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RESEARCH

Mapping Net Blotch Resistance in 'Nomini' and CIho 2291 Barley

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ABSTRACT

Net blotch (*Pyrenophora teres*) is one of the most devastating diseases of barley (*Hordeum vulgare* L.) worldwide. Identification of diagnostic molecular markers associated with genes and quantitative trait loci (QTL) for net blotch resistance will facilitate pyramiding of independent genes. Linkage mapping was used to identify chromosomal locations of the independent, dominant genes conditioning net blotch resistance in the winter barley 'Nomini' (PI 566929) and spring barley CIho 2291. The F_2 populations of 238 and 193 individuals, derived from crosses between the susceptible spring barley parent 'Hector' (CIho 15514) and the resistant parents Nomini and CIho 2291, respectively, were used to map the genes governing resistance in the resistant parents. The dominant gene governing resistance in Nomini, temporarily designated *Rpt-Nomini*, was mapped to a 9.2-cM region of barley chromosome 6H between the flanking microsatellite markers *Bmag0344a* (r^2 = 0.7) and *Bmag0103a* (r^2 = 0.9), which were 6.8 and 2.4 cM away from *Rpt-Nomini*, respectively. The dominant gene governing resistance in CIho 2291, temporarily designated *Rpt-CIho2291*, was mapped to a 34.3-cM interval on the distal region of barley chromosome 6H between the flanking microsatellite markers *Bmag0173* (r^2 = 0.65) and *Bmag0500* (r^2 = 0.26), which were 9.9 and 24.4 cM away from *Rpt-CIho2291*, respectively. Identification of the chromosomal location of *Rpt-Nomini* and *Rpt-CIho2291* will facilitate efforts in pyramiding multiple genes for net blotch resistance.

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Abbreviations: BSA, bulked segregant analysis; DH, doubled haploid; MAS, marker-assisted selection; NTNB, net-type net blotch; PCR, polymerase chain reaction; QTL, quantitative trait loci; SSR, simple sequence repeat or microsatellite marker; STNB, spot-type net blotch.

Net blotch (*Pyrenophora teres*) is one of the most widespread foliar diseases of barley (*Hordeum vulgare* L.), occurring in most regions where barley is grown. Net blotch epidemics can cause yield losses ranging from a trace to 100%, but typically cause losses from 10 to 40% (Mathre, 1997). The disease occurs in two forms: *Pyrenophora teres* f. *teres* Smedeg. causes the net-type of net blotch (NTNB) and *P. teres* f. *maculata* Smed.-Pet. causes the spot-type of net blotch (STNB). The NTNB-causing isolates have been reported as more virulent than STNB-causing isolates (Wu et al., 2003).

Resistance to NTNB has been characterized in several studies. The NTNB resistance genes *Rpt1a*, *Rpt3d*, *Rpt1b*, and *Rpt2c* were assigned to barley chromosomes 3H, 2H, 3H, and 5H, respectively, using trisomic analysis (Bockelman et al., 1977). The donor parents of these resistance genes are 'Tifang' (PI 69426, *Rpt1a*), CIho

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Table 1. Summary of net blotch resistance genes or major quantitative trait loci (QTL) (R^2 > 0.50) previously mapped to barley chromosome 6H.

Gene/QTL	Flanking markers	Interval†	Resistance source	Isolate‡	Reference
		cM			
Unnamed gene	*HVM65* *HVM14*	2.0	CIho 9819	P8	Manninen et al. (2000)
QRpt	*Bmag0173*	1.7–4.8	ND11231 and Kaputar	NB77	Cakir et al. (2003)
Rpt	*Xksua3b* *Xwg719d*	36.7	Chevron	ND89-19	Ma et al. (2004) and Emebiri et al. (2005)
Unnamed QTL	*EBmac0874* *M49-P40-650*	9.3	SM89010	15A, 0-1, and ND89-19	Friesen et al. (2006)
rpt.k	*ABC02895/Bmag0173* *GBS0468/ABC01797*	5.9	Kombar	6A	Abu Qamar et al. (2008)
rpt.r	*ABC02895/Bmag0173* *GBS0468/ABC01797*	5.9	Rika	15A	Abu Qamar et al. (2008)
QRpt6	*HVM74* *Bmag0496/Bmag0009*	3.0	TR251	WRS858 and WRS1607	Grewal et al. (2008)
Rpt-Nomini	*Bmag0344a* *Bmag0103a*	9.2	Nomini	ND89-19	Current study
Rpt-CIho2291	*Bmag0500* *Bmag0173*	34.3	CIho 2291	ND89-19	Current study

