Molecular Mapping of Loci Conferring Susceptibility to Spot Blotch and Resistance to Powdery Mildew in Barley Using the Sequencing-Based Genotyping Approach

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ABSTRACT

Spot blotch (SB) caused by *Bipolaris sorokiniana* and powdery mildew (PM) caused by *Blumeria graminis* f. sp. *hordei* are two important diseases of barley. To map genetic loci controlling susceptibility and resistance to these diseases, a mapping population consisting of 138 recombinant inbred lines (RILs) was developed from the cross between Bowman and ND5883. A genetic map was constructed for the population with 852 unique single nucleotide polymorphism markers generated by sequencing-based genotyping. Bowman and ND5883 showed distinct infection responses at the seedling stage to two isolates (ND90Pr and ND85F) of *Bipolaris sorokiniana* and one isolate (Race I) of *Blumeria graminis* f. sp. *hordei*. Genetic analysis of the RILs revealed that one major gene (*Scs6*) controls susceptibility to *Bipolaris sorokiniana* isolate ND90Pr, and another major gene (*Mla8*) confers resistance to *Blumeria graminis* f. sp. *hordei* isolate Race I, respectively. *Scs6* was

mapped on chromosome 1H of Bowman, as previously reported. *Mla8* was also mapped to the short arm of 1H, which was tightly linked but not allelic to the *Rcs6/Scs6* locus. Quantitative trait locus (QTL) analysis identified two QTLs, *QSbs-1H-P1* and *QSbs-7H-P1*, responsible for susceptibility to spot blotch caused by *Bipolaris sorokiniana* isolate ND85F in ND5883, which are located on chromosome 1H and 7H, respectively. *QSbs-7H-P1* was mapped to the same region as *Rcs5*, whereas *QSbs-1H-P1* may represent a novel allele conferring seedling stage susceptibility to isolate ND85F. Identification and molecular mapping of the loci for SB susceptibility and PM resistance will facilitate development of barley cultivars with resistance to the diseases.

Keywords: genetics and resistance

Spot blotch (SB) and powdery mildew (PM) of barley are caused by *Bipolaris sorokiniana* (syn. *Cochliobolus sativus*) and *Blumeria graminis* f. sp. *hordei*, respectively. These two fungal pathogens have different lifestyles. *Bipolaris sorokiniana* is considered hemibiotrophic (Kumar et al. 2002), whereas *Blumeria graminis* f. sp. *hordei* is biotrophic (Both and Spanu 2004). Epidemics of the two diseases can significantly reduce yield and quality of the crop. SB occurs mainly in North America, especially in the northern Great Plains of the United States and Canadian prairies, and 33% of yield loss has been reported for the disease (Mathre 1997; Wilcoxson et al. 1990). PM is commonly observed in almost all regions of the world where barley is grown; it is the most prevalent barley disease in Europe (Dreiseitl 2011; Jørgensen 1994) and the second most important disease in Australia, with the potential to reduce annual production by \$103 million (Murray and Brennan 2010). Yield losses to PM range from 5 to 20% and reach 40% under favorable conditions (Chaure et al. 2000). Use of host resistance is the most effective method for managing these two important diseases.

In the past several decades, a number of resistance sources for SB have been identified, and major genes and quantitative trait loci (QTLs) for resistance to the disease have been mapped. For

example, Steffenson et al. (1996) and Bilgic et al. (2005) identified a gene (*Rcs5*) on chromosome 7H conferring seedling SB resistance to pathotype 1 (ND85F) of *Bipolaris sorokiniana* by using a doubled haploid (DH) population derived from the cross between Steptoe and Morex. Further studies indicated that *Rcs5* is a recessive allele for resistance to SB, and it is the dominant allele at the same locus that confers susceptibility to the disease (Ameen 2019). Bilgic et al. (2006) identified a gene (designated as *Rcs6*) on chromosome 1H conferring resistance to pathotype 2 (ND90Pr) of *Bipolaris sorokiniana* by using a DH population from the Calicuchima-sib/Bowman-BC cross. Our recent study indicated that *Rcs6* is a recessive allele, and the corresponding dominant allele (designated as *Scs6*) at the same locus is responsible for susceptibility to pathotype 2 of *Bipolaris sorokiniana* in cultivar Bowman (Leng et al. 2018). Other genes or QTLs for SB resistance have been identified and mapped on different barley chromosomes (Berger et al. 2013; Bilgic et al. 2005, 2006; Bovill et al. 2010; Castro et al. 2012; Grewal et al. 2012; Wang et al. 2017, 2019; Zhou and Steffenson 2013).

