Reaction of barley seedlings with different stem rust resistance genes to *Puccinia graminis* f. sp. *tritici* and *Puccinia graminis* f. sp. *secalis*

Y. Sun and B.J. Steffenson

Abstract: Seedling tests are desirable for disease resistance screening in breeding programs because a large number of lines can be evaluated in a short period of time and with modest space requirements. This study was undertaken to identify pathotypes of the wheat stem rust pathogen (*Puccinia graminis* f. sp. *tritici*) and cultures of the rye stem rust pathogen (*Puccinia graminis* f. sp. *secalis*) that might be useful for detecting resistance genes in barley (*Hordeum vulgare*) at the seedling stage. Barley accessions with different resistance genes (*Rpg1*, *Rpg2*, *Rpg3*, *rpg4*, and *rpgBH*) were assessed for their infection types (ITs) to 13 pathotypes of *P. graminis* f. sp. *tritici* and two cultures of *P. graminis* f. sp. *secalis* at two temperature regimes (18–21 °C and 24–25 °C). The *P. graminis* f. sp. *tritici* pathotypes HKHJ and QCCJ were effective for specifically detecting *Rpg1* and *rpg4*, respectively, and will facilitate resistance breeding efforts. No cultures were found to be effective for specifically detecting the resistance of Hietpas-5, PI 382313, or Black Hulless at the seedling stage. Some pathotypes or cultures did, however, produce low ITs on these sources and will therefore be useful for genetic studies involving crosses with susceptible barleys.

Key words: disease resistance, *Hordeum vulgare*, wheat stem rust, rye stem rust.

Résumé : Dans les programmes d'amélioration génétique, les tests sur plantules sont indiqués pour le tri pour la résistance aux maladies parce qu'un grand nombre de lignées peut être évalué dans un court laps de temps et dans peu d'espace. La présente étude fut entreprise pour identifier des pathotypes de l'agent pathogène de la rouille des tiges du blé (*Puccinia graminis* f. sp. *tritici*) et des cultures de l'agent pathogène de la rouille des tiges du seigle (*P. graminis* f. sp. *secalis*) qui pourraient être utiles pour détecter des gènes de résistance dans l'orge (*Hordeum vulgare*) au stade plantule. Des obtentions d'orge possédant différents gènes de résistance (*Rpg1*, *Rpg2*, *Rpg3*, *rpg4* et *rpgBH*) furent évaluées en regard de leurs types d'infection (TI) en réaction à 13 pathotypes de *P. graminis* f. sp. *tritici* et à deux cultures de *P. graminis* f. sp. *secalis* selon deux régimes de température (18–21 °C et 24–25 °C). Les pathotypes HKHJ et QCCJ du *P. graminis* f. sp. *tritici* furent efficaces à détecter spécifiquement *Rpg1* et *rpg4*, respectivement, et faciliteront les efforts d'amélioration génétique pour la résistance. Aucune culture n'a permis de détecter spécifiquement la résistance de Hietpas-5, PI 382313 ou Black Hulless au stade plantule. Cependant, quelques pathotypes ou cultures ont produit des TI peu développés sur ces plantes et seront, par conséquent, utiles pour des études de génétique impliquant des croisements avec des orges sensibles.

Mots clés : résistance aux maladies, *Hordeum vulgare*, rouille des tiges du blé, rouille des tiges du seigle.

Introduction

Stem rust, caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn. and *Puccinia graminis* Pers.:Pers. f. sp. *secalis* Eriks. & E. Henn., has historically been an important disease of barley in the Upper Midwest region of the United States. During the first half of the 20th century, stem rust epidemics were relatively frequent and yield losses ranging from trace to 15% were reported for barley (Roelfs 1978). Since 1942, losses to stem rust in barley have been minimal due to the planting of cultivars with the durable resistance gene *Rpg1* (Steffenson 1992). Pathotypes with virulence to *Rpg1* have been reported periodically in the Upper Midwest region since 1942 (Steffenson 1992). In 1989, a pathotype (QCCJ) with virulence for *Rpg1* became widespread in the northern Great Plains, damaging some barley fields in both the United States and Canada (Harder and Dunsmore 1991; Roelfs et al. 1991). Pathotype QCCJ is still a threat to barley production in the region. To protect against possible yield losses in the future, it is important

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that other effective resistance genes be identified and incorporated into commercial barley cultivars.

In barley, several genes for resistance to *P. graminis* f. sp. *tritici* have been identified: *Rpg1* derived from the cultivars Peatland (CIho 5267), 'Chevron' (CIho 1111), and 'Kindred' (CIho 6969) (Powers and Hines 1933; Shands 1939; Steffenson 1992); *Rpg2* from Hietpas-5 (CIho 7124) (Patterson et al. 1957); *Rpg3* from PI 382313 (Jedel 1990; Jedel et al. 1989); and *rpg4* from Q21861 (PI 584766) (Jin et al. 1994). Another gene, provisionally designated as *RpgU*, was identified in Peatland and reported to confer moderate resistance to pathotype QCCJ in the field (Fox and Harder 1995). Genes conferring resistance to *P. graminis* f. sp. *secalis* also have been described. A single dominant gene for resistance was identified in the barley accession Skinless (Luig 1957), and a single recessive gene (*rpgBH*, formerly designated as *S* gene) was found in Black Hulless (CIho 666) (Steffenson et al. 1984). Another dominant gene for resistance to *P. graminis* f. sp. *secalis* was reported in Q21861 (PI 584766) by Sun et al. (1996). This gene, provisionally designated as *RpgQ*, was thought to be the same gene as *rpg4* (Sun et al. 1996); however, recent molecular studies indicate that it is a different resistance locus closely linked with *rpg4* (B. Steffenson and A. Kleinhofs, unpublished data).

In breeding programs, seedling tests are desirable for disease resistance screening because a large number of lines can be evaluated in a short period of time and with modest space requirements. Unfortunately, it is sometimes difficult to detect genes for resistance to stem rust of barley because many accessions exhibit mesothetic reactions (a mixture of different infection types on the same leaf) in response to *P. graminis* at the seedling stage (Miller and Lambert 1955; Steffenson et al. 1985). Success in reliably detecting some resistance genes at the seedling stage has been reported. Steffenson et al. (1993) discovered that pathotypes MCC and HPH of *P. graminis* f. sp. *tritici* could clearly differentiate accessions with *Rpg1* from those without this gene at the seedling stage. Jin et al. (1994) found that *rpg4* could be reliably detected at 18–21 °C with pathotype QCC of *P. graminis* f. sp. *tritici*. Moreover, Sun et al. (1996) found culture 92-MN-90 of *P. graminis* f. sp. *secalis* useful for differentiating progenies with and without *RpgQ*. Stem rust resistance genes *Rpg2*, *Rpg3*, and *rpgBH* were originally described as adult plant resistance genes in genetic studies (Jedel 1990; Jedel et al. 1989; R. Shands and G. Wiebe (unpublished data) as cited in Patterson et al. 1957; Steffenson et al. 1984) and may be valuable in programs breeding for stem rust resistance; however, no systematic evaluation has been conducted to detect these three genes with various pathotypes or cultures of *P. graminis* at the seedling stage. Thus, the objective of this study was to determine the seedling reaction of barley accessions with different stem rust resistance genes to pathotypes of *P. graminis* f. sp. *tritici* and cultures of *P. graminis* f. sp. *secalis*.

