Mapping of major spot-type and net-type net-blotch resistance genes in the Ethiopian barley line CI 9819

O.M. Manninen, M. Jalli, R. Kalendar, A. Schulman, O. Afanasenko, and J. Robinson

Abstract: Net blotch of barley (*Hordeum vulgare* L.), caused by the fungal phytopathogen *Pyrenophora teres* Drechs. f. *teres* Smedeg., constitutes one of the most serious constraints to barley production worldwide. Two forms of the disease, the net form, caused by *P. teres* f. *teres*, and the spot form, caused by *P. teres* f. *maculata*, are differentiated by the type of symptoms on leaves. Several barley lines with major gene resistance to net blotch have been identified. Earlier, one of these was mapped in the Rolfi × CI 9819 cross to barley chromosome 6H, using a mixture of 4 Finnish isolates of *P. teres* f. *teres*. In this study, we used the same barley progeny to map resistance to 4 spot-type isolates and 4 net-type isolates of *P. teres*. With all net-type isolates, a major resistance gene was located on chromosome 6H, in the same position as described previously, explaining up to 88% of the phenotypic variation in infection response in the progeny. We designate this gene *Rpt5*. Several minor resistance genes were located on chromosomes 1H, 2H, 3H, 5H, and 7H. These minor genes were not genuinely isolate-specific, but their effect varied among isolates and experiments. When the spot-type isolates were used for infection, a major isolate-specific resistance gene was located on chromosome 5H, close to microsatellite marker HVLEU, explaining up to 84% of the phenotypic variation in infection response in the progeny. We designate this gene *Rpt6*. No minor gene effects were detected in spot-type isolates. The Ethiopian 2-rowed barley line CI 9819 thus carries at least 2 independent major genes for net-blotch resistance: *Rpt5*, active against net-type isolates; and *Rpt6*, active against specific spot-type isolates.

Key words: hordeum vulgate, pyrenophora teres, net blotch resistance, seedling resistance, QTL mapping.

Résumé : La rayure réticulée chez l'orge (*Hordeum vulgare* L.), causée par le pathogène fongique *Pyrenophora teres* Drechs. f. *teres* Smedeg., constitue l'une des plus importantes contraintes à la production d'orge au niveau mondial. Deux formes de la maladie, la forme réticulée causée par le *P. teres* f. *teres* et la forme tachetée causée par le *P. teres* f. *maculata*, présentent des symptômes différents sur les feuilles. Plusieurs lignées d'orge affichant une résistance à la rayure réticulée conférée par des gènes majeurs ont été identifiées. Précédemment, ces auteurs ont cartographié l'un de ces gènes sur le chromosome 6H au sein d'un croisement Rolfi × CI 9819 à l'aide de quatre isolats finlandais du *P. teres* f. *teres*. Dans le présent travail, les auteurs ont employé la même population pour cartographier la résistance à quatre isolats du type tacheté et à quatre isolats du type réticulé du *P. teres*. Avec tous les isolats de type réticulé, un gène majeur de résistance a été identifié sur le chromosome 6H, au même locus que rapporté antérieurement et expliquant jusqu'à 88 % de la variation phénotypique chez la progéniture. Les auteurs désignent ce gène *Rpt5*. Plusieurs gènes de résistance mineurs ont été observés sur les chromosomes 1H, 2H, 3H, 5H et 7H. Ces gènes à effet mineur n'étaient pas véritablement spécifiques d'un isolat, mais leur effet variait selon l'isolat et l'expérience. Lorsque les isolats de type tacheté ont été employés pour les inoculations, un gène de résistance majeur spécifique de l'isolat a été observé sur le chromosome 5H, à proximité du microsatellite HVLEU et expliquant jusqu'à 84 % de la variation phénotypique. Les auteurs désignent ce gène *Rpt6*. Aucun effet attribuable à des gènes mineurs n'a été détecté pour les isolats de type tacheté. L'orge éthiopienne à deux rangs CI 9819 porte ainsi au moins deux gènes majeurs différents conférant la résistance à la rayure réticulée : *Rpt5* offrant une résistance aux isolats de type réticulé et *Rpt6* contre certains isolats de type tacheté.

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Introduction

Net blotch of barley (*Hordeum vulgare* L.), caused by the fungal phytopathogen *Pyrenophora teres* Drechs. f. *teres* Smedeg., constitutes a serious constraint to barley production worldwide (Shipton et al. 1973), reaching as far north as the Arctic Circle in Finland (Mäkelä 1975). Severe infections can lead to grain-yield losses of up to 40% (Steffenson 1997). Two forms of the net-blotch disease, the net form, caused by *P. teres* f. *teres*, and the spot form, caused by *P. teres* f. *maculata*, are differentiated by the type of symptom expression on leaves (Smedegård-Petersen 1971).