For PM, a number of major resistance genes and QTLs also have been identified and reported in barley. The described major genes for PM resistance include *Mla*, *Mlat*, *MLk*, *Mlnn*, *Mlra*, *MLGa*, and *Mlp* on chromosome 1H (Jørgensen 1994; Wei et al. 1999), *MILa* on 2H (Jørgensen 1994), *mlo* and *Mlg* on 4H (Baker et al. 1997; Buschges et al. 1997), *Mlj* on 5H (Schonfeld et al. 1996), *Mlh* on 6H (Jørgensen 1994), and *mlt* and *Mlf* on 7H (Schonfeld et al. 1996). Among them, *Mla* and *mlo* are the most effective and widely deployed PM resistance genes in barley breeding programs. *Mla* is a complex locus that comprises ≥30 alleles with various specificities to different races of *Blumeria graminis* f. sp. *hordei* (Seeholzer et al. 2010; Wei et al. 1999). The gene *mlo*, which was derived from

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loss-of-function of the gene at the *Mlo* locus, exhibits broad-spectrum resistance to *Blumeria graminis* f. sp. *hordei* and thus has been widely used in European countries to protect barley from PM for almost four decades (Büschges et al. 1997; Kusch and Panstruga 2017). However, several studies indicated that the *mlo*-based resistance led to more susceptibility to hemibiotrophic and necrotrophic pathogens such as *Magnaporthe oryzae*, *Ramularia collo-cygni*, and *Bipolaris sorokiniana* in barley (Brown and Rant 2013; Jarosch et al. 1999; Kumar et al. 2001; McGrann et al. 2014), raising the issue of trade-off between resistance to biotrophic pathogens and increased susceptibility to nonbiotrophic pathogens. In addition to the major genes, QTLs for PM resistance have been identified in wild and cultivated barley populations through genome-wide association studies (Ames et al. 2015; Bengtsson et al. 2017).

To map genes or QTLs for traits of interest in plants, a number of marker technologies have been used, including restriction fragment length polymorphisms, amplified fragment length polymorphisms, simple sequence repeats, and single nucleotide polymorphisms (SNPs). Among them, SNPs have become increasingly favored because of their abundance, relative ease of discovery, and low cost per data point (Kumar et al. 2012). To genotype SNP markers, high-throughput Illumina arrays have been developed for cereal crops, including wheat (Wang et al. 2014; Winfield et al. 2016), rice (Chen et al. 2014), maize (Ganal et al. 2011), and barley (Bayer et al. 2017; Close et al. 2009; Comadran et al. 2012; Fan et al. 2003). With the decreasing costs of sequencing, the sequencing-based genotyping (SBG) approach has become more popular. SBG has been used to construct genetic maps and identify several genes or QTLs in barley (Liu et al. 2014; Poland et al. 2012). Especially with the availability of a new barley genome assembly (Mascher et al. 2017; Monat et al. 2019), discovery of SNP markers by SBG in a mapping population and construction of marker-saturated genetic map for gene mapping have become more popular and easier.

In a previous study (Leng et al. 2018), we fine-mapped and physically anchored the dominant SB susceptibility gene *Scs6* to a 125-kb genomic region containing the *Mla* locus on barley chromosome 1H of Morex, but it is still not known whether *Scs6* is allelic to any of the *Mla* alleles. Therefore, the objectives of this study were to identify and map loci conferring susceptibility to SB and resistance to PM in a recombinant inbred line (RIL) mapping population derived from a cross between barley cultivar Bowman and barley line ND5883 through the SBG approach, and unravel the relationship between *Scs6* and a gene for PM resistance at the *Mla* locus.