Materials and methods

Plant materials

Eleven barley accessions were selected for this study (Table 1). 'Chevron' and 'Kindred' are different sources of the resistance gene *Rpg1*. Accessions 80-TT-29 and 80-tt-30 are near-isogenic for *Rpg1* and were selected after 29 backcross generations to the recurrent parent (Steffenson et al. 1985). They were included to assess the reaction of the *Rpg1* and *rpg1* alleles without the influence of major genetic background differences. Hietpas-5 and PI 382313 are the sources of *Rpg2* and *Rpg3*, respectively. Q21861 is the source of *rpg4*, but it also carries *Rpg1* (Jin et al. 1994). To assess the reaction of accessions with *rpg4* alone, a Q21861-derived line (QSM16) carrying only *rpg4* was also included. Black Hulless is the source of *rpgBH*. Seed of Skinless and the Peatland selection carrying only *RpgU* were not available for this study. 'Hiproly' and 'Steptoe' do not possess any known stem rust resistance gene and were included as susceptible controls. Four to five seeds of each barley accession were sown in plastic cones (21 cm deep and 3.8 cm in diameter) filled with a 3:1 mixture of peat moss and perlite (*v*/*v*; No. 1 Sunshine Mix, Fisons Horticulture, Vancouver, British Columbia, Canada) and grown in a greenhouse at 22–26 °C. Fertilizer in a controlled-release formulation of 14–14–14 (N–P–K) was applied at planting at a rate of 2–3 g per cone. Natural sunlight was supplemented by light from 1000 W metal halide bulbs (530–710 mmol photon·m^{-2}·s^{-1}) for 13 h·day^{-1}.

Table 1. List of barley accessions tested and their recognized alleles for stem rust resistance.

Accession	PI or CIho No.	Recognized resistance alleles
'Chevron'	CIho 1111	*Rpg1*
'Kindred'	CIho 6969	*Rpg1*
80-TT-29	CIho 16129	*Rpg1*
80-tt-30	CIho 16130	None
'Hiproly'	CIho 3947	None
'Steptoe'	CIho 15229	None
Hietpas-5	CIho 7124	*Rpg2*
PI 382813	PI 382813	*Rpg3*
QSM16	—*	*rpg4*
Q21861	PI 584766	*Rpg1* and *rpg4*
Black Hulless	CIho 666	*rpgBH*

*QSM16 is a doubled haploid line derived from the cross Q21861 × SM89010. No PI number has been assigned to this accession.

Rust pathotypes and cultures

Thirteen pathotypes of *P. graminis* f. sp. *tritici* and two cultures of *P. graminis* f. sp. *secalis* were selected for this study based on their virulence pattern or historical importance to North American wheat and barley production. All were derived from single uredinia. The 13 pathotypes of *P. graminis* f. sp. *tritici* were inoculated onto the 16 standard wheat stem rust differentials to verify their virulence (pathotype) pattern and purity. A four-letter pathotype code (i.e., Pgt-code) was assigned (Table 2) to each *P. graminis* f. sp. *tritici* pathotype based on its reaction across the wheat differential lines (Roelfs and Martens 1988). A set of rye lines with different stem rust resistance genes has been developed (Tan et al. 1976), but seed was not available for this study. Thus, cultures of *P. graminis* f. sp. *secalis* were not characterized for their virulence (pathotype) pattern. Pathotypes of *P. graminis* f. sp. *tritici* were increased on seed-

Table 2. Virulence phenotypes of 13 pathotypes of *Puccinia graminis* f. sp. *tritici* on 16 differential wheat lines carrying single genes for stem rust resistance.

	Infection phenotypes of *P. graminis* f. sp. *tritici* pathotypes[†]												
Differential genes*	gb-121	orge	111	A-12	A-21	CRL-5	CRL-1	MCC	CRL-8	CRL-4	CRL-3	QCC-2	CRL-6
Sr 5	L	L	H	H	H	H	L	H	H	L	H	H	H
Sr 21	H	H	L	H	H	H	H	L	H	H	H	H	H
Sr 9e	H	H	L	H	H	L	L	L	H	L	L	L	L
Sr 7b	L	L	L	H	H	L	H	H	H	H	H	L	H
Sr 11	L	L	L	L	H	L	L	L	L	H	L	L	L
Sr 6	L	L	L	L	L	L	H	L	L	H	H	L	L
Sr 8a	L	L	L	L	L	L	H	L	L	H	H	L	L
Sr 9g	H	H	H	H	H	H	H	H	H	H	H	H	H
Sr 36	H	L	L	H	H	H	L	L	H	H	H	L	H
Sr 9b	L	L	L	H	L	L	H	L	H	L	H	L	H
Sr 30	L	L	L	H	L	L	L	L	L	L	L	L	L
Sr 17	H	H	L	H	H	H	L	H	L	H	L	H	H
Sr 9a	H	L	H	H	L	H	L	L	L	L	H	L	H
Sr 9d	L	H	L	H	H	H	H	L	H	H	H	H	H
Sr 10	H	H	L	H	H	H	H	H	H	H	L	H	H
Sr Tmp64	L	L	L	L	L	L	L	H	H	L	L	L	L
Pgt-code[‡]	JCMN	JCCJ	LCBL	TCTS	TMMJ	QCMS	HKHJ	MCCF	TCQK	HTMJ	RKQQ	QCCJ	RCRS

*The differential genes (*Sr*) are according to Roelfs and Martens (1988).

[†]Infection phenotypes were based on the infection types (assessed on the 0–4 scale of Stakman et al. (1962)) observed on each wheat differential line. Infection types 0, ;, 1, and 2 were considered indicative of a low (L) infection phenotype and infection types 3 and 4 were considered indicative of a high (H) infection phenotype.

[‡]A four-letter Pgt-code was assigned to isolates based on their virulence phenotype according to the nomenclature of Roelfs and Martens (1988).

lings of the susceptible wheat accession Line E (PI 357308), and cultures of *P. graminis* f. sp. *secalis* (92-MN-90 and HQ) were increased on the susceptible rye accession Prolific (PI 535177). Urediniospores were collected from the susceptible hosts, placed in a desiccator for 2 days, sealed in glass ampoules, and kept frozen at –80 °C until needed for inoculation.