The most cost-effective and environmentally friendly way to control net-blotch disease is with resistant cultivars (Jalli and Robinson 1999, Robinson and Jalli 1996). Several barley lines with major gene resistance to net blotch have been identified, most controlling resistance to net-type isolates only. The results of classic genetic analyses indicate that resistance is controlled by 1 to 3 loci, depending on the barley accession and the net-blotch isolate used for testing (Douiyssi et al. 1996, Ho et al. 1996, Mode and Schaller 1958, Wilcoxson et al. 1992). At least 3 independently segregating resistance genes, *Rpt1*, *Rpt2*, and *Rpt3*, have been localized, by trisomic analysis on chromosomes 3H, 1H, and 2H, respectively, (Bockelman et al. 1977). Quantitative resistance for net blotch has also been reported (Arabi et al. 1990, Robinson and Jalli 1997, Steffenson et al. 1996, Steffenson and Webster 1992*a*).

Molecular markers have been used to map genes affecting net-blotch resistance in barley. A single dominant gene for resistance to 1 of the 6 tested pathogen isolates was localized in cv. Igri on chromosome 3H (Graner et al. 1996). In a cv. Steptoe × Morex cross, 1 locus for seedling resistance was identified on chromosome 4H, and 2 loci were identified on chromonsome 6H; adult plant resistance is controlled by 7 quantitative trait loci (QTLs) on chromosomes 2H, 3H (2 loci), 4H, 5H, 6H, and 7H (Steffenson et al. 1996). In another study, 12 QTLs were identified, with the response depending on the leaf used for testing and the elapsed time after infection (Richter et al. 1998). Research on resistance genes to spot-type net blotch has been scarce. Major resistance genes conferring resistance on spot-type net blotch have been identified on chromosome 7H with restriction fragment length polymorphism (RFLP) markers (Williams et al. 1999), and on chromosome 2H with random amplified polymorphic DNA (RAPD) markers (Molnar et al. 2000). Recently, Friesen et al. (2006) located a major resistance gene for spot-type net blotch on chromosome 4H.

A major seedling resistance gene for Finnish net-type *P. teres* isolates, accounting for 65% of the phenotypic variation in infection response (IR) in a cross between the resistant line CI 9819 and the susceptible variety Rolfi has been identified (Manninen et al. 2000). The resistance gene was mapped to chromosome 6H, with the aid of 2 recently developed systems of retrotransposon-based markers. Several groups studying different barley accessions have recently found a major net-type net-blotch resistance gene on chromosome 6H (Cakir et al. 2003, Ma et al. 2004, Emebiri et al. 2005, Friesen et al. 2006), which suggests a multigenic region controlling reaction to *P. teres* f. *teres*. Contrasting results on the number, location, and isolate specificity of the net-blotch resistance genes in CI 9819 have been published (Bockelman et al. 1977, Afanasenko et al. 1999). In this study, we tested the infection response of the Rolfi × CI 9819 doubled-haploid mapping progeny to geographically distant pathogen isolates. The CI 9819 line has been shown to be resistant to both net-type and spot-type net blotch on a wide geographic scale (Khan and Boyd 1969, Sato and Komatsuda 1993, Robinson and Jalli 1996, Wu et al. 2003). The collection of isolates used in this study contained both net- and spot-type isolates, and genetic mapping was done separately for each isolate.

Material and methods

Net-blotch isolates

Four net-type isolates of *P. teres*, originating from different parts of the world, were used: 84-28-1 (USA, from B. Steffenson), 92-46/15 (Canada, from A.Tekauz), UK 80-12 (United Kingdom, from B. Steffenson), and 27-36 (Australia, from R. Loughman). Two of these, UK80-12 and 84-28-1, have been described as differing somewhat in their pathotype (Wu et al. 2003). Four spot-type isolates were used: P1332 and P1333 (Finland), P51 (Czech Republic), and P1340 (South Australia). Information on the pathotypes of these isolates was not available. Conidia were placed on 2.3% lima-bean agar (Difco, BD Microbiology Systems, Franklin Lakes, N.J.) plates and kept under near-UV light (with a 12 h photoperiod) at 18 °C for 2 weeks. Petri dishes were then flooded with 100 mL of distilled water to produce a spore suspension for inoculation.

