 (0.0)	0 (0.0)	8 (100.0)	8
Egypt	0 (0.0)	0 (0.0)	13 (100.0)	13
Eritrea	0 (0.0)	0 (0.0)	4 (100.0)	4
Ethiopia	0 (0.0)	0 (0.0)	12 (100.0)	12
Kenya	0 (0.0)	0 (0.0)	16 (100.0)	16
Libya	0 (0.0)	0 (0.0)	10 (100.0)	10
Morocco	0 (0.0)	0 (0.0)	25 (100.0)	25
South Africa	0 (0.0)	0 (0.0)	7 (100.0)	7
Tunisia	0 (0.0)	0 (0.0)	9 (100.0)	9
Continent total	0 (0.0)	0 (0.0)	104 (100.0)	104
Asia				
Afghanistan	0 (0.0)	0 (0.0)	10 (100.0)	10
Armenia	0 (0.0)	0 (0.0)	7 (100.0)	7
Azerbaijan	0 (0.0)	0 (0.0)	2 (100.0)	2
Bangladesh	0 (0.0)	0 (0.0)	2 (100.0)	2
China	3 (8.1)	0 (0.0)	34 (91.9)	37
Cyprus	0 (0.0)	0 (0.0)	1 (100.0)	1
Georgia	0 (0.0)	0 (0.0)	10 (100.0)	10

(*continued on next page*)

[a] PD = predominantly and Seg = segregating. A general reaction was assigned to the infection type (IT) mode where 0 or 0; is highly resistant (HR); 1 is resistant (R); 2 is moderately resistant (MR); 3– is moderately susceptible (MS); and 3, 3+, or 4 is susceptible (S). Then, accessions were categorized into three groups: those exhibiting only HR, R, or MR reactions across all experiments; those exhibiting predominantly HR, R, or MR reactions within the IT mode across experiments or in individual plants of accessions that were phenotypically mixed due to possible segregation or seed admixtures; and those exhibiting only or predominantly MS to S reactions within the IT mode across experiments.

[b] Total number evaluated.

functional *Rpg1* allele of RPG1-N-F (CGGCTAATCACATCAAG TAA) versus RPG1-N-R (AGCCCATCATCAATAGACAA) and the primer pair for the nonfunctional *rpg1* allele of RPG1-S-F (GGCTAATCACATCAAGGTT) and RPG1-S-R (CCACGACCA ATTATGTTCTG), as described by Derevnina et al. (2014). The primer combinations produce dominant markers consisting of amplicons in either the functional or nonfunctional PCR assay.

Molecular assays and sequencing for *Rpg5* and *Rpg1* were done by Functional Biosciences, Madison, WI.

RESULTS

Seedling evaluations in the greenhouse. *Reaction of susceptible controls and accessions with recognized stem rust resistance genes.* The data presented in Table 1 provide an overall summary of the degree of central tendency and range observed for ITs of the controls and carriers of known resistance genes across experiments. Susceptible controls were included in multiple replicates in all experiments to monitor the infection level and virulence phenotype of pathotype TTKSK. Moderate to high infection levels were observed in all experiments, allowing for reliable scoring of ITs on the *Hordeum* accessions. Barley (Hiproly and PI 532013) and wheat (McNair 701 and Line E) controls all exhibited the expected compatible IT modes of 3 and 4, respectively (Table 1; Fig. 1).

Hietpas-5 (*Rpg2*) and Black Hulless (*rpgBH*) exhibited S ITs (IT mode 3), similar to the susceptible controls Hiproly and PI 532013 (Table 1). Chevron (*Rpg1*), GAW-79 (*Rpg3*), and 212Y1 (*rpg6*) all exhibited predominantly MS ITs but with some MR types at lower frequency (IT mode 3–, 2). Accessions carrying the gene complex of *rpg4/Rpg5* (Q21861 and Q/SM20) were HR to R, exhibiting IT modes of 0;, 1, and 1, 0;, respectively. Q21861, carrying *Rpg1* in addition to *rpg4/Rpg5*, exhibited a higher proportion of HR (i.e., hypersensitive IT 0;) reactions (Fig. 1) than Q/SM20, carrying only the *rpg4*/*Rpg5* complex (Table 1).

Reaction of cultivated barley accessions. Of the 1,924 cultivated barley accessions evaluated to pathotype TTKSK, only 32 (1.7%) exhibited consistently HR to MR reactions (IT modes 0; to 2) across all experiments (Table 2). Many of these accessions exhibited the full range of resistant class ITs from 0; to 2 across the three experiments (Table 3). However, some exhibited predominantly IT 0; (e.g., 'Anakin'), whereas others exhibited predominantly IT 2 (e.g., ND23821, CLE 202, BHS 248, Cantala, and Tallon). Resistance was identified from 13 of the 59 (22.0%) countries from which germplasm was tested, located on the continents of North America, South America, Asia, Europe, and Australia (Table 2). Nine countries had more than one resistant accession: Canada with nine, United States with two, Uruguay with two, China with three, Denmark with four, France with two, Russian Federation with two, United Kingdom with two, and Australia with two. Nearly all of the resistant accessions identified were cultivars or breeding lines from different barley improvement programs around the world, the exception being Brandham II (Fig. 1), a landrace from Austria (Table 3; Supplementary Table S1).

TABLE 2. (*continued from preceding page*)