Inoculation, infection, and incubation of plants

Prior to inoculation, frozen urediniospores were heat-shocked (Rowell 1985) by placing the glass ampoules into a water bath at 46 °C for 6 to 7 min. The ampoules were then opened and the urediniospores were rehumidified by placing over a saturated salt solution of NH_4SO_4 (approx. 80% relative humidity) for at least 3 h. Germination of urediniospores was assessed in plates containing 1% water agar. All pathotypes or cultures had a germination rate greater than 90%.

Plants were inoculated with rust 7 days after planting when the primary leaves were fully expanded. A concentration of 5.4 mg urediniospores·mL^{-1} of mineral oil (Soltrol 170, Phillips Petroleum, Bartlesville, Oklahoma) was applied at a rate of approximately 0.035 mg·plant^{-1} with an atomizer pressured at 25–30 kPa. After inoculation, the plants were placed in front of a small electric fan for 3–4 minutes to hasten the evaporation of the oil carrier from leaf surfaces, thereby reducing phytoxicity. The protocol followed for the infection period of *P. graminis* was similar to that described by Rowell (1985). Plants were placed in mist chambers at 21 °C for 16 h in the dark. During this time, the plants were kept moist by periodic misting from ultrasonic humidifiers. Then, light (150–250 mmol photon·m^{-2}·s^{-1}) was provided by 400-W high pressure sodium lamps. The chamber doors were opened halfway when the lights were turned on to reduce the buildup of heat. These final steps allowed the plant surface to dry slowly over a period of about 2–3 h. When leaf surfaces were completely dried up, plants were transferred to growth chambers set at 18–21 °C or 24–25 °C. A 14-h photoperiod was provided by 115-W cool-white bulbs (150–350 mmol photon·m^{-2}·s^{-1}) in each chamber.

Disease assessment

Twelve to 14 days after inoculation, the ITs on each accession were assessed using a 0–4 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). Mesothetic reactions are common on barley infected with both *P. graminis* f. sp. *tritici* and *P. graminis* f. sp. *secalis*. All of the ITs observed in each treatment combination (host accession × rust pathotype or culture) were recorded in order of their prevalence. To simplify the presentation of data, the two most common ITs are presented together with the lowest and highest ITs observed in parenthesis (Table 3). The range of ITs observed was included to indicate the degree of variation in reaction. In this study, ITs of 0, 0;, 1, 2, or combinations thereof (e.g., 00;, 0;1, 12, and 21) were considered a low host reaction (low ITs); IT 23– was considered an intermediate host reaction (intermediate ITs); and ITs of 3–2, 3–, 3–3, 33–, or 3 were considered a high host reaction (high ITs). The experiment was conducted in a completely randomized design and repeated once. Infection types for each treatment (host accession × rust pathotype or culture × temperature regime) within an experiment were based on 8–10 plants. Differences among treatments were analyzed by inspection of the two most common ITs. Comparisons were made on this basis because the two most common ITs usually comprised about 70%–90% of the total ITs observed in each treatment.

Results

The inoculum concentration used in this study resulted in a moderate density of infection sites on plants. This allowed individual uredinia to develop fully without overcrowding and facilitated the unambiguous classification of ITs. In general, the ITs observed on host accessions in experiment one were similar to those observed in experiment two; however, there were notable exceptions where large IT differences (e.g., from low ITs to intermediate or even high ITs) were observed (see below). The ITs of accessions with different genes or gene combinations to pathotypes or cultures of *P. graminis* are summarized below.

Reaction of barley accessions with *Rpg1*

'Chevron' exhibited low ITs (00;, 0;, 0;1, 12, and 21) to all stem rust pathotypes or cultures, except JCCJ and QCCJ. Low (21) and intermediate (23–) ITs were observed on 'Chevron' to pathotype JCCJ at the low and high temperatures, respectively, whereas intermediate ITs (23–) were observed to pathotype QCCJ at both temperatures. 'Kindred' reacted similarly to 'Chevron' and exhibited low ITs (00;, 0;1, 12, and 21) to all pathotypes or cultures, except LCBL, JCCJ, and QCCJ at both temperatures. High ITs (3–2 and 3–3) predominated on 'Kindred' in response to pathotype QCCJ. Infection types varied from low to intermediate (21 to 23–) in response to the other two pathotypes at one or both temperatures. Accession 80-TT-29 exhibited low ITs (0;1, 12, and 21) to TCTS, TMMJ, MCCF, HKHJ, RKQQ, RCRS, 92-MN-90, and HQ at both temperatures, and to HTMJ, TCQK, JCMN, and QCMS at the high temperature only. This accession exhibited predominately high ITs (3–2, 3–3, and 33–) to pathotypes JCCJ and QCCJ at both temperatures. Intermediate to high ITs (23– to 3–2) were observed on 80-TT-29 to pathotypes HTMJ, TCQK, and JCMN at the low temperature. Low to intermediate (21 to 23–) ITs were observed on 80-TT-29 to pathotype LCBL at both temperatures and to QCMS at the low temperature only.

Reaction of a barley accession with *Rpg2*

Hietpas-5 exhibited low ITs (21) in response to culture HQ at both temperatures. To pathotypes HTMJ and JCMN and culture 92-MN-90, Hietpas-5 exhibited low ITs (21) at the low temperature and low to intermediate or intermediate to high ITs (21 to 23– or 23– to 3–2) at the high temperature. Predominately high ITs (3–2 to 3) were observed to pathotypes TCQK, TCTS, TMMJ, QCMS, MCCF, JCCJ, HKHJ, QCCJ, RKQQ, and RCRS at both temperatures. Hietpas-5 gave low to intermediate ITs (21 to 23–) to

Table 3. Infection types of barley accessions in response to pathotypes of *Puccinia graminis* f. sp. *tritici* and cultures of *P. graminis* f. sp. *secalis* at 18–21 °C and 24–25 °C.