Plant material

Anther-culture-derived doubled haploids (119 lines) from the Rolfi × CI 9819 cross were used for linkage map construction and for mapping the resistance genes. Rolfi is a Finnish 6-rowed spring barley susceptible to net blotch, and CI 9819 is an Ethiopian 2-rowed line resistant to net blotch (Jalli and Robinson 1999). For resistance tests, seeds of each doubled-haploid line were sown in pots of nutrient-supplemented peat, 1 seed per pot. The pots were arranged, in a randomized block design of 4 replicate blocks, in a greenhouse maintained at 16–20 °C, with a 12 h photoperiod. The pots were watered by hand.

Inoculation and scoring

For inoculation, the conidial density was assessed in a haemocytometer, and aqueous suspension was prepared at 1×10^4 conidia/mL. Two weeks after sowing, when seed-

lings were at the GS12/13 growth stage (Tottman and Makepeace 1979), the relative humidity in the greenhouse was raised to 100%, and seedlings were inoculated with the conidial suspension, at 0.5 mL per pot, using a compressed air sprayer. The high humidity was maintained for 2 d, until the first net-blotch symptoms appeared. The IR was recorded on the second leaf of single seedlings from each pot, using the 10-point scale described by Tekauz (1985). The response was recorded when the entire IR range was evident in the material. The seedling-resistance test was done twice for the 4 net-type isolates and once for the spot-type isolates. An IR lower than 5 was considered resistant and above 5 considered susceptible, as defined in Tekauz (1985).

DNA analyses

DNA was extracted from 10- to 14-day-old barley seedlings, using a modified cetyltrimethylammonium bromide (CTAB) method (Poulsen et al. 1993), as described elsewhere (Manninen et al. 2000). A previously published linkage map (Manninen et al. 2000), containing 6 different types of markers (retrotransposon-microsatellite amplified polymorphic DNA (REMAP), inter-retrotransposon amplified polymorphism (IRAP), inter-simple sequence repeat (ISSR), simple sequence repeat (SSR), RAPD, and RFLP), was used to locate resistance genes. There was 1 major difference between our linkage map and the published linkage map: 1 small linkage group was assigned to chromosome 5H instead of chromosome 7H, as previously reported. Details of the marker analyses are given in Manninen et al. (2000).

Mapping resistance genes

A corrected version of the previously published linkage map, containing 306 molecular markers (Manninen et al. 2000), was used in QTL analyses. MQTL, version 0.98, software (Tinker and Mather 1995*a*) was used for the QTL analysis. The average of the IR scores from 4 replicate blocks was used as the phenotypic value for each doubled-haploid line. Both simple interval mapping (SIM) and simplified composite interval mapping (sCIM) were used. Epistatic interactions were studied with SIM. A genome-wide 5% error-rate threshold for the test statistics was computed, using 1000 permutations. The test statistics used were converted to logarithm of odds (LODs) by dividing them by 2 ln(10) (Tinker and Mather 1995*b*), and the 2-LOD support intervals (Lander and Botstein 1989) were calculated for the major QTLs. Estimated effects of QTLs were calculated, with the MQTL program, as differences between 2 homozygous individuals. Thus, the estimated effect represents the effect of 2 allele substitutions (Tinker and Mather 1995*b*).

Results

Resistance in CI 9819 to net-type isolates of *P. teres*

The resistant parent, CI 9819, was rated as resistant (IR < 3.7) to all net-type isolates used, whereas Rolfi was rated as susceptible (IR > 6.0). The difference in IR between the parents ranged from 3.25 to 7.50, depending on the experiment and the isolate used. In all 4 net-type isolates tested, the major seedling resistance gene was located on chromosome 6H (Fig. 1). For all net-type isolates, the peak values for test statistics were located within a 1.1 cM region. The 2-LOD

support intervals for the location of resistance QTLs in different isolates overlapped, and covered a total distance of 6.1 cM. Eighteen markers were mapped in this region of the genome. The estimated effect of this locus, when homozygous, varied, with scores from 2.01 to 5.10 on the IR scale, depending on the isolate used; the CI 9819 allele had a positive effect on resistance. This locus explained 60%–88% of the phenotypic variation in the mean IR scores among the doubled-haploid lines from the Rolfi × CI 9819 cross (Table 1). This major net-blotch resistance gene on chromosome 6H is designated *Rpt5*.

Minor loci in CI 9819 affecting net-type net-blotch resistance

Several minor resistance loci to net-type isolates of net blotch were found (Table 2). In most cases, the effect of these loci was epistatic; the effect on resistance was evident only when the allele of *Rpt5* was inherited from the susceptible parent (Rolfi). In all but 1 case, the CI 9819 allele in the minor loci had a positive effect on resistance. Minor resistance loci were located on chromosomes 1H, 2H, 3H, 5H, and 7H (Fig. 1). Between 1 and 4 minor genes were detected with each isolate. The differences in detection of these minor loci were dependent on pathogen isolates, as well as on experiments.