† Genetic distance between the two closest markers flanking the gene/QTL as reported in the study in which the gene/QTL was mapped.

‡ *Pyrenophora teres* f. sp. isolate used in mapping study.

7584 (*Rpt3d*), and CIho 9819 (*Rpt1b* and *Rpt2c*). An additional gene for NTNB resistance derived from the winter barley cultivar Igri (PI 428488) was mapped to chromosome 3H and was assigned the temporary designation *Pt,,a* until an allelism test is conducted with *Rpt1a* and *Rpt1b* to determine the uniqueness of these independently mapped genes (Graner et al., 1996).

Several linkage mapping studies have reported genes or major quantitative trait loci (QTL) for NTNB on barley chromosome 6H (Table 1). One study reported a single gene for NTNB resistance, which was mapped to chromosome 6H using retrotransposon markers (Manninen et al., 2000). This gene accounted for 65% of the phenotypic variation for net blotch resistance in a doubled-haploid (DH) population derived from a cross between the resistant Ethiopian barley line CIho 9819 and the susceptible parent 'Rolfi'. It is unknown whether the gene derived from CIho 9819 is identical to previously reported net blotch resistance genes, as common markers were not used or allelism tests conducted. Cakir et al. (2003) identified a major QTL for NTNB resistance that mapped to chromosome 6H, and explained 83 and 66% of the phenotypic variation for resistance to NTNB in DH populations derived from the crosses 'Tallon' (PI 573731) × 'Kaputar' (PI 591928) and VB9524 × ND11231, respectively. A gene for NTNB in the barley cultivar Chevron (PI 38061), temporarily designated *Rpt*, was mapped to chromosome 6H (Ma et al., 2004). A major QTL that explained 89% of the phenotypic variation for NTNB resistance in a DH population derived from a cross between resistant SM89010 and susceptible Q21861 (PI 584766) mapped to chromosome 6H (Friesen et al., 2006). Segregation analysis of an F_2 population derived from the same cross confirmed that NTNB resistance in the population was governed by a single dominant gene. This gene was linked to the simple sequence repeat (SSR) marker *Bmag0173*, suggesting it is the same gene reported by Cakir et al. (2003) in ND11231 and Kaputar. A major QTL for net blotch resistance derived from the resistant barley genotype TR251, designated *QRpt6*, was mapped to chromosome 6H and explained 65 and 60% of the phenotypic variation for resistance to *P. teres* f. sp. *teres* isolates WRS858 and WRS1607, respectively (Grewal et al., 2008).

Several studies have also reported minor-effect QTL for net blotch resistance that mapped to barley chromosome 6H. Steffenson et al. (1996) identified a QTL on chromosome 6H that explained 14% of the phenotypic variation for seedling resistance to NTNB in a DH population derived from a cross between the resistant cultivar Steptoe (CIho 15229) and the susceptible cultivar Morex (CIho 15773). An additional QTL on chromosome 6H that explained 10% of the phenotypic variation for adult plant resistance to NTNB was derived from Steptoe (Steffenson et al., 1996). A QTL analysis using an F_2 population derived from a cross between the resistant parent 'Hor 9088' and the susceptible cultivar Arena identified four QTL on chromosome 6H that conditioned resistance to the NTNB-causing isolate 04/6T and accounted for 10.3 to 26.9% of the phenotypic variation, depending on the leaf used in phenotypic assessments (Richter et al., 1998). A QTL conditioning net blotch resistance that explained 21% of the phenotypic variation was mapped to chromosome 6H, using a DH population derived from a cross between the resistant parent TR306 and the susceptible parent 'Harrington' (Spaner et al., 1998). Two QTL for NTNB resistance were mapped to chromosome 6, using

