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ORIGINAL ARTICLE

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Genome-wide association studies in a barley (*Hordeum vulgare*) diversity set reveal a limited number of loci for resistance to spot blotch (*Bipolaris sorokiniana*)

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Abstract

Spot blotch caused by *Bipolaris sorokiniana* is an important disease in barley worldwide, causing considerable yield losses and reduced grain quality. In order to identify QTL conferring resistance to spot blotch, a highly diverse worldwide barley set comprising 449 accessions was phenotyped for seedling resistance with three isolates (*No 31*, *SH 15* and *SB 61*) and for adult plant resistance at two locations (Russia and Australia) in two years. Genotyping with the 50 k iSelect barley SNP genotyping chip yielded 33,818 informative markers. Genome-wide association studies (GWAS) using a compressed mixed linear model, including population structure and kinship, revealed 38 significant marker-trait associations (MTA) for spot blotch resistance. The MTA corresponded to two major QTL on chromosomes 1H and 7H and a putative new minor QTL on chromosome 7H explaining between 2.79% and 13.67% of the phenotypic variance. A total of 10 and 14 high-confidence genes were identified in the respective major QTL regions, seven of which have a predicted involvement in pathogen recognition or defence.

KEYWORDS

Bipolaris sorokiniana, genetic diversity, Genome-wide Association Study, *Hordeum vulgare*, resistance, spot blotch

1 | INTRODUCTION

The fungal pathogen *Bipolaris sorokiniana* (Sacc.) Shoem. (teleomorph: *Cochliobolus sativus* (Ito &Kurib.) Drechs. ex Dastur) is present in all cereal growing regions with warm and humid conditions, but its importance is also increasing in the Americas and Europe (Gupta et al., 2018). *Bipolaris sorokiniana* is the causal agent of a number of diseases such as common root rot, seedling blight, black point and spot blotch (Kumar et al., 2002). This hemi-biotrophic fungus has a wide host range and is pathogenic on a number of plant species, such as bread and durum wheat (*Triticum aestivum* and *T. durum*), barley (*Hordeum vulgare*), triticale (x *Triticosecale*), rye (*Secale cereal*), maize (*Zea mays*), rice (*Oryza sativa*), pearl and fox millet (*Pennisetum glaucum* and *Setaria italica*) and several other wild grasses (Acharya, Dutta, & Pradhan, 2011; Gupta et al., 2018; Kumar et al., 2002). First reported in 1914, it became an important pathogen mainly with the beginning of the Green Revolution when semi-dwarf wheat cultivars turned out to be highly susceptible

(Gupta et al., 2018). Yield losses between 4% and 43% in South Asia, 18% to 22% in India and 10% to 20% in Scotland, Canada and Brazil have been reported (Murray et al., 1998; Sharma, Duveiller, & Sharma, 2006; Singh et al., 1998). In Nepal in wheat–rice growing systems, yield losses went up to 70% to 100% (Sharma & Duveiller, 2007), showing that short crop rotations or crop rotations with high proportions of cereal crops foster the disease. Apart from yield losses, the pathogen also has a negative effect on grain quality, which is of special importance with respect to malting barley. The disease severity is greatly affected by crop management practices, soil fertility, plant density and developmental stage, and abiotic conditions (Acharya et al., 2011; Gupta et al., 2018). Due to this and the wide host range, it is difficult to control the disease solely by agricultural practices.

In barley, the most important disease caused by *B. sorokiniana* is spot blotch. Symptoms appear on all aboveground plant parts as long, dark-brown necrotic blotches with chlorosis in later stages and are up to several centimetres in length (Acharya et al., 2011; Mathre, 1997). The fungus survives as conidia on plant debris and volunteer plants in the field as well as in soil and on seeds or as mycelium in infected plant tissue. Infected seeds are considered the primary source of inoculum. Primary infection starts with conidia germinating on the leaf (within 4 hr), formation of an appressorium (8 hr) and the penetration of the cuticle by infection hyphae (12 hr). The fungus multiplies and spreads into the intercellular space of the mesophyll from where further plant cells are infected. The hyphae eventually produce conidiophores, which appear through the stomata carrying new conidia. Under optimal conditions, a new generation of conidia is produced within 48h which makes it a highly epidemic disease with several infection cycles within one season (Acharya et al., 2011; Gupta et al., 2018). The sexual stage is of no importance in the disease cycle and has only been observed under natural conditions in Zambia (Raemaekers, 1988). Nonetheless, the existence of two mating types (*A* and *a*) was shown (Tinline, 1951) and isolates show high variability especially in the interaction with *H. vulgare* (Gupta et al., 2018).

The presence of pathotypes was first described by Valjavec-Gratian and Steffenson (1997). In their study, they evaluated the virulence patterns of 33 isolates from the United States, China and Japan on three barley genotypes, ND 5883, Bowman and ND B112, and found three pathotypes designated 0, 1 and 2. Leng, Wang, Ali, Zhao, and Zhong (2016) screened over 2000 barley accessions with isolate ND4008 from North Dakota and identified a new pathotype they designated pathotype 7. Arabi and Jawhar (2002, 2004) identified three different pathotypes among over 120 *B. sorokiniana* isolates from Syria. Meldrum, Platz, and Ogle (2004) identified six pathotypes among 34 Australian isolates and Ghazvini and Tekauz (2007) identified eight virulence groups among 92 Canadian isolates belonging to one of the three pathotypes (0, 1, 2) described by Valjavec-Gratian and Steffenson (1997).

Quantitative trait loci (QTL) for resistance against spot blotch have been identified on all seven barley chromosomes. Many have been identified via traditional bi-parental mapping (Bilgic, Steffenson, & Hayes, 2005, 2006; Bovill et al., 2010; Grewal, Rossnagel, & Scoles, 2012; Haas, Menke, Chao, & Steffenson, 2016; Steffenson, Hayes, & Kleinhofs, 1996; Yun et al., 2006, 2005) and others through the use of genome-wide association studies (GWAS) (Berger et al., 2013; Bykova, Lashina, Efimov, Afanasenko, & Khlestkina, 2017; Gutiérrez et al., 2015; Gyawali et al., 2018; Roy et al., 2010; Wang, Leng, Ali, Wang, & Zhong, 2017; Zhou & Steffenson, 2013). To date, three resistance genes have been fine-mapped. Resistance gene *Rcs 5* was initially described by Steffenson et al. (1996) and verified by Bilgic et al. (2005). Drader, Johnson, Brueggeman, Kudrna, and Kleinhofs (2009) narrowed the interval down to 2.8 cM located within bin 3 on chromosome 7H of the Morex genome. Bilgic et al. (2006) identified a resistance gene; they designated *Rcs 6* on chromosome 1H in a double-haploid population of Calicuchima-sib × Bowman-BC. Just recently, Leng et al. (2018) identified the corresponding susceptibility gene *Scs 6* and were able to anchor it to a 125 kb region on the short arm of chromosome 1H between 63,571 and 192,067 bp. Based on their data, Leng et al. (2018) postulated that *Rcs 6* and *Scs 6* are located at the same locus and that *Scs 6* is the dominant allele. In a GWAS study with 1,480 barley accessions, Wang et al. (2017) identified, among others, a QTL on the short arm of chromosome 6H for resistance against pathotype 7 using isolate ND4008. This was later anchored to an interval between 13,136,710 and 13,370,566 bp and designated *Rbs 7* (Wang, Leng, Zhao, & Zhong, 2019). This interval contains five low-confidence and ten high-confidence genes.

