Theor Appl Genet (2008) 117:1261–1270
DOI 10.1007/s00122-008-0860-x

ORIGINAL PAPER

A region of barley chromosome 6H harbors multiple major genes associated with net type net blotch resistance

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Received: 1 April 2008 / Accepted: 30 July 2008 / Published online: 19 August 2008

Abstract Net type net blotch (NTNB), caused by *Pyrenophora teres* f. *teres* Drechs., is prevalent in barley growing regions worldwide. A population of 118 doubled haploid (DH) lines developed from a cross between barley cultivars 'Rika' and 'Kombar' were used to evaluate resistance to NTNB due to their differential reaction to various isolates of *P. teres* f. *teres*. Rika was resistant to *P. teres* f. *teres* isolate 15A and susceptible to isolate 6A. Conversely, Kombar was resistant to 6A, but susceptible to 15A. A progeny isolate of a 15A × 6A cross identified as 15A × 6A#4 was virulent on both parental lines. The Rika/Kombar (RK) DH population was evaluated for disease reactions to the three isolates. Isolate 15A induced a resistant:susceptible ratio of 78:40 (R:S) whereas isolate 6A induced a resistant:susceptible ratio of 40:78. All but two lines had opposite disease reactions indicating two major resistance genes linked in repulsion. Progeny isolate 15A × 6A#4 showed a resistant:susceptible ratio of 1:117 with the one resistant line also being the single line that was resistant to both 15A and 6A. An RK F_2 population segregated in a 1:3 (R:S) ratio for both 15A and 6A indicating that resistance is recessive. Molecular markers were used to identify a region on chromosome 6H that harbors the two NTNB resistance genes. This work shows that multiple NTNB resistance genes exist at the locus on chromosome 6H, and the recombinant DH line harboring the resistance alleles from both parents will be useful for the development of NTNB-resistant barley germplasm.

Communicated by A. Graner.

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Introduction

Net blotch of barley (*Hordeum vulgare* L.), caused by *Pyrenophora teres* Drechs. Smedeg. [Anamorph: *Drechslera teres* (Sacc.) Shoem.] is present in many different regions of the world including North America, Australia, Asia, Africa, Europe, and the Middle East (Shipton et al. 1973; Mathre 1997). The pathogen is most prevalent in areas where barley is planted under cool, wet conditions, but it can be found in warmer, dry areas as well (Shipton et al. 1973).

Yield and quality losses resulting from net blotch of barley have the potential to reach 100% if the disease occurs under favorable environmental conditions on susceptible cultivars (Mathre 1997), but typical losses are between 10 and 40% (Mathre 1997; Douiyssi et al. 1998; Ma et al. 2004). Two types of net blotch have been identified on barley including net type net blotch (NTNB) caused by *P. teres* f. *teres*, and spot type net blotch (STNB) caused by

P. teres f. *maculata*. NTNB symptoms consist of elongated lesions containing dark brown blotches and longitudinal and transverse striations with a net like appearance (Mathre 1997; Steffenson et al. 1999), whereas STNB symptoms consist of dark brown or elliptical lesions surrounded by chlorotic zones (Mathre 1997).

Several studies have concluded that NTNB resistance genes are present on various barley chromosomes; however a few recurring locations have been identified. Steffenson et al. (1996) evaluated populations at the seedling stage and found three major quantitative trait loci (QTL) on barley chromosomes 4H, 6HS, and 6HL. The QTLs identified on 4H and 6HS together explained 47% of the phenotypic variation. The third QTL identified on the long arm of chromosome 6H by itself accounted for 10% of the phenotypic variation, but this locus only increased the explanation of the total phenotypic variation to 49.6%. Richter et al. (1998) identified one resistance gene on chromosome 6HL, while Ma et al. (2004) identified two QTLs, one on chromosome 6HS explaining 60% of the phenotypic variation, and a second on chromosome 2HS which explained 7% of the phenotypic variation. Together, the two QTL explained 69.2% of the phenotypic variation. Raman et al. (2003) identified a major NTNB resistance QTL on chromosome 4H explaining 64% of the disease variation, and four additional QTLs explained 9–17% of the variation. Cakir et al. (2003) identified a major QTL associated with resistance to NTNB in the VB9524 × ND11231-12 (VN) population on chromosome 6H explaining 83% of the phenotypic variation. Emebiri et al. (2005) also identified a major QTL associated with NTNB resistance on the 6H chromosome in the same population, explaining 75% of the phenotypic variation. Friesen et al. (2006) identified a major gene for NTNB seedling resistance on chromosome 6H. This gene was mapped with simple sequence repeat (SSR) markers common to the Cakir et al. (2003) study and was shown to be in a similar location. Grewel et al. (2008) also identified a major 6H seedling resistance QTL in a similar location in the cross CDC Dolly/TR251. Collectively, these studies have shown that either chromosome 6H has a single major resistance gene that is present in various barley backgrounds, or chromosome 6H has several linked resistance genes that are effective against various *P. teres* f. *teres* pathotypes.