a recombinant inbred line population derived from a cross between the NTNB-resistant breeding line M120 and the Septoria speckled leaf blotch (*Septoria passerinii* Sacc.)–resistant breeding line Sep2-72. These QTL mapped to separate locations on chromosome 6 and accounted for 19 to 48% and 25 to 44% of the phenotypic variation, respectively. Interestingly, the second of these QTL was derived from the NTNB-susceptible parent (St. Pierre et al., 2010).

A lack of common markers between mapping studies reporting genes for net blotch resistance on barley chromosome 6H, as well as a lack of allelism tests between the resistant sources from which these genes were derived prohibits direct comparison of genes for uniqueness. Another possibility, however, is that chromosome 6H may contain several distinct gene loci or multiple alleles governing net blotch resistance. Abu Qamar et al. (2008) mapped two recessive genes for net blotch resistance in a DH population derived from a cross between 'Rika' (PI 467748) and 'Kombar' (CIho 15694). These genes, designated *rpt.r* and *rpt.k*, were linked in repulsion at approximately 1.8 cM apart, and each condition resistance to different isolates of *P. teres*. Previously mapped net blotch resistance genes on chromosome 6H were dominant, indicating that this region either contains multiple net blotch resistance genes, or the other genes mapped to this region are allelic variants of *rpt.r* or *rpt.k* having different modes of gene action depending on the *P. teres* isolates. Results from other studies (St. Pierre et al., 2010) confirm the presence of multiple distinct loci for net blotch resistance on chromosome 6H.

Previous results (O'Boyle et al., 2011) indicate that the winter barley cultivar Nomini and the spring barley genotype CIho 2291 each have a different single dominant gene conditioning NTNB resistance. The relationship to other sources of net blotch resistance could not be inferred until the approximate chromosomal location of these resistance genes was known. Based on this information, appropriate follow-up allelism studies could be designed to further examine the relationship and novelty or lack thereof between the resistance genes in Nomini and CIho 2291 compared to other reported resistance sources.

Identification of tightly linked molecular markers flanking the NTNB resistance genes in Nomini and CIho 2291 would facilitate the transfer and pyramiding these genes into common barley breeding lines. As new marker technologies are constantly being adapted by breeding programs, the identification of flanking markers would also facilitate future endeavors to saturate the resistance loci with additional markers to more accurately pinpoint the location of the resistance genes. The objective of this research was to utilize SSR markers to map the genes governing NTNB resistance in Nomini and CIho 2291, using F_2 populations derived from crosses between resistant parents Nomini and CIho 2291 and the susceptible parent Hector.

MATERIALS AND METHODS

Plant Materials

Crosses between the resistant parents Nomini (['Boone' × 'Henry'] × VA77-12-41) and CIho 2291 (selection from CIho 1326), and susceptible parent Hector ('Betzes' × 'Palliser') were made at Virginia Tech in 1998 to 1999. The breeding line VA77-12-41 was derived from a composite of crosses including CIho 9623, CIho 9658, CIho 9708, and 'Atlas', each crossed to a (['Cebada Capa' × 'Wong'] × Awnletted 'Hudson' selection) (Starling et al., 1994). The F_1 seeds were planted in a field at Langdon, ND, in 1999 to produce F_2 seed, which was kept in cold storage before use in these experiments. The Hector × Nomini F_2 mapping population consisted of 238 individuals and the Hector × CIho 2291 F_2 mapping population consisted of 193 individuals.