	Most common infection types (infection type range)*											
	LCBL				HTMJ				TCQK			
Accessions	Exp L1	Exp L2	Exp H1	Exp H2	Exp L1	Exp L2	Exp H1	Exp H2	Exp L1	Exp L2	Exp H1	Exp H2
'Chevron'(*Rpg1*)	21(1/2)	21(1/2)	0;1(0;/1)	0;1(0;/1)	21(1/2)	21(1/3–)	00;(0/0;)	12-(1/2–)	21(1/2)	21(1/3–)	0;1–(0;/1–)	0;1(0;/1)
'Kindred' (*Rpg1*)	21(1/3–)	23–(1/3–)	21(1/3–)	23–(1/3–)	0;1(0;/1)	12(1/2)	0;1(0;/1)	0;1(0;/1)	21(1/3–)	21(1/3–)	0;1(0;/1)	0;1(0;/1)
80-TT-29 (*Rpg1*)	21(1/3–)	23–(2/3–)	21(1/3–)	23–(1/3–)	23–(2/3–)	3–2(2/3–)	21(1/2)	21(1/3–)	3–2(2/3)	3–2(2/3)	10;(0;/2–)	12-(0;/2–)
80-tt-30	3–(3–/3–)	3–2(2/3–)	3–2(2/3–)	3–2(2/3)	3–2(2/3–)	3–2(2/3–)	3–2(2/3–)	3–2(2/3)	3–2(2/3–)	3–2(2/3–)	3–3(2/3)	3–3(3–/3)
'Hiproly'	3–2(2/3–)	3–(3–/3–)	3–(3–/3–)	3–3(3–/3)	3–(3–/3–)	33–(3–/3)	3–(3–/3–)	3–3(3–/3)	23–(1/3–)	3–(3–/3–)	3–3(3–/3)	3–3(3–/3)
'Steptoe'	21(1/3–)	21(1/3–)	3–2(2/3–)	3–3(3–/3)	21(1/3–)	21(1/3–)	3–2(2/3–)	3–(3–/3–)	3–(3–/3–)	3–2(2/3–)	3–3(3–/3)	3–(3–/3–)
Hietpas-5 (*Rpg2*)	21(1/3–)	23–(2/3–)	3–3(3–/3)	3–2(2/3)	21(1/3–)	21(1/3–)	3–2(2/3–)	23–(2/3–)	23–(2/3–)	3–2(2/3–)	3–2(2/3–)	3–2(2/3–)
PI 382813 (*Rpg3*)	23–(2/3–)	3–2(2/3–)	3–2(2/3–)	3–2(2/3)	21(1/3–)	23–(2/3–)	23–(1/3–)	23–(2/3–)	23–(1/3–)	23–(2/3–)	3–2(2/3–)	23–(2/3–)
QSM 16 (*rpg4*)	00;(0/0;)	00;(0/0;)	12-(0;/2–)	0;1(0;/2–)	23–(2/3–)	3–2(2/3–)	3–2(2/3–)	3–(3–/3–)	00;(0/0;)	00;(0/0;)	0;1(0;/2-)	0;1(0;/2)
Q21861 (*Rpg1, rpg4*)	00;(0/0;)	0;1-(0;/1–)	0;1(0;/2–)	0;1(0;/1)	21(0;/3–)	21(1/3–)	21(1/3–)	3–2(2/3–)	00;(0/0;)	00;(0/0;)	0;1(0;/1)	0;1(0;/1)
Black Hulless (*rpgBH*)	23–(2/3–)	3–2(2/3–)	33–(3–/3)	33–(3–/3)	3–2(2/3–)	3–2(2/3)	3–2(2/3–)	3–(3–/3–)	3–3(3–/3)	3–2(2/3–)	33–(3–/3)	3–3(3–/3)

Note: Exp L1, experiment 1 at low temperature (18–21 °C); Exp L2, experiment 2 at low temperature (18–21 °C); Exp H1, experiment 1 at high temperature (24–25 °C); Exp H2, experiment 2 at high temperature (24–25 °C).

*Infection types are based on the 0–4 scale of Stakman et al. (1962) as modified for barley by Miller and Lambert (1955). The two most common infection types observed on accessions are given in addition to the lowest and highest types observed (i.e., the range in parenthesis).

†Cultures of *P. graminis* f. sp. *secalis* have not been assigned a pathotype designation.

‡Data not available.

Table 3 (*continued*).

	Most common infection types (infection type range)*											
	TCTS				TMMJ				JCMN			
Accessions	Exp L1	Exp L2	Exp H1	Exp H2	Exp L1	Exp L2	Exp H1	Exp H2	Exp L1	Exp L2	Exp H1	Exp H2
'Chevron'(*Rpg1*)	21(1/2)	10;(0;/1)	0;1(0;/1)	0;1(0;/1)	0;1(0;/1)	0;1(0;/1)	00;(0/0;)	00;(0/0;)	21(1/3–)	21(1/3–)	00;(0/0;)	0;1(0;/1)
'Kindred' (*Rpg1*)	12(1/2)	0;1(0;/2–)	00;(0/0;)	00;(0/0;)	00;(0/0;)	0;1(0;/1)	00;(0/0;)	00;(0/0;)	2(2/2)	21(1/3–)	00;(0/0;)	0;1(0;/1)
80-TT-29 (*Rpg1*)	21(1/3–)	21(0;/3–)	0;1-(0;/1–)	0;1-(0;/1–)	0;1(0;/1)	12(0;/2)	0;1-(0;/1–)	0;1(0;/1)	23–(2/3–)	23–(2/3–)	12-(1/2–)	12(1/2)
80-tt-30	33–(3–/3)	3–2(2/3–)	33–(2/3)	3–2(2/3)	3–2(1/3–)	33–(3–/3)	3–2(2/3)	3–2(2/3)	3–2(2/3–)	23–(1/3–)	3–2(2/3–)	3–2(2/3–)
'Hiproly'	3–2(2/3–)	3–(3–/3–)	33–(3–/3)	3–3(3–/3)	3–2(2/3–)	3–2(2/3–)	3–(3–/3–)	3–(3–/3–)	3–3(3–/3)	3–(3–/3–)	3–(3–/3–)	3–(3–/3–)
'Steptoe'	3–3(3–/3)	3–2(2/3–)	3–(3–/3–)	3–2(2/3)	3–2(2/3–)	3–2(2/3–)	3–2(2/3–)	3–(3–/3–)	23–(1/3–)	23–(2/3–)	23–(2/3–)	3–2(2/3–)
Hietpas-5 (*Rpg2*)	3–2(2/3–)	3–2(2/3–)	3–2(2/3–)	3–2(2/3–)	3–2(2/3–)	3–(3–/3–)	3–2(2/3)	3–2(2/3–)	21(1/3–)	21(1/3–)	21(1/3–)	23–(2/3–)
PI 382813 (*Rpg3*)	23–(2/3–)	3–2(2/3–)	21(1/3–)	23–(2/3–)	21(1/3–)	21(1/3–)	21(1/3–)	21(1/3–)	23–(2/3–)	23–(2/3–)	21(1/2)	21(1/3–)
QSM 16 (*rpg4*)	0;1-(0;/1-)	00;(0/0;)	3–2(2/3–)	3–2(2/3–)	3–2(2/3)	3–2(2/3–)	3–2(2/3–)	3–2(2/3–)	00;(0/0;)	0;1(0;/1)	0;1(0;/1)	0;1(0;/1)
Q21861 (*Rpg1, rpg4*)	00;(0/0;)	0;1(0;/1)	00;(0/0;)	0;1-(0;/1–)	0;1(0;/1)	12(0;/2)	00;(0/0;)	00;(0/1-)	00;(0/0;)	0;1(0;/1)	0;1(0;/1)	0;1(0;/1)
Black Hulless (*rpgBH*)	23–(1/3–)	3–2(2/3)	23–(2/3–)	23–(2/3–)	3–2(1/3–)	3–3(2/3)	3–2(2/3–)	3–2(2/3–)	3–2(2/3–)	3–2(2/3–)	23–(2/3–)	3–2(2/3–)

Table 3 (*continued*).