MATERIALS AND METHODS

Plant materials. A mapping population consisting of 138 RILs developed from a cross between Bowman and ND5883 were used in the study. Bowman is a two-rowed barley cultivar released by North Dakota State University (NDSU) (Franckowiak et al. 1985), which is resistant to the pathotype 1 isolate ND85F of *Bipolaris sorokiniana* but susceptible to the pathotype 2 isolate ND90Pr of the fungus (Valjevec-Gratian and Steffenson 1997a; Zhong and Steffenson 2001). ND5883 is an experimental barley line also released by NDSU, which is susceptible to isolate ND85F but resistant to isolate ND90Pr (Valjevec-Gratian and Steffenson 1997a; Zhong and Steffenson 2001). Bowman and ND5883 and another NDSU experimental barley line, NDB 112, are commonly used as differentials for identification of pathotypes of *Bipolaris sorokiniana* in North Dakota (Valjevec-Gratian and Steffenson 1997a, b; Zhong and Steffenson 2001).

SB phenotyping. The method described by Leng et al. (2016) was used to evaluate the infection responses (IRs) of the barley parents (Bowman and ND5883) and the mapping population to SB at the seedling stage. Two *Bipolaris sorokiniana* isolates, ND85F and ND90Pr, were used in this study. ND85F (pathotype 1) and

ND90Pr (pathotype 2) (Valjevec-Gratian and Steffenson 1997a, b; Zhong and Steffenson 2001) are routinely used in our studies on the barley–*Bipolaris sorokiniana* system (Leng et al. 2011; Leng and Zhong 2012, 2015; Wang et al. 2015, 2016). The isolates were grown on V8 PDA (150 ml of V8 juice, 3 g of CaCO₃, 39 g of potato dextrose agar, and 850 ml of distilled water) under 14 h fluorescent light each day at approximately 23°C. After 7 days of growth, conidia were harvested, and conidial suspension was prepared for spray inoculation as described by Leng et al. (2016). The 1 to 9 rating scale of Fetch and Steffenson (1999) was used to rate the IR of each barley plant. Range of IR (minimum and maximum) and the most common IR (mode of IR) were recorded for each barley genotype, and each genotype was classified as low (with mode 1 to 3), intermediate (with mode 4 to 5), or high (with mode 6 to 9) IR. Three inoculation experiments were conducted for all barley genotypes. Mean disease rating scores were calculated for each genotype and used for further analysis.

PM evaluation. Bowman and ND5883 were initially evaluated with 50 reference *Blumeria graminis* f. sp. *hordei* isolates collected in all nonpolar continents over a period of 63 years (1953 to 2016), including an old Japanese *Blumeria graminis* f. sp. *hordei* isolate designated as Race I (Hiura and Heta 1955) to postulate their specific resistance genes against PM. Before inoculation, all *Blumeria graminis* f. sp. *hordei* isolates were checked for their purity and verified for their correct pathogenicity phenotypes on standard barley differential lines (Kølster et al. 1986). The isolates were multiplied on leaf segments of susceptible cultivar Stirling (Dreiseitl and Platz 2012). The method for PM inoculation and evaluation was previously described (Dreiseitl 2014). Briefly, 20 seeds of each parent and 5 seeds of each RIL were sown in plastic pots (80 mm diameter) filled with a gardening peat substrate and placed in a mildew spore-proof greenhouse under natural daylight. Leaf segments (15 mm long) were cut from the central part of healthy, fully expanded primary leaves when the second leaves were emerging. Three leaf segments taken from three plants of each RIL were placed with adaxial surfaces upward in a 150-mm Petri dish on water agar (0.8%) containing benzimidazole (40 mg/l), a leaf senescence inhibitor. In each Petri dish, leaf segments from each of the two parents (Bowman and ND5883) were placed as checks. The dishes with leaf segments were placed at the bottom of a metal inoculation tower and inoculated at a concentration of about 8 conidiospores per square millimeter. The dishes with inoculated leaf segments were incubated at 18.0 ± 2.0°C under artificial light (cool-white fluorescent lamps providing 12 h light at 30 ± 5 μmol/m²/s) with a 12-h photoperiod under artificial light supplied by cool-white fluorescent lamps (30 ± 5 μmol/m²/s). At 6 and 8 days after inoculation, IRs (phenotype of genotype × isolate interaction) on the central part of the adaxial side of leaf segments were scored on a 0 to 4 scale including intertypes, where 0 = no mycelium and sporulation, and 4 = strong mycelial growth and sporulation (Torp et al. 1978; Supplementary Table S1). Each genotype was tested in two independent experiments, and mean IR score was calculated for further analysis. IRs of 0 to 3 were classified as resistant, and IRs of 4 were classified as susceptible. For postulation of PM resistance genes, an IR array (IRA) was generated for each line according to its IRs to the *Blumeria graminis* f. sp. *hordei* isolates. By comparing the IRA with previously determined IRAs of standard barley differentials possessing known resistance genes, we postulated the resistance genes in each tested line based on the gene-for-gene model.