Growth Chamber Inoculations and Classification of Barley Reaction to *Pyrenophora teres* f. sp. *teres*

The phenotyping of the Hector × Nomini and Hector × CIho 2291 populations was previously reported in O'Boyle et al. (2011). The NTNB-causing isolate ND89-19 is one of the most virulent isolates in North America (Wu et al., 2003; Fetch et al., 2008) and has the pathotype 1-2-6-7-10-13-16-18-25 (Wu et al., 2003). Therefore, this isolate was used in all growth chamber inoculations. The parents and F_2 populations were planted approximately 2 wk before inoculation in square plastic pots (6 by 6 by 5.5 cm) with 4 seeds pot^{-1} and 32 pots $flat^{-1}$. Resistant (Nomini and CIho 2291) and susceptible (Hector) parents and the susceptible check 'Stander' (PI 564743) were included and randomized within each flat. Establishment, fertilization, inoculum culture, and preparation and inoculation of plants were described in O'Boyle et al. (2011). Ratings were conducted using the 1-to-10 scale described by Tekauz (1985). Categorization of disease reaction was described in O'Boyle et al. (2011). Plants that received a rating of 1 through 5 were categorized as resistant (R), and plants receiving a rating of 6 through 10 were categorized as susceptible (S) for χ^2 of all phenotypic data. Data for F_2 plants derived from different F_1 plants were tested for homogeneity using a χ^2 test before pooling data.

DNA Isolation and Polymerase Chain Reaction

Barley leaf tissue was harvested from young leaves from F_2 plants of both Hector × Nomini and Hector × CIho 2291 populations. Leaf tissue was bulked from 3 to 6 plants of each parent because of the demand for a higher volume of DNA of the parental lines due to their inclusion as checks in all reactions. Leaf tissue from both populations and their respective parents was stored at −80°C before grinding using a GenoGrinder (Spex CertiPrep, Metuchen, NJ). DNA was isolated from parental materials and the Hector × Nomini F_2 population using the protocol described by Saghai Maroof et al. (1984). DNA was isolated from the Hector × CIho 2291 F_2 population using the protocol described by Pallotta et al. (2003), which allows for faster DNA isolation but with lower yields, which satisfied the demand for the current study while improving the efficiency.

Polymerase chain reaction (PCR) was conducted using two comparable methods differing only in the technique used

to fluorescently label primers, and both types of primers were used in both mapping populations. Primers obtained from ABI (Applied Biosystems Inc., Foster City, CA) were synthesized to directly contain a fluorophore (PET, 6-FAM, VIC, or NED), and are referred to as *direct-labeled primers*. The PCR amplifications using direct-labeled primers were multiplexed and performed in 12-μL reactions including 1.2 μL of 10× PCR buffer (containing 1.5 mM magnesium chloride), 0.97 μL of pooled dNTPs (2.5 mM each dNTP), 0.15 μL of each forward and reverse primer (10 μM μL^{-1}), and approximately 25 ng of DNA. Primers ordered from IDT (Integrated DNA Technologies Inc., Coralville, IA) were utilized via the nested PCR method reported by Schuelke (2000) and are referred to as *M13-labeled primers*. The PCR amplifications using M13-labeled primers were completed in 12-μL reactions including 1.2 μL of 10× PCR buffer (containing 1.5 mM magnesium chloride), 0.97 μL of pooled dNTPs (2.5 mM each dNTP), 0.96 μL of the forward primer (1 μM μL^{-1}) with an M13 tail at its 5′ end, 0.72 μL of the reverse primer (10 μM μL^{-1}), 0.72 μL of a fluorescent-labeled M13 primer (either PET, 6-FAM, VIC, or NED), and approximately 20 ng of DNA. All PCRs were performed in either an Eppendorf Mastercycler (Brinkmann Instruments, Inc., Westburg, NY) or a Bio-Rad C1000 Thermal Cycler (Bio-Rad, Hercules, CA). Amplification conditions for all primers except *GBM1215* consisted of an initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 1 min, 55 to 58°C (primer dependent) for 1 min, 72°C for 2 min, and a final extension at 72°C for 10 min. Amplification conditions for *GBM1215* consisted of an initial denaturation at 95°C for 3 min, followed by 10 cycles of 95°C for 30 s, 60°C for 40 s (decreasing by 1°C $cycle^{-1}$), 72°C for 90 s, and an additional 25 cycles of 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 50°C for 40 s, 72°C for 90 s, and a final extension at 72°C for 10 min.