Continent, region, country	Number (%) exhibiting reactions[a]			Total[b]
	Only HR to MR	PD R to MR or Seg	Only or PD MS to S	
India	1 (5.6)	1 (5.6)	16 (88.8)	18
Iran	0 (0.0)	0 (0.0)	9 (100.0)	9
Iraq	0 (0.0)	1 (10.0)	9 (90.0)	10
Israel and adjacent territories	0 (0.0)	1 (8.3)	11 (91.7)	12
Japan	0 (0.0)	0 (0.0)	19 (100.0)	19
Jordan	0 (0.0)	0 (0.0)	10 (100.0)	10
Kazakhstan	0 (0.0)	0 (0.0)	9 (100.0)	9
Kyrgyzstan	0 (0.0)	0 (0.0)	10 (100.0)	10
Nepal	0 (0.0)	0 (0.0)	2 (100.0)	2
Pakistan	0 (0.0)	0 (0.0)	10 (100.0)	10
South Korea	0 (0.0)	1 (5.0)	19 (95.0)	20
Syria	0 (0.0)	0 (0.0)	21 (100.0)	21
Tajikistan	0 (0.0)	0 (0.0)	10 (100.0)	10
Turkey	0 (0.0)	0 (0.0)	16 (100.0)	16
Turkmenistan	0 (0.0)	0 (0.0)	9 (100.0)	9
Uzbekistan	0 (0.0)	0 (0.0)	7 (100.0)	7
Yemen	0 (0.0)	1 (10.0)	9 (90.0)	10
Continent total	4 (1.5)	5 (1.8)	262 (96.7)	271
Europe				
Austria	1 (11.1)	0 (0.0)	8 (88.9)	9
Czech Republic	0 (0.0)	0 (0.0)	1 (100.0)	1
Denmark	4 (19.1)	2 (9.5)	15 (71.4)	21
Finland	0 (0.0)	0 (0.0)	1 (100.0)	1
France	2 (3.1)	1 (1.6)	61 (95.3)	64
Germany	1 (1.9)	0 (0.0)	52 (98.1)	53
Italy	0 (0.0)	0 (0.0)	47 (100.0)	47
Netherlands	0 (0.0)	0 (0.0)	16 (100.0)	16
Norway	0 (0.0)	0 (0.0)	8 (100.0)	8
Russian Federation	2 (1.7)	6 (5.3)	107 (93.0)	115
Spain	1 (2.4)	0 (0.0)	41 (97.6)	42
Sweden	0 (0.0)	0 (0.0)	27 (100.0)	27
Switzerland	0 (0.0)	0 (0.0)	7 (100.0)	7
United Kingdom	2 (0.8)	10 (4.1)	231 (95.1)	243
Continent total	13 (2.0)	19 (2.9)	622 (95.1)	654
Oceania				
Australia	2 (2.0)	0 (0.0)	97 (98.0)	99
New Zealand	0 (0.0)	0 (0.0)	11 (100.0)	11
Region total	2 (1.8)	0 (0.0)	108 (98.2)	110
Overall totals	32 (1.7)	48 (2.5)	1,844 (95.8)	1,924

In addition to these 32 consistently resistant accessions (Table 2), 48 (2.5%) others exhibited predominately R to MR reactions. However, this group also exhibited MS to S reactions within the IT mode across experiments or in individual plants as possible phenotypically mixed accessions (light green and blue highlighted accessions in Supplementary Table S1). Considering the two general groups together, 80 (4.2%) cultivated accessions exhibited exclusively or predominantly R to MR reactions across experiments.

Reaction of wild barley accessions. Only 13 (1.4%) of the 934 wild barley (*H. vulgare* subsp. *spontaneum*) accessions tested exhibited consistently HR to MR reactions (IT modes ranging from 0; to 2) across experiments (Table 4). Similar to cultivated barley, many of the select wild barleys exhibited the full range of R class ITs from 0; to 2 but some had predominantly IT 0; (WBDC119) (Fig. 1) or predominantly IT 2 (WBDC225) (Table 5). Most of the resistant accessions identified (9 of 13) were from the Central Asian countries of Afghanistan (one accession), Kazakhstan (one), Turkmenistan (one), Tajikistan (two), and Uzbekistan (four) (Table 4). Although accessions from the Fertile Crescent comprised more than 85% of the wild barley germplasm evaluated in this study, only four accessions were found resistant from this region: three from Israel and one from Syria (Table 4).

In addition to the 13 consistently R wild barley accessions mentioned above (Table 4), 11 (1.2%) others exhibited predominately R to MR reactions—but with MS to S reactions within the IT mode across experiments or in individual plants as possible phenotypically mixed accessions (light green and blue highlighted accessions in Supplementary Table S2). Considering the two general groups together, 24 (2.6%) wild barley accessions exhibited exclusively or predominantly R to MR reactions across experiments.

Reaction of lines with confirmed or putative introgressions from wild Hordeum *spp.* Of the 55 barley lines with characterized or putative introgressions from various wild *Hordeum* spp., none was resistant to race TTKSK (Supplementary Table S3). The majority of these lines exhibited ITs of 3– to 3+ but some gave ITs 3–, 2.

Adult plant evaluations in the field. *Reaction of susceptible controls and accessions with recognized stem rust resistance genes.* The data presented in Table 1 provide a summary of the overall average and range observed for rust severity across experiments, plus the central tendency and variability of IRs for the controls and carriers of known resistance genes. These aggregated data provide an assessment of the consistency of rust reactions across locations and years. The susceptible controls Hiproly and PI 532013 exhibited overall average severities of 23 and 53%, respectively, in the Kenya nursery and 36 and 71% in the South Africa nursery. Both of these controls also exhibited predominately S IRs. In contrast, the resistant controls Q21861 and Q/SM20 exhibited much lower overall average severities of 2 and 1% in the Kenya nursery, respectively, and 4 and 14% in the South Africa nursery. These two controls also exhibited mostly MR to R IRs.

Chevron (*Rpg1*), Hietpas-5 (*Rpg2*), GAW-79 (*Rpg3*), and Black Hulless (*rpgBH*) exhibited rust severities intermediate (15 to 22%) to those of the resistant and susceptible control groups in Kenya (Table 1). Similar results were found in South Africa, the exceptions being Hietpas-5, which exhibited a lower severity (10%) than resistant control Q/SM20 (14%), and Black Hulless (66%), which exhibited a higher severity than susceptible control Hiproly (36%). These sources of *Rpg1*, *Rpg2*, *Rpg3*, and *rphBH* exhibited mostly MS to S IRs, the exception being Hietpas-5, which had mostly MR IRs in South Africa.

The severity of rust epidemics varied greatly across the different nurseries in Africa. Therefore, the average severity and IRs of susceptible and resistant controls within individual experiments were also presented to provide a proper comparison for the performance of selected cultivated and wild barley accessions.

