Mapping Net Form Net Blotch and Septoria Speckled Leaf Blotch Resistance Loci in Barley

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ABSTRACT

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Septoria speckled leaf blotch (SSLB), caused by *Septoria passerinii* Sacc., and net form net blotch (NB), caused by *Pyrenophora teres* f. *teres* Drechsler, are fungal diseases that decrease the yields of barley in the Upper Midwest. An effective way to manage these diseases is to plant resistant cultivars. To characterize the genetics of resistance to both pathogens, two advanced barley breeding lines, one resistant to NB (M120) and another resistant to SSLB (Sep2-72), were crossed, creating a population of 115 recombinant inbred lines. The two parents and the population were evaluated in three greenhouse seedling assays for each pathogen and for simple-sequence repeat and diversity arrays technology markers. Composite interval mapping revealed two major quantitative trait loci (QTL) associated with NB on chromosome 6H, located in bins 2 and 6. The QTL located in bin 6 explained 19 to 48% of the phenotypic variation and the QTL located in bin 2 explained 25 to 44% of the phenotypic variation. A new locus for resistance to SSLB, *Rsp4*, was identified on chromosome 6H, located in bins 3 to 4. Mapping these genes in elite breeding germplasm will accelerate the development and utilization of marker-assisted selection to enhance resistance to these diseases.

Additional keywords: disease resistance, *Hordeum vulgare*.

Septoria speckled leaf blotch (SSLB), caused by *Septoria passerinii* Sacc., and the net form of net blotch (NB), caused by *Pyrenophora teres* f. *teres* Drechsler (anamorph: *Drechslera teres* f. *teres* (Sacc.) Shoemaker), are residue-borne diseases that affect barley (*Hordeum vulgare* L.) in many parts of the world. In the Upper Midwest region of the United States, development of both diseases is favored by warm temperatures (20 to 24°C) and high humidity. Dispersal of *S. passerinii* spores is facilitated by splashing rain, whereas conidia of *D. teres* f. *teres*, the repeating spore stage of the pathogen, are disseminated primarily by wind. Both diseases can reduce yields by as much as 10 to 40% (14). Methods to manage SSLB and NB are tillage and crop rotation to avoid planting into residue, application of fungicides, and planting resistant cultivars (15). Because many farmers have adopted reduced-tillage practices, and fungicides are not economical on a large scale, substantial effort has been invested in breeding for disease resistance to SSLB and NB.

Breeding for resistance to both of these diseases can be accomplished either by selection in greenhouse seedling assays or by adult-plant screening in the field (24,29). Although these methods are generally effective, field screening, in particular with SSLB, can be affected by weather and results can be inconsistent (B. Steffenson, *unpublished*). From a breeding perspective, it would be useful to have marker-assisted selection (MAS) strategies that would permit screening in early generations (i.e., F_2) and on single plants. Genetic mapping studies have identified resistance loci for NB on all seven chromosomes (2,4,5,7,10–13,16, 17,23,24,35). Similarly, several loci have been identified for resistance to SSLB: *Rsp1* located on chromosome 3H (9), *Rsp2* and *Rsp3* on 1H (9,37), and two unnamed loci on chromosomes 2H and 6H bin 6 (36).

A major obstacle to utilizing information from quantitative trait loci (QTL) studies is that mapping parents are often chosen to be genetically diverse compared with the relevant breeding germplasm and lack genes necessary for regional adaptation and intended end use. This "wide cross" approach serves to ensure that there is adequate marker polymorphism to generate complete linkage maps as well as sufficient phenotypic variation to map the trait of interest. A disadvantage to this approach is that segregation for many other traits in the mapping population, such as maturity, may complicate accurate assessment of disease phenotypes. In addition, it may turn out that the resistance gene or QTL that is mapped is not useful for breeding because it is tightly linked to undesirable alleles (linkage drag) or the gene itself may have an undesirable effect. Thus, it may require several generations of breeding, even with the aid of markers, to produce useful cultivars. One way to avoid this situation is to conduct mapping in elite breeding germplasm in which resistance has already been introduced. This ensures that the disease resistance is already functional in the relevant germplasm and is free of undesirable linkages. However, a disadvantage of mapping in elite germplasm is that there may be insufficient marker polymorphism to conduct whole-genome scans for QTL. This latter issue has been made less important in barley with the recent development of numerous high-throughput polymerase chain reaction (PCR)-based markers (25,28,33,34).