Bulked Segregant Analysis

Bulked segregant analysis (BSA), as described by Michelmore et al. (1991), was initially used to screen 182 SSR markers in both Hector × Nomini and Hector × CIho 2291 F_2 populations. The level of polymorphism between the parents was 59% (108/182) between Hector and Nomini and 49% (89/182) between Hector and CIho 2291. The initial BSA only identified 10 polymorphic SSR markers in the Hector × Nomini F_2 population and three in the Hector × CIho 2291 F_2 population. In a subsequent BSA, the two resistant bulks and two susceptible bulks were comprised of equal amounts of DNA from 10 to 15 homozygous F_2 individuals (based on $F_{2:3}$ data) per bulk. An additional 60 SSR primer pairs were screened using BSA for the Hector × Nomini population of which 35 (58%) were polymorphic. The BSA for the Hector × CIho 2291 population consisted of 80 SSR primer pairs of which 40 (50%) were polymorphic. Microsatellite markers that were polymorphic between the respective parents and the corresponding susceptible and resistant bulks were then used to genotype the two F_2 populations.

Linkage Mapping

A consensus map developed for barley was used in the current study and consists of 775 SSR loci distributed across all seven chromosomes, averaging 111 SSR markers per chromosome (Varshney et al., 2007). Although chromosome 6H was the most sparsely mapped chromosome, 93 markers spanned 139.9 cM and averaged 1.5 markers cM^{-1}. Phenotypic variation explained by each marker was estimated by the coefficient of determination (r^2) value.

The PCR products were resolved using an ABI Prism 3130XL Genetic Analyzer (Applied Biosystems, Foster City, CA) following the manufacturer's instructions using GeneScan −500 LIZ (Applied Biosystems, Foster City, CA) as an internal size standard for each sample. Fragment analysis was conducted with GeneMarker v1.4 (SoftGenetics LLC, State College, PA). Linkage maps were generated using MAPMAKER 3.0 software (Lander et al., 1987). Graphical depiction of linkage maps were generated using MapChart (Voorrips, 2002). The Kosambi function was used to determine centimorgan estimates between adjacent markers. Based on results from a previous study (O'Boyle et al., 2011), classical mapping was conducted using two discrete phenotypic classifications (resistant and susceptible), as opposed to a QTL analysis.

RESULTS

Phenotyping of the Hector × Nomini and Hector × CIho 2291 Mapping Populations

All phenotyping for the current study was previously reported in O'Boyle et al. (2011). Inoculated plants were screened using the 1-to-10 scale. Screening of the F_2 populations derived from a partial diallel indicated that each resistant parent (Nomini and CIho 2291) has a distinct single dominant gene for resistance to NTNB. Phenotyping of the $F_{2:3}$ families of these populations confirmed that Nomini and CIho 2291 each have a single dominant NTNB resistance gene (O'Boyle et al., 2011).

Linkage Mapping in a Hector × Nomini F_2 Population

The BSA identified 45 microsatellite markers that were polymorphic in the Hector × Nomini F_2 population. These markers had been previously mapped to each of the barley chromosomes, with the exception of chromosome 3H. A set of 28 microsatellite primer pairs ultimately selected to screen the entire F_2 population included markers that had been previously mapped to each of the barley chromosomes, again with the exception of chromosome 3H. Results of preliminary linkage analysis (not shown) indicated the gene governing NTNB resistance in Nomini was on chromosome 6H; therefore, emphasis was placed on screening markers that had previously been mapped to this chromosome.

The linkage map of chromosome 6H developed using the Hector × Nomini F_2 population included 10 SSR markers and spanned a total of 84.1 cM, averaging 8.4 cM between adjacent markers. Fragment size amplified by each parent for each marker on the Hector × Nomini linkage map is presented in Table 2. The gene governing NTNB resistance in Nomini was mapped to a 9.2-cM region of chromosome 6H flanked by SSR markers *Bmag0344a* and *Bmag0103a*, which were 6.8 and 2.4 cM from the gene and explained 70 and 90% of the phenotypic variation, respectively. The linkage map of barley chromosome 6H developed for the Hector × Nomini F_2 population is presented in Fig. 1.