The objectives of this study were to (1) develop a DH mapping population from a cross between barley cultivars 'Rika' (PI 269154) and 'Kombar' (CIho 15694) in order to further characterize and evaluate resistance to NTNB using 15A, 6A and the progeny isolate 15A × 6A#4 derived from a cross of 15A and 6A, and (2) map and identify chromosomal locations for resistance genes using simple sequence repeat (SSR), sequence tagged site (STS), cleaved amplified polymorphism (CAP), and restriction fragment length polymorphism (RFLP) markers. Rika and Kombar were chosen due to their common usage in several sets of differential host lines used in the evaluation of *P. teres* f. *teres* field populations (Steffenson et al. 1991; Steffenson and Webster 1992; Gupta and Loughman 2001; Cromey and Parkes 2003; Wu et al. 2003).

Materials and methods

Biological materials

Pyrenophora teres f. *teres* isolates 15A, 6A, and the progeny isolate 15A × 6A#4 (derived from a cross of 15A and 6A) were used to characterize the genetics of resistance to the pathogen. Isolates 15A and 6A were both collected from California barley fields with isolate 6A (84-28-1) being collected from Fresno county (Wu et al. 2003) and isolate 15A from Solano county (Steffenson and Webster 1992). Isolates 15A and 6A were designated as pathotypes 11-22 and 10-15-19, respectively, and were therefore shown to have different virulences on barley lines Rika and Kombar (Steffenson and Webster 1992). The progeny isolate of a 15A by 6A cross designated as 15A × 6A#4, shown to be virulent on both Rika and Kombar, was also used for genetic analysis.

A population of 118 anther-culture derived-DH lines from the cross of barley cultivars Rika and Kombar, hereafter referred to as the RK population, were used. Rika was shown to be resistant to isolate 15A, while it was susceptible to isolate 6A. Conversely, Kombar was susceptible to isolate 15A and resistant to isolate 6A indicating that Rika and Kombar harbor different resistance genes or alleles.

In addition, F_2 populations derived from a cross between Rika and Kombar were used to determine the gene action of resistance to *P. teres* f. *teres* isolates 15A and 6A. A total of 92 F_2 individuals were used to evaluate the reaction to isolate 6A, and 48 individuals were used to evaluate the reaction to isolate 15A.

All *P. teres* f. *teres* isolates were grown in the dark on V-8 PDA (150 ml V-8 juice, 10 g Difco PDA, 3 g $CaCO_3$, 10 g agar, and 850 ml distilled water) for 5–7 days at 20°C, followed by continuous light for 24 h at room temperature and finally 24–48 h in the dark at 15°C. Once spores were present, the plates were flooded with sterile distilled water and an inoculating loop was used to harvest the conidia. Inoculum was adjusted to 2,000 spores/ml using sterile distilled water, and two drops of Tween 20 (polyoxyethylene-20-sorbitan monolaurate) were added per 100 ml of inoculum to reduce spore clumping.

Fungal progeny were obtained as described by Weiland et al. (1999). Briefly, barley straw was inoculated with parental isolates 15A and 6A and placed onto water agar

plates at 14°C under a 12-h photoperiod. *P. teres* is heterothallic and therefore this fungus is not self fertile. After approximately 3–5 weeks, ascospores were released and progeny of single ascospore isolates were collected and cultured on V8-PDA medium. Mycelium of single ascospore progeny were dried in 8 mm diameter plugs and stored at −80°C for future use.

Inoculation and disease evaluation procedures

Individual DH lines of the RK population were planted along with the parents using three SC10 super cell cone-tainers (Stuewe and Sons. Inc., Corvallis, OR, USA) per line and three seeds per cone-tainer. Plants were placed in racks of 98 cone-tainers consisting of 20 lines surrounded by a border of barley plants used to eliminate any edge effect. Inoculations were done as described by Friesen et al. (2006). Plants were inoculated with conidia of *P. teres* f. *teres* at the two- to three-leaf stage. Inoculum was sprayed on to plants until a heavy mist had covered all the leaves but before runoff. Following inoculation, plants were placed in 100% relative humidity in the light at 21°C for 24 h, and then placed in a controlled growth chamber under a 12-h photoperiod at 21°C. Disease reactions were evaluated 7 days post-inoculation. Disease evaluations were done using a 1–10 scale as described by Tekauz (1985) where reaction type 1 is resistant and reaction type 10 is susceptible. Three replicates of three cone-tainers each were completed for all lines of the population and the parents, where nine plants (3 cones, 3 plants per cone) were evaluated collectively for each replicate. Due to space limitations, individual replicates were planted and inoculated at different times. Populations were planted, inoculated, and scored for reaction to each of the three isolates 15A, 6A, and the progeny isolate 15A × 6A#4 as described above.

Molecular mapping and marker analysis

Previously published genetic maps of barley containing SSR markers detected by Bmac and Bmag SSR primer sets (Ramsay et al. 2000) were surveyed to select several SSR markers from each of the seven barley chromosomes for mapping NTNB resistance in the RK DH population. DNA was extracted from fresh leaf tissue of each of the 118 DH lines and parents as described by Aljanabi and Martinez (1997). Polymerase chain reaction (PCR) for SSR markers was performed in a 10 µl volume in 96-well plates in a GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA, USA). Each PCR reaction consisted of 200 ng of template DNA, 1 µl of 10× buffer, 0.5 units of *Taq* polymerase (New England BioLabs, Ipswich, MA, USA), 4.0 pmol of forward and reverse primers, and 0.8 µl of 2.5 mM dNTPs, as described by Ramsay et al. (2000). Amplified products were either run on an ABI 3130x1 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) as described by Chao et al. (2007), or they were separated on 6% polyacrylamide gels (CBS Scientific, Del Mar, IA, USA) according to Friesen et al. (2006), stained with SYBR Green II (Sigma, St. Louis, MO, USA) for 30 min, and scanned with a Typhoon model 9410 variable mode imager (GE Healthcare, Waukesha, WI, USA).