Resistance in CI 9819 to spot-type isolates of *P. teres*

Our resistance source, CI 9819, was resistant to the 2 Finnish spot-type isolates (P1332 and P1333), but not to the Czech isolate (P51) (Table 1). The Australian isolate (P1340) was not clearly virulent on either of the parental barley lines. A major resistance gene conferring resistance to the Finnish spot-type isolates was located on chromosome 5H, near microsatellite marker HVLEU. The peak values for test statistics were located within a 1.5 cM region for both isolates. The 2-LOD support intervals for the location of resistance QTLs in these isolates overlapped, and covered a total distance of 2.5 cM. Six markers were mapped in this region of the genome. The estimated effect of this locus, when homozygous, varied, with scores from 4.21 to 4.94 on the IR scale; the CI 9819 allele had a positive effect on resistance. This locus explained 65%–84% of the variation in the mean IR scores in the doubled-haploid progeny from the Rolfi × CI 9819 cross (Table 2). The observed IR frequency distributions for the 2 homozygotes at the HVLEU locus are shown in Fig. 2. We designate this resistance gene to the spot-type isolate of net blotch as *Rpt6*. No effect from this locus was seen in the Australian or Czech isolates. Interestingly, the chromosomal region derived from CI 9819 at the *Rpt5* locus, which conferred resistance to net-type isolates, had a small but negative influence on resistance to spot-type isolates. No other minor loci affecting resistance were found with the spot-type isolates.

Discussion

The genetics of net-blotch resistance in barley has been studied in segregating progenies, from crosses between resistant and susceptible parents or between 2 resistant barley lines, by several authors (Afanasenko et al. 1999, Schaller 1955, Mode and Schaller 1958, Bockelman et al. 1977,

Fig. 1. Location of major net-blotch resistance genes *Rpt5* and *Rpt6* and minor net-blotch resistance genes on barley chromosomes. Regions with a significant minor effect are shown separately for each isolate of *Pyrenophora teres*. Only chromosomes with significant effects are shown.
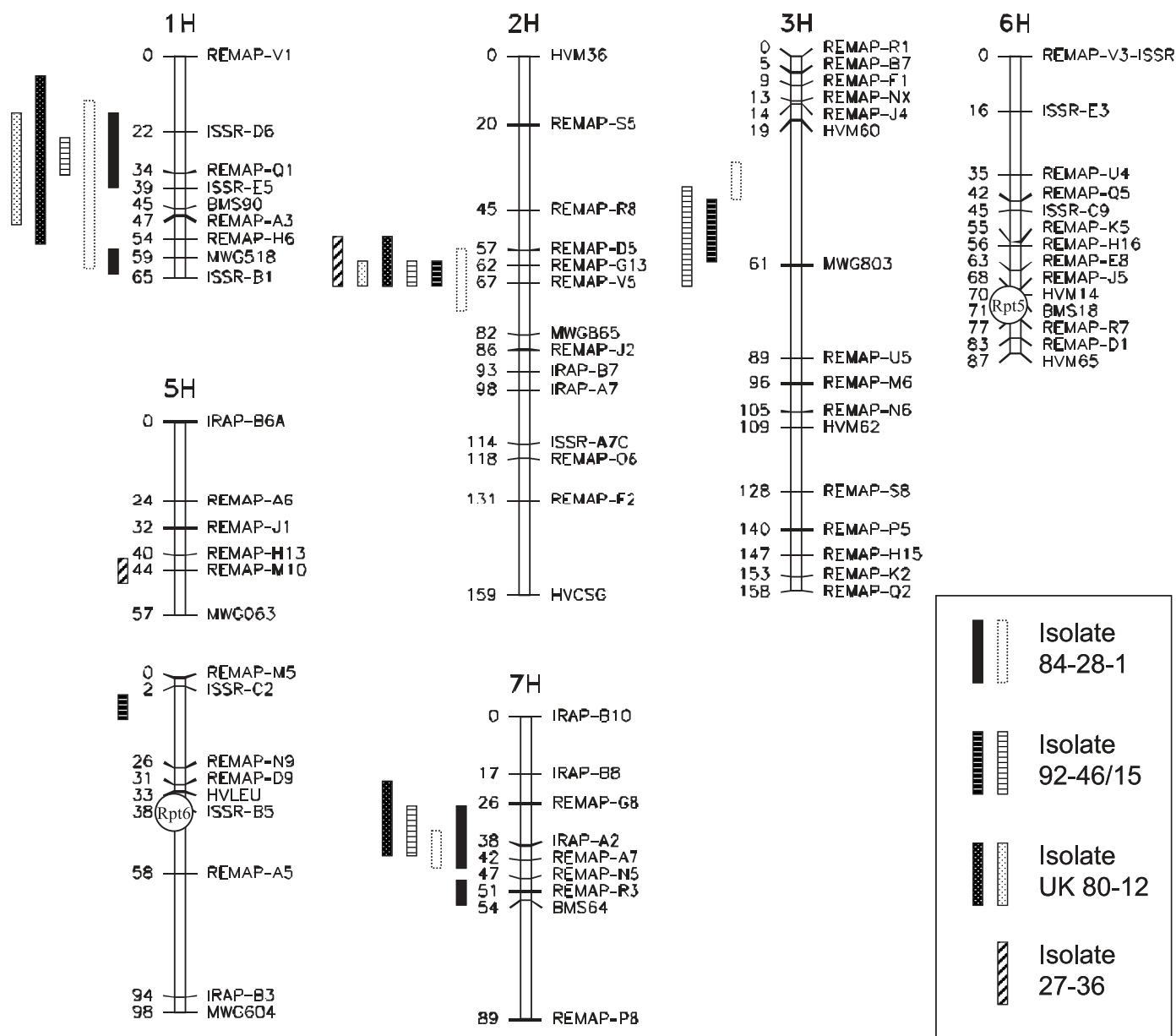
Douiyssi et al. 1996). Typically, the presence of 1 to 3 resistance genes has been proposed, depending on the barley lines used. The pathogen isolate used for resistance tests was also important, and the number of resistance genes revealed depended on the isolate used for testing (Afanasenko et al. 1999, Ho et al. 1996). In our study, we confirmed the major gene effect of the *Rpt5* locus mapped on chromosome 6H with net-type isolates from diverse parts of the world. The pathotypes of the isolates were not described but, according to available information, at least 2 of them had somewhat different pathotypes (Wu et al. 2003). However, it is possible that diversity in geographic origin does not reflect diversity in pathotypes. With all net-type isolates used, the major resistance locus was located in the same region on chromosome 6H, and explained up to 88% of the variation