DNA extraction and construction of SBG libraries. DNAs were extracted from 2-week-old seedling plants of the parents and 138 RILs with a DNA purification kit and quantified with a fluorometer according to the manufacturers' protocols. The DNA samples were diluted and normalized at a concentration of 50 ng/μl. The 140 samples were split into two 96-well plates, one with 72 samples and the other with 68 samples. The SBG libraries were constructed according to the methods described by Saintenac

et al. (2013). Briefly, DNAs were digested in 30- μ l reactions containing 200 ng of genomic DNA, 1 $\times$ New England Biolabs buffer 4, 8 U of *Pst*I-HF, and 8 U of *Mse*I, incubated at 37°C for 3 h, then 80°C for 20 min to inactivate the enzymes. For ligation, 4 ng of annealed barcoded adapter (Santenac et al. 2013) and 200 ng of annealed common Y-adapter (Santenac et al. 2013) were added along with 1 $\times$ T4 DNA ligase buffer and 200 U of T4 ligase in a total volume of 50 μ l. All ligation reactions were incubated at 22°C for 2 h, then 65°C for 20 min. An aliquot (5 μ l) was removed from each ligation reaction, pooled, purified with a PCR purification kit, and eluted in 30 μ l of dH₂O. PCR amplification was conducted in 50- μ l reactions containing 4 μ l of pooled and purified library DNA, 1 $\times$ high-fidelity DNA polymerase buffer, 0.2 μ M of dNTP, 0.2 μ M of primer 1 (complementary to barcode adapter), 0.2 μ M of primer 2 (complementary to common Y-adapter), and 1 U of Taq polymerase. PCR was conducted as follows: 98°C for 30 s for one cycle; 20 cycles of 98°C for 10 s, 65°C for 20 s, and 68°C for 20 s; and one cycle of 75°C for 5 min, cool to 4°C. The PCR-enriched library was gel purified, with the 200- to 500-bp size fraction selected with a gel extraction kit, eluted in 12 μ l of ddH₂O, and the quality and quantity of the library were measured with a spectrophotometer and bioanalyzer.

Sequencing and processing raw SBG data. Single-read sequencing from the *Pst*I site was carried out, and 150 bp reads were generated. The two parents, Bowman and ND5883, were originally included in the library construction and sequencing, but not enough reads were generated for them. To have higher coverage for the parents, the DNA samples were sent to BGI (Cambridge, MA) for whole-genome sequencing in the BGI500 sequencing platform, where paired-end 100-bp reads were generated. All sequence reads were submitted to the Sequence Read Archive section of the NCBI (accession no. PRJNA514287).

SNP discovery and genotype calling. SNP discovery and genotype calling were performed using the TASSEL-GBS pipeline (Glaubitz et al. 2014), and the Morex genome pseudomolecule and contigs sequences (version 2.0, http://plants.ensembl.org/Hordeum_vulgar/Info/Index) were used as the references.

Linkage map construction and QTL identification. Linkage analysis was performed in MapDisto (Lorieux 2012) with a logarithm of the odds (LOD) threshold of 3.0 and theta value of 0.4. For cosegregating SNP markers in the mapping population, only one was selected for constructing the genetic map. Recombination fraction was converted into map distance via the Kosambi function. The genetic linkage maps were generated in MapChart version 2.1 (Voorrips 2002). QTL interval mapping was used to locate QTLs for susceptibility to SB in QGene, version 4.3.10 (Joehanes and Nelson 2008). A permutation test with 1,000 permutations was used to establish the LOD threshold.