Resistant cultivars are pivotal for controlling this disease and the emergence of new pathotypes renders the identification of new resistance sources an ongoing task. Therefore, the aims of this study were (a) to screen a diverse barley set for resistance against *B. sorokiniana* under controlled and field conditions, (b) to identify QTL for resistance by employing genome-wide association studies and (c) to compare the detected regions with previously described QTL to identify putatively new loci and closely linked markers.

2 | MATERIAL AND METHODS

2.1 | Plant material

The association panel set comprised 449 *H. vulgare* (L.) accessions, including 277 barley landraces and 172 commercial cultivars, which were obtained from the N. I. Vavilov Research Institute of Plant Genetic Resources (VIR). The accessions are derived from different regions of the world and express different levels of resistance to *B. sorokiniana*. The panel includes 178 two-rowed and 271 six-rowed accessions. A total of 51 accessions have naked kernels, 28 have black kernels, and 20 are winter-types. For more detailed information on the accessions, see Novakazi et al. (2019).

2.2 | Fungal isolates

Four single-spore *B. sorokiniana* isolates were used in this study. Isolates *No 31* and *Cher 3* were collected in 2012 and 2015,

respectively, near Volosovo in the Leningrad region in the northwest of Russia. *Cher 3* was previously used by Bykova et al. (2017) for its high aggressiveness. Isolate *SH 15* was collected in 2015 on fields in Quedlinburg (JKI site) in Germany. Isolate *SB 61* was collected in 1998 from the fields in Monto, Queensland, Australia, and was used in glasshouse and field trials.

Spot blotch isolates *No 31* and *SH 15* were grown on SNA medium containing (g per 1L): 1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4$*$7H_2O$, 0.5 g KCl, 0.5 g glucose, 0.5 g sucrose, 15 g phytoagar and 75 g cellulose. Isolates were grown at 23°C under UV-light (12 hr/day) for 12 to 14 days. The culture was then flooded with distilled water, and conidia were harvested with a sterile spatula and filtered through gauze to remove mycelial fragments. Conidia concentration was adjusted to 6,000 conidia/ml using a haemocytometer. Isolate *SB 61* was grown as described by Bovill et al. (2010), and conidial concentration was adjusted to 6,500 conidia/ml for phenotyping seedlings in the glasshouse.

The inoculum for the field trial with isolate *SB 61* was propagated in the laboratory and applied to blocks of very susceptible varieties in the field, which were sown in early to mid-April. When necessary infection was promoted by sprinkler irrigation at least twice a week, these blocks provided heavily infected plant material as inoculum for the subsequent field screening (Martin et al., 2018).

2.3 | Glasshouse experiments

Glasshouse trials with isolates *No 31* and *SH 15* were conducted at the Julius Kuehn-Institute in Quedlinburg, Germany, in 2016, and set up in four replications as complete randomized blocks. Accessions were grown in plastic pots (8 × 8 × 8 cm) with three seeds per accession at 16–18°C with alternating light/darkness periods of 12 hr (5,000 lux). When the second leaf was fully expanded (BBCH 12–13), the plants were spray-inoculated with approximately 1 ml spore suspension/pot and immediately covered with plastic foil for 48 hr to ensure 100% humidity. Inoculated plants were grown at 22–24°C and 70% humidity for another 7 to 10 days until symptoms were clearly developed.

Isolate *SB 61* was tested at the Hermitage Research Facility in Warwick, Queensland, Australia, in 2017, in two replications as incomplete blocks, with pots corresponding to blocks with three lines per block. Four to five seeds were sown at 0, 120 and 240° around the circumference of each pot (10 cm diameter, 17 cm tall) in commercial potting mix (Searles Premium Potting Mix) and grown at 15/25°C. At BBCH, 12–13 plants were inoculated from four directions using a WallWick® commercial spray gun applying an average 3 ml inoculum/pot. Inoculated plants were kept at 19°C in a dark fogging chamber for 24 hr. Incubated plants were moved to the glasshouse and grown at 15/25°C for another nine days.

Infection response type was assessed on the second leaf of each plant following the scale of Fetch and Steffenson (1999).

2.4 | Field experiments

Field experiments were conducted at two locations in Russia, that is Pushkin and Volosovo in 2016 and 2017, and at one location – Cleveland, in Queensland, Australia in 2017.

Experiments in Russia were conducted at the N. I. Vavilov Research Institute of Plant Industry (VIR) in Pushkin, Saint Petersburg, and at the Federal State Budget Institution "State Commission of the Russian Federation on Testing and Protection of Selection Achievements" in Volosovo, Leningrad Region, in 2016 and 2017. Accessions were sown in rows of 1 m with 15–20 seeds per row and a spacing of 0.3 m between rows. The trials were set up in a complete randomized block design with three replications. The susceptible cultivar 'Cherio' was sown around the trials as a border and after every 10th accession to support *B. sorokiniana* infection. To increase infection, all accessions were spray inoculated at the seedling stage with a mix of two spot blotch isolates (*No 31* and *Cher 3*) with a spore concentration of 20,000 conidia/ml. The percentage of leaf area infected was assessed at three time points during the growing period. The first assessment was conducted at BBCH 32–33, the second at BBCH 69–71 and the third at BBCH 83–85. The area under disease progress curve (AUDPC) and the average ordinate (AO) were calculated as described by Vatter et al. (2017).

Field experiments in Australia were conducted at the Redlands Research Facility, Cleveland, in 2017 with one distinct isolate (*SB 61*). Accessions were sown in hill plots with 0.5 m and 0.76 m in-row and between-row spacing. Spreaders were sown between every other plot-row about 2–3 weeks before the plots. Infected green plant material from the inoculum increase blocks was used as inoculum when the spreaders were at about BBCH 30. To ensure infection and enhance epidemics, overhead sprinkler irrigation was applied in the late afternoon and/or early evening two or more nights per week when conditions were favourable for infection; so that the nurseries remained wet overnight. Infection responses were taken on a whole plot basis using a variant of the scale by Saari and Prescott (1975) (0 to 9 scale) at BBCH stages 70–73. It takes into account the plant response (infection type; IT), and the amount of disease per plot and therefore correlates very well with the standard leaf area diseased measurement.

2.5 | Statistical analysis

Statistical analyses and analysis of variance (ANOVA) were performed using the software package SAS 9.4 (SAS Institute Inc.) using *proc glimmix* and *proc mixed*. For field trials in Pushkin and Volosovo, the least square means (lsmeans) of the average ordinates (AO) across years were calculated for each location separately. The genotype was treated as a fixed effect, the year and the year*genotype interaction were set as random effects. For glasshouse trials and the field trial in Cleveland, the means of the infection responses were calculated for each isolate separately. Lsmeans and means for each location and isolate, respectively, were used as phenotypic input data for subsequent genome-wide

association studies (GWAS). Broad sense heritability across years was calculated using the formula:

$$h^2 = V_G/(V_G + V_{GY}/y + (V_R/yr)$$

as described by Vatter et al. (2017), where V_G is genotypic variance, V_{GY} is genotype *x* year variance, V_R is residual variance, and *y* and *r* are the number of years and replicates, respectively.