In the 2014 Kenya nursery infected with races TTKSK and TTKST, disease pressure was moderate because Hiproly and PI 532013 exhibited an average severity of 20 and 53%, respectively, with IRs of S-MS (Table 3). Disease pressure in the South Africa nurseries infected with race PTKST was low and extremely high in 2012 and 2015, respectively, because Hiproly exhibited an average severity of 5% (IRs S-MS) and 78% (IR S), respectively (Table 3). PI 532013 was not included in the 2012 nursery but exhibited an average severity of 100% (IR S) in the 2015 nursery (Supplementary Fig. S1). Susceptible local wheat controls were heavily infected in all nurseries: Red Bobs exhibited an average severity of 71% (IR S) in the 2014 Kenya nursery, whereas L37-07 had an average severity of 80% (IR S) and 95% (IR S) in the 2012 and 2015 South Africa nurseries, respectively (data not included in tables). The resistant control Q21861 (carrying the *rpg4*/*Rpg5* complex plus *Rpg1*) exhibited very low severities and mostly R IRs (range of 0 R to trace R-MR) in the 2014 Kenya and 2012 South Africa nurseries (Table 3). Under extremely high disease pressure in the 2015 South Africa nursery, Q21861 exhibited a higher average severity of 10%, with IRs of MR to R (Table 3; Supplementary Fig. S1). Q/SM20 (carrying the *rpg4*/*Rpg5* complex only) exhibited slightly higher rust severities than Q21861 in the nurseries where both accessions were included and, in some cases, higher IRs. Chevron (*Rpg1* only) exhibited very low (2% severity, with IRs MR-MS), low (14%, with IRs MS-S), and moderate (36%, with IRs S-MS) (Table 3; Supplementary Fig. S1) rust severities corresponding to the relative disease pressure in the 2012 South Africa, 2014 Kenya, and 2015 South Africa nurseries, respectively.

In the 2014 Kenya and 2012 South Africa nurseries with moderate and low disease pressure, the 32 seedling-resistant selections of cultivated barley ranged in reaction from trace R-MR to 15 S-MS and from 0% R to 10% S, respectively (Table 3). Under extreme disease pressure in the 2015 South Africa nursery, nearly all of these 32 selections were overwhelmed, exhibiting severities of 35 to 85% and IRs of MS to S. The two most resistant accessions identified were BM 9723-53 and Anakin, both exhibiting severities of 15% with IRs of MR-MS. Other accessions exhibiting lower severities included County (15%), Paramount (20%), and BHS 248 (20%) but they all had MS and S IRs.

Reaction of selected wild barley accessions. Adult plant assessments for many of the selected wild barley accessions could not be obtained in the Kenya nursery because they did not develop to the heading stage. This was likely due to the photoperiod sensitivity of the germplasm growing at an equatorial site. Data were obtained, however, in the South Africa nurseries. In the 2012 South Africa nursery with low disease pressure, the seedling-resistant wild barley selections exhibited reactions ranging from 0% R to 20% S (Table 5). As was the case for cultivated barleys, most of the selected wild barley accessions were overwhelmed in the 2015 South Africa nursery, giving reactions ranging from 35% S or MS-S to 60% MS-S. The most resistant wild barley accessions identified in this nursery were WBDC 220, 224, and 225, which had lower severities of 20 to 25% and mostly MR IRs. WBDC 333 exhibited a severity of 25% but had an S IR.

Molecular assays for *Rpg5* and *Rpg1* in selected *Hordeum* accessions with seedling resistance. *Molecular assay for* Rpg5 *to detect a functional* rpg4/Rpg5 *gene complex.* To determine whether any of the selected resistant *Hordeum* accessions might carry the *rpg4*/*Rpg5* complex known to be effective against pathotype TTKSK, molecular assays were conducted for a functional *Rpg5* gene. In all, 12 of the 32 (37.5%) selected cultivated accessions tested positive for a functional *Rpg5* gene (Table 3). Accessions with a functional *Rpg5* gene generally had a higher frequency of very low ITs (i.e., the hypersensitive 0;) across experiments than those without the gene (Table 3). However, there were exceptions to this trend: Zang Qing 80, possessing a functional *Rpg5* gene, exhibited no 0; reactions, and TR 02272 and BM 8923-30, lacking a functional *Rpg5* gene, exhibited some 0; ITs in two of the three experiments. With respect to adult plant reactions, accessions with a functional *Rpg5* gene generally had low rust severities under low (2012 South Africa) and moderate (2014 Kenya) disease pressure; however, other accessions

lacking the gene also exhibited comparably low rust severities (Table 3).

In total, 11 of the 13 (84.6%) selected wild barley accessions tested positive for a functional *Rpg5* gene (Table 5). No clear trends were observed in the seedling or adult plant reactions between groups of accessions carrying *Rpg5* and those lacking the gene. This comparison was rather limited because the latter group consisted of only two accessions.

The 59 *Hordeum* accessions (48 cultivated and 11 wild barley) that exhibited predominantly R to MR reactions or were phenotypically mixed with respect to seedling ITs were also assayed for *Rpg5* (light green and blue highlighted accessions in Supplementary Tables S1 and S2). Five cultivated (NB08410, Danuta, BM 9238-15, RD-583, and LAND09-155) and five wild (ICARDA112787, WBDC026, WBDC125, WBDC348, and WBDC349) barley accessions within this group tested positive for the functional gene, indicating the robustness of the seedling evaluations for detecting *Rpg5*.

Molecular assay to detect a functional Rpg1 *gene.* The selected cultivated accessions were also assayed for the presence of resistance gene *Rpg1* to assess whether it might possibly confer residual resistance against a virulent pathotype like TTKSK. Of the 12 accessions carrying a functional *Rpg5* gene, 7 also had a functional *Rpg1* gene (Table 3). There were no consistent IT trends between accessions carrying both *Rpg5* and *Rpg1* and those carrying only *Rpg5*. Five other accessions were found to carry only *Rpg1*. Again, no consistent IT trends were observed between accessions carrying only *Rpg1* and those lacking the two assayed resistance genes. With respect to the adult plant reactions in the field, no strong consistent trends were noted among accessions carrying different combinations of the resistance genes or those lacking the two assayed resistance genes because the range of severities overlapped for the different

TABLE 3. List of cultivated barley accessions exhibiting only resistant (R) to moderately resistant (MR) infection types (IT) to *Puccinia graminis* f. sp. *tritici* pathotype TTKSK at the seedling stage, their corresponding adult plant reactions in the field, and results of molecular assays for functional resistance genes