The Minnesota breeding program routinely conducts phenotypic selection for resistance to NB and SSLB. Here, we report the use of an elite breeding population to map the location of genes that have already been shown to be effective in the breeding program and appear to be free of undesirable linkages. The specific objectives were to (i) characterize the segregation of resistance to SSLB and NB in a barley breeding population and (ii) map the location of resistance loci for the two diseases.

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MATERIALS AND METHODS

Plant material. Two advanced barley breeding lines were crossed: one resistant to SSLB and susceptible to NB (Sep2-72) and another resistant to NB and susceptible to SSLB (M120). The F_1 was self pollinated to produce a segregating F_2 population, which was advanced by single-seed descent to the F_4 generation, creating a population (SNB1) of 115 recombinant inbred lines (RILs). The pedigree of the parents used to generate the SNB1 mapping population is shown in Figure 1. Both of the parents (M120 and Sep2-72) have the common parent Lacey, which is a six-rowed spring barley cultivar developed for malting quality (19). PC-84 is the original source of SSLB resistance (8) and Heartland is the presumed source of NB resistance (3,27). Sep2-72 was identified as an SSLB-resistant progeny from the cross between M94-172 and Lacey. M120 was identified as an NB-resistant progeny from the cross between CDC Tisdale and Lacey.

Disease assessment. To evaluate resistance to SSLB and NB, three experiments for each disease were conducted in a greenhouse at the Plant Growth Facility on the St. Paul campus of the University of Minnesota. Each experiment consisted of a randomized complete block design with two replications. The population, parents, and resistant and susceptible controls were planted in each experiment. For the NB experiments, the resistant and susceptible controls were CDC Tisdale and Robust (20), respectively, and, for the SSLB experiment, the controls were CIho4780 (30) and Stander (21).

For the NB experiments, five seeds of each line were sown in conetainers (3.8 cm in diameter by 21.6 cm in height) containing MetroMix 200 (Sun Gro Horticulture CM Ltd., Canada) (50 to 60% vermiculite, Canadian sphagnum peat moss, perlite, dolomitic limestone, wetting agent). The plants were grown in the greenhouse at 18 to 27°C; days were extended to 16 h with 1,000-W sodium vapor lights (530 to 710 $\mu Em^{-2}s^{-1}$). Plants were fertilized with a 20-20-20 (N-P-K) fertilizer at second-leaf emergence (≈10 days after planting). After the third leaf emerged (≈19 days after planting), the seedlings were inoculated with *P. teres* f. *teres* (24) using a mixture of the five isolates 3010001, 30190005-2, 30199019-1, 30199012-2, and 30199010-3. Conidia were suspended in sterile water (25,000 conidia/ml) with one drop of Tween 20 per 100 ml of the conidial suspension. Plants were inoculated using a 1.5-liter handheld pump sprayer until runoff. Plants were then placed in the dew chamber with 100% humidity at 22°C for 24 h and returned to the greenhouse. Seven days after inoculation, the reaction of plants to NB was assessed using the 1-to-10 rating scale of Tekaus (26), with the higher number representing greater susceptibility. Analysis of variance for NB data was performed with PROC GLM using SAS (22).

For the SSLB experiment, four seeds of each line were grown in 10-by-10-cm square plastic pots with MetroMix 200. The plants were grown in the greenhouse at 19 to 26°C with 14 h of light per day using 1,000-W sodium vapor lamps (530 to 710 $\mu Em^{-2}s^{-1}$). Osmocote 14-14-14 (Scott's Co., Marysville, OH) and Peters Dark Weather 15-0-15 (Scott's Co.) fertilizers were applied at planting. In addition, Peters (20-10-20) fertilizer was applied every 2 weeks. Inoculum of *S. passerinii* was prepared following the procedures of Toubia-Rahme and Steffenson (29) using isolate Sp97-15. One drop of Tween 20 was added per 100 ml of the conidial suspension (2.5×10^6 conidia/ml). Inoculum was applied to plants at the two-and-a-half-leaf stage (15 days after planting) at a rate of 1.7 ml per pot using an artist's airbrush pressurized at 0.138 MPa. During the infection period, plants were placed in mist chambers with temperatures of 19 to 24°C at nearly 100% humidity with various periods of darkness and light. Plants were initially incubated in the dark for 40 h, then 5 h of light, 16 h of darkness, 5 h of light, 2 h of darkness, and finally 5 h of light while the plant surfaces dried slowly with the chamber doors opened. Plants were then moved to the greenhouse for incubation, and disease evaluations were done 18 days after inoculation. To obtain a phenotypic score for each line, the four plants were scored individually using a 0-to-5 rating scale, with the higher number indicating greater susceptibility (29). The four individual plant scores were then averaged to obtain an overall score for the line. Lines giving a score of 0 to 2 were considered resistant and those with a score of 3 to 5 were considered susceptible. Lines with plants exhibiting clear resistant and susceptible reactions were considered segregating. Resistance to SSLB was mapped as a single-gene trait using the phenotypic data and classifying lines as resistant, susceptible, or segregating. Resistant lines were designated as homozygous for the Sep 2-72 allele and susceptible lines homozygous for the M120 allele.