Once the chromosomal region harboring a NTNB resistance locus was identified, more DNA markers including SSR, STS, CAP, and RFLP markers were developed to saturate the region and refine the location of the resistance loci based on the previously published RFLP and EST-based maps (Künzel et al. 2000, Rostoks et al. 2005; Stein et al. 2007).

Primer sequences for RFLP probes and EST-based markers reported in previously published maps are available at http://wheat.pw.usda.gov/GG2/index.shtml and (http://bioinf.scri.ac.uk/barley_snpdb/index.html). If previously designed primers were not available for a given marker, the relevant sequence was downloaded from the above websites or from http://pgrc.ipk-gatersleben.de/cr-est and new primers were designed using the web-based program Primer3 (http://frodo.wi.mit.edu/, Rozen and Skaletsky, 2000) (Table 1). To develop STS or CAP markers, all pairs of primer were amplified from parental lines using a touchdown PCR program that included denaturing at 94°C for 2 min, 10 cycles of 30 s at 94°C, 30 s at a gradient from 65 to 55°C with each cycle decreasing 1°C, and 1 min at 72°C, followed by 30 cycles of 30 s at 94°C, 30 s at 55°C and 1 min at 72°C and finally a 10 min extension at 72°C. The PCR reaction was set up in a 10 µl volume containing 200 ng DNA, 4 pmol of forward and reverse primers, 0.8 µl of 2.5 mM dNTPs and 0.5 units of *Taq* polymerase (New England BioLabs, Ipswich, MA, USA). The PCR products were added to 5 µl 3× loading buffer, denatured for 10 min, and separated on 6% denaturing polyacylamide gels. Gels were run at a constant 70 W for 2 h followed by staining with SYBR Green II (Sigma, St. Louis, MO, USA). Stained gels were scanned using a Typhoon 9410 variable mode imager (GE Healthcare, Waukesha, WI, USA).

For CAP marker analysis, PCR products were produced as described above but were digested before being loaded onto the gel. Digestions were done by adding 1.5 µl of 10× buffer, 0.15 µl of 10 mg/ml BSA and 1 unit of the restriction enzymes *Rsa*I or *Hae*III. The resulting 15 µl volume was incubated at 37°C for 2 h.

Two RFLP markers were developed from wheat ESTs that have been mapped within the chromosome 6AL4-0.55 and 6BL5-0.4-1.00 bins (Randhawa et al. 2004), respectively (Table 1). The RFLP protocol was done as described by Faris et al. (2000) with minor modifications. The main difference was that 10 µg of barley genomic DNA was

Table 1 EST-based markers mapped on chromosome 6H in the Rika/Kombar barley doubled haploid population

Marker	Original marker or bin location[a]	Reference	Primer sequence	Marker type[b]
MWG652	MWG652 (RFLP)	Künzel et al. 2000	*F: GAGCTGCTCGTTCTCGTTGA* *R: CACACCTTCTTCTTCCTCTT*	CAP (*Hae*III)
MWG916	MWG916 (RFLP)	Künzel et al. 2000	*F: GCGGACCAGATCAATATCGA* *R: CGACGTAGGGAAACACGCAT*	CAP (*Hae*III)
ABG388	ABG388 (RFLP)	Künzel et al. 2000	*F: GCACTGGCATAGTCTCACAA* *R: CGATGCTGGTTCGGTCATAC*	STS
cMWG2029	cMWG2029 (RFLP)	Künzel et al. 2000	*F: CCAGTTATCCGAATCCGGAA* *R: GTGGTCAGGTACATACGAAT*	STS
MWG2137	MWG2137 (RFLP)	Künzel et al. 2000	*F: CCCGTCGATCGATCGATCAA* *R: GCTACTGTTTCGCGGTTGCT*	STS
cMWG679	cMWG679 (RFLP)	Künzel et al. 2000	F: TCAAGGCTAACCCCATGTTC R: CCCATGAAGATGAGTGCAT	CAP (*Rsa*I)
ABG458	ABG458 (RFLP)	Künzel et al. 2000	F: CCCTTTCCTCCTCGTCCTTT R: CTTGAACCAAACGGCCTCTC	CAP (*Rsa*I)
GBM1075	GBM1075 (EST-SSR)	Stein et al. 2007	F: CCCGACCAAGCTTTTCTCAC R: TGATGGTGGGCTTCTTGTTG	STS
GBM1423	GBM1423 (EST-SSR)	Stein et al. 2007	F: CAAATCCCCAAGCCAATCT R: CTTGCCTGTCAACGTCTTCA	STS
GBS0468	GBS0468 (SNP)	Stein et al. 2007	F: TGAACATCAGTCAAACACCAACA R: CATCCTTCCTGACAGCTTAAACC	STS
ABC06204	Scsnp06204 (SNP)	Rostok et al. 2005	**F: TCAAAGTGGGCAGGCATCAA** **R: ATCATGACCCGATGCGGTG**	STS
ABC02895	Scsnp02895 (SNP)	Rostok et al. 2005	**F: TGATCGGTCCAGTTCACCCA** **R: GGAATCGCAAGCACTACGGG**	CAP (*Hae*III)
ABC01719	Scsnp01797 (SNP)	Rostoks et al. 2005	F: GGAGACCTCCATCTTCGCCA R: GGCAGCGGAAAAACAACAGC	STS
ABC14681	Scsnp14681 (SNP)	Rostoks et al. 2005	**F: TTGCCGTTGGAGAGTAATTTTGAC** **R: CAGGCGCGAGATCGAACAC**	STS
BE636841	6AL4-0.55	Randhawa et al. 2004	RFLP probes	RFLP
BF293263	6BL5-0.4-1.00	Randhawa et al. 2004	RFLP probes	RFLP