2.6 | Genotyping, population structure, kinship and linkage disequilibrium

Genomic DNA was extracted from 14-day-old plantlets according to Stein, Herren, and Keller (2001). Accessions were genotyped on the Illumina iSelect 50k Barley SNP Chip at Trait Genetics GmbH. SNPs with failure rates >10%, heterozygous calls >12.5% and a minor allele frequency (MAF) <5% were excluded from the analyses, as well as unmapped SNPs, leaving 33,818 SNPs for subsequent GWAS. Further filtering of the SNPs was done with the software PLINK 1.9 (www.cog-genomics.org/plink/1.9/) (Chang et al., 2015). The tool *LD prune* was used with the following parameters: indep pairwise window size 50, step 5 and r^2 threshold 0.5 (Campoy et al., 2016). The resulting 8,533 markers were used to calculate the kinship and population structure. With the web-based platform Galaxy (Afgan et al., 2016) using the tool *Kinship* and the Modified Roger's Distance, the kinship was calculated (Reif, Melchinger, & Frisch, 2005). Population structure was determined with the software STRUCTURE v2.3.4 (Pritchard, Stephens, & Donnelly, 2000) with a burn-in of 50,000, followed by 50,000 Monte Carlo Markov chain (MCMC) replications for k = 1 to k = 10 with 10 iterations. The optimal k was identified using STRUCTURE HARVESTER (Earl & vonHoldt, 2012), followed by a new STRUCTURE analysis with a burn-in of 100,000 and 100,000 MCMC iterations at the optimal k value. Accessions with membership probabilities <80% were considered as admixtures (Richards, Friesen, & Brueggeman, 2017). Physical positions of markers were obtained from Bayer et al. (2017), which is based on the barley pseudomolecule assembly by Mascher et al. (2017). The tool *linkage disequilibrium* in the web-based platform Galaxy was used to calculate the linkage disequilibrium (LD) as squared allele frequency correlations (R^2) between all intra-chromosomal marker pairs. Genome-wide LD decay was plotted as R^2 of a marker against the corresponding genetic distance, and a Loess regression was computed. For R^2, the default settings were used (Novakazi et al., 2019; Sannemann, Huang, Mathew, & Léon, 2015).

2.7 | Association analyses

Genome-wide association studies (GWAS) were performed as described in Novakazi et al. (2019) using the Galaxy implemented tool *GAPIT*, which uses the *R* package GAPIT (Lipka et al., 2012). A compressed mixed linear model (CMLM) (Zhang et al., 2010) including the population structure (*Q*) and kinship (*K*) was used. A Bonferroni corrected significance threshold was determined, based on the reduced marker set of 8,533 SNPs and a significance level of p = .2 (Muqaddasi et al., 2017; Storey & Tibshirani, 2003). This resulted in a threshold of logarithm of odds (LOD) ≥ 4.63. GWAS for field trials in Pushkin, Russia, was conducted across years. GWAS for glasshouse trials and the field trial in Australia were conducted for each isolate separately. Manhattan plots were generated with the *R* v.3.4.4 package *qqman*.

The databases GrainGenes (https://wheat.pw.usda.gov/GG3/) and BARLEX (https://apex.ipk-gatersleben.de/apex/f?p=284:10) were used to identify physical positions of previously published QTL in order to compare them with QTL identified in the present study. If the previously described QTL were identified based on iSelect markers, the physical positions were obtained from Bayer et al. (2017).

Predicted genes, their locations and annotations were retrieved from the BARLEYMAP website (Cantalapiedra, Boudiar, Casas, Igartua, & Contreras-Moreira, 2015) (http://floresta.eead.csic.es/barleymap/).

3 | RESULTS

3.1 | Phenotypic evaluation

Analysis of variance (ANOVA) showed significant differences among the barley genotypes for all glasshouse and field experiments (Table 1). For field experiments in Volosovo, no significant differences among the barley genotypes were detected; hence, these data were excluded from further analyses.

Disease severity scores for field trials in Pushkin ranged between 3.09% and 16.77% (mean 9.67%), with seven accessions showing <5% and five accessions showing> 15% of leaf area diseased (Figure 1). The heritability for this location was h^2 = 0.46.

The infection response type (IRT) for isolate *No 31* ranged between 3 and 8 (mean 4.89) (Figure 1). Most genotypes were moderately susceptible, with 204 and 164 accessions expressing IRT 5 and 6, respectively. Only two accessions showed IRT ≤3 and 22 accessions showed IRT ≥7.

Isolate *SH 15* showed IRT between 3 and 8 (mean 5.23), with 137 and 201 accessions expressing IRT 5 and 6, respectively (Figure 1).

TABLE 1 Analysis of variance (ANOVA) for spot blotch (*Bipolaris sorokiniana*) severity for 449 barley genotypes evaluated under glasshouse and field conditions

Isolate (glasshouse)	Effect	*F*-value	*p*-value	CV%[a]
No 31	Genotype	3.11	<.0001	14.71
SH 15	Genotype	3.67	<.0001	16.35
SB 61	Genotype	9.44	<.0001	27.16
Field location	**Effect**	***F*-value**	***p*-value**	**CV%**
Pushkin	Genotype	1.26	.0085	23.53
Volosovo	Genotype	0.89	.8829	55.80
Cleveland (SB 61)	Genotype	9.95	<.0001	20.07

[a]Coefficient of variation.