Marker data. Leaf tissue (≈2 cm in length) was harvested from seedlings of the parents and lines to isolate genomic DNA using a cetyltrimethylammonium bromide mini-prep protocol (6). Each DNA sample was generated from a bulk of four plants from each parent or line. PCRs were performed using published simple-sequence repeat (SSR) and reaction conditions (18). The PCR products were separated on 6% polyacrylamide gels and visualized by silver staining (1). A total of 144 SSR markers was screened on the parents to identify polymorphisms. The DNA of the parents and lines was sent to Triticarte (Yarralumla, Australia) to screen for diversity arrays technology (DArT) (34) markers across the whole genome.

Resistance gene mapping. Linkage maps were constructed using linear regression analysis with JoinMap 4.0 (32). Maps, which included SSLB scored as a single gene, were constructed using lines having <17% missing data and SSR and DArT markers containing <15% missing disease data. When markers co-segregated, the marker with the lower χ^2 was selected for inclusion in the map. We selected the genetic map with the most markers and a log of the likelihood ratio (LOD) score >3.0 for subsequent QTL analysis.

We conducted composite interval mapping, using PlabQTL (31), to identify QTL for NB. The LOD score threshold of 2.45 for detection of a QTL was set using an experiment-wide error rate of $P < 0.05$, which corresponds to a comparison-wise error rate of $P = 0.0036$. The data from each NB experiment were analyzed separately.

Comparisons with previous NB studies. Several previous studies have reported QTL for NB resistance in the centromeric region of chromosome 6H. To better examine our findings in the context of this work, we located previously mapped NB resistance QTL on two consensus maps (33,35). The QTL were placed on the consensus maps by using markers that are both located on the consensus map and associated with the QTL in the published study. From this analysis, we were able to place 10 previously mapped QTL on the consensus maps (Fig. 2). The QTL from Spaner et al. (23) was not placed on a consensus map due to lack of common markers.

RESULTS

Disease response. The parents of the mapping population Sep2-72 and M120 were resistant (overall score 1.29) and

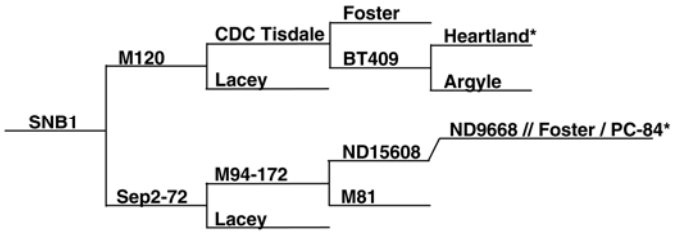

Fig. 1. Pedigree for the SNB1 population. Heartland is presumed as the original source of net form net blotch resistance (3,27), and PC-84 is the original source of Septoria speckled leaf blotch resistance (8).

susceptible (overall score 4.25) to SSLB, respectively. In the population, 81 of the 115 lines were given an identical score (resistant, segregating, and susceptible) in all three experiments. The rest of the lines exhibited more variable reactions across the experiments and needed to be analyzed in more detail to achieve a consensus score. These lines fell into three categories. The first category was when a line was scored consistently (i.e., either resistant or susceptible) in two experiments and segregating in the third. The consensus score for lines in this category was the consistent score. The second category occurred when a line was scored as segregating in two or more experiments. The consensus score for this category was segregating. The final category was when plants within a line were scored as resistant and susceptible in separate experiments. Only six lines fit this category and were considered segregating.