The sequences of primers in italics were obtained from http://wheat.pw.usda.gov/GG2/index.shtml, those in bold were obtained from http://bioinf.scri.ac.uk/barley_snpdb/index.html. The remaining primers were designed by the authors

F forward primer, *R* reverse primer

[a] Information in parentheses indicates the type of marker that was developed in the previously published papers including restriction fragment length polymorphism (RFLP), expressed sequence tag–simple sequence repeat (EST–SSR), and single nucleotide polymorphism (SNP)

[b] Sequence tagged site (STS), cleavage amplified polymorphism (CAP). Information in brackets indicates which enzyme was used to digest the PCR product

digested instead of 25 µg DNA. To prepare an RFLP probe, the bacterial clone containing a specific wheat EST was inoculated into 1.0 ml Luria Bertani (LB) liquid medium containing 50 µg/ml ampicillin in a 1.5-ml micro centrifuge tube and grown overnight at 37°C. A 1 µl amount of the overnight-grown bacterial culture was used as the DNA template to conduct PCR with the M13 forward and reverse primers under the same PCR conditions as those used for SSRs with an annealing temperature of 55°C. The PCR product was run on a 0.9% agarose gel and the resolved single band was excised from the gel and purified using the QIAquick gel extraction kit (Qiagen, Valencia, CA, USA).

Molecular and phenotypic markers were assembled together into linkage maps using the computer program MAPMAKER v2.0 for Macintosh (Lander et al. 1987) with a minimum logarithmic of the odds (LOD) threshold of 3.0 and the Kosambi mapping function as described by Liu et al. (2005). The final marker order was validated using the 'RIPPLE' command. Markers not mapping at an LOD of 3.0 were placed in their most likely positions along the map. The segregation ratios of molecular and phenotypic marker genotypes were tested for fit to the expected 1:1 ratio using Chi-squared analysis with the computer program QGENE (Nelson 1997).

Results

Isolate 15A induced a differential reaction when inoculated onto parental lines Rika and Kombar, with Rika being resistant (average disease reaction 2.0) and Kombar being susceptible (average disease reaction 7.0) (Table 2; Fig. 1). The RK DH population segregated in a ratio of 78:40 (resistant: susceptible) for reaction to *P. teres* f. *teres* isolate 15A (Table 3). The 78 resistant lines showed reaction types ranging from 1.0 to 4.8 with an overall average of 2.1, whereas the 40 susceptible lines showed reaction types ranging from 6.3 to 7.8 with an overall average of 7.0 (Table 2).

When inoculated with isolate 6A, parental lines Rika and Kombar showed opposite disease reaction responses compared to isolate 15A, with Rika showing susceptibility (7.8) and Kombar showing resistance (1.7) (Table 2; Fig. 1). The RK population segregated in a ratio of 40:78 (resistant: susceptible), which was nearly the complete opposite to that of isolate 15A with only two exceptions. Line 85 was resistant to both isolates (average disease reactions were 1.5, and 3.0 for 15A and 6A, respectively) and line 99 was susceptible to both isolates (average disease reactions were 6.7 and 7.0 for 15A and 6A, respectively). This result indicated that there were at least two NTNB resistance genes segregating in the RK DH population, and they were closely linked in repulsion (Table 3; Fig. 1). The 40 lines that were resistant to NTNB caused by 6A showed reaction types ranging from 1.2 to 4.3 with an overall average of 2.7, whereas the 78 susceptible lines showed reaction types ranging from 6.0 to 8.8 with an overall average of 7.6 (Table 2).

A bimodal distribution was observed for both isolates, indicating at least one major gene was conferring resistance to isolate 15A, and at least one distinct major gene was conferring resistance to isolate 6A (Fig. 2). The resistant:susceptible segregation ratio observed in the RK DH population for reaction to isolate 15A was approximately 2:1, whereas the resistant:susceptible segregation ratio observed for isolate 6A was approximately 1:2. Chi-squared values for reaction to both 15A and 6A differed significantly from a 1:1 ratio ($\chi^2 = 12.2$, $P = 0.05$). Also, reactions to 15A differed significantly from a 3:1 (R:S) ratio ($\chi^2 = 4.98$, $P = 0.026$), and reactions to 6A differed significantly from a 1:3 ratio (R:S) ($\chi^2 = 4.98$, $P = 0.026$) (Table 3).