			Seedling reaction to race TTKSK[a]									Adult plant reaction[b]			Assay[c]	
			Experiment 1			Experiment 2			Experiment 3			Severity (%) IR				
Accession	Origin[d]	Donor[e]	IT1	IT2	GR	IT1	IT2	GR	IT1	IT2	GR	K 2014	SA 2012	SA 2015	*Rpg5*	*Rpg1*
Checks																
Q21861	Mexico via Western Australia	CDC	0;	1	HR-R	1	0;	R-HR	0;	1	HR-R	Tr R-MR	0 R	10 MR-R	+	+
Q/SM20	Saskatoon, SK, Canada	CDC	1	0;	R-HR	1	0;	R-HR	1	2	R-MR	1 MR-MS	…	14 MR-R	+	–
Chevron	Lucerne, Switzerland	USDA	3–	2	MS-MR	3–	2	MS-MR	2	3–	MR-MS	14 MS-S	2 MR-MS	36 S-MS	–	+
Hiproly	Ethiopia	USDA	3	3–	S-MS	3–		MS	3		S	20 S-MS	5 S-MS	78 S	–	–
PI 532013	Egypt	USDA	3		S	3	3+	S	3	3+	S	53 S-MS	…	100 S	–	–
Experimental lines																
BM 9723-53	Brandon, Canada	AAFC	1	0;	R-HR	1	0;	R-HR	0;	1	HR-R	…	Tr MS	15 MR-MS	+	+
SB97197	Saskatoon, SK, Canada	CDC	0;	1	HR-R	2	1	MR-R	2	1	MR-R	…	0 R	45 MS-S	+	+
SH98073	Saskatoon, SK, Canada	CDC	0;	1	HR-R	0;	1	HR-R	2	1	MR-R	Tr MR-R	0 R	50 S-MS	+	+
SH98076	Saskatoon, SK, Canada	CDC	0;	1–	HR-R	0;	1	HR-R	2	1	MR-R	Tr R-MR	0 R	45 S-MS	+	+
MC0181-11	Saskatoon, SK, Canada	CDC	1	2	R-MR	1	0;	R-HR	0;	1	HR-R	15 S-MS	Tr MR	50 MS-S	+	+
MC0181-31	Saskatoon, SK, Canada	CDC	1	0;	R-HR	2	1	MR-R	1	0;	R-HR	5 MS-MR	2 MS-MR	50 S	+	+
Fusion	Horsens, Denmark	SEJET	1	0;	R-HR	0;	1	HR-R	0;	1	HR-R	5 MR-R	0 R	40 MR-MS	+	+[f]
Brandham II	Austria	ICARDA	1	0;	R-HR	1	0;	R-HR	1	0;	R-HR	…	…	35 MR-MS	+	–
Anakin	Horsens, Denmark	SEJET	0;	1	HR-R	0;	1	HR-R	0;	1	HR-R	Tr R-MR	0 R	15 MR-MS	+	–
Otira	Horsens, Denmark	SEJET	2		MR	1	0;	R-HR	0;	1	HR-R	…	1 S	85 S-MS	+	–
Zang Qing 148	Xizang, China	USDA	1	2	R-MR	1	0;	R-HR	1	2	R-MR	…	2 MS	45 S	+	–
Zang Qing 80	Xizang, China	USDA	2	1	MR-R	1	2	R-MR	2	1	MR-R	…	3 S-MS	70 S	+	–
H94035132[h]	Lacombe, Canada	FCDC	2	1	MR-R	2	1	MR-R	1	2	R-MR	…	10 S	60 S-MS	–	+
TR 02272	Brandon, Canada	AAFC	0;	1	HR-R	1	2	R-MR	0;	1	HR-R	…	2 MS	40 S-MS	–	+
2ND26373	Fargo, ND	NDSU	2	1	MR-R	2	1	MR-R	1	2	R-MR	3 MS	1 S	60 S-MS	–	+
ND23821	Fargo, ND	NDSU	2		MR	2	1	MR-R	2	1	MR-R	3 MS	2 MS	35 MS-S	–	+
CLE 202[h]	Colonia, Uruguay	INIA	2	1	MR-R	2	1	MR-R	2	1	MR-R	15 MS-S	5 S	60 S-MS	–	+
BM 8923-30	Brandon, Canada	AAFC	1	0;	R-HR	2	1	MR-R	0;	1	HR-R	1 MR-MS	0 R	35 S-MS	–	–
CLE 250	Colonia, Uruguay	INIA	2	1	MR-R	2		MR	1	2	R-MR	…	2 S	50 S-MS	–	–
Power	Horsens, Denmark	SEJET	0;	1	HR-R	2	1	MR-R	2	1	MR-R	Tr MR-MS	1 MS	35 S-MS	–	–
Magaly	Froissy, France	UNISIGMA	1	2	R-MR	2	1	MR-R	2	1	MR-R	…	0 R	30 S-MS	–	–
Marigold	Froissy, France	UNISIGMA	1	2	R-MR	2	1	MR-R	2	1	MR-R	…	2 S	50 S	–	–
Onyx	Bergen, Germany	KWS	1	2	R-MR	2	1	MR-R	1	2	R-MR	3 MR-MS	…	70 S-MS	–	–
BIOS 1	Russia	Vavilov	2	1	MR-R	2	1	MR-R	1	2	R-MR	…	1 S	45 S-MS	–	–
Zadonskij 8	Russia	Vavilov	2	1	MR-R	2	1	MR-R	1	2	R-MR	10 MS	…	50 S-MS	–	–
County	Cambridge, England	Syngenta	1	2	R-MR	2	1	MR-R	2	1	MR-R	10 MS	0 R	15 S-MS	–	–
Macarena	Cambridge, England	LS Plant	1	2	R-MR	1	0;	R-HR	2	1	MR-R	1 MR-MS	…	35 MS-S	–	–
Paramount	Cambridge, England	LS Plant	1	0;	R-HR	2	1	MR-R	1	2	R-MR	…	…	20 MS-S	–	–
BHS 248	Himachal Pradesh, India	USDA	2	1	MR-R	2		MR	2	1	MR-R	…	2 MS-MR	20 S-MS	–	–
Mano	Jiangsu, China	USDA	2		MR	1	2	R-MR	1	2	R-MR	Tr R-MR	3 S	75 S	–	–
Cantala	Melbourne, Australia	USDA	2	1	MR-R	2	1	MR-R	2	1	MR-R	Tr R-MR	…	65 S	–	–
Tallon	Warwick, Australia	USDA	2		MR	2	1	MR-R	2	1	MR-R	5 MS-MR	1 MS	50 S-MS	–	–