RESULTS

Phenotype data. Bowman and ND5883 exhibited low IRs (1 to 3) and high IRs (7 to 9) when infected with the pathotype 1 isolate ND85F of *Bipolaris sorokiniana*. Conversely, they exhibited high IRs (7 to 9) and low IRs (1 to 3) when infected with the pathotype 2 isolate ND90Pr of *Bipolaris sorokiniana*. The distribution pattern of the RIL population to pathotype 1 was continuous (Fig. 1). With pathotype 2, the RIL population segregated into 67 resistant and 61 susceptible, fitting a 1:1 ratio ($\chi^2 = 0.28$, $P = 0.60$) and indicating a single gene locus controlling susceptibility and resistance. The recessive resistance allele was previously designated as *Rcs6* (Bilgic et al. 2006), and the dominant susceptibility allele was designated as *Scs6* (Leng et al. 2018).

Evaluation of Bowman and ND5883 along with standard barley differential lines carrying known PM resistance genes using the 50 *Blumeria graminis* f. sp. *hordei* isolates revealed that no specific PM resistance genes were present in Bowman, whereas *Mla8* and *MLK1* were identified in ND5883 (Table 1). Among the 50 *Blumeria graminis* f. sp. *hordei* isolates tested, Race I showed avirulence on ND5883 with a rating of 0, 0 to 1, or 1 (with hypersensitive flecks) and virulence on Bowman with a rating of 4, and thus it was selected to evaluate 133 of the 138 RILs derived from the cross between Bowman and ND5883. The 133 RILs segregated in a 1:1 ratio (72 resistant, 61 susceptible) ($\chi^2 = 0.91$; $P = 0.34$), suggesting that a single gene (*Mla8*) controls the resistance to PM in ND5883.

Sequence reads from the mapping population derived from the Bowman $\times$ ND5883 cross. In the BGI500 sequencing platform, 712.05 and 708.20 million 100-bp reads were generated from Bowman and ND5883, respectively. A total of 389.77 million 150-bp reads were generated from the 138 RILs with a high-throughput sequencer. The number of reads per RIL ranged from 9,921 to 4,946,336 (Supplementary Table S2). All sequence reads generated from the population were submitted to the Sequence Read Archive section of the NCBI (accession no. PRJNA514287).

Identification of SNP markers and construction of linkage map. The TASSEL-GBS pipeline was used to identify the SNP markers in the mapping population. The sequence reads were first filtered to keep only those uniquely mapped to the reference genome, leading to 70.03% of the 389.76 million reads left for SNP discovery and genotype calling. A total of 5,215 SNP markers were identified according to the following parameters: read depth >3, minor allele frequency >0.05, and missing data <20% (Supplementary Table S3). SNP markers were named based on their physical positions on the pseudomolecules corresponding to seven barley chromosomes (1H to 7H) (Mascher et al. 2017). For example, 1H_51926 represents a SNP marker located at position 51926 bp on pseudomolecule of 1H.

The 5,215 SNP markers were analyzed in MapDisto, and only 4,905 high-confidence SNP markers were left for construction of linkage maps. After removal of the cosegregating markers, 852 unique SNP markers were used in the final construction of the genetic map. Nine linkage groups were generated, with the number of markers per group ranging from 14 to 201 (Supplementary Fig. S1; Supplementary Table S3).

Mapping of the gene and QTLs for susceptibility to SB and the gene for resistance to PM. As expected, the gene (*Scs6*) for susceptibility to the pathotype 2 isolate ND90Pr, contributed by Bowman, was mapped on the short arm of chromosome 1H (Fig. 2A). The gene (*Mla8*) for PM resistance was also mapped to the short arm of chromosome 1H, which was tightly linked to *Scs6* (Fig. 2A). The distance between *Scs6* and *Mla8* was 0.4 cM.

QTL analysis identified two QTLs (*QScs-1H-P1* and *QScs-7H-P2*) for susceptibility to the pathotype 1 isolate ND85F at the seedling stage, both contributed by ND5883 and mapped on chromosome 1H and 7H, respectively (Fig. 2). *QScs-1H-P1* peaked at marker 1H_256259151, flanked by 1H_20693748 and 1H_486185365, and

Fig. 1. Frequency distributions for the mean of the most common infection response (IR) to spot blotch among the recombinant inbred lines derived from cross between Bowman and ND5883 at the seedling stage. The IR of each barley plant was rated using the 1 to 9 rating scale of Fetch and Steffenson (1999).