	Most common infection types (infection type range)*											
	QCMS				MCCF				JCCJ			
Accessions	Exp L1	Exp L2	Exp H1	Exp H2	Exp L1	Exp L2	Exp H1	Exp H2	Exp L1	Exp L2	Exp H1	Exp H2
'Chevron'(*Rpg1*)	21(1/3–)	21(1/3–)	0;(0;/0;)	00;(0/0;)	0;1(0;/1)	0;1(0;/1)	0;1-(0;/1–)	0;1(0;/1)	21(1/3–)	21(1/3–)	23–(2/3–)	23–(2/3–)
'Kindred' (*Rpg1*)	21(1/2)	21(1/3–)	0;1-(0;/1–)	00;(0/0;)	0;1(0;/1)	12-(1/2–)	0;1-(0;/1–)	0;1-(0;/1–)	23–(2/3–)	21(1/3–)	23–(1/3–)	23–(2/3–)
80-TT-29 (*Rpg1*)	21(1/3–)	23–(2/3–)	0;1-(0;/1–)	00;(0;/1–)	21(1/2)	21(0;/2)	0;1(0;/1)	0;1(0;/1)	23–(0;/3–)	3–3(2/3)	3–2(2/3–)	3–2(2/3–)
80-tt-30	33–(3–/3)	3–2(2/3–)	33–(2/3)	23–(2/3–)	3–2(2/3–)	3–2(2/3–)	33–(3–/3)	33–(3–/3)	23–(2/3–)	3–2(2/3–)	3–2(2/3–)	3–(3–/3–)
'Hiproly'	23–(2/3–)	3–2(2/3–)	3–2(2/3–)	3–(3–/3–)	3–2(2/3–)	3–3(3–/3)	33–(3–/3)	3–3(3–/3)	3–3(3–/3)	3–(3–/3–)	3–2(2/3–)	3–2(2/3–)
'Steptoe'	23–(2/3–)	23–(1/3–)	3–2(2/3–)	23–(2/3–)	23–(2/3–)	33–(3–/3)	3–2(2/3–)	3–2(2/3)	3–(3–/3–)	3–2(2/3–)	3–2(2/3–)	3–(3–/3–)
Hietpas-5 (*Rpg2*)	3–2(2/3–)	3–2(2/3–)	3–3(3–/3)	3–2(2/3–)	23–(2/3–)	33–(3–/3)	3–2(2/3–)	3–2(2/3–)	3–3(3–/3)	3–2(2/3–)	3–(3–/3–)	3–2(2/3–)
PI 382813 (*Rpg3*)	3–2(2/3–)	3–(3–/3–)	21(1/2)	23–(2/3–)	21(1/3–)	21(1/2)	23–(2/3–)	3–2(2/3)	23–(2/3–)	3–2(2/3–)	3–2(2/3–)	3–2(2/3)
QSM 16 (*rpg4*)	23–(2/3–)	3–2(2/3–)	23–(2/3–)	3–2(2/3–)	00;(0/0;)	0;1(0;/1)	12(0;/2)	21(0;/2)	0;1-(0;/1–)	0;1(0;/1)	3–2(2/3–)	3–2(2/3)
Q21861 (*Rpg1, rpg4*)	23–(1/3–)	23–(2/3–)	00;(0/0;)	0;1-(0;/1–)	00;(0/0;)	00;(0/0;)	00;(0/0;)	00;(0/0;)	0;1(0;/1)	12(0;/2)	21(1/3–)	23–(2/3–)
Black Hulless (*rpgBH*)	3–(3–/3–)	33–(3–/3)	3–2(2/3)	3–2(2/3–)	23–(2/3–)	3–2(2/3–)	3–3(2/3)	3–3(2/3)	3–2(2/3–)	3–(3–/3–)	3–2(2/3–)	33–(3–/3)

Table 3 (*continued*).

	Most common infection types (infection type range)*											
	HKHJ				QCCJ				RKQQ			
Accessions	Exp L1	Exp L2	Exp H1	Exp H2	Exp L1	Exp L2	Exp H1	Exp H2	Exp L1	Exp L2	Exp H1	Exp H2
'Chevron'(*Rpg1*)	21(1/2)	10;(0;1)	0;1–(0;/1–)	0;1(0;/1)	23–(2/3–)	23–(2/3–)	23–(2/3–)	23–(2/3–)	21(1/2)	2(2/2)	0;1(0;/1)	12–(1/2–)
'Kindred' (*Rpg1*)	21(1/2)	21(1/2)	00;(0/0;)	0;1–(0;/1–)	3–3(2/3)	23–(2/3)	3–2(2/3–)	3–2(2/3–)	12(1/2)	2(2/2)	12(1/2)	21(1/2)
80-TT-29 (*Rpg1*)	21(0;/2)	21(1/2)	0;1(0;/1)	0;1(0;/1)	33–(3–/3)	3–2(2/3)	3–3(3–/3)	3–3(3–/3)	21(1/3–)	21(1/3–)	21(1/3–)	21(1/3–)
80-tt-30	23–(1/3–)	3–3(3–/3)	3–3(3–/3)	3–3(3–/3)	3–(3–/3–)	3–3(3–/3)	33–(3–/3)	3–3(3–/3)	21(1/3–)	21(1/3–)	3–2(2/3–)	3–2(2/3)
'Hiproly'	3–3(3–/3)	3–3(3–/3)	3–2(2/3–)	3–3(3–/3)	33–(3–/3)	3–3(3–/3)	33–(3–/3)	33–(3–/3)	3–2(2/3–)	3–(3–/3–)	3–(3–/3–)	3–3(3–/3)
'Steptoe'	3–2(2/3–)	3–2(2/3–)	3–2(2/3–)	3–2(2/3)	3–3(2/3)	33–(3–/3)	33–(3–/3)	33–(3–/3)	23–(1/3–)	23–(2/3–)	3–(3–/3–)	3–3(3–/3)
Hietpas-5 (*Rpg2*)	3–2(2/3–)	3–2(2/3–)	3–2(2/3–)	3–2(2/3–)	3–3(2/3)	3–2(2/3–)	3(3/3)	3–2(2/3)	23–(2/3–)	3–2(2/3–)	3–2(2/3–)	3–2(2/3)
PI 382813 (*Rpg3*)	3–2(2/3–)	3–2(2/3–)	21(1/3–)	3–(3–/3–)	21(1/3–)	3–2(2/3–)	23–(1/3–)	3–2(2/3–)	21(1/2)	21(1/3–)	21(1/3–)	23–(2/3–)
QSM 16 (*rpg4*)	3–2(2/3–)	3–2(2/3–)	3–2(2/3–)	3–2(2/3–)	00;(0/0;)	00;(0/1–)	23–(1/3–)	23–(1/3–)	00;(0/0;)	0;1–(0;/2)	0;1(0;/2)	0;1(0;/2–)
Q21861 (*Rpg1, rpg4*)	0;1(0;/1)	21(1/2)	00;(0/1–)	0;1(0;/1)	0;1–(0;/1–)	00;(0/0;)	12(1/2)	21(0;/3–)	00;(0/0;)	0;1(0;/1)	10;(0;/2)	0;1(0;/1)
Black Hulless (*rpgBH*)	3–2(2/3–)	3–3(3–/3)	3–2(2/3–)	3–(3–/3–)	3–2(2/3)	3–2(2/3–)	3–3(3–/3)	3–3(3–/3)	23–(2/3–)	3–2(2/3–)	3–3(3–/3)	3–3(3–/3)