[a] ITs were scored based on the wheat-stem rust system of Stakman et al. (1962), as modified by Miller and Lambert (1955) for barley. IT1 and IT2 represent the most common and the next most common IT observed on accessions within an experiment. Symbols + and – denote more or less sporulation of classically described uredinia, respectively. A general reaction (GR) was assigned for the one or two most common ITs observed, where 0 or 0; is highly resistant (HR); 1 is R; 2 is MR; 3– is moderately susceptible (MS); and 3, 3+, or 4 is susceptible (S).

[b] Adult plant reaction was assessed at the mid- to hard-dough stage of development as the percentage of stem and leaf sheath tissue infected by stem rust as estimated using the modified Cobb scale (0 to 100%) (Peterson et al. 1948) and also as the type of uredinia (i.e., infection response [IR]) observed, where R = resistant, MR = moderately resistant, MS = moderately susceptible, and S = susceptible (Roelfs et al. 1992). The mean severity is given if more than one replicate was included in the nursery. Trace (Tr) represents a severity of less than 1%. The most common types of uredinia observed on accessions are given in order of their frequency. K 2014 = the 2014 Kenya nursery off season, which had a mixture of pathotypes TTKSK and TTKST, and SA = South Africa nurseries in the years indicated, which were infected with pathotype PTKST; … indicates data missing or not applicable.

[c] Molecular assays to determine a functional or nonfunctional *Rpg5* gene were conducted according to the methods of Arora et al. (2013) and Mamo et al. (2015), and those for *Rpg1* were done according to Eckstein et al. (2003) as modified by Derevnina et al. (2014). Detailed descriptions of the assays are given in the Materials and Methods section. Symbols: + indicates the presence of the functional gene and – indicates the lack of the functional gene.

[d] Location of origin for accession.

[e] Donor institutions: CDC = Crop Development Centre, USDA = United States Department of Agriculture–Agricultural Research Service National Small Grains Collection, AAFC = Agriculture and Agri-Food Canada, SEJET = SEJET Plant Breeding, ICARDA = International Center for Agricultural Research in the Dry Areas, FCDC = Field Crop Development Centre, NDSU = North Dakota State University, INIA = Instituto Nacional de Investigación Agropecuaria, KWS = KWS LOCHOW GmbH, Vavilov = N. I. Vavilov Institute of Plant Industry, Syngenta = Syngenta Seeds, and LS Plant = LS Plant Breeding Ltd.

[f] Marker is heterozygous.

groups (Table 3). BM9723-53 and Anakin were the most resistant accessions identified in the high-disease-pressure nursery in South Africa in 2015. The former carries both *Rpg5* and *Rpg1* whereas the latter carries only *Rpg5*. None of the selected wild barley accessions tested positive for a functional *Rpg1* gene (Table 5).

DISCUSSION

Pathotypes of *P. graminis* f. sp. *tritici* in the Ug99 race group represent one of the greatest biotic threats to wheat production in more than 50 years. An extensive international research effort by members of the Borlaug Global Rust Initiative has led to the identification of many new resistance genes effective against these dangerous pathotypes, especially TTKSK (http://www.globalrust.org/). This work has contributed greatly to alleviating the threat posed by these dangerous virulence types to wheat. In contrast, comparatively little research has been advanced on barley even though it is known that pathotype TTKSK also attacks the crop. The present study was undertaken to assess the vulnerability of barley to pathotype TTKSK at the seedling stage and to identify possible sources of resistance. From the evaluation of more than 1,924 cultivated barley accessions to pathotype TTKSK, more than 95% (1,844) were susceptible (Table 2). Similar results were found in a large panel (>3,000 accessions) of breeding germplasm from the United States (Zhou et al. 2014). Only 32 (1.7%) cultivated accessions exhibited consistently HR to MR reactions across all experiments (Tables 2 and 3). Evaluation of the wild progenitor (*H. vulgare* subsp. *spontaneum*) of cultivated barley revealed a similar high frequency (910 of 934 = 97.4%) of susceptibility to pathotype TTKSK at the seedling stage. Only 13 (1.4%) wild barley accessions exhibited consistently HR to MR reactions across all experiments (Tables 4 and 5). Finally, 55 barley lines with characterized or putative introgressions from various wild *Hordeum* spp. were also tested against pathotype TTKSK but none was found to be resistant. Considering all of the *Hordeum* accessions tested (2,913) in this investigation, over 96% (2,809) were susceptible as seedlings, indicating the extreme vulnerability of the crop to the African pathotypes of *P. graminis* f. sp. *tritici*.

The *rpg4*/*Rpg5* gene complex is the only one described in barley that is highly effective against pathotype TTKSK at the seedling (Steffenson et al. 2009) and adult plant stages. To determine whether any of the resistant *Hordeum* accessions identified from the seedling phenotyping assays might carry this gene complex, molecular assays were conducted for a functional *Rpg5* gene. In all, 12 of the 32 (37.5%) resistant cultivated accessions and 11 of the 13 (84.6%) resistant wild barley accessions tested positive for a functional *Rpg5* gene (Tables 3 and 5). This result clearly shows that the overall diversity for resistance in *Hordeum* is very narrow because more than 51% of the TTKSK-resistant accessions carry the *rpg4*/*Rpg5* complex. Breeding line Q21861 is the original described source of the *rpg4*/*Rpg5* gene complex (Brueggeman et al. 2008; Jin et al. 1994; Steffenson et al. 2009). However, subsequent studies have documented the presence of the complex in both Swiss landraces (Mamo et al. 2015; Steffenson et al. 2016) and also the wild progenitor *H. vulgare* subsp. *spontaneum* (Mamo et al. 2015). Steffenson et al. (2016) found a high frequency (approximately 43%) of *rpg4/Rpg5* in landraces from the mountainous areas of eastern Switzerland based on a sample of 73 accessions. Although no additional landraces from Switzerland were tested in this study, the *rpg4*/*Rpg5* complex was confirmed in one landrace (Brandham II) from nearby Austria (Table 3). Thus, this area of central Europe (eastern Switzerland and adjacent Austria) is particularly rich with regard to landraces carrying this gene complex. The *rpg4*/*Rpg5* complex was extremely common in TTKSK-resistant wild barley accessions because more than 84% were carriers. Accessions with the gene complex were found widely across the *H. vulgare* subsp. *spontaneum* geographic range from the Fertile Crescent region to Central Asia. However, the highest percentage of resistance, notwithstanding the large differences in sample sizes, was found in accessions from the Central Asian Republics of Tajikistan and Uzbekistan. The relatively common presence of the *rpg4*/*Rpg5* complex in landraces from Switzerland and in wild barley accessions from Central Asia is intriguing. One