Table 2. Marker name, fragment-size amplified by the net blotch susceptible parent 'Hector' and resistant parents 'Nomini' and CIho 2291, fluorescence method, and annealing temperature of markers used to map *Rpt-Nomini* and to barley chromosome 6H.

Marker name	cM‡	Allele†			Fluorescence§	Annealing temperature¶
		Hector	Nomini	CIho 2291		
		bp				°C
Bmag0500	31.65	181	167	183	M13 tailed	58
GBM1215	39.54	237	229	n/a#	M13 tailed	Touchdown
Bmag0173	57.79	170	n/a	172	M13 tailed	58
GMS006	57.88	173	n/a	171	M13 tailed	58
hvm65	62.11	124	122	n/a	Direct labeled	58
Bmac0018	61.79	137	131	n/a	Direct labeled	58
Bmag0496	63.76	202	190	196	Direct labeled	58
Bmag0009	62.21	170	172	n/a	Direct labeled	58
hvm14	62.28	162	160	n/a	Direct labeled	58
Bmgtttttt0001	71.86	225	207	222	Direct labeled	58
Bmag0344a	67.83	176	180	182	M13 tailed	58
Bmag0103a	66.05	166	164	n/a	M13 tailed	58
Bmag0040a	UNK	215	n/a	244	M13 tailed	58

† Base pairs (bp) amplified by the susceptible parent 'Hector' and the resistant parents 'Nomini' or CIho 2291. Polymerase chain reaction (PCR) products were resolved using an ABI Prism 3130XL Genetic Analyzer (Applied Biosystems, Foster City, CA).

‡ Marker position in centimorgans (cM) based on 2007 simple sequence repeat consensus map (Varshney et al., 2007); unknown position (UNK).

§ Designates the method used for fluorescent labeling of amplified fragments. Direct labeled = primers ordered from ABI (Applied Biosystems Inc., Foster City, CA) with a fluorescent dye label. M13 tailed = primers ordered from IDT (Integrated DNA Technologies Inc., Coralville, IA) and labeled using a nested PCR method (Schuelke, 2000).

¶ Indicates the annealing temperature used in amplification. *GBM1215* was amplified using a touchdown PCR method with the initial annealing temperature of 60°C and a final annealing temperature of 50°C.

Some markers were not polymorphic in one population or the other and were not used to screen both populations (were used only in either the Hector × Nomini or the Hector × CIho 2291 population).

Linkage Mapping in a Hector × CIho 2291 F_2 Population

The BSA identified 43 microsatellite markers that were polymorphic in the Hector × CIho 2291 F_2 population. A set of 25 microsatellite primer pairs ultimately selected to screen the entire F_2 population included markers that had been previously mapped to all seven of the barley chromosomes. Results of linkage analysis indicated the gene governing NTNB resistance in CIho 2291 was on chromosome 6H.

The linkage map of chromosome 6H developed using the Hector × CIho 2291 F_2 population included seven SSR markers and spanned 83.9 cM, averaging 12.0 cM between markers. The fragment size amplified by each parent is presented in Table 2. The gene governing NTNB resistance in CIho 2291 was mapped to a 34.3-cM region on the short arm of chromosome 6H with the flanking markers *Bmag0500* and *Bmag0173*, which were 24.4 and 9.9 cM from *Rpt-CIho2291*, and explained 26 and 65% of the phenotypic variation, respectively. The only other SSR markers that had been previously mapped to the *Bmag0500–Bmag0173* interval are from the Gatersleben Barley Microsatellite set (Varshney et al., 2007) but did not amplify in the Hector × CIho 2291 mapping population, preventing the identification of markers that are more tightly linked to *Rpt-CIho2291*. The linkage map of chromosome 6H developed for the Hector × CIho 2291 population is presented in Fig. 1.