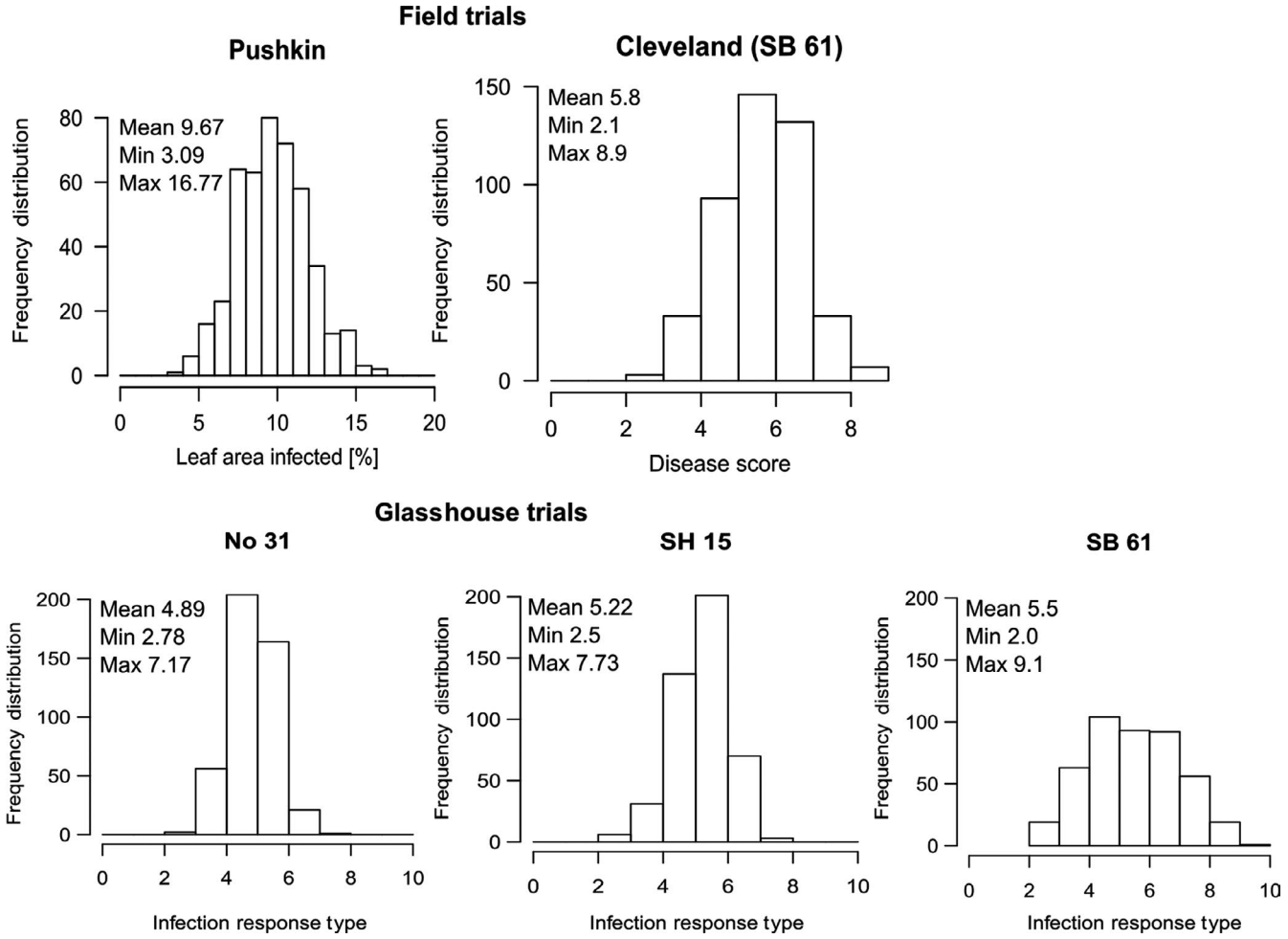

FIGURE 1 Frequency distribution of 449 barley accessions after inoculation with *Bipolaris sorokiniana* in field trials (Pushkin, Russia and Cleveland, Australia with isolate *SB 61*) and in glasshouse trials with three isolates (*No 31*, *SH 15* and *SB 61*). Disease assessment in Pushkin was based on leaf area infected, in Cleveland on a 0–9 scale and in the glasshouse on the scale of Fetch and Steffenson (1999)

Six accessions were highly resistant (IRT ≤ 3), and three accessions were highly susceptible (IRT ≥ 8).

The IRT for isolate *SB 61* tested under glasshouse conditions ranged between 3 and 10 (mean 5.55) (Figure 1). Twenty accessions were highly susceptible and showed IRT ≥ 9; 19 accessions were highly resistant (IRT ≤ 3). Most accessions expressed a moderately susceptible to susceptible reaction, with 104, 93 and 92 accessions expressing IRT of 5, 6 and 7, respectively.

Under field conditions, resistance to isolate *SB 61* varied between disease scores of 3 and 9 (1 to 9 scale, mean 5.8), with only three accessions being highly resistant (disease score ≤3), 33 being moderately resistant (disease score ≤4) and 40 accessions being highly susceptible (disease scores ≥8) (Figure 1).

3.2 | Linkage disequilibrium and population structure

Genome-wide linkage disequilibrium (LD) decay was estimated at 167 kb. Analysis with the software STRUCTURE identified three sub-populations. One-hundred and sixty-one accessions had membership probabilities of less than 80% and were considered admixtures, while 58, 139 and 91 individuals belonged to sub-population one, two and three, respectively. Sub-populations one and two comprised mainly 6-rowed accessions, whereas sub-population three comprised mainly 2-rowed accessions. For more information, see Novakazi et al. (2019).

3.3 | Genome-wide association studies

For isolate *No 31*, fifteen significant marker-trait associations (MTA) were detected—all located on chromosome 1H between 31 and 36 Mbp (40.63–41.02 cM) (Table 2, Figure 2). LOD scores ranged from 4.96 to 11.96. The two peak markers JHI-Hv50k-2016–17526 and SCRI_RS_153785 explained 10.22 and 9.49% of the phenotypic variance, respectively.

A total of seven MTA were detected for resistance to isolate *SH 15* (Table 2, Figure 2). All markers were located on chromosome 7H between 26 and 28 Mbp (24.22 – 26.56 cM), with LOD scores

TABLE 2 Significant marker-trait associations identified for resistance to *Bipolaris sorokiniana* (spot blotch) in a set of 449 barley accessions

Marker	Chr	Position [MB][a]	cM[b]	*p*-value	LOD	MAF	R^{2b}
No 31							
JHI-Hv50k-2016-17275	1H	31.354357	N/A	1.09E-05	4.963	0.289	.0377
JHI-Hv50k-2016-17277	1H	31.354447	N/A	1.09E-05	4.963	0.289	.0377
JHI-Hv50k-2016-17526	1H	32.102667	40.63	1.09E-12	11.962	0.467	.1022
JHI-Hv50k-2016-17533	1H	32.178059	40.63	1.10E-09	8.959	0.392	.0738
JHI-Hv50k-2016-17683	1H	33.444712	N/A	5.36E-06	5.270	0.146	.0404
SCRI_RS_153785	1H	33.444893	40.63	6.39E-12	11.194	0.487	.0949
JHI-Hv50k-2016-17765	1H	34.086518	41.02	1.09E-06	5.964	0.219	.0465
BOPA1_5381-1950	1H	34.087694	41.02	1.47E-07	6.833	0.384	.0543
JHI-Hv50k-2016-17885	1H	35.724537	41.02	1.62E-06	5.790	0.220	.0450
SCRI_RS_189483	1H	35.725028	41.02	1.09E-06	5.964	0.219	.0465
JHI-Hv50k-2016-17892	1H	35.72625	41.02	1.62E-06	5.790	0.220	.0450
JHI-Hv50k-2016-17905	1H	35.728954	41.02	1.62E-06	5.790	0.220	.0450
JHI-Hv50k-2016-17907	1H	35.729187	41.02	1.94E-06	5.712	0.221	.0443
SCRI_RS_140837	1H	36.073804	41.02	4.08E-06	5.389	0.441	.0414
JHI-Hv50k-2016-17967	1H	36.074648	41.02	5.82E-06	5.235	0.483	.0401
SH 15							
BOPA1_8365-454	7H	26.44753	N/A	9.39E-06	5.027	0.446	.0363
JHI-Hv50k-2016-454168	7H	26.540553	N/A	3.28E-07	6.484	0.321	.0486
JHI-Hv50k-2016-454253	7H	26.737545	24.22	1.40E-06	5.854	0.489	.0433
JHI-Hv50k-2016-454328	7H	26.816315	N/A	1.77E-06	5.752	0.489	.0424
JHI-Hv50k-2016-454931	7H	27.770934	26.56	4.01E-06	5.397	0.273	.0394
JHI-Hv50k-2016-455261	7H	28.116204	N/A	1.14E-05	4.944	0.115	.0357
JHI-Hv50k-2016-455308	7H	28.146486	N/A	6.70E-06	5.174	0.110	.0376
SB 61 (seedling)							
SCRI_RS_139762	7H	26.541829	N/A	2.58E-06	5.588	0.132	.0346
JHI-Hv50k-2016-454253	7H	26.737545	24.22	2.83E-07	6.548	0.490	.0415
JHI-Hv50k-2016-454263	7H	26.738361	24.22	2.86E-06	5.543	0.133	.0343
JHI-Hv50k-2016-454328	7H	26.816315	N/A	1.04E-05	4.984	0.488	.0304
JHI-Hv50k-2016-454422	7H	27.122714	N/A	6.66E-06	5.177	0.267	.0317
JHI-Hv50k-2016-454931	7H	27.770934	26.56	1.13E-10	9.947	0.272	.0667
JHI-Hv50k-2016-454991	7H	27.775336	26.56	6.55E-10	9.184	0.125	.0609
JHI-Hv50k-2016-455015	7H	27.776943	26.56	6.36E-07	6.196	0.173	.0390
JHI-Hv50k-2016-455016	7H	27.777032	26.56	1.40E-09	8.853	0.139	.0584
JHI-Hv50k-2016-455041	7H	27.862823	26.56	3.06E-10	9.514	0.126	.0634
JHI-Hv50k-2016-455261	7H	28.116204	N/A	1.81E-19	18.742	0.115	.1367
JHI-Hv50k-2016-455308	7H	28.146486	N/A	2.38E-19	18.623	0.111	.1357
JHI-Hv50k-2016-455437	7H	28.772177	N/A	2.14E-05	4.671	0.200	.0282
SB 61 (adult plant)							
JHI-Hv50k-2016-455261	7H	28.116204	N/A	6.11E-06	5.2143	0.115	.0307
JHI-Hv50k-2016-455308	7H	28.146486	N/A	1.61E-05	4.7939	0.111	.0279
Pushkin							
JHI-Hv50k-2016-467659	7H	68.476333	N/A	8.96E-06	5.0478	0.490	.0395

Note: Adult plant resistance was tested in field experiments in Pushkin, Russia, and Cleveland, Australia (with isolate *SB 61*). Seedling resistance was tested under glasshouse conditions with isolates *No 31*, *SH 15* and *SB 61*.