Table 3 (*concluded*).

	Most common infection types (infection type range)*											
	RCRS				Culture 92-MN-90†				Culture HQ†			
Accessions	Exp L1	Exp L2	Exp H1	Exp H2	Exp L1	Exp L2	Exp H1	Exp H2	Exp L1	Exp L2	Exp H1	Exp H2
'Chevron' (*Rpg1*)	12(1/2)	12(0;/2)	00;(0/0;)	00;(0/0;)	12(1/2)	10;(0;/1)	12(1/2)	21(1/3–)	21(1/3–)	21(1/3–)	12–(0;/2–)	12(0;/2)
'Kindred' (*Rpg1*)	0;1(0;/1)	21(0;/2)	0;1–(0;/1–)	0;1–(0;/1–)	0;1(0;/2)	10;(0;/1)	21(1/3–)	21(1/3–)	0;1(0;/1)	12(0;/2)	12–(0;/2–)	21(0;/2)
80-TT-29 (*Rpg1*)	0;1(0;/1)	21(0;/2)	0;1–(0;/1–)	0;1(0;/2)	21(1/3–)	21(0;/3–)	21(1/3–)	21(1/3–)	21(1/3–)	21(1/2)	21(1/2)	12(1/3–)
80-tt-30	3–2(2/3–)	3–2(2/3–)	3–2(2/3–)	3–2(2/3)	21(0;/3–)	21(1/3–)	23–(1/3–)	23–(1/3–)	23–(1/3–)	23–(2/3)	21(1/3–)	21(1/3–)
'Hiproly'	3–(3–/3–)	3–2(2/3–)	3–2(2/3–)	3–3(3–/3)	23–(2/3–)	3–3(3–/3)	3–3(2/3)	3–3(3–/3)	3–3(3–/3)	3–(3–/3–)	3–2(2/3–)	3–3(3–/3)
'Steptoe'	3–2(2/3–)	3–2(2/3–)	3–(3–/3–)	23–(1/3–)	23–(2/3–)	23–(1/3–)	3–2(2/3–)	3–2(2/3)	21(1/3–)	21(1/3–)	21(1/3–)	21(1/3–)
Hietpas-5 (*Rpg2*)	33–(3–/3)	23–(2/3–)	3–3(3–/3)	3–2(2/3–)	21(1/2)	21(1/2)	21(1/3–)	23–(1/3–)	21(0;/2)	21(0;/3–)	21(1/2)	21(1/3–)
PI 382813 (*Rpg3*)	—‡	23–(2/3–)	23–(2/3–)	3–2(2/3–)	12–(1/2–)	21(1/2)	12(1/2)	21(1/2)	10;(0;/1)	10;(0;/2)	21(1/3–)	23–(1/3–)
QSM 16 (*rpg4*)	0;1(0;/1)	0;1–(0;/1–)	12(0;/3–)	21(1/3–)	00;(0/0;)	00;(0/0;)	0;1(0;/1)	0;1(0;/1)	00;(0/0;)	00;(0/0;)	12(0;/2)	21(1/2)
Q21861 (*Rpg1, rpg4*)	0;1–(0;/1–)	00;(0/0;)	0;1–(0;/1–)	0;1(0;/2–)	00;(0/0;)	00;(0/0;)	0;1–(0;/1–)	0;1(0;/1)	00;(0/0;)	0;1–(0;/1–)	0;1(0;/1)	0;1(0;/1)
Black Hulless (*rpgBH*)	3–2(2/3–)	3–2(2/3–)	3–2(2/3–)	3–2(2/3–)	3(3/3)	3–2(2/3)	23–(1/3–)	3–2(2/3–)	21(1/3–)	21(1/3–)	3–3(3–/3)	3–3(2/3)

pathotype LCBL at the low temperature and high ITs (3–2 to 3–3) at the high temperature.

Reaction of a barley accession with *Rpg3*

PI 382313 exhibited low ITs (10; to 21) to pathotype TMMJ and culture 92-MN-90 at both temperatures, to pathotypes MCCF and RKQQ and culture HQ at the low temperature only, and to pathotype JCMN at the high temperature only. The lowest IT (10;) observed on this accession was to culture HQ of *P. graminis* f. sp. *secalis* at the low temperature. Intermediate to high ITs (23– to 3–2 and 3–) were observed on PI 382313 in response to pathotypes LCBL, TCQK, JCCJ, and RCRS at both temperatures, to pathotypes TCTS, JCMN, QCMS, and HKHJ at the low temperature only, and to pathotypes HTMJ, MCCF, and QCCJ at the high temperature only. The level of variation in ITs between experiments was relatively high for PI 382313. Low to intermediate (e.g., HTMJ and TCTS at the low and high temperatures, respectively), intermediate to high (e.g., LCBL and TCQK at the low and high temperatures, respectively), and even low to high ITs (e.g., HKHJ and QCCJ at the high and low temperatures, respectively) differences were observed between experiments in nearly half of the treatments.

Reaction of a barley accession with *rpg4*

QSM16 exhibited low ITs (00; to 21) to pathotypes LCBL, TCQK, JCMN, MCCF, RKQQ, and RCRS, and cultures 92-MN-90 and HQ at both temperatures. To pathotypes TCTS, JCCJ, and QCCJ, QSM16 exhibited very low ITs (00; to 0;1) at the low temperature and intermediate to high ITs (23– to 3–2) at the high temperature. QSM16 exhibited intermediate to high ITs (from 23– to 3–2 and 3–) to pathotypes HTMJ, TMMJ, QCMS, and HKHJ at both temperatures.

Reaction of a barley accession with both *Rpg1* and *rpg4*

Q21861 exhibited low ITs (00;, to 21) to all stem rust pathotypes or cultures, except HTMJ, QCMS, and JCCJ. Predominately low ITs (0;1 to 21) were also observed in response to pathotypes HTMJ and JCCJ; however, some intermediate or high ITs (23– to 3–2) were observed in one of the experiments at the high temperature. Q21861 exhibited intermediate and low ITs (23– and 00; to 0;1-) to pathotype QCMS at the low and high temperatures, respectively.