in IR scores among the doubled-haploid progeny lines from the Rolfi × CI 9819 cross. In addition, a major QTL locus was recently mapped on chromosome 6H in the Chevron × Stander cross (Ma et al. 2004). Because of a lack of common markers, a comparison of the location of this QTL with our results is difficult. However, the region where we mapped *Rpt5* on chromosome 6H was recently found to determine net-type net-blotch resistance in 5 other barley accessions (Cakir et al. 2003, Emebiri et al. 2005). Pompadour, WPG8412, Tallon, Kaputar, and ND11231*12 all possess resistance genes on chromosome 6H, close to SSR markers Bmag0173 and EBmac0874. In the barley SSR map (Ramsay et al. 2000), EBmac0874 is located within 2 cM of Bmac0018 (synonym BMS18), HVM65, and HVM14, which flank the *Rpt5* locus in our study.

Table 1. Net-blotch infection responses of parentals and the homozygous effects of the CI 9819 allele at the major resistance quantitative trait loci (QTLs) in the Rolfi × CI 9819 doubled-haploid (DH) barley progeny.

Isolate	Origin	Exp.	Infection response			Effect of major QTL in DH projeny				
			CI 9819	Rolfi	Parental difference	Location of major QTL	Observed homozygous effect	% parental difference explained	Estimated homozygous effect (sCIM)	R^2
Net type										
84-28-1	USA	1	0.50	8.00	7.50	6H	3.48	46.4	3.18	0.60
		2	3.00	10.00	7.00	6H	4.73	68.0	4.23	0.80
92-46/15	Canada	1	2.75	6.00	3.25	6H	3.24	99.7	2.89	0.74
		2	1.50	6.00	4.50	6H	2.20	48.9	2.01	0.67
UK 80-12	UK	1	3.67	10.00	6.33	6H	5.65	89.3	5.10	0.85
		2	1.50	6.75	5.25	6H	3.08	58.7	2.72	0.69
27-36	Australia	1	2.25	6.00	3.75	6H	5.47	145.9	4.71	0.88
		2	1.25	6.00	4.75	6H	3.04	64.0	2.69	0.70
Spot type										
P51	Czech Republic	3	6.00	6.25	0.25	—	—	—	—	—
P1332	Finland	2	2.00	8.75	6.75	5H	4.94	73.2	4.94	0.84
P1333	Finland	3	4.25	8.75	4.50	5H	4.00	88.9	4.21	0.65
P1340	Australia	3	4.75	4.00	–0.75	—	—	—	—	—

Note: Exp., experiment number; sCIM, simplified composite interval mapping.