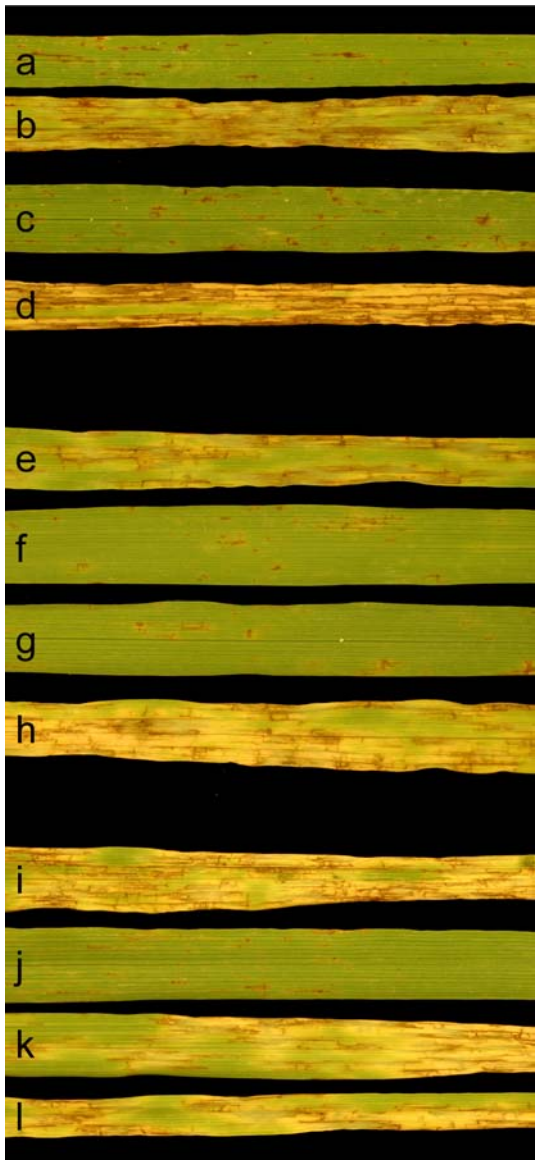

Fig. 1 Reaction types of *Pyrenophora teres* f. *teres* isolates 15A, 6A, and 15A × 6A progeny isolate #4 (15A × 6A#4) on parental lines Rika and Kombar, and two RK population recombinant lines RK 85 (resistant to both isolates), and RK 99 (susceptible to both isolates). From *top* to *bottom*: isolate 15A on Rika (*a*), Kombar (*b*), RK85 (*c*), and RK99 (*d*), Isolate 6A on Rika (*e*), Kombar (*f*), RK85 (*g*), and RK99 (*h*). Progeny isolate 15A × 6A#4 on RK99 (*i*), RK85 (*j*), Kombar (*k*), and Rika (*l*)

Table 2 Average disease reaction to *P. teres* f. *teres* isolates 15A, 6A and 15A × 6A#4 in the RK DH population

	Average disease reaction		
	15A	6A	15A × 6A#4
Rika	2.0 ± 1.0	7.8 ± 1.0	7.8 ± 0.3
Kombar	7.0 ± 0	1.7 ± 0.8	7.5 ± 0.5
Resistant Lines	2.1 ± 0.8	2.7 ± 0.8	3.5 ± 0
Susceptible Lines	7.0 ± 0.3	7.6 ± 0.7	7.9 ± 0.7

Progeny isolate 15A × 6A#4 was virulent on both Rika and Kombar and was therefore used to further characterize the genetics of resistance in the RK DH population. The RK population segregated in a resistant:susceptible ratio of 1:117 with line RK85 being the only line with resistance to this isolate having an average disease reaction score of 3.5. Line 85 was also the only line resistant to both 15A and 6A

Table 3 Segregation of Rika × Kombar F_2 individuals and doubled haploid (DH) lines into resistant and susceptible reactions to *P. teres* f. *teres* isolates 15A and 6A

Isolate	Resistant F_2	Susceptible F_2	χ^2 (1:3)[a]	Resistant DH	Susceptible DH	χ^2 (1:1)[b]
15A	14	34	0.44	78	40	12.2
6A	24	68	0.58	40	78	12.2

[a] Not significantly different from 1:3 ($P = 0.05$)

[b] Significantly different from a 1:1 ($P = 0.05$)

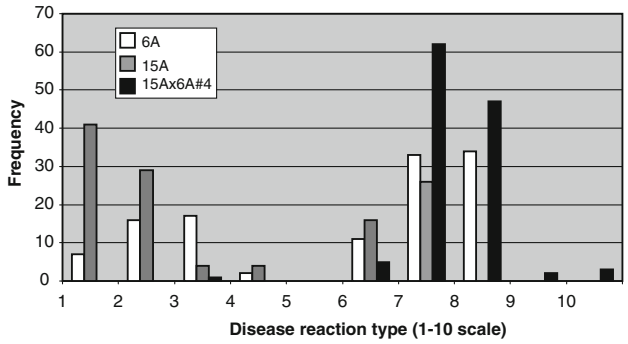

Fig. 2 Histograms of average disease reaction types caused by *Pyrenophora teres* f. *teres* isolates 15A, 6A, and 15A × 6A#4 on the Rika × Kombar barley doubled haploid mapping population

parental isolates. Disease reactions on the remaining 117 susceptible lines ranged from 6.5 to 10 with an overall average of 7.9.