[a]Physical positions based on Bayer et al. (2017).

[b]Genetic positions based on RIL population of Golden Promise × Morex by Bayer et al. (2017).

[c]Explained phenotypic variance per marker.

between 4.94 and 6.48 explaining 3.57% to 4.86% of the phenotypic variance.

For glasshouse experiments with isolate *SB 61*, 13 significant MTAs were detected, which are located on chromosome 7H between 26 and 28 Mbp (24.22–26.56 cM) (Table 2, Figure 2). The two peak markers JHI-Hv50k-2016–455261 and JHI-Hv50k-2016-455308 with LOD scores of 18.74 and 18.62 explained 13.67 and 13.57% of the phenotypic variance, respectively. Under field conditions, two significant MTAs were detected for isolate *SB 61* (Table 2, Figure 2). The two markers are the same as the peak markers under glasshouse conditions (JHI-Hv50k-2016-455261 and JHI-Hv50k-2016-455308) located on chromosome 7H at 28 Mbp and explaining 3.07% and 2.79% of the phenotypic variance in this case.

For field trials in Pushkin, only one significant MTA was detected on chromosome 7H at 68 Mbp, with a LOD score of 5.05 (Table 2, Figure 2). Marker JHI-Hv50k-2016-467659 explains 3.95% of the phenotypic variance.

In the interval identified on chromosome 1H between 31,354,357 bp and 36,074,648 bp, there are four low-confidence (LC) genes with undescribed protein annotations and ten high-confidence (HC) genes (Table 3). Of the ten HC genes, one (HORVU1Hr1G013490) has no designated function and three are directly involved in pathogen recognition or defence. They belong to the UDP-glycosyltransferase superfamily, tetraspanin family and lateral organ boundary (LOB) domain (HORVU1Hr1G012680, HORVU1Hr1G012690, HORVU1Hr1G012720). The remaining genes are a ribosome biogenesis regulatory protein homolog, magnesium-chelatase subunit, 4'-phosphopantetheinyl transferase, ubiquitin-conjugating enzyme, isoleucine–glutamine (IQ)-domain and a sugar transporter (Table 3).

In the detected regions on chromosome 7H, between 26,447,530 to 28,772,177 bp and at 68,476,333 bp, three LC genes and twenty-one HC genes are located (Table 3). Two of the LC genes have undescribed protein annotations, whereas the other is probably a transposon Ty1-PL Gag-Pol polyprotein. The 21 HC genes belong to different transporters (sulphate transporter, magnesium transporter), kinases (ATP-dependent 6-phosphofructokinase, receptor kinase, receptor-like protein kinase), oxidases (peroxidase superfamily, Fe superoxide dismutase), proteins (pentatricopeptide repeat-containing protein, DNA-repair protein, nodulin-related proteins), polygalacturonase-1 non-catalytic subunit β, coatomer subunit beta', carbonic anhydrases, fatty acyl-CoA reductase, Cadmium tolerant, myosin-J heavy chain and protein arginine methyltransferase (Table 3).

4 | DISCUSSION

The fungal pathogen *B. sorokiniana* has a wide host range and induces a number of diseases, such as common root rot, seedling blight, black point and spot blotch (Acharya et al., 2011; Gupta et al., 2018; Kumar et al., 2002). One of the hosts of *B. sorokiniana* is barley (*H. vulgare*), a crop used worldwide for animal feed, human consumption and malting. The most important disease in barley induced by *B. sorokiniana* is spot blotch. The symptoms are dark-brown, necrotic blotches that appear mostly on the leaves, but also on stems, awns and glumes (Acharya et al., 2011; Mathre, 1997). The damage is based on a reduced photosynthesis, which leads to reduced yields, but also to a decrease in grain quality. The pathogen prefers warm, humid conditions, which occur for example in South Asia, the Middle East, Upper Midwest of the USA and Central Canada (Acharya et al., 2011; Chatrath, Mishra, Ferrara, Singh, & Joshi, 2007; Fetch & Steffenson, 1999). However, with increasing temperatures in temperate climate zones due to climate change, the incidence of spot blotch infection and epidemics will increase. For example, in the north-west region of European Russia, epidemics of barley spot blotch have occurred every 2–3 years for the past two decades (Lashina & Afanasenko, 2019).

In the United States, resistance derived from the 6-rowed barley line ND B112 has provided effective control of spot blotch since the late 1950s. This resistance was effective against pathotypes 0, 1 and 2 (Valjavec-Gratian & Steffenson, 1997) and was used in the 6-rowed malting barley breeding programmes (Fetch & Steffenson, 1994; Wilcoxson, Rasmusson, & Miles, 1990). Meanwhile, two-rowed barleys generally remained susceptible. Eventually, the durable resistance of ND B112 was overcome by the emergence of a new pathotype identified by Leng et al. (2016) and designated pathotype 7. The occurrence of spot blotch pathotypes has been reported in several studies from different regions of the world (Arabi & Jawhar, 2002, 2004; Ghazvini & Tekauz, 2007; Meldrum et al., 2004). Breeding for resistance is an effective mean for controlling the disease and so far three major resistance loci have been mapped, namely *Rcs 6/Scs 6*, *Rbs 7* and *Rcs 5* located on chromosomes 1H, 6H and 7H, respectively (Drader et al., 2009; Leng et al., 2018; Wang et al., 2019). Additionally, several minor QTL have been identified on all seven barley chromosomes (Berger et al., 2013; Bilgic et al., 2005, 2006; Bovill et al., 2010; Bykova et al., 2017; Grewal et al., 2012; Gutiérrez et al., 2015; Gyawali et al., 2018; Haas et al., 2016; Roy et al., 2010; Steffenson et al., 1996; Wang et al., 2017; Yun et al., 2006, 2005; Zhou & Steffenson, 2013).