The ratio of lines resistant/segregating/susceptible to SSLB in the population based on the consensus phenotype for the three experiments was 49:12:54, which fits the expected segregation ratio for a single gene (53.9:7.2:53.9) in the F_4 generation (χ^2 = 2.21, P = 0.16 for χ^2 test). For NB, significant variation (P < 0.0001) was detected for resistance among the lines in the breeding population in each of the three experiments (Table 1). When the data were pooled across all three experiments, we found a significant experiment–line interaction (P = 0.0005). The heritability, as calculated on an entry-mean basis for each experiment, was 0.56 to 0.78.

Marker linkage map. Among the 144 SSR markers screened on the parents, 37 (25.7%) were polymorphic and were subsequently evaluated on the population. The DArT platform we used to screen our population features 2,000 markers, of which 155

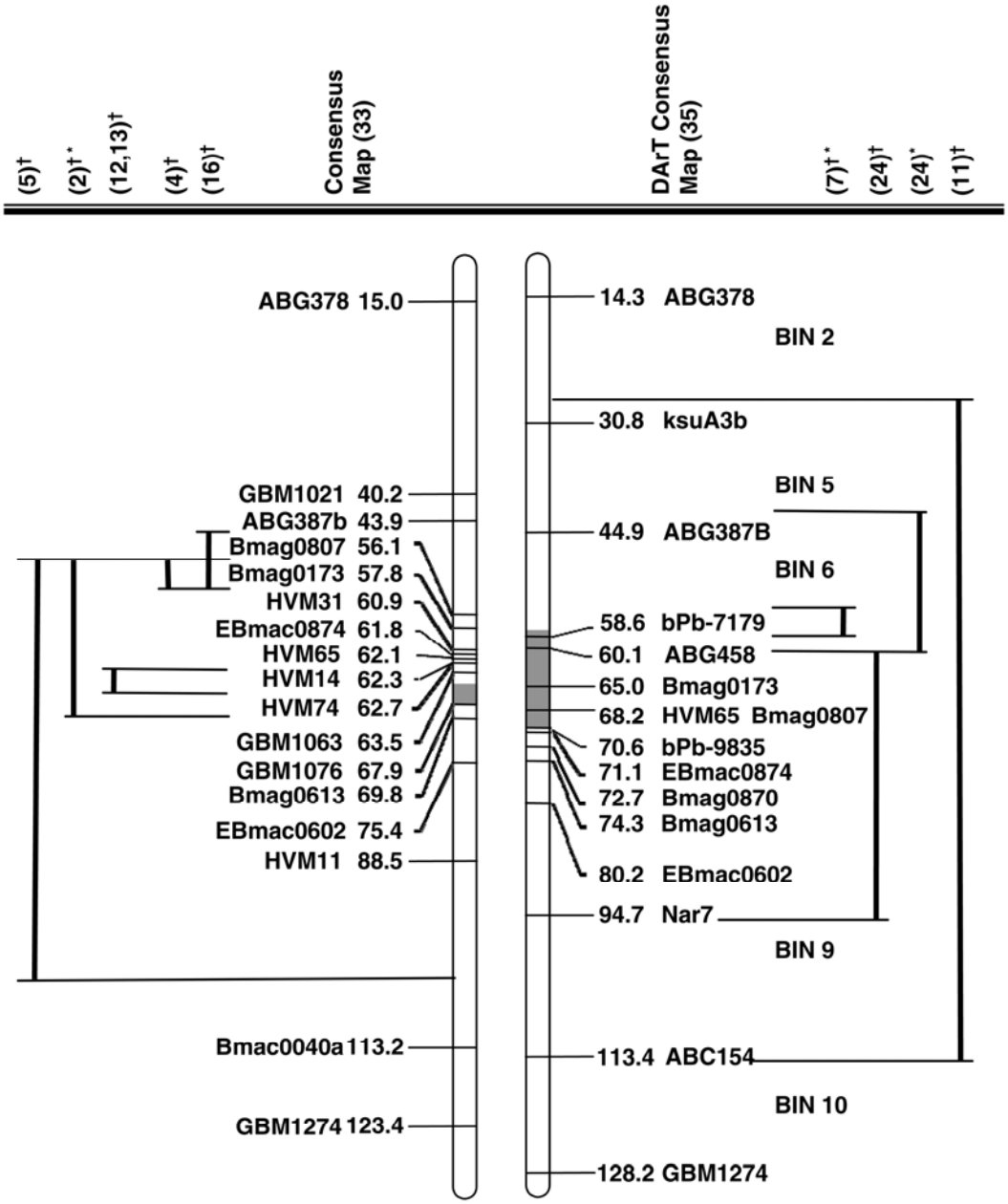

Fig. 2. Quantitative trait loci (QTL) regions for net form net blotch (NB) resistance on chromosome 6H extending from bins 2 to 6 identified in the SNB1 population and previously published studies. Two consensus maps that contain common markers are aligned to display the QTL regions (33,35). QTL identified in previous studies were placed on the consensus map using markers in common between the QTL study and one of the consensus maps. Bin assignments were determined using the diversity arrays technology (DArT) consensus (35) and barley Bin map (http://barleygenomics.wsu.edu/all-chr.pdf). Each QTL is labeled with the citation and the plant stage in which resistance was assessed: seedling (†) or adult (*). The shaded region on the consensus maps indicates the position of the bin 6 QTL identified in the SNB1 population.

produced a useful signal and also segregated in the population. In total, 111 lines with <17% missing genomic data were used and 95 markers containing <15% missing data were included in the map. There were 7, 20, 29, 18, 15, and 6 markers mapped on chromosomes 1H, 2H, 3H, 4H, 6H, and 7H, respectively. Marker numbers for chromosome 5H were insufficient to produce a linkage map. The overall average distance between markers was 3.6 centimorgans (cM). By comparing common markers on our map and the published DArT map (35), we estimate ≈40% coverage of the genome in the Sep2-72/M120 population.

Resistance loci. A new locus, *Rsp4*, associated with resistance to SSLB was identified on chromosome 6H, bins 3 to 4 (Fig. 3). Composite interval mapping was used to identify QTL for NB resistance. The data from each environment were analyzed separately, and there were between two and four co-factors included in the models. Two major QTL associated with NB resistance were identified on chromosome 6H, located in bins 2 and 6. The resistance allele for the NB QTL located in 6H bin 6 was contributed by the NB-resistant parent (M120), explained 19 to 48% of the phenotypic variation, and was detected in all three experiments. The resistance allele for the NB QTL located in 6H bin 2 was contributed by the NB-susceptible parent (Sep2-72), explained 25 to 44% of the phenotypic variation for NB, and was detected in two of the three experiments.