The RK F_2 population segregated in a 1:3 (resistant:susceptible) ratio for each isolate, indicating that a single gene, or closely linked genes, conferred resistance to NTNB caused by 15A (14:34) ($\chi^2 = 0.44$, $P = 0.505$), and 6A (24:68) ($\chi^2 = 0.58$, $P = 0.81$) (Table 3). In addition, F_1 plants of the Rika/Kombar cross were susceptible to isolates 15A (reaction types 7–10) and 6A (reaction types 8–10), indicating that resistance was recessive in nature.

Forty-five SSR markers specific to all chromosome arms and polymorphic between Rika and Kombar were mapped in the RK DH population. Among these 45 markers, the SSR markers *Bmag0807* and *Bmag0173* were found to be linked to the genetic loci conferring resistance to NTNB caused by isolates 15A and 6A (Fig. 3). Hereafter, the loci conferring resistance to 15A and 6A will be referred to as *rpt.r* and *rpt.k*, respectively. *Bmag0807* and *Bmag0173* are known to be on chromosome 6H and were shown to be linked to NTNB resistance loci on 6H in other research (Friesen et al. 2006; Cakir et al. 2003; Emebiri et al. 2005). Therefore, chromosome 6H was targeted with additional SSR and STS markers.

A total of 23 markers were mapped to chromosome 6H in the RK DH population, including the two phenotypic markers (*rpt.r* and *rpt.k*), five SSRs, 9 STSs, 5 CAPs, and 2 RFLPs (Table 1; Fig. 3). The linkage map of 6H spanned a genetic distance of 128.9 cM, and eight of the 24 markers mapped at an LOD < 3.0. Chi-squared analysis of the marker segregation ratios indicated that, with the exception of one marker (*cMWG679*), all had ratios that deviated significantly from the expected 1:1 ratio (Fig. 3). The markers with the most severely distorted segregation ratios lay within a 28 cM segment delineated by *ABG458* and *ABG388*.

The NTNB resistance loci *rpt.r* and *rpt.k* mapped 1.8 cM apart and were flanked by the CAP marker *ABC02895* and the locus detected by STS markers *GBS0468* and *ABC01797* (Fig. 3). These flanking loci delineate *rpt.r* and *rpt.k* to a 5.9 cM interval, which also contained three markers (*Bmag0173*, *BE636841*, and *Bmag0496*) that did not map at an LOD > 3.0.

The accumulated data indicate that the RK DH population segregates for at least two major recessive resistance genes. The genes are closely linked in repulsion on chromosome 6H and they confer resistance to different pathotypes of *P. teres* f. *teres*.

Discussion

Previous research has indicated that although QTL are present (Steffenson and Webster 1992), the NTNB pathosystem is at least partially controlled by several major resistance or susceptibility genes. Products of these genes theoretically interact directly or indirectly with corresponding avirulence/virulence gene products produced by *P. teres* f. *teres* (Weiland et al. 1999; Lai et al. 2007). Other studies have shown that resistance in the barley—*P. teres* f. *teres* pathosystem may be dominant (Friesen et al. 2006), incompletely dominant (Schaller 1955; Bockelman et al. 1977), or recessive (Ho et al. 1996), indicating the presence of complex interactions between the host and pathogen. Here, we identified on barley chromosome 6H two recessive resistance genes, each of which confer resistance to specific pathotypes of *P. teres* f. *teres*.

Although there was an obvious separation between resistant and susceptible lines, several lines showed reactions in the 3–4 (moderately resistant) and the 6–7 (moderately susceptible) reaction type range, indicating that minor genes