Overall, the barley accessions tested in our study revealed a large diversity in all experiments and phenotypic reactions varied from highly resistant to highly susceptible, with IRT ranging from three to 10. Disease levels in field trials in Pushkin ranged between 3% and 16.7%. However, these scores are AO values and based on AUDPC values of three scoring dates assessed during the growth period in two years (2016 and 2017). The AUDPC takes into account the development and intensity of the disease over time. The average ordinate (AO) describes the mean disease severity at every point in time. In our case, the unit of the curve is per cent. Disease severities in 2016 were quite high and ranged between 10% and 68% with some accessions occasionally expressing disease severities of 80%–100% (data not shown). Infection pressure and environmental conditions were less favourable for disease development in 2017. Mean disease severities ranged only between 4% and 38%. The variation

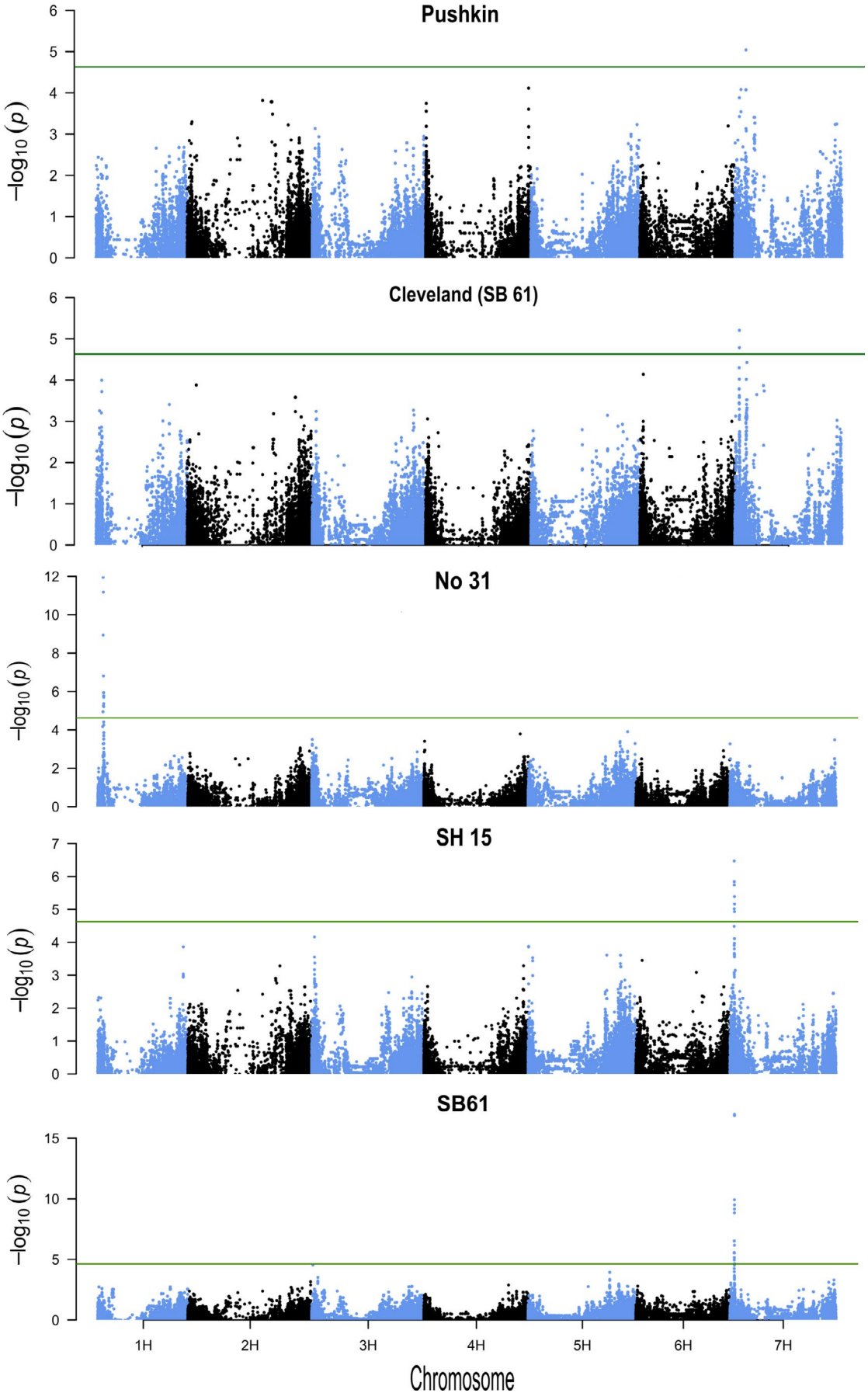
Pushkin
Cleveland (SB 61)
No 31
SH 15
SB61
−log10 (p)
1H
2H
3H
4H
5H
6H
7H
Chromosome

FIGURE 2 Genome-wide association analyses for resistance to *Bipolaris sorokiniana* in field trials (Pushkin, Russia and Cleveland, Australia with isolate *SB 61*) and in glasshouse trials with isolates *No 31*, *SH 15* and *SB 61*. The *x-axis* shows the seven barley chromosomes, positions are based on the physical map, and the −log10(*p*) value is displayed on the *y-axis*. The *green horizontal line* represents the significance threshold of −log10(*p*) = 4.63

of disease severity between the two years might explain the low heritability (h^2 = 0.46) for this location. Steffenson et al. (1996) and Grewal et al. (2012) reported heritabilities for spot blotch resistance in barley of 0.91 and 0.73–0.96, respectively. Heritability for spot blotch resistance in wheat was reported to be between 0.65 and 0.89 (Ayana et al., 2018; Kumar, Joshi, Kumar, Chand, & Röder, 2010; Lillemo, Joshi, Prasad, Chand, & Singh, 2013; Singh et al., 2016; Zhu et al., 2014).

A total of 38 marker-trait associations (MTA) were detected in the present study corresponding to two major QTL located on chromosome 1H and 7H, respectively, and one minor QTL on chromosome 7H. In several other studies, a major QTL was reported on the short arm of chromosome 3H for seedling and adult plant resistance (Bilgic et al., 2005, 2006; Bovill et al., 2010; Grewal et al., 2012; Haas et al., 2016; Roy et al., 2010; Wang et al., 2017; Zhou & Steffenson, 2013). This QTL explained phenotypic variations between 1% and 60% (Bilgic et al., 2005; Grewal et al., 2012; Zhou & Steffenson, 2013). However, most of those studies analysed germplasm from the USA and Canada. In the present study, the association panel was of diverse origin and out of 449 accessions only 19 originated from the USA and eleven from Canada. This emphasizes the importance of screening germplasm from a wide range of origins in order to identify new QTL for resistance.

The region detected on chromosome 1H is located between 31,354,357 bp (JHI-Hv50k-2016–17275) and 36,074,648 bp (JHI-Hv50k-2016–17967) and confers resistance in the seedling stage. Zhou and Steffenson (2013) screened 3,840 breeding lines and cultivars in glasshouse and field trials for spot blotch resistance with isolate ND85F (pathotype 1). On chromosome 1H, they identified a region conferring seedling and adult plant resistance located between 34 and 37 Mbp. One of their significant markers (11_10764) was also significantly associated with disease resistance in our study (BOPA1_5381-1950). This very same marker was identified as a peak marker in a bi-parental mapping study by Afanasenko et al. (2015). In their study, they tested a DH population of Ranniy 1 x Zernogradsky 813 for spot blotch resistance with several isolates. Finally, Wang et al. (2017) studied a barley set consisting of 621 two-rowed and 857 six-rowed accessions with three isolates (ND85F, ND90Pr and ND4008) representing the three pathotypes 1, 2 and 7, respectively. They detected a QTL for resistance against pathotype 1 on chromosome 1H named *QRcs-1H-P1*, which is located between 31 and 35 Mbp at a LOD score of up to 25.34 explaining between 17.3% and 24% of the phenotypic variance. Three of their peak markers (SCRI_RS_153785, SCRI_RS_189483 and BOPA1_5381-1950) were also significant in the present study. Bilgic et al. (2006) identified a region on the short arm of chromosome 1H conferring seedling and adult plant resistance to spot blotch pathotype 2 in a DH population of Calicuchima-sib × Bowman-BC (C/B). Based on their results, the resistance was contributed by the resistant parent Calicuchima-sib based on a single gene they designated *Rcs 6*. In a more recent study, Leng et al. (2018) using the same DH population were able to show that the susceptible parent Bowman contributed a dominant susceptibility gene, *Scs 6*, which was located at the same locus as the resistance gene *Rcs 6*. Further fine mapping in F_2 recombinants of Bowman × ND 5,883 and Bowman x ND B112 narrowed the interval down to a 125 kb region physically located between 64 and 192 Mbp (Leng et al., 2018). Thus, the QTL detected in our study does not correspond to the resistance/ susceptibility locus *Rcs 6/ Scs 6*, but represents another major resistance QTL against pathotype 1. In particular, marker BOPA1_5381-1950, located on chromosome 1H at 34,087,694 bp, may be of special importance as it turned out to be significantly associated with disease resistance in the present study as well as in the studies of Zhou and Steffenson (2013), Afanasenko et al. (2015) and Wang et al. (2017).