Fig. 2. Regions of linkage maps for **A**, chromosome 1H and **B**, chromosome 7H harboring *Mla8* for powdery mildew resistance, *Scs6* and quantitative trait loci (QTLs) for spot blotch susceptibility detected in the mapping population derived from the cross between barley line ND5883 and cultivar Bowman. *Mla8* was identified in ND5883, and *Scs6* was detected in Bowman. Both QTLs on 1H and 7H for spot blotch susceptibility were derived from the susceptible barley parent ND5883. The centimorgan (cM) distances between marker loci and the positions of marker loci are on the right and left sides of the linkage maps, respectively. The logarithm of the odds (LOD) significance threshold 3.0 is represented by a vertical dotted line. The markers that are most closely linked to the QTLs are marked in dark gray (red). The markers flanking the QTL region are underlined and bold.

TABLE 1. Infection response arrays of 4 barley genotypes to 10 selected *Blumeria graminis* f. sp. *hordei* isolates

Barley genotype	Genes	Isolate									
		GH ^a	3-33	Z-6	EmA30	120	J-462	Race I	65	KM18	MN-B
		A ^b	CH	CZ	DK	G	ISR	JAP	JAR	SW	USA
		2005 ^c	2003	2014	Before 1976	Before 1999	1979	1953	2004	1975	2016
Bowman	None	4	4	4	4	4	4	4	4	4	4
Pallas ^d	<i>Mla8</i>	4	4	4	4	4	4	0–1	4	4	4
Abee (selection) ^d	<i>Mlk1</i>	4	2	2	2	2	4	2	4	4	4
ND5883	<i>Mla8</i> , <i>Mlk1</i>	4	2	2	2	2	4	0–1	4	4	4

^a Isolate name. The origin and collection year of the isolate are indicated under the isolate name. Virulence and avirulence arrays of the *Blumeria graminis* f. sp. *hordei* isolates to 35 standard barley differential genotypes are shown in Dreiseitl (2019).

^b Country where the isolate was collected: A, Australia; CH, China; CZ, Czech Republic; DK, Denmark; G, Germany; ISR, Israel; JAP, Japan; JAR, Republic of South Africa; SW, Sweden; USA, United States of America (Minnesota).

^c Year when the isolate was collected.

^d Standard differential barley genotypes used to postulate the genes in ND5883.

explained 27% of the total phenotypic variation, whereas *QScs-7H-P1* peaked at marker 7H_30702241, flanked by 7H_18088775 and 7H_45702908, and explained 20.8% of the variation (Table 2).

Effects of individual and combined QTLs on SB susceptibility were further investigated by grouping the RILs according to genotypes of marker loci, 1H_256259151 and 7H_30702241, closely linked to the two QTLs on 1H and 7H, respectively. RILs carrying one or both susceptibility QTL alleles showed a significantly higher IR than those with resistance alleles (Fig. 3). The result indicated that both QTLs increased susceptibility to SB, and their effects were additive.

DISCUSSION

A previous study identified a single gene (*Rcs6*) for resistance to pathotype 2 isolate ND90Pr in a DH population derived from the Calicuchima-sib/Bowman-BC cross (Bilgic et al. 2006). The *Rcs6* allele contributed by Calicuchima-sib was mapped on the short arm of chromosome 1H and effectively confers both seedling and adult plant resistance (Bilgic et al. 2006). Using the same DH population from the Calicuchima-sib/Bowman-BC cross and a large population of F₂ individuals from the Bowman/ND5883 cross, we showed that *Rcs6* is a recessive allele, whereas the corresponding allele (*Scs6*) at the same locus is dominant and responsible for susceptibility to SB in Bowman (Leng et al. 2018). *Scs6/Rcs6* was finely mapped to the PM resistance *Mla* locus within a 125-kb region of chromosome 1H of Morex (Leng et al. 2018). Among the six markers cosegregating with *Scs6*, four contained sequences encoding NBS-LRR proteins, which led us to hypothesize that *Scs6* may represent an *Mla* allele, conferring resistance to PM but susceptibility to SB (Leng et al. 2018). In the present study, we showed that *Scs6* and *Mla8* were not cosegregating, although they were tightly linked together on chromosome 1H, suggesting that *Scs6* and *Mla8* are not allelic. However, considering the fact that the genome structure of the *Mla* locus is complex and highly diversified across different barley genotypes, and there are multiple copies of *Mla* alleles at this locus (Seeholzer et al. 2010; Wei et al. 1999, 2002; Y. Leng and S. Zhong, unpublished data), the possibility that *Scs6* represents one of the *Mla* homologs at the *Mla* locus cannot be ruled out.