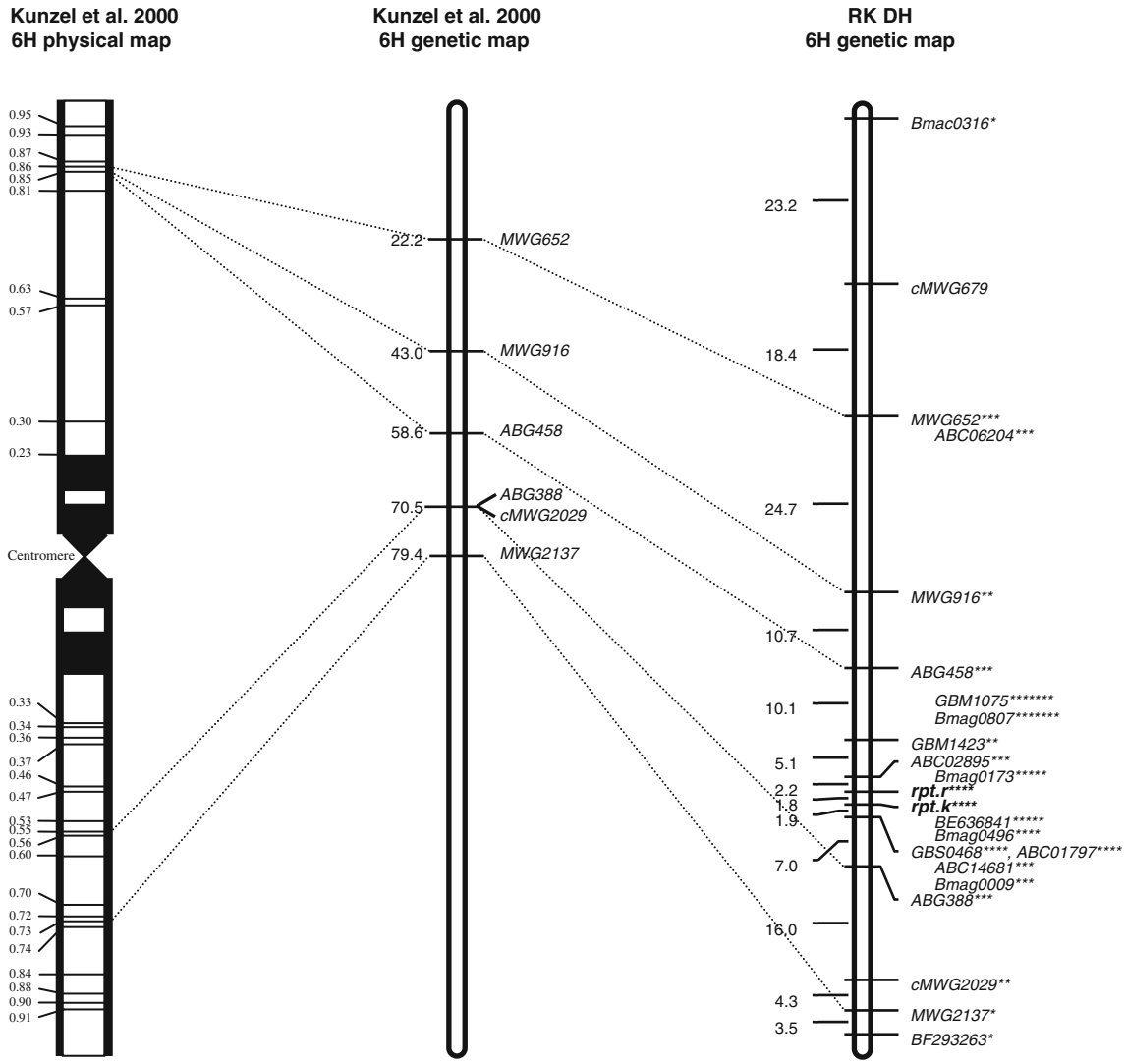

Fig. 3 Comparison of the barley chromosome 6H physical map (*left*) and the corresponding genetic map (*middle*) (Kunzel et al. 2000) with the chromosome 6H map developed in the Rika/Kombar doubled haploid population (*right*). Maps are oriented with short arms on *top*. Numbers to the *left* of the physical map are fraction lengths of translocation break points as described by Kunzel et al. (2000), numbers to the *left* of the corresponding genetic map (*middle*) are marker positions in centimorgans as described in Kunzel et al. (2000). For the Rika/Kombar 6H map (*right*), map distances are given in centimorgans (cM) to the *left* and markers are shown along the *right*. *Asterisks* behind marker names indicate the degree deviation from a 1:1 ratio where *, **, ***, ****, *****, ******, and *******, indicate significance at the 0.05, 0.01, 0.005, 0.001, 0.0005, 0.0001, and 0.00005 levels of probability, respectively

may have an effect on the level of resistance present in the population. It is also possible that this is due to experimental error, but reaction types were highly repeatable, indicating that genetic effects, rather than error, were likely responsible.

Barley chromosome 6H has been identified in several studies as harboring major and minor genes conferring resistance to *P. teres* f. *teres* (Manninen et al. 2000; Cakir et al. 2003; Ma et al. 2004; Emebiri et al. 2005; Friesen et al. 2006; Grewal et al. 2008). These previous studies have not been able to adequately show whether the resistance is conferred by one or multiple genes present in this region. In the current work, we demonstrated that at least two different genes are present within close proximity to each other on barley chromosome 6H. The SSR markers (*Bmag0173* and *Bmag0807*) used to map *P. teres* f. *teres* resistance in at least two other studies using different barley populations (Cakir et al. 2003; Friesen et al. 2006) were also used in our study, and the location of the resistance loci on chromosome 6H coincide among the three studies. Friesen et al. (2006) showed that at least one dominant resistance gene accounting for 84–89% of the disease variation was present at the 6H region. This together with the two recessive genes identified in our study suggest that at

least three resistance genes are present at the 6H region, one dominant and two recessive. It is interesting to note that Friesen et al. (2006) also used isolate 15A in their analyses, and found that resistance to 15A in the barley line SM89010 was conferred by a dominant gene. The dominant gene identified by Friesen et al. (2006) and the recessive gene identified in this work for resistance to isolate 15A are either different genes or are different alleles of the same gene.

The order of markers along our map agreed well with the previously published 6H maps, including an RFLP map (Künzel et al. 2000), an SNP map (Rostoks et al. 2005) and a highly saturated EST-based map (Stein et al. 2007). Only the marker *cMWG679* has a different position compared to the RFLP map. This may be due to the fact that the new primer we designed can amplify several fragments visible on a polyacrylamide gel, and the fragment we scored may be different from the original RFLP fragments.