In the interval identified between 31,354,357 bp and 36,074,648 bp, there are four low-confidence (LC) genes with undescribed protein annotations and ten high-confidence (HC) genes (Table 3). Out of the ten HC genes, one has so far no designated function (HORVU1Hr1G013490) and three are involved in pathogen recognition or defence (HORVU1Hr1G012680, HORVU1Hr1G012690, HORVU1Hr1G012720). UDP-glycosyltransferase proteins (HORVU1Hr1G012680) are involved in the biosynthesis of, for example, phenolics and glucosinolates, but also in the glycosylation of phytohormones and other plant metabolites and have long been shown to be involved in plant defence against biotic stress (Vogt & Jones, 2000). Rehman et al. (2018) showed several UDP-glycosyltransferase genes to be upregulated in *Arabidopsis thaliana* after infection with fungal pathogens such as *Alternaria brassiciola*, *Blumeria graminis*, *E. coli*, *Rhizoctonia solani*and and *Xanthomonas campestris*. Tetraspanins (HORVU1Hr1G012690) are a family of proteins found in all eukaryotic organisms located in the cell membrane and involved among others in cell adhesion, growth, fusion and migration (Reimann, Kost, & Dettmer, 2017). However, they also have been linked to be involved in pathogen recognition and to be upregulated in *A. thaliana* after treatment with pathogen elicitors (Wang et al., 2015). Lateral organ bounding (LOB) domains (LBD) are transcription factors with key roles in plant organ development, but have also been shown to be involved in plant regeneration, pollen development, nitrogen and anthocyanin metabolisms as well as pathogen response (Xu, Luo, & Hochholdinger, 2016). So far, 24 LBD genes have been described in barley located on all seven barley chromosomes, four of which are located on chromosome 1H (Guo et al., 2016). None of the barley LBDs have been linked to pathogen resistance or recognition yet; however, several LBD genes were identified to show differential expression levels after pathogen attack in, for example *Arabidopsis thaliana* (*Fusarium oxysporum*), *Vitis vinifera* (*Botrytis cinerea*, *Plasmopara viticola*) and *Malus domestica* (*Pseudomonas*

TABLE 3 Predicted genes located on chromosomes 1H and 7H at 31–36 Mbp and 26–28 Mbp and at 68 Mbp, respectively, and their respective functional annotations

Gene ID [a]	Gene class [b]	Chrom	Physical location [bp]		Annotation
HORVU1Hr1G012470	HC_G	1H	31,351,339	31,355,135	Ribosome biogenesis regulatory protein homolog
HORVU1Hr1G012600	HC_G	1H	31,622,380	31,623,341	Magnesium-chelatase subunit ChlH, chloroplastic
HORVU1Hr1G012620	LC_u	1H	31,630,058	31,630,407	Undescribed protein
HORVU1Hr1G012680[c]	**HC_G**	1H	**31,672,910**	**31,677,138**	**UDP-Glycosyltransferase superfamily protein**
HORVU1Hr1G012690	**HC_G**	1H	**31,684,461**	**31,688,423**	**Tetraspanin family protein**
HORVU1Hr1G012720	**HC_G**	1H	**32,101,798**	**32,102,986**	**Lateral organ boundary domain-containing protein 11**
HORVU1Hr1G012730	HC_G	1H	32,173,373	32,186,590	4'-Phosphopantetheinyl transferase superfamily
HORVU1Hr1G012750	LC_u	1H	32,177,144	32,182,714	Undescribed protein
HORVU1Hr1G013040	HC_G	1H	33,441,856	33,445,024	Ubiquitin-conjugating enzyme 37
HORVU1Hr1G013210	HC_G	1H	34,083,562	34,088,731	Isoleucine–glutamine (IQ)-domain 2
HORVU1Hr1G013480	HC_G	1H	35,679,610	35,683,434	Sugar transporter 1
HORVU1Hr1G013490	HC_U	1H	35,723,711	35,729,110	Unknown function
HORVU1Hr1G013560	LC_u	1H	36,073,128	36,078,899	Undescribed protein
HORVU1Hr1G013570	LC_u	1H	36,073,270	36,074,655	Undescribed protein
HORVU7Hr1G019680	**HC_G**	7H	**26,446,831**	**26,449,799**	**Polygalacturonase 1 non-catalytic β subunit**
HORVU7Hr1G019720	LC_TE	7H	26,534,413	26,542,035	Transposon Ty1-PL Gag-Pol polyprotein
HORVU7Hr1G019730	HC_G	7H	26,546,229	26,549,975	Pentatricopeptide repeat-containing protein
HORVU7Hr1G019810	HC_G	7H	26,736,047	26,740,148	ATP-dependent 6-phosphofructokinase 7
HORVU7Hr1G019830	HC_G	7H	26,812,633	26,817,294	Acyl-ACP thioesterase
HORVU7Hr1G019880	LC_u	7H	26,920,657	26,921,300	Undescribed protein
HORVU7Hr1G019890	**HC_G**	7H	**26,920,897**	**26,926,149**	**Sulphate transporter 3;4**
HORVU7Hr1G019930	HC_G	7H	27,119,476	27,129,693	Coatomer, beta' subunit
HORVU7Hr1G019990	HC_G	7H	27,155,214	27,161,528	DNA-repair protein XRCC1
HORVU7Hr1G020190	HC_G	7H	27,478,173	27,480,352	Carbonic anhydrase
HORVU7Hr1G020270	HC_G	7H	27,505,060	27,507,837	Fatty acyl-CoA reductase 1
HORVU7Hr1G020300	**HC_G**	7H	**27,546,603**	**27,556,612**	**Peroxidase superfamily protein**
HORVU7Hr1G020370	HC_G	7H	27,657,951	27,659,978	Carbonic anhydrase
HORVU7Hr1G020580	HC_G	7H	27,768,879	27,774,101	Cadmium tolerant 1
HORVU7Hr1G020590	HC_G	7H	27,771,905	27,777,772	Fe superoxide dismutase 3
HORVU7Hr1G020610	HC_G	7H	27,775,540	27,775,880	Myosin-J heavy chain
HORVU7Hr1G020620	HC_G	7H	27,861,260	27,864,514	Protein arginine methyltransferase 10
HORVU7Hr1G020660	HC_G	7H	27,958,533	28,145,362	Receptor kinase 3
HORVU7Hr1G020720	HC_G	7H	27,986,777	27,992,005	Receptor-like protein kinase 4
HORVU7Hr1G020730	LC_u	7H	27,989,246	27,990,451	Undescribed protein
HORVU7Hr1G020770	**HC_G**	7H	**28,104,777**	**28,112,667**	**Early nodulin-related**
HORVU7Hr1G020780	**HC_G**	7H	**28,111,982**	**28,112,637**	**Early nodulin-related**
HORVU7Hr1G020830	**HC_G**	7H	**28,203,216**	**28,204,125**	**Early nodulin-related**
HORVU7Hr1G033370	HC_G	7H	68,395,174	68,477,165	Magnesium transporter protein 1

[a]The predicted genes and their respective annotations were obtained from BARLEYMAP (Cantalapiedra et al., 2015).