Table 2. Estimated effects (simple interval mapping) of the minor QTLs possessing CI 9819 alleles. Effects followed by the letter "a" are additive effects; effects followed by the letter "e" are epistatic; the homozygous effect shown is only for genotypes possessing Rolfi alleles at the *Rpt5* locus.

| Net-type isolate	Origin	Exp.	Estimated effect of minor QTLs						
			1H	2H	3H	4H	5H	6H	7H
84-28-1	USA	1	2.43e	—	—	—	—	—	2.27e
		2	2.38e	2.01e	4.14e	—	—	—	2.07e
92-46/15	Canada	1	—	1.25e	1.56e	—	–0.62a	—	—
		2	1.21e	1.09e	1.12a	—	—	—	1.07e
UK 80-12	UK	1	2.24e	1.60e	—	—	—	—	2.05e
		2	1.95e	1.55e	—	—	—	—	—
27-36	Australia	1	—	—	—	—	—	—	—
		2	—	1.68e	—	—	2.30a	—	—

Note: Exp., experiment number

Bmag0173 is located a little bit further on the SSR map (Ramsay et al. 2000); it is 24 cM from Bmac0018 (synonym BMS18), HVM65, and HVM14. In a recent paper by Friesen et al. (2006), net-blotch resistance to 3 different net-type isolates was mapped on chromosome 6H very close to SSR marker EBmac0874 and, thus, very close to the location of *Rpt5*. It is clear that on barley chromosome 6H, there is either a group of closely linked net-blotch resistance loci or 1 locus possessing several alleles.

The minor effects detected differed to some extent among different net-type isolates, but greater differences were observed in different experiments. For example, in the first experiment, 2 minor gene effects were seen on chromosomes 1H and 7H, using isolate 84-28-1, and 3 minor gene effects were seen on chromosomes 2H, 3H, and 5H, using isolate 92-46/15. To verify the isolate specificity of the genes, we repeated the experiment and found 4 minor gene effects on chromosomes 1H, 2H, 3H, and 7H, with both isolates (84-28-1 and 92-46/15). The minor gene locations shown in Fig. 1 are those with statistical significance. It was common to have a peak in test statistics in other cases, but the peak did not exceed the significance limit and, thus, the location is not shown on the map. We concluded that differences in the detection of minor resistance genes were caused by small differences in environmental conditions rather than by the isolates used for infection.

The genetics of net-blotch resistance in the Ethiopian line CI 9819 has been studied previously. Khan and Boyd (1969) found that resistance in CI 9819 was controlled by 2 genes, one of which was designated *Pta*. Bockelman et al. (1977) used an F_2 progeny of Betzes primary trisomics and CI 9819, and concluded from reaction-class frequences that 1 resistance gene, *Rpt1b*, was located on chromosome 3H, and another, *Rpt2c*, was probably located on chromosome 1H. No indication of a resistance gene on chromosome 6H was seen in their study. This is in opposition to our results. Whether this is because of differences in isolates remains unclear. To date, we have used 9 net-type isolates from different parts of the world (data not shown), and in each case, we found the same location on chromosome 6H for the major net-type resistance gene. Only epistatic minor gene effects were found on chromosomes 1H and 3H.