DISCUSSION

The gene for resistance to SSLB detected in this study appears to be new. Previous studies have identified several loci conferring resistance to *S. passerinii* (i.e., *Rsp* loci): *Rsp1* on chromosome 3H (9) and *Rsp2* and *Rsp3* on 1H (9,37). Two unnamed loci are located on chromosomes 2H bins 7 to 11 and 6H bins 10 to 14 (36). This is the first report of a gene for SSLB resistance in bin 3 of chromosome 6H. Therefore, this new gene is given the locus designation of *Rsp4* and allele designation of *Rsp4.d*.

It is not clear whether the NB resistance QTL identified in this study is new. Previous studies have positioned NB resistance QTL using different germplasm lines (Fig. 2). Major QTL for NB resistance at the seedling growth stage have been mapped to chromosome 6H bin 6 (2,4,5,7,11–13,16,24). Major QTL for adult plant resistance to NB also have been mapped to the same region (2,7,23,24). For NB of barley, different genes control resistance at the seedling and adult plant stages (11). An objective in the study by Steffenson et al. (24) was to locate QTL for NB resistance at both the seedling and adult plant stages. Seedling resistance QTL were located on chromosomes 4H and 6H. For both of these chromosomes, adult plant resistance also was identified in the same chromosome regions. It should be noted that QTL conferring resistance at different ontogenetic stages were located in the same chromosome region, indicating a possible cluster of genes. A recent study has shown that there are at least two resistance genes that are tightly linked in repulsion in this region of chromosome 6H (16).

By developing a mapping population using elite lines that each carried resistance to a different disease, we were able to identify resistance loci to both SSLB and NB in the same population. In addition, because the two elite parents have been used in breeding, the identified genes are likely segregating in other populations in the breeding program. Furthermore, these parents are three cycles of breeding from the sources of resistance and are advanced lines in the breeding program. Multiple years of testing for both of these parents show that they are agronomically competitive and have acceptable malting quality (data not shown). This suggests that there will not be any linkage drag associated with these resistance alleles when used in breeding. One promising progeny in the SNB1 population, SNB1-66, carries the resistance alleles at both NB QTL and *Rsp4,* was scored as resistant to SSLB in all three experiments, and had the lowest average NB score.