[b]HC_G, high-confidence gene with predicted function, HC_U, high-confidence gene without predicted function, LC_u, low-confidence gene without predicted function.

[c]Genes in bold are involved in pathogen defence or recognition.

syringae) (Grimplet, Pimentel, Agudelo-Romero, Martinez-Zapater, & Fortes, 2017; Thatcher, Kazan, & Manners, 2012; Wang, Zhang, Su, Liu, & Hao, 2013).

The second region identified in this study is located on chromosome 7H at 26,447,530 to 28,772,177 bp and was associated with seedling and adult plant resistance. Steffenson et al. (1996)

studied 150 DH lines of a cross of Steptoe × Morex for spot blotch resistance using isolate ND85F and identified a major QTL on chromosome 7H active at the seedling and adult plant stage, which they designated *Rcs 5*. Bilgic et al. (2005) and Bovill et al. (2010) screened four DH populations each for seedling and adult plant resistance, and identified a QTL that co-located with the *Rcs 5* locus. In the former study, isolate ND85F was used and in the latter study isolate *SB 61*, which was also used in the present study. Yun et al. (2005) developed 104 recombinant inbred lines (RILs) and 98 advanced backcross lines (Yun et al., 2006) from a cross between OUH 602 (*H. vulgare* subsp. *spontaneum*) and barley cultivar Harrington, and as well identified the resistance locus *Rcs 5* using isolate ND85F. Furthermore, in GWA studies, Roy et al. (2010) screened 318 wild barley accessions with isolate ND85F and identified a QTL on chromosome 7H named *Rcs-qtl-7H-bPb-4584* located between 16 and 22Mbp that coincides with *Rcs 5*. Berger et al. (2013) studied 329 lines and cultivars from the Virginia Tech programme again with isolate ND85F and identified significant MTAs for seedling resistance on chromosome 7H located at 22 to 31 Mbp. Zhou and Steffenson (2013) identified a region located at 26 to 32 Mbp via GWAS. The BOPA marker 11_20162 was associated with resistance in all their trials. This marker was also significantly associated with disease resistance against pathotype 1 (isolate ND85F) in a GWA study by Wang et al. (2017). In an association study with 336 genotypes and an isolate mixture of 19 Moroccan isolates, Gyawali et al. (2018) identified a region associated with seedling and adult plant resistance located on chromosome 7H at 26 to 27 Mbp. Drader et al. (2009) developed a saturated map of the *Rcs 5* locus and postulated it to be flanked by markers BF263248 and BG414713 with a genetic interval of 2.8 cM. Drader et al. (2009) hypothesized that the spot blotch resistance on chromosome 7H in barley is similar or even the same gene as in wheat. This hypothesis was confirmed by Ayana et al. (2018), who conducted GWAS with 294 hard winter wheat accessions and identified a significant QTL (*QSb.sdsu-7B.1*) on wheat chromosome 7B, which corresponded to the resistance QTL *Rcs 5* in barley.

The second region identified on chromosome 7H is located at 68,476,333 bp, where no overlapping QTL have yet been described in previous studies. Hence, based on the data available we presume this to be a new QTL.

In the region detected between 26,447,530 to 28,772,177 bp and at 68,476,333 bp, there are three low-confidence genes and twenty-one high-confidence genes located (Table 3). Polygalacturonase 1 non-catalytic β subunit (HORVU7Hr1G019680) is part of the polygalacturonase, which is involved in pectin degradation. Pectin is a macromolecule and is a major component of plant cell walls. It contributes to cell wall stability, surface charge, ion balance, porosity and pH (Voragen, Coenen, Verhoef, & Schols, 2009). Pectin degradation in plants is important for fruit ripening (Liu et al., 2014). Phytopathogenic fungi, bacteria and nematodes produce polygalacturonase in order to penetrate and colonize plant tissue (Gomathi & Gnanamanickam, 2004). It was shown that increased polygalacturonase levels, and in particular an increased activity of the polygalacturonase 1 non-catalytic β subunit in plant tissue, lead to increased susceptibility towards abiotic and biotic stress in rice (Liu et al., 2014). In fact, markers SCRI_RS_139762, JHI-Hv50k-2016-454253, JHI-Hv50k-2016-454263 and JHI-Hv50k-2016-454328 showed positive allelic effects and therefore increased susceptibility (data not shown).

Sulphur is vital for plant growth and development, since it is essential for certain amino acids, hormones and secondary metabolites. Sulphate transporters (HORVU7Hr1G019890) are therefore important in every plant species (Gigolashvili & Kopriva, 2014; Takahashi, 2019). The role of glucosinolates in the *Brassicaceae* family against herbivorous and fungal pathogens has long been known (Bednarek et al., 2009; Radojčić Redovniković, Glivetić, Delonga, & Vorkapić-Furač, 2008). Glutathione is another sulphur containing essential molecule in every plant species, with vital roles in the primary metabolism, detoxification and redox signalling (Noctor et al., 2012). It was also shown to enhance susceptibility towards biotrophic and resistance towards necrotrophic fungal pathogens (Dubreuil-Maurizi & Poinssot, 2012; Gullner, Zechmann, Künstler, & Király, 2017; Noctor et al., 2012).

Besides catalysing the oxidoreduction between hydrogen peroxide and reductants, plant peroxidases are also involved in lignification, suberization, phytoalexin synthesis, the metabolism of auxin, reactive oxygen and nitrogen species, and cross-linkage of cell wall components (Almagro et al., 2008; Hiraga, Sasaki, Ito, Ohashi, & Matsui, 2001). Furthermore, it was demonstrated that peroxidases (HORVU7Hr1G020300) play a role in pathogen recognition and defence, by strengthening the cell wall through, for example, increased lignification, increasing levels of reactive oxygen species and levels of phytoalexin (Hiraga et al., 2001).

Nodules are root organs formed by legumes in order to go into symbiosis with nitrogen-fixing bacteria (Wagner, 2011). Nodulin genes were first described in soya bean (*Glycine max* L.) to be involved in the nodule formation (Legocki & Verma, 1980). However, nodulin-like proteins (HORVU7Hr1G020770, HORVU7Hr1G020780 and HORVU7Hr1G020830) were also described in non-nodulating plant species and classified into seven families (Denancé, Szurek, & Noël, 2014). They act as transporters among other functions for sugars, amino acids, auxin and nutrients or as virulence factors of pathogens (Chen et al., 2010; Denancé et al., 2014).

The aim of this study was to screen a diverse barley set for their response towards *B. sorokiniana*, the causal agent of the spot blotch disease in barley, and to identify QTL for resistance employing genome-wide association studies. The detected MTA corresponded to two major QTL located on chromosome 1H and 7H, respectively. Even though the two QTL on chromosome 1H (31 – 36 Mbp) and 7H (26 – 28 Mbp) have been described in previous studies, further research is necessary to narrow down and fine-map the intervals of interest and characterize the genes underlying resistance. Additionally, a putative new QTL identified on chromosome 7H at 68 Mbp represents a potentially interesting source of quantitative resistance for barley breeding.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest in the reported research.

AUTHOR CONTRIBUTION

OA and FO planned and managed the project. NL and IL conducted the field screenings in Russia. GJP was in charge of the screenings conducted in Australia (field and glasshouse). OA, FO, GJP and RS contributed to the interpretation and discussion of the results. FN conducted glasshouse screenings in Germany, analysed the data and wrote the manuscript.

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