TABLE 4. Summary of general reactions of wild barley accessions from different countries to *Puccinia graminis* f. sp. *tritici* pathotype TTKSK at the seedling stage

Country	Number (%) exhibiting reactions[a]			Total[b]
	Only HR to MR	PD R to MR or Seg	Only or PD MS to S	
Afghanistan	1 (9.1)	0 (0.0)	10 (90.9)	11
Armenia	0 (0.0)	0 (0.0)	5 (100.0)	5
Azerbaijan	0 (0.0)	0 (0.0)	17 (100.0)	17
China	0 (0.0)	0 (0.0)	8 (100.0)	8
Cyprus	0 (0.0)	0 (0.0)	3 (100.0)	3
Egypt	0 (0.0)	0 (0.0)	1 (100.0)	1
Iran	0 (0.0)	2 (4.8)	40 (95.2)	42
Iraq	0 (0.0)	0 (0.0)	21 (100.0)	21
Israel	3 (0.7)	2 (0.4)	444 (98.9)	449
Jordan	0 (0.0)	0 (0.0)	136 (100.0)	136
Kazakhstan	1 (14.3)	0 (0.0)	6 (85.7)	7
Lebanon	0 (0.0)	0 (0.0)	26 (100)	26
Libya	0 (0.0)	0 (0.0)	11 (100.0)	11
Pakistan	0 (0.0)	0 (0.0)	2 (100.0)	2
Russian Federation	0 (0.0)	0 (0.0)	4 (100.0)	4
Syria	1 (1.0)	2 (2.1)	93 (96.9)	96
Tajikistan	2 (25.0)	1 (12.5)	5 (62.5)	8
Turkey	0 (0.0)	1 (2)	38 (97.4)	39
Turkmenistan	1 (3.4)	2 (6.9)	26 (89.7)	29
Uzbekistan	4 (21.1)	1 (5.3)	14 (73.7)	19
Totals	13 (1.4)	11 (1.2)	910 (97.4)	934

[a] PD = predominantly and Seg = segregating. A general reaction was assigned to the infection type (IT) mode where 0 or 0; is highly resistant (HR); 1 is resistant (R); 2 is moderately resistant (MR); 3– is moderately susceptible (MS); and 3, 3+, or 4 is susceptible (S). Then, accessions were categorized into three groups: those exhibiting only HR, R, or MR reactions across all experiments; those exhibiting predominantly HR, R, or MR reactions within the IT mode across experiments or in individual plants of accessions that were phenotypically mixed due to possible segregation or seed admixtures; and those exhibiting only or predominantly MS to S reactions within the IT mode across experiments.

[b] Total number evaluated.

possible explanation for this is that barberry, the alternate host of *P. graminis* f. sp. *tritici*, is present and often infected in these regions (Berlin et al. 2014; Steffenson et al. 2016) (M. Rahmatov, personal communication). The persistent presence of the pathogen over a long period of time may have contributed to the evolution of stem rust resistance in these two germplasm pools. With respect to cultivated barley from breeding programs, the *rpg4*/*Rpg5* complex was found in six lines (SB97197, SH98073, SH98076, MC0181-11, MC0181-31, and BM 9723-53) from two improvement programs in Canada, three cultivars ('Fusion', Anakin, and 'Otira') from one breeding company in Denmark, and two cultivars ('Zang Qing 80' and 'Zang Qing 148') from one institute in China (Table 3). In central and western Canada, stem rust resistance is an important target trait, and use of the *rpg4/Rpg5* donor Q28161 was intentional and well-documented (B. Rossnagel and W. Legge, personal communication). In Denmark and China, stem rust is not an important breeding target; thus, the transfer of *rpg4/Rpg5* was likely unintentional and the donors unknown. Most of the Canadian breeding lines and all three of the Danish cultivars carrying *rpg4/Rpg5* possess good agronomic traits and, therefore, would be useful parents for initiating a program in breeding for resistance to pathotypes of *P. graminis* f. sp. *tritici* in the Ug99 race group. All of the Canadian lines with *rpg4/Rpg5* also carry *Rpg1* (Table 3), a gene that has protected barley from significant stem rust losses for more than 70 years in North America (Steffenson 1992). The pyramiding of these genes together in cultivars could provide greater durability for stem rust resistance.