Using a DH population derived from the Steptoe and Morex (S/M) cross, Steffenson et al. (1996) detected one major gene (*Rcs5*) for seedling resistance and two QTLs for adult plant resistance to SB caused by pathotype 1 isolate ND85F, with the major effect QTL on chromosome 1H explaining 62% of the phenotypic variance and the minor effect QTL mapping to the *Rcs5* region on chromosome 7H explaining 9% of the phenotypic variance. Later, Bilgic et al. (2005) confirmed these two QTLs by using the same S/M population and designated them as *Rcs-qt1-1H-6-7* and *Rcs-qt1-7H-2-4*, respectively. In the present study, we detected two QTLs (*QScs-1H-P1* and *QScs-7H-P1*) for susceptibility to SB caused by ND85F at the seedling stage in the RIL population derived from the Bowman/ND5883 cross. The 7H QTL, *QScs-7H-P1* contributed by ND5883, was mapped at the same region as *Rcs5* (Bilgic et al. 2005; Steffenson et al. 1996), suggesting that *QScs-7H-P1*, *Rcs-qt1-7H-2-4*, and *Rcs5* may be allelic or closely linked together at the same

locus. Recently, Ameen (2019) showed that *Rcs5* is a recessive allele carried by Morex, whereas the corresponding susceptibility allele carried by Steptoe is dominant, which contains two functional genes (*Rbs1* and *Rbs2*) controlling the susceptibility to pathotype 1 isolate ND85F. The peak marker (1H_256259151) of *QScs-7H-P1* is homologous to *HvWak1* (HORVU7Hr1G020700), which is only 56 kb from the susceptibility gene (*Sbs1*) at the *Rcs5* locus (Ameen 2019). It is possible that *QScs-7H-P1* contributed by ND5883 contains the same susceptibility genes as Steptoe, but PCR amplification and sequencing of *Rbs1* and *Rbs2* from ND5883 should be done to confirm this hypothesis. The 1H QTL, *QScs-1H-P1*, also contributed by ND5883, was mapped to the same region as *Rcs-qt1-1H-6-7* in Morex (Bilgic et al. 2005). However, it is not known whether *QScs-1H-P1* and *Rcs-qt1-1H-6-7* are allelic to each other or closely linked at the same locus because *Rcs-qt1-1H-6-7* conferred adult plant resistance to SB in the S/M population, whereas *QScs-1H-P1* controlling SB susceptibility was detected at the seedling stage in the Bowman/ND5883 population. Additional studies are needed to determine whether *QScs-1H-P1* also controls the susceptibility at the adult plant stage.

In a genetic study with two other DH populations derived from crosses of Harrington/Morex (H/M) and Dicktoo/Morex (D/M), Bilgic et al. (2005) failed to detect the 1H QTL (*Rcs-qt1-1H-6-7*) originally identified by Steffenson et al. (1996) in the S/M population although all three mapping populations (H/M, D/M, and S/M) shared the same resistant parent Morex. The reason that *Rcs-qt1-1H-6-7* was detected in S/M but not in H/M and D/M could not be well explained at that time because of the assumption that SB resistance is controlled by dominant gene alleles. With more evidence indicating that the barley-*Bipolaris sorokiniana* interactions fall mostly under the inverse gene-for-gene interaction

Fig. 3. Box plot distributions of recombinant inbred lines according to allele combinations at markers associated with quantitative trait loci (QTLs) for susceptibility to spot blotch caused by the isolate ND85F of *Bipolaris sorokiniana* in the mapping population. The infection response represents the mean disease ratings based on the 1 to 9 rating scale of Fetch and Steffenson (1999). Medians are indicated by solid lines, x represents mean, the dot represents an outlier, + represents presence of the QTL, and – represents absence of the QTL. The number of RILs for each group is also indicated.