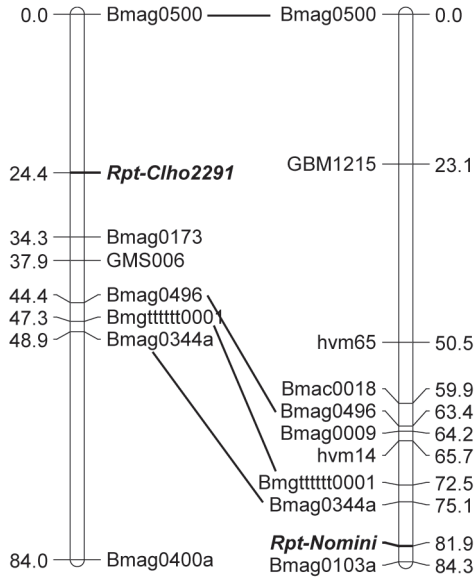

Figure 1. Linkage map of barley chromosome 6H based on simple sequence repeat markers screened in F_2 populations derived from a cross between the net blotch susceptible parent 'Hector' and the resistant parents 'Nomini' and CIho 2291.

DISCUSSION

Several authors previously reported barley net blotch resistance genes at different locations on chromosome 6H (Steffenson et al., 1996; Manninen et al., 2000; Cakir et al., 2003; Ma et al., 2004; Emebiri et al., 2005; Friesen et al., 2006; Grewal et al., 2008; Abu Qamar et al., 2008; St. Pierre et

al., 2010). While it is difficult to ascertain whether these studies have mapped a common gene or multiple loci for net blotch resistance on chromosome 6H, Abu Qamar et al. (2008) demonstrated that at least two independent recessive net blotch resistance genes are on 6H. Other studies have identified dominant genes conferring net blotch resistance on chromosome 6H (Table 1), suggesting that these genes may be independent of those mapped by Abu Qamar et al. (2008). It is therefore possible that several independent loci conditioning net blotch resistance are on chromosome 6H. In the current study, two net blotch resistance genes were mapped to chromosome 6H. These two genes are temporarily designated *Rpt-Nomini* and *Rpt-CIho2291*, until their relationship with net blotch resistance genes previously mapped to barley chromosome 6H is determined in allelism tests.

Genetic analysis of an F_2 population derived from a cross between CIho 2291 and Nomini segregated 285R:11S, fitting a 15:1 ratio (O'Boyle et al., 2011). This indicated that these parents each have a single dominant gene governing NTNB resistance. Although the NTNB resistance genes in both CIho 2291 and Nomini mapped to chromosome 6H, they segregated with a recombination frequency of approximately 40%. Based on their relationship to the SSR marker *Bmag0344a*, which was mapped in both Hector × CIho 2291 and Hector × Nomini F_2 populations, it can be inferred that *Rpt-Nomini* and *Rpt-CIho2291* are at least 30 cM apart on chromosome 6H. A more accurate assessment of the distance between the two genes is complicated due to the low number of markers that were polymorphic in both populations for this linkage group. The only markers that were mapped to chromosome 6H and were polymorphic in both populations were *Bmag0500*, *Bmag0496*, *Bmgtttttt0001*, and *Bmag0344a*.

Previous studies mapping genes for net blotch resistance have reported the presence of loci conditioning resistance in the region of barley chromosome 6H to which *Rpt-Nomini* was mapped. Grewal et al. (2008) identified a major QTL governing NTNB resistance in the resistant barley line TR251, designated as *QRpt6*, on chromosome 6H, between the flanking SSR markers *Bmag0009* and *Bmag0496*. These markers were both mapped in the current study and were 17.7 and 18.5 cM distal to *Rpt-Nomini*, respectively. Additionally, both *Bmag0009* and *Bmag0496* were within 2 cM of the net blotch QTL mapped by St. Pierre et al. (2010). The gene conditioning NTNB resistance in the barley line CIho 9819 was also mapped to barley chromosome 6H (Manninen et al., 2000); however, retrotransposon-based markers were used for linkage mapping in the study, which prevented a direct comparison.