Although the resistance conferred by *rpg4/Rpg5* is quite effective in protecting barley against stem rust, its expression can vary depending on various factors such as genetic background, the presence of other resistance genes, disease pressure, and temperature. Seedling ITs of *Hordeum* accessions carrying only the *rpg4*/*Rpg5* complex ranged from predominately 0; (e.g., Anakin and WBDC119) to 2 (e.g., Zang Qing 80 and WBDC225) (Tables 3 and 5). A similar result for *rpg4/Rpg5* carriers was found with respect to adult plant resistance, where rust severities ranged from 15% (Anakin) to 85% (Otira) in cultivated barley and from 20% (WBDC225) to 60% (WBDC032) in wild barley in the 2015 South Africa nursery where disease pressure was very high (Tables 3 and 5). These data demonstrate a moderate to strong effect of genetic background on the expression of *rpg4/Rpg5*. Comparison of seedling rust phenotypes between controls Q21861 (*rpg4/Rpg5* plus *Rpg1*) and Q/SM20 (*rpg4/Rpg5* only) in this and many other previous experiments revealed that the former consistently exhibits a higher proportion of IT 0; (usually 0;1) than the latter (usually 10;). A similar trend was observed at the adult plant stage in nurseries with heavier rust infection, where Q/SM20 typically exhibits a higher rust severity (Tables 1 and 3). Q/SM20 is a doubled haploid line derived from a cross involving Q21861 (Steffenson et al. 1995). These results suggest a possible epistatic effect of *Rpg1* with *rpg4/Rpg5* in Q21861 or perhaps the influence of other undescribed resistance genes in this line. Other cultivated barley accessions having the same gene combinations (Table 3) as these controls did not exhibit such strong tendencies with respect to either seedling ITs or adult plant rust severity. Previous studies have clearly demonstrated the temperature sensitivity of *rpg4/Rpg5* at the seedling stage: at 18 to 20°C, the genes confer a high level of resistance (mostly IT 0;) to pathotypes such as QCCJB and TTKSK whereas, at temperatures exceeding 27°C, the genes are rendered completely ineffective (Jin et al. 1994; Sun and Steffenson 1997). In this study, temperature or disease pressure had a marked effect on the level of resistance conferred by *rpg4/Rpg5* at the adult plant stage in South Africa. In 2012, temperatures were near normal during

TABLE 5. List of wild barley accessions exhibiting only highly resistant to moderately resistant infection types (IT) to *Puccinia graminis* f. sp. *tritici* race TTKSK at the seedling stage, their corresponding adult plant reactions in the field, and results of molecular assays for functional resistance genes.

					Seedling reaction to race TTKSK[a]												
					Experiment 1			Experiment 2			Experiment 3			Adult plant[b]		Assays[c]	
Accession	Other[d]	Location[e]	Country[f]	Donor[g]	IT1	IT2	GR	IT1	IT2	GR	IT1	IT2	GR	2012	2015	*Rpg5*	*Rpg1*
Checks																	
Q21861	PI 584766	Mexico via Western Australia	Mexico	CDC	0;	1	HR-R	1	0;	R-HR	0;	1	HR-R	0 R	10 MR-R	+	+
QSM20		Saskatoon	Canada	CDC	1	0;	R-HR	1	0;	R-HR	1	2	R-MR	…	14 MR-R	+	–
Chevron	PI 38061	Lucerne	Switzerland	USDA	3-	2	MS-MR	3-	2	MS-MR	2	3-	MR-MS	2 MR-MS	36 S-MS	–	+
Hiproly	CIho 3947	–	Ethiopia	USDA	3	3-	MS-S	3-		MS	3		S	5 S-MS	78 S	–	–
PI 532013	2794	–	Egypt	USDA	3		S	3	3+	S	3	3+	S	…	100 S	–	–
Experimental lines																	
ICARDA039	38936	West Bank	Israel	ICARDA	1	0;	R-HR	2	1	MR-R	1	0;	R-HR	1 MR-MS	35 MS-S	+	–
WBDC 014	38659	Baghlan	Afghanistan	ICARDA	2	1	MR-R	1	2	R-MR	1	2	R-MR	3 MS	50 MS-S	+	-
WBDC 032	38869	Hazafon	Israel	ICARDA	1	0;	R-HR	0;	1	HR-R	0;	1	HR-R	2 MR	60 MS-S	+	-
WBDC 119	40108	Dzhizak	Uzbekistan	ICARDA	0;	1	MR-R	0;	1	HR-R	0;	1	HR-R	7 MS-S	35 S	+	–
WBDC 209	123972	Dzhizak	Uzbekistan	ICARDA	1	2	R-MR	2	1	MR-R	2	1	MR-R	5 MS-S	40 S-MS	+	–
WBDC 213	124035	Samarkand	Uzbekistan	ICARDA	0;	1	HR-R	2	1	MR-R	2		MR	0 R	…	+	–
WBDC 214	124046	Samarkand	Uzbekistan	ICARDA	2	1	MR-R	1	0;	R-HR	0;	1	HR-R	2 MR	35 S	+	–
WBDC 220	131642	Chimkent	Kazakhstan	ICARDA	1	2	R-MR	1	2	R-MR	1	2	R-MR	20 S	25 MR-MS	+	–
WBDC 224	131790	Dushanbe	Tajikistan	ICARDA	2	1	MR-R	0;	1	HR-R	1	0;	R-HR	2 MS-MR	25 MR-MS	+	–
WBDC 225	131792	Dushanbe	Tajikistan	ICARDA	2	1	MR-R	2	1	MR-R	2	1	MR-R	0.5 MR-MS	20 MR-MS	+	–
WBDC 333	135478	Garygalla	Turkmenistan	ICARDA	2	1	MR-R	2	1	MR-R	1	2	R-MR	2 MR-MS	25 S	+	–
ICARDA040	38947	Yerushalayim	Israel	ICARDA	0;	1	HR-R	2	1	MR-R	1	0;	R-HR	0.5 MR	50 MR-MS	–	–
WBDC 302	38635	Damascus	Syria	ICARDA	1	2	MR-R	0;	1	HR-R	0;	1	HR-R	10 MS	50 MS-S	–	–

[a] ITs were based on the wheat-stem rust system of Stakman et al. (1962), as modified by Miller and Lambert (1955) for barley. IT1 and IT2 represent the most common and the next most common IT observed on accessions within an experiment. Symbols + and – denote more or less sporulation of classically described uredinia, respectively. A general reaction (GR) was assigned for the one or two most common ITs observed, where 0 or 0; is highly resistant (HR); 1 is resistant (R); 2 is moderately resistant (MR); 3– is moderately susceptible (MS); and 3, 3+, or 4 is susceptible (S).

[b] Adult plant reaction to pathotype PTKST in South Africa in the years indicated was assessed at the mid- to hard-dough stage of development as the percentage of stem and leaf sheath tissue infected by stem rust, estimated using the modified Cobb scale (0 to 100%) (Peterson et al. 1948) and also as the type of uredinia (i.e., infection response or IR) observed where R = resistant, MR = moderately resistant, MS = moderately susceptible, and S = susceptible (Roelfs et al. 1992). The mean severity is given for the controls as more than one replicate was included in the nursery; … indicates data missing or not applicable.