The challenge of whole-genome mapping in a genetically narrow biparental population is identifying sufficient polymorphic markers. In total, 144 SSR markers and 2,000 DArT markers (34) were screened on the parents to identify polymorphisms. Only 192 (9%) markers were polymorphic throughout the genome. This is very low compared with typical mapping populations that use diverse parents. A study using a wild × cultivated barley mapping population reported 296 out of 395 (75%) SSR markers polymorphic between the parents (36).

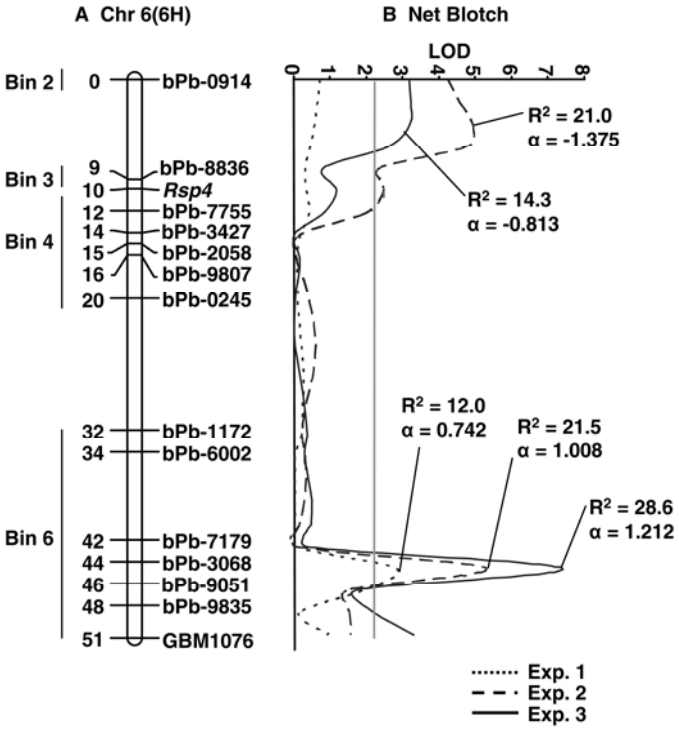

Fig. 3. A, Linkage map of chromosome 6H extending from bins 2 to 6 and showing quantitative trait loci (QTL) for net form net blotch resistance. Bin assignments were determined by diversity arrays technology (DArT) consensus map (35) and the barley Bin map (http://barleygenomics.wsu.edu/all-chr.pdf). **B,** QTL log of the likelihood ratio (LOD) scan for net blotch on chromosome 6H. The threshold for detection was 2.45, which corresponds to an experiment-wise error rate of $P = 0.05$ and a comparison-wise error rate of $P = 0.003$. Peaks of the QTL are located at 4 and 44 centimorgans.

TABLE 1. Net blotch scores for parents (M120 and Sep2-72), recombinant inbred line (RIL) SNB1-66, and the mean and ranges for the RIL population[a]

	Parents			M120 × Sep2-72 RIL population		
Experiment	M120	Sep2-72	SNB1-66	Mean	Range	LSD[b]
1	7.0	9.5	5.0	7.0	5.0–9.5	0.26
2	4.0	7.0	2.0	4.9	2.0–8.5	0.34
3	2.8	6.5	1.0	3.8	1.0–10.0	0.25

[a] Disease resistance readings based on a 1-to-10 scale (26).
[b] Least significant difference ($P = 0.05$).

The use of MAS will help to identify lines carrying resistance alleles at these loci without phenotypic screening for disease resistance. All of the markers that mapped to the regions of interest were DArT markers. Although DArT markers are very efficient and cost effective to scan the genome, they are not directly useful for MAS strategies to target one or a few QTL. To use DArT markers for MAS, it is necessary to sequence them and develop primers that can detect insertions, deletions, or single-nucleotide polymorphisms. Alternatively, one can use consensus maps to identify other markers that are more appropriate for MAS. We have identified a single SSR marker within 6 cM of *Rsp4* (Bmag500) based on consensus maps. Similarly, we identified 13 SSR markers within 2 cM of the 6H NB resistance QTL (Ebmac787, Bmac040, Bmag807, Ebmac624, Bmac018, Ebmac639, GBM1389, HVM65, Bmag009b, Bmag496, Ebmac607b, Ebmac767, and Ebmac874). These markers, if polymorphic in the populations of interest, should permit selection for resistance to NB and SSLB in early generations and without the use of greenhouse assays or field disease nurseries.

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