Reaction of a barley accession with *rpgBH*

Black Hulless exhibited low ITs (21) only to culture HQ of *P. graminis* f. sp. *secalis* at the low temperature. Intermediate to high ITs (23– to 3–2 and 33–) were observed on this cultivar in response to the other *P. graminis* f. sp. *secalis* culture 92-MN-90 and to all pathotypes of *P. graminis* f. sp. *tritici* at both temperatures.

Reaction of barley accessions lacking known resistance genes

'Hiproly' exhibited predominantly high ITs (3–2 to 33–) to all stem rust pathotypes or cultures at both temperatures. Similar results were found for 80-tt-30 with a few notable exceptions. Low (21) ITs were observed on 80-tt-30 to

pathotype RKQQ at low temperature. Also, to *P. graminis* f. sp. *secalis* cultures 92-MN-90 and HQ, this accession exhibited low and intermediate ITs (21 and 23–) across the temperature regimes. 'Steptoe' exhibited predominantly high ITs (3–2 to 33–) in response to pathotypes TCQK, TCTS, TMMJ, JCCJ, HKHJ, QCCJ, and RCRS at both temperatures. Low ITs (21) were observed on 'Steptoe' to culture HQ at both temperatures. Low (21) and high (3–2 to 3–3) ITs were observed in response to pathotypes LCBL and HTMJ at the low and high temperatures, respectively. Intermediate (23–) to high (3–2 to 3–3) ITs were observed in response to pathotypes JCMN, QCMS, MCCF, and RKQQ and culture 92-MN-90 at low and high temperature.

Discussion

Screening barley for resistance to stem rust at the seedling stage can be difficult because many accessions exhibit mesothetic reactions in response to *P. graminis* (Miller and Lambert 1955; Patterson et al. 1957; Steffenson et al. 1985, 1993). In this study, we observed mesothetic reactions in a number of host accession × rust pathotype (or culture) combinations; however, some stem rust pathotypes were found effective for detecting certain resistance alleles. Accessions with *Rpg1* ('Chevron', 'Kindred', and 80-TT-29) and those without any known gene (80-tt-30, 'Hiproly', and 'Steptoe') exhibited predominantly very low and high ITs (mostly 00; to 0;1 vs. 3–2 to 33–), respectively, to pathotypes TCQK, TCTS, QCMS, MCCF, HKHJ, and RCRS at the high temperature and to pathotype TMMJ at both temperatures (Table 3). Thus, any one of these seven cultures may be useful for detecting *Rpg1* in crosses with susceptible cultivars. Pathotype TMMJ has the added advantage of being able to differentiate cultivars with and without *Rpg1* under a wider range of temperatures, which may eliminate the need for a growth chamber for incubation. The results of this study confirm and extend those of Steffenson et al. (1993) who found pathotype MCC (the same culture used in this study) effective for clearly differentiating barley accessions with and without *Rpg1*. One limitation of using this pathotype is that it cannot reliably differentiate *Rpg1* from *rpg4* in barley because the latter gene also confers resistance to MCCF. The same is true for most of the other pathotypes mentioned above (i.e., TCQK, TCTS, and RCRS) with respect to *rpg4* and (or) other resistance alleles. In this regard, pathotype HKHJ is particularly noteworthy because it produced predominantly high ITs (3–2 to 3–3) on all accessions, except those carrying *Rpg1*. In contrast, accessions with *Rpg1* exhibited very low ITs (00; to 0;1) to this pathotype at the high temperature. These results were confirmed in several double-blind experiments conducted in the growth chamber at different temperatures as well as in the greenhouse (Y. Sun and B. Steffenson, unpublished data). Thus, pathotype HKHJ may be useful for specifically detecting *Rpg1* alone or in combination with other resistance alleles over a range of temperatures. Further studies using additional barley accessions with different gene combinations should be made to corroborate this result.

Accessions carrying *rpg4* (Q21861 and QSM16) gave low ITs (00; to 0;1) to pathotype QCCJ at the low temperature, whereas all other accessions gave predominately intermediate (23–) or high ITs (3–2 to 33–). Thus, pathotype QCCJ may be useful for specifically detecting *rpg4* in crosses with susceptible cultivars as well as with cultivars carrying other resistance genes, such as Q21861 with *Rpg1* (Table 3). That QCCJ can detect accessions with the *rpg4* resistance at low temperature is in agreement with a previous report by Jin et al. (1994).

Hietpas-5, the source of *Rpg2*, exhibited low ITs to only a few stem rust pathotypes or cultures (e.g., HTMJ, JCMN, 92-MN-90, and HQ) at one or both temperatures (Table 3). If studies on the genetics of seedling resistance are made in the future, it would be best to cross Hietpas-5 with a highly susceptible accession like 'Hiproly' to achieve the largest phenotypic difference in the resulting progeny. In this regard, pathotypes HTMJ and JCMN and culture HQ may be best for detecting the resistance in Hietpas-5. It is not known whether *Rpg2* is responsible for the seedling resistance observed in Hietpas-5 since the gene was originally reported to confer adult plant resistance in the field by R. Shands and G. Wiebe (unpublished data) as cited in Patterson et al. (1957). No pathotype or culture was identified for specifically differentiating the resistance gene(s) in Hietpas-5 from all other genes as was found for *Rpg1* and *rpg4*.

PI 382313, the source of *Rpg3*, exhibited a relatively high level of variation for ITs between experiments. The reason for this variation is not known, but it certainly made the detection of resistance in this accession difficult with some stem rust pathotypes or cultures. Still, there were several pathotypes or cultures to which PI 382313 exhibited consistently low ITs at one or both temperatures (e.g., TMMJ, JCMN, MCCF, RKQQ, 92-MN-90, and HQ) (Table 3). The lowest IT observed on this accession was 10; to culture HQ at the low temperature. If studies on the genetics of seedling resistance are made with PI 382313 in the future, it would be best to cross it with a highly susceptible accession like 'Hiproly' and use culture HQ at the low incubation temperature to achieve the largest phenotypic difference in the resulting progeny. Other suitable pathotypes or cultures for this purpose include TMMJ (at both temperatures), JCMN (at the high temperature), RKQQ (at the low temperature), and 92-MN-90 (at both temperatures). Jedel et al. (1983) reported that PI 382313 was resistant or moderately resistant to six different stem rust pathotypes from Canada. It is not known for certain whether *Rpg3* is responsible for the seedling resistance observed in PI 382313 because the gene was originally described on the basis of the adult plant reaction in the field (Jedel et al. 1989). Pathotype TMMJ may be useful for differentiating the resistance gene(s) in PI 382313 from those in Hietpas-5, QSM16, and Black Hulless. PI 382313 exhibited low ITs (21), whereas the other three accessions exhibited high ITs (3–2 to 3–) to pathotype TMMJ. It may also be possible to differentiate PI 382313 from *Rpg1* sources as the former consistently exhibited higher ITs (21) than the latter (ITs of 00; to 0;1). Additional experiments should be made to confirm whether these differences can be reliably detected over a range of environmental conditions.