Most of the markers are clustered with the two resistance loci because we only selected markers targeting that region. The markers *ABG485* and *ABG388* on the previous RFLP map (Künzel et al. 2000) were separated by about 13 cM in genetic distance, but physically encompassed a large portion of chromosome 6H. Therefore, the region between the two markers was defined as a region with low recombination frequency. However, in our map the genetic distance between the two markers was expanded to 28 cM, indicating an increased recombination frequency. The resistance loci were close to the RFLP marker developed from wheat EST BE636841, which was physically mapped to the 6AL bin, and the STS marker ABC01797, which has high homology to the wheat group 6 bin-mapped EST BE637763. This suggests the resistance loci may be located on the long arm of 6H but very close to the centromere. Unfortunately, we still cannot be certain since both of the markers were mapped on the same side of the resistance loci. More EST markers in this region are needed to verify on which arm the genes reside. Several STS and SSR markers flank the two resistance loci (Fig. 3), which will serve as good markers for marker assisted selection.

Within the RK DH population, all but two lines showed opposite disease reactions when inoculated with isolate 6A as compared to inoculations done with isolate 15A. The DH line RK99 was susceptible to both 15A and 6A indicating that it harbors dominant alleles at both the *rpt.r* and *rpt.k* loci, and the DH line RK85 was resistant to both 15A and 6A, indicating that it harbors the recessive alleles at both loci. When inoculated with 15A × 6A#4, the progeny isolate that was virulent on both parental lines, RK85 was resistant but RK99 was susceptible. This indicates that the presence of dominant alleles at either or both the *rpt.r* and *rpt.k* loci on chromosome 6H are sufficient to cause high levels of disease susceptibility.

Due to the high level of variability in the natural *P. teres* f. *teres* population, pyramiding of genes is critical for maintaining durable resistance to this pathogen. The DH line RK85 and the molecular markers identified in this study will be useful in producing barley cultivars resistant to this disease. However, the "pyramiding" approach will potentially need to focus on both dominant and recessive resistance genes in order to obtain the highest levels of durable resistance. Continued saturation mapping of this region is also underway and will aid in producing markers more closely linked to this gene region.

Phenotyping of the RK population with isolates 15A and 6A produced a segregation ratio of 78:40 and 40:78 (R:S), respectively, which was neither significant for a 1:1 (single gene) nor a 3:1 (two gene) ratio; however, molecular markers specific to the 6H region also segregated in an approximately 2:1 ratio indicating a significant segregation distortion occurred in this region in the RK population. This was also the case in the Friesen et al. (2006) study, where an approximately 2:1 (R:S) phenotypic ratio was observed and markers associated with the locus were also distorted in a similar ratio. Segregation distortion is a commonly reported phenomenon, especially in anther-culture derived-DH populations (Graner et al. 1991; Heun et al. 1991; Devaux et al. 1995). In comparative mapping experiments between an anther-culture derived-DH population and a DH population derived from the *H. bulbosum* method, Devaux et al. (1995) observed a much stronger degree of segregation distortion in the anther-culture derived population. They identified RFLP markers with severely distorted segregation ratios in multiple genomic regions, including the short arm of chromosome 6H, but map distances were not affected by the distortions. It is difficult to determine if the distorted region of 6H in the RK population is the same as that observed in the population investigated by Devaux et al. (1995) because the two maps do not harbor common markers. However, CMap (http://rye.pw.usda.gov/cmap/) comparisons of distorted markers from both maps with maps generated in other barley populations that unite common marker loci strongly suggest the region of segregation distortion along 6H in both populations is the same. Therefore, a simplistic explanation is that regions of segregation distortion represent loci that govern anther-culture-driven gamete selection, or that are involved in overcoming stress caused by the tissue culture conditions.

In the classical gene-for-gene hypothesis (Flor 1942), dominant resistance gene products interact either directly or indirectly with pathogen produced avirulence gene products signaling a cascade of events leading to host resistance. Conversely, several necrotrophic type fungal pathogen systems involving virulence factors such as host-selective toxins work in an inverse gene-for-gene manner

(Wolpert et al. 2002; Lamari et al. 2003; Friesen et al. 2007, 2008). In this case, virulence gene products produced by the pathogen interact directly or indirectly with dominant susceptibility gene products in the host to stimulate a cascade of events leading to susceptibility rather than resistance. Closely related fungal pathogens in the *Alternaria*, *Pyrenophora*, *Cochliobolus*, and *Stagonospora* genera have all been shown to produce virulence factors (e.g., host-selective toxins) that correspond with dominant susceptibility genes in the host (Wolpert et al. 2002; Friesen et al. 2008). Although additional characterization of the net form of net blotch system still needs to be done, it is likely that, in addition to classical dominant gene-for-gene resistance, dominant susceptibility genes exist in barley and are being used by the pathogen to incite disease. An additional possibility that seems less likely is that pathotype-specific suppressors at the 6H locus are suppressing resistance and therefore conferring susceptibility. This research shows that two distinct loci segregating in the RK population are conferring recessive resistance (or dominant susceptibility). These genes have also been shown to be closely linked in repulsion on barley chromosome 6H close to the centromere.

Acknowledgments Authors would like to thank Danielle Holmes for technical assistance. This research was supported by the US Barley Genome Project, the American Malting Barley Association and by USDA-ARS CRIS projects 5442-22000-043-00D and 5442-22000-030-00D.

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