[c] Molecular assays to determine a functional or nonfunctional *Rpg5* gene were conducted according to the methods of Arora et al. (2013) and Mamo et al. (2015), and those for *Rpg1* were done according to Eckstein et al. (2003), as modified by Derevnina et al. (2014). Detailed descriptions of the assays are given in the Materials and Methods section. Symbols: + indicates the presence of the functional gene and – indicates the lack of the functional gene.

[d] Other designation.

[e] Province, region, city, or site.

[f] Country of origin.

[g] Donor institutions: CDC = Crop Development Centre, USDA = United States Department of Agriculture–Agricultural Research Service National Small Grains Collection, and ICARDA = International Center for Agricultural Research in the Dry Areas.

the logarithmic phase of the rust epidemic (October to early November) and inoculum pressure was light. In contrast, temperatures were markedly higher in 2015 during this critical time interval and disease pressure was extraordinarily heavy, due to the late-planted barley crop succumbing to the peak inoculum load coming from adjacent plots of heavily rusted wheat. As a result, severities for cultivated and wild barleys carrying only *rpg4/Rpg5* ranged from just 0 to 3% and 0 to 7% in 2012 up to 15 to 85% and 20 to 60% in 2015, respectively. Although the *rpg4*/*Rpg5* complex generally confers a high level of adult plant resistance in barley based on this and other studies (Zhou et al. 2014), the 2015 South Africa nursery revealed a startling vulnerability: that the resistance genes can be rendered ineffective at elevated temperatures or high inoculum pressure. These factors should be considered when deploying cultivars with the *rpg4*/*Rpg5* complex.

In addition to accessions with the *rpg4*/*Rpg5* complex, others tested in this study may be useful sources of all-stage or adult plant resistance. With respect to sources of all-stage resistance, there were a number of cultivated and wild barley accessions that exhibited low seedling ITs and rust severities under moderate epidemics and were not carriers of the *rpg4*/*Rpg5* complex based on the molecular assay (Tables 3 and 5). Other potentially useful sources of all-stage resistance may be present in the germplasm group, giving predominantly R to MR or phenotypically mixed reactions (Supplementary Tables S1 and S2), but critical adult plant resistance evaluations still need to be done. These potential sources of all-stage resistance are from diverse geographic regions (Tables 2). Adult plant resistance is a particularly important breeding target against stem rust because the disease rarely infects barley until after the heading stage (Zhou et al. 2014). Moreover, this type of resistance may be more durable than all-stage resistance, which is often controlled by one or two genes of major effect (Dyck and Kerber 1985; Ellis et al. 2014). Repeated attempts to obtain robust adult plant resistance data on the entire germplasm panel in both Kenya and South Africa failed several times due to low infection (see below). Therefore, more emphasis was placed on seedling tests because they provide more reliable and consistent stem rust phenotypes than those collected on adult plants in the field and with higher throughput. Although seedling assays may provide useful data on the potential vulnerability of barley to stem rust in the field and also identify accessions with all-stage resistance, they will not reveal accessions carrying only adult plant resistance. It is likely that useful sources of adult plant resistance are present in some of the seedling-susceptible accessions evaluated in this study. This assertion is based on several biparental and genome-wide association mapping studies where quantitative trait loci contributing to low rust severity in adult plants were detected in seedling-susceptible accessions from U.S. breeding programs as well as in carriers of the genes *Rpg2* (Hietpas-5) and *Rpg3* (GAW 79-3), which are largely ineffective at the seedling stage (Case 2017; Zhou et al. 2014) (unpublished data).

An important factor to consider before using sources of all-stage or adult plant resistance in breeding is whether they will be uniformly effective against all of the widely virulent pathotypes reported in the world. The *rpg4*/*Rpg5* complex is effective against a number of pathotypes in the Ug99 race group at the seedling stage (Case 2017) and to pathotypes TTKSK, TTKST, and PTKST at the adult plant stage under moderate disease pressure. Our current research is focused on screening selected resistant accessions against a suite of diverse pathotypes of *P. graminis* f. sp. *tritici* and elucidating the genetics of resistance.

The results from this study highlight the potential vulnerability of barley to widely virulent pathotypes like TTKSK. Genetically, barley is more vulnerable to disease outbreaks and other biotic threats than wheat because it is a diploid crop with few wild *Hordeum* spp. from which to readily transfer new resistance genes (von Bothmer et al. 1995). Bread wheat is an allo-hexaploid species and, therefore, can benefit from the rich diversity of genes in the different genome donors comprising the crop as well as in allied species. It has been genetically enriched through the transfer of many important resistance genes from progenitor and allied species using conventional or specialized cytogenetic techniques (Jiang et al. 1994). Yet, compared with wheat, barley is inherently more resistant to stem rust. This contention is based on the fact that barley frequently exhibits mesothetic reactions, whereby most uredinia are surrounded by chlorosis or necrosis (Sun and Steffenson 2005) and never the fully compatible diamond-shaped uredinia characteristic of IT 4 on wheat (compare PI 532013 and Hiproly versus McNair 701 in Figure 1). Additionally, in rust nurseries where both crops are planted together in the field, barley seldom rusts as heavily as wheat. This inherent or basal resistance may have contributed to the low rust infection observed on barley in most of the African stem rust nurseries where adjacent susceptible wheats exhibited rust severities greatly exceeding 65%. Under commercial production, this basal resistance may protect the barley crop from most epidemics. Still, as clearly demonstrated in this study, many barley accessions, including those carrying the *rpg4*/*Rpg5* complex, can succumb to very high levels of stem rust infection in the field under certain environmental conditions and disease pressure (i.e., South Africa in 2015).

In conclusion, seedling assays revealed the extreme vulnerability of barley to African stem rust pathotypes such as TTKSK. Although most of the resistant *Hordeum* accessions were found to carry the *rpg4*/*Rpg5* gene complex, other potentially useful sources of resistance were identified in germplasm from diverse regions. Barley possesses a basal level of stem rust resistance in comparison with wheat. Enhancing this basal resistance with *rpg4/Rpg5* and other genes in newly discovered resistance sources should provide more durable resistance against the array of different virulence types in the Ug99 race group.

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