Fig. 2. Frequency of different infection-response classes in Rolfi × CI 9819 DH progeny lines when tested with Finnish *P. teres* f. *maculata* isolates P1332 and P1333. Marker genotypes at the HVLEU locus are shaded grey or black.
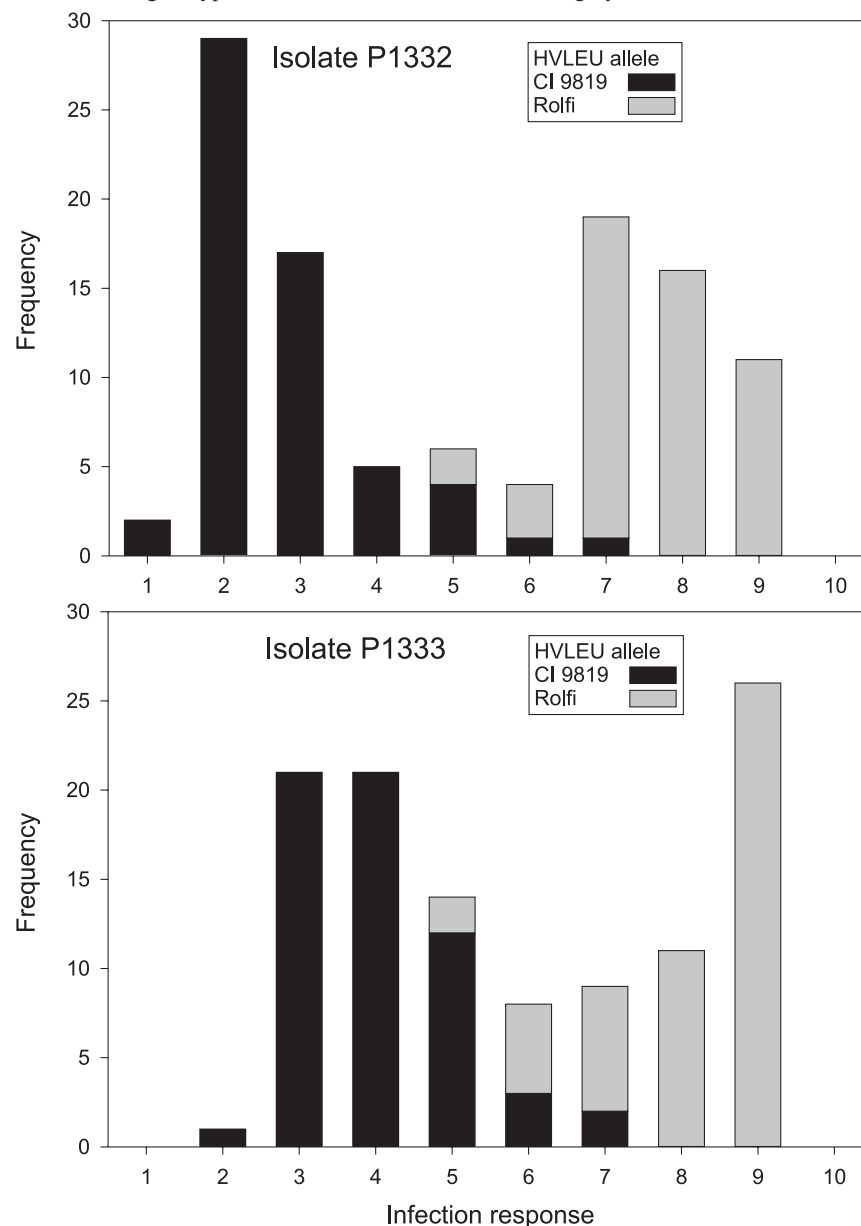
Using a Mendelian analysis of F$_2$ progeny, Afanasenko et al. (1999) studied the number of genes controlling resistance to net-blotch isolates, and concluded that different resistance genes in CI 9819 protected against particular pathogen strains. Two of the net-type isolates used in their experiment, 27-36 and UK 80-12, were the same as the ones we used. Afanasenko et al. (1999) found 1 dominant and 1 recessive gene (AAcc) to isolate 27-36, and 2 recessive genes (ccdd) to isolate UK 80-12. None of the genes found in their study was effective against all 5 of the pathogen isolates they tested. In our study, the *Rpt5* locus was effective against *P. teres* isolates 27-36 and UK 80-12. In addition, we found 2 minor genes on chromosomes 2H and 5H, using isolate 27-36, and 3 minor genes on chromosomes 1H, 2H, and 7H, using isolate UK 80-12.

Mendelian analyses based on segregating progenies have

2 drawbacks: only relatively simple genetic models can be tested; and tests are based on the division of progenies into susceptible and resistant classes. In our study, we identified 1 major gene to net-type isolates, as well as several epistatic minor genes. Epistasis is not commonly included in the genetic models tested with classic Mendelian analysis. Complex genetic modes of resistance might not be detected with simple Mendelian analysis. We did not divide our doubled haploids into susceptible and resistant classes, but instead used the actual IRs, although they were measured using the qualitative Tekauz scale, in a quantitative manner. The aggressiveness of net-blotch isolates might vary among experiments, making a distinction between susceptible and resistant classes difficult.

The effects of the epistatic minor genes varied between experiments. When an effect was observed on a particular

chromosome, it was located at the same region of the chromosome, no matter which isolate was used. However, there was much more variation in its location than was found on the major gene. The estimated effects of the minor loci varied, from 1.07–4.14, depending on the *P. teres* net-type isolate and the experiment. These effects were manifested only when *Rpt5* on chromosome 6H possessed alleles from the susceptible parent (Rolfi). It is important to note that even if these are considered epistatic minor genes, in some cases their effect on resistance was notable.