TABLE 2. Chromosome locations, flanking markers, and peak markers of the quantitative trait locus (QTL) conferring susceptibility to spot blotch caused by isolate ND85F of *Bipolaris sorokiniana* at the seedling stage

Chromosome	QTL	Marker interval	Peak marker	LOD value ^a	R ² value ^b	Add ^c
1H	<i>QScs-1H-P1</i>	1H_20693748–1H_486185365	1H_256259151	9.224	0.270	–0.712
7H	<i>QScs-7H-P1</i>	7H_18088775–7H_45702908	7H_30702241	6.841	0.208	–0.589

^a Logarithm of the odds (LOD) value was calculated through a 1,000-iteration permutation test at the 0.01 level of probability.
^b R² value represents the percentage of phenotypic variance explained by the QTL.
^c Negative value indicates that the ND5883 allele increases the trait (disease severity) value relative to the Bowman allele.

scenario (Wolpert et al. 2002), where disease susceptibility is induced by the interaction between a dominant susceptibility gene in the plant and the effector produced by the pathogen (Ameen 2019; Leng et al. 2018; Singh et al. 2014; Valjavec-Gratian 1996; Valjavec-Gratian and Steffenson 1997a), it is not a surprise that a resistance QTL allele was detected in one DH population but not in other DH populations derived from the same resistant parent. In the S/M population, Steptoe contributed the dominant susceptibility allele (*Rcs-qt1-1H-6-7S*), whereas Morex contributed the recessive resistance allele (*Rcs-qt1-1H-6-7s*) at the locus, thus the QTL could be detected regardless of alleles (Bilgic et al. 2005; Steffenson et al. 1996). In the H/M population, if both Harrington and Morex carried the recessive resistance allele (*Rcs-qt1-1H-6-7s*) at the locus, this QTL would not be detected at the locus in this population, as observed by Bilgic et al. (2005). The same is true for the D/M population because Dicktoo and Morex carried the same recessive resistance allele (*Rcs-qt1-1H-6-7s*). However, Bilgic et al. (2005) detected several other different resistance QTLs for SB resistance in H/M and D/M populations because the susceptible parents Harrington and Dicktoo carried the corresponding susceptibility alleles for the fungal isolate ND85F. We suggest that dominant and recessive relationship of resistance and susceptibility for SB should be considered during QTL analysis, and the dominant alleles of the loci should be designated whenever the relationship is known.

A number of genes or QTLs for SB resistance have been reported in barley, and they are distributed on all seven barley chromosomes (Berger et al. 2013; Bilgic et al. 2005, 2006; Bovill et al. 2010; Castro et al. 2012; Grewal et al. 2012; Wang et al. 2017, 2019; Zhou and Steffenson 2013). Some genes or QTLs are responsible for the seedling stage resistance and some control the resistance at the adult plant stage, whereas some others are effective for resistance throughout the whole growth stages. The majority of these genes or QTLs were detected by biparental mapping with DH and RIL populations or by genome-wide association studies, and thus the dominant/recessive relationship between alleles at each locus is not known. Considering that a resistance gene can be recessive (Ameen 2019; Leng et al. 2018) or dominant (Wang et al. 2019), further efforts are needed to determine which QTLs or genes confer susceptibility and which QTLs or genes control resistance to SB. This has practical significance in breeding program because susceptible dominant alleles should be removed to develop resistant cultivars, whereas resistance alleles should be selected no matter whether they are dominant or recessive.

In summary, we constructed a genetic map of barley with SNP markers generated by SBG and identified one major gene (*Scs6*) and two QTLs (*QScs-1H-P1* and *QScs-7H-P1*) responsible for SB susceptibility to two different pathotype isolates in the population from a cross between Bowman and ND5883. We showed that the *Scs6* gene was not the *Mla8* gene, although it could be one of the other *Mla* homologs at the *Mla* locus. The 7H QTL, *QScs-7H-P1*, may be the same as the susceptible gene allele (*rcs5*) carrying *Sbs1* and *Sbs2* in Steptoe, whereas the 1H QTL, *QScs-1H-P1*, may represent a novel gene allele at the *Rcs-qt1-1H-6-7* locus. Identification of genes or QTLs controlling susceptibility or resistance to SB and their closely linked DNA markers will facilitate the development of durable resistance to the disease by eliminating susceptible gene alleles and keeping resistance gene alleles.

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