Black Hulless, the source of *rpgBH*, was susceptible to all but one culture (i.e., HQ) of *P. graminis* tested in this study, and the resistance was expressed at the low temperature only (Table 3). If studies on the genetics of seedling re-

sistance are made in the future, it would be best to cross Black Hulless with a highly susceptible accession like 'Hiproly' to achieve the largest phenotypic difference in the resulting progeny. It is not known whether *rpgBH* is responsible for the seedling resistance observed in Black Hulless because the gene was originally described on the basis of the adult plant reaction in the greenhouse by Steffenson et al. (1984). No pathotype or culture was identified for specifically differentiating the resistance gene(s) in Black Hulless from all other known genes.

Several previous studies have documented the effect of temperature on the reaction of barley seedlings to stem rust (Jin et al. 1994; Patterson et al. 1957; Steffenson et al. 1985, 1993; Sun and Steffenson 1997). In this study, accessions with *Rpg1* exhibited similar ITs at both low and high temperatures to most stem rust pathotypes or cultures. The exceptions were with pathotypes TCQK, JCMN, QCMS, and HKHJ, where markedly lower ITs were observed at the high temperature than at the low temperature. In the case of 80-TT-29 with pathotype TCQK, the IT change was from high (3–2) to low (10; to 12–) at 18–21 °C and 24–25 °C, respectively. This dramatic change in IT did not occur with 'Chevron' or 'Kindred', suggesting that they may contain additional resistance gene(s) not present in 80-TT-29. In general, *rpg4* in QSM16 was more effective at the low temperature than at the high temperature in this study; however, the degree of change in the resistance level was pathotype or culture specific. To some stem rust pathotypes, the IT differences across the two temperature regimes were only minor (e.g., 00; to 0;1 for TCQK), whereas to other pathotypes they were quite large (e.g., 0;0 to 3–2 for TCTS and 0;1 to 3–2 for JCCJ). Results from previous studies have indicated the extreme temperature sensitivity of *rpg4* to stem rust, in particular to pathotype QCC (Jin et al. 1994; Sun and Steffenson 1997). Differences in IT across temperature regimes were not as dramatic and consistent with Hietpas-5 or PI 382313. Some differences were, however, observed with Hietpas-5 to pathotype HTMJ (21 vs. 23– to 3–2) and with PI 382313 to pathotypes JCMN (23– vs. 21) and MCCF (21 vs. 23– to 3–2). The seedling susceptibility of Black Hulless was uniform to most pathotypes or cultures across temperature regimes. The only marked IT difference observed between temperatures was with culture HQ (IT 21 vs. 3–3). It is clear from these results that incubation temperature is as critical as the selection of the stem rust pathotype or culture for assessing the resistance gene(s) in these sources.

Q21861, which carries both *Rpg1* and *rpg4*, was resistant to all pathotypes or cultures of *P. graminis* tested in this study, except QCMS at low temperature. This result suggests that broader and more effective resistance might be obtained in cultivars bred with both *Rpg1* and *rpg4*. The incorporation of just one resistance gene may not provide adequate protection because some pathotypes or cultures of *P. graminis* are virulent on accessions with either *Rpg1* (e.g., QCCJ) (Steffenson 1992) or *rpg4* (e.g., HTMJ, TMMJ, QCMS, and HKHJ) (this study) alone. Q21861 and QSM16 showed a high level of resistance to both cultures of *P. graminis* f. sp. *secalis*. This resistance was initially thought to be due to *rpg4* (Sun et al. 1996), but recent genetic data indicate that the rye stem rust resistance in these lines is due to a closely linked, dominant-acting gene designated *RpgQ* (B. Steffenson and A. Kleinhofs, unpublished data). Thus, the use of Q21861 or QSM16 as a parent in breeding programs will provide resistance to both *P. graminis* f. sp. *tritici* and *P. graminis* f. sp. *secalis* in barley.

The resistance genes *Rpg2*, *Rpg3*, and *rpgBH* were originally shown to confer adult plant resistance in genetic studies involving Hietpas-5, PI 382313, and Black Hulless, respectively (Jedel et al. 1989; R. Shands and G. Wiebe (unpublished data) as cited in Patterson et al. 1957; Steffenson et al. 1984). In this study, stem rust pathotypes or cultures were identified that produced low ITs on each of these three accessions at the seedling stage. An important study for the future will be to determine whether *Rpg2*, *Rpg3*, and *rpgBH* are responsible for this seedling resistance. The chromosome locations of *Rpg2*, *Rpg3*, and *rpgBH* have not yet been determined. The pathotypes or cultures producing low ITs on Hietpas-5, PI 382313, and Black Hulless may be useful in molecular mapping studies provided that the appropriate susceptible parent (i.e., 'Hiproly') is used.

The three accessions with no known resistance genes (80-tt-30, 'Hiproly', and 'Steptoe') differed markedly in their responses to various pathotypes or cultures of stem rust. 'Hiproly' exhibited high ITs to all pathotypes or cultures and appears to be universally susceptible to stem rust (Steffenson et al. 1985, 1993). This attribute makes 'Hiproly' useful as a susceptible control or a susceptible parent in genetic studies. In contrast, 80-tt-30 and 'Steptoe' exhibited low ITs to a few pathotypes or cultures of *P. graminis*. The differential reactions observed on 80-tt-30 and 'Steptoe' to pathotypes LCBL and HTMJ and culture 92-MN-90 suggest that they carry a different resistance gene(s). Both accessions apparently possess resistance to rye stem rust because low ITs were observed to culture HQ at one or both temperatures.

The development of methods for reliably phenotyping barley accessions with specific resistance genes is important in breeding, molecular mapping, and the map-based cloning of resistance genes. The *P. graminis* f. sp. *tritici* pathotype MCC was found to be very effective for detecting *Rpg1* at the seedling stage (Steffenson et al. 1993). This discovery facilitated the efficient transfer of *Rpg1* in breeding programs, the precise positioning of *Rpg1* on chromosome 1 (7H) using molecular markers (Kilian et al. 1994), and ultimately the isolation of the gene by a map-based approach (Brueggeman et al. 2002). Progress along these same lines has been made with *rpg4* and *RpgQ* using pathotype QCCJ and culture 92-MN-90, respectively, (Borovkova et al. 1995; Sun et al. 1996; B. Steffenson and A. Kleinhofs, unpublished data).

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