Resistance to spot-type isolates of net blotch was governed by a different gene than resistance to net-type isolates. This gene, *Rpt6*, was located on chromosome 5H, and explained 65%–84% of the IR when the 2 Finnish spot-type isolates were used. The effect of this locus was isolate-specific; no response was recorded with isolates from Australia or the Czech Republic. It has been reported that barley line CI 9819 is moderately resistant to spot-type isolates originating from Denmark, Norway, New Zealand, Australia, and Canada (Wu et al. 2003). Whether this resistance is based on *Rpt6* remains to be determined. Major resistance genes to spot-type isolates of net blotch have been previously assigned to chromosomes 2H (Ho et al. 1996) and 7H (*Rpt4*) (Williams et al. 1999). Williams et al. (1999) located QTLs for seedling resistance to spot-type isolates of net blotch on chromosome 7H in 5 different barley crosses, explaining 27%–74% of phenotypic variation. However, in only 2 of the crosses did the same chromosomal region explain a significant amount of adult-stage resistance. In our study, we did not verify the effect of *Rpt6* beyond the seedling stage. Recently, a resistance gene to spot-type isolates of net blotch was mapped on chromosome 4H, explaining 64% of phenotypic variation in response to *P. teres* f. *maculata* in a cross between barley lines Q21861 and SM89010 (Friesen et al. 2006). Although many of the resistance genes to net-type isolates of net blotch map on chromosome 6H, resistance to spot-type isolates of net blotch has been mapped on different chromosome in every study (2H in Ho et al. 1996; 7H in Williams et al. 1999; 4H in Friesen et al. 2006; and 5H in this study). Whether this reflects diversity in the spot-type resistance sources or the spot-type pathogen isolates used for mapping remains to be determined.

Microsatellite markers HVM14 and HVM65, which flank *Rpt5* on chromosome 6H, can be used as anchors when locating the resistance gene on published linkage maps. From the maps of Liu et al. (1996), Qi et al. (1996), and Künzel et al. (2000), it can be concluded that *Rpt5* is located at the very centromeric region of chromosome 6H. The centromere is located between HVM14 and HVM65 on the map of Liu et al. (1996), and the distance between the microsatellite markers is less than 10 cM. However, when the consensus barley map (Qi et al. 1996) is used to bridge the maps of Liu et al. (1996) and Künzel et al. (2000), it can be concluded that this region covers more than half of the physical distance of chromosome 6H. The spot-type resistance gene, *Rpt6*, was mapped close to HVLEU, which is located near the centromeric region of the long arm of chromosome 5H (Liu et al. 1996). For practical use in breeding programs, the genetic distance between the target gene and the marker locus is more important than the physical distance. However, information on the physical location of the target gene

might also be important, particularly if other relevant genes are located close to the centromeric resistance gene, in which case it might be difficult to find recombinants between the 2 unless very large progeny populations are used.

Lack of polymorphism at marker loci might hinder the effective use of marker-assisted selection in breeding programs. For both the net-blotch resistance genes (*Rpt5* and *Rpt6*), a group of genetic markers exists close to the target gene. Almost 30 markers were located within 10 cM of the *Rpt5* locus, and 11 markers were within 10 cM of *Rpt6*. Several of these markers are codominant, indicating that manipulation of these loci in backcross breeding programs should be relatively straightforward. Both microsatellite and retrotransposon-based markers map close to the resistance genes, and such marker types are known to be highly polymorphic in barley (Saghai Maroof et al. 1994, Vicient et al. 1999). The source of resistance we used was barley line CI 9819, which is effective against a wide spectrum of net-type isolates from the United States, Mexico, Europe, Australia, and New Zealand (Steffenson and Webster 1992*b*, Robinson and Jalli 1996, Wu et al. 2003, Cromey and Parkes 2003), and thus is useful in barley breeding. It also possesses moderate resistance to several spot-type isolates of *P. teres* (Wu et al. 2003). This wide spectrum of resistance in line CI 9819 promises potentially good durability of resistance when used in barley breeding programs.

In this study, we designated 2 net-blotch resistance genes: *Rpt5* on chromosome 6H, controlling resistance to net-type net blotch; and *Rpt6* on chromosome 5H, controlling resistance to 2 Finnish spot-type isolates. In addition, several minor genes affecting resistance were identified. Interestingly, resistance genes active against the net-type isolates were totally different from those active against spot-type isolates in the resistance source CI 9819. Further research is needed to determine whether these resistance genes in barley reflect a difference in the infection mechanisms of the net and spot forms of *P. teres*.

Acknowledgements

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