Previous studies mapping net blotch resistance genes have also reported the presence of loci conditioning resistance near the region of barley chromosome 6H where *Rpt-CIho2291* was mapped in the current study. The closely linked recessive net blotch resistance genes *rpt.r* and *rpt.k* are both linked at <5 cM to the SSR marker *Bmag0173* (Abu Qamar et al., 2008), which is one of the markers flanking *Rpt-CIho2291*. The location of *Rpt-CIho2291* on chromosome 6H is 9.9 cM distal to *Bmag0173*, while both *rpt.r* and *rpt.k* were proximal in relation to *Bmag0173*. This suggests that a third gene for net blotch resistance may be located in this region of chromosome 6H, or a chromosomal inversion or deletion may have occurred. Allelism tests will be necessary to further examine the relationship of *Rpt-CIho2291* with *rpt.r* and *rpt.k*. A dominant gene conditioning resistance to three *P. teres* isolates in the barley line SM89010 also mapped to chromosome 6H and was proximal to *Bmag0173* (Friesen et al., 2006). It is unknown whether the gene conditioning NTNB resistance in SM89010 is either a dominant allele of *rpt.r* or *rpt.k*, or an independent net blotch resistance gene. Cakir et al. (2003) also identified a major QTL linked to *Bmag0173* in two distinct DH mapping populations. This QTL accounted for 83 and 66% of the phenotypic variation for NTNB resistance in Tallon × Kaputar and VB9524 × ND11231 DH populations, respectively.

In conclusion, the dominant net blotch resistance genes *Rpt-Nomini* and *Rpt-CIho2291* were mapped to distinct regions of barley chromosome 6H and were at least 30 cM apart. The approximate chromosomal location of these genes is based on flanking SSR markers that have been previously reported to be associated with net blotch resistance. Future research that would facilitate pyramiding of multiple genes for net blotch resistance includes conducting allelism tests between barley lines that reportedly have net blotch resistance genes on chromosome 6H, and saturation of the *Rpt-CIho2291* region with additional molecular markers to identify markers that are tightly linked to *Rpt-CIho2291* and could be used in marker-assisted selection (MAS). Many techniques have been used in recent years to increase marker saturation in linkage mapping studies. In particular, Diversity Arrays Technology (DArT) markers (Wenzl et al., 2004) have been used extensively in barley to develop high-density maps. The practical use of both genes described in the current study would benefit from further marker saturation using such a technique. Linkage maps derived from DArT can be integrated with linkage maps derived from other types of markers to achieve optimum marker density (Wenzl et al., 2006). As the chromosomal location of *Rpt-Nomini* and *Rpt-CIho2291* has been identified in the current study, an emphasis could be placed on saturating this region as opposed to a whole-genome scan. Marker-assisted selection for NTNB resistance derived from Nomini can be conducted using the flanking microsatellite markers *Bmag0103a* and *Bmag0344a*, as in the current study these markers accounted for 90 and 70% of the phenotypic variation for net blotch resistance, respectively. In the event of monomorphism of these markers in a breeding population, the microsatellite marker *Bmgtttttt0001* may provide an alternative for MAS based on its location 2.6 cM

distal to *Bmag0344a*. Additionally, MAS for NTNB resistance based on the microsatellite marker *Bmag0173*, which accounted for 65% of the phenotypic variation in the current study, may be applied in breeding populations with NTNB resistance derived from CIho 2291, ND11231 (Cakir et al., 2003; Emebiri et al., 2005), Kaputar (Cakir et al., 2003), or SM89010 (Friesen et al., 2006). While allelism tests have not been conducted to compare the resistance genes in these resistant parents, *Bmag0173* was linked to net blotch resistance derived from each of these parents. Further studies to facilitate the adoption of these genes in barley breeding programs would include examining their effectiveness across different breeding populations with diverse genetic backgrounds, if possible, using markers that are more tightly linked and flanking the resistance genes. Marker–trait associations are not always conserved across populations, making widespread adoption of molecular markers for MAS complicated. Once the effectiveness of MAS across populations using these markers can be demonstrated, pyramiding of *Rpt-Nomini* and *Rpt-CIho2291* into elite backgrounds will provide a sound net blotch control strategy and help ensure the durability of net blotch resistance.

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