Quantitative Trait Loci Mapping for Spot Blotch Resistance in Two Biparental Mapping Populations of Bread Wheat

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ABSTRACT

Spot blotch (SB), caused by *Bipolaris sorokiniana*, is a major fungal disease of wheat in South Asia and South America. Two biparental mapping populations with 232 F_{2.7} progenies each were generated, with CIMMYT breeding lines CASCABEL and KATH as resistant parents and CIANO T79 as the common susceptible parent. The two populations were evaluated for field SB resistance in CIMMYT's Agua Fria station for three consecutive cropping seasons, with artificial inoculation. Genotyping was done with the DArTseq platform and approximately 1,500 high quality and nonredundant markers were used for quantitative trait loci (QTL) mapping. In both populations, a major QTL was found on chromosome 5A in the *Vm-A1* region, explaining phenotypic variations of 13.5 to 25.9%, which turned up to be less- or nonsignificant when days to heading and plant height were used as covariates in

the analysis, implying a disease escape mechanism. Another major QTL was located on chromosome 5B in CASCABEL, accounting for 8.9 to 21.4% of phenotypic variation. Minor QTL were found on 4A and 4B in CASCABEL; 1B, 4B, and 4D in KATH; and 1B, 2B, and 4B in CIANO T79. Through an analysis of QTL projection onto the IWGSC Chinese Spring reference genome, the 5B QTL in CASCABEL was mapped in the *Sb2* region, delimited by the single nucleotide polymorphism marker *w SNP_Ku_c50354_55979952* and the simple sequence repeat marker *gwm213*, with a physical distance of about 14 Mb to the *Tsn1* locus.

Keywords: *Bipolaris sorokiniana*, *Cochliobolus sativus*, disease resistance, genetics and resistance, *Sb2*, *Triticum aestivum*, *Tsn1*

Spot blotch (SB), caused by *Bipolaris sorokiniana* (telemorph *Cochliobolus sativus*), is a globally important disease of wheat (*Triticum aestivum*), especially in warm and humid regions in South Asia and South America (Navathe et al. 2020). SB usually causes symptoms on leaf, sheath, and stem, however, under conducive conditions it also infects spikes resulting in shriveled kernels with black point (Gupta et al. 2018). The disease affects about 25 million hectares of wheat-growing areas globally, causing average yield reduction from 15 to 20%, which can be as high as 40 to 70% during severe epidemics (Duveiller and Sharma 2009; Sharma et al. 2007). Climate change has promoted the further spread of the disease in South Asia, especially the Indo-Gangetic and Trans-Gangetic plains (Gupta et al. 2018), and the widely adopted rice-wheat rotation system is favorable for the survival and multiplication of *B. sorokiniana* (Saari 1998). Among the SB management strategies, host resistance has been regarded as an indispensable component due to its economical and environmentally friendly nature. This is especially true for small scale farmers who have very limited access to fungicides and other advanced disease management tools (Dubin and Rajaram 1996).

Resistance to SB of wheat in field experiments is a quantitative trait, which is highly influenced by environmental conditions (Singh et al. 2018). Although no line exhibited immunity to SB, varieties with good resistance combined with early maturity and high yielding were identified in South Asian germplasm, like NL644 and NL297 (Dubin et al. 1998). Exotic sources with higher

degree of resistance have been found in germplasm from Brazil (BH 1146), China (Yangmai 6 and Ning 8201), CIMMYT (Chirya 3 and Saar) etc. (Duveiller and Sharma 2009; Lillemo et al. 2013). Genetic studies on such resistance sources all indicated a quantitative mode of inheritance, with quantitative trait loci (QTL) dispersing on 12 of the 21 chromosomes, as reviewed by Gupta et al. (2018). Soon afterward, more QTL have been reported in biparental mapping and genome-wide association studies, where high density of molecular markers were applied (Ahirwar et al. 2018; Ayana et al. 2018; Jamil et al. 2018; Singh et al. 2018). So far, SB resistance QTL have been reported on all 21 wheat chromosomes, except 3D and 5D. Of these QTL, four with major and stable effects have been designated as SB resistance genes. They are *Sb1* on chromosome 7DS (Lillemo et al. 2013), *Sb2* on 5BL (Kumar et al. 2015), *Sb3* on 3BS (Lu et al. 2016), and *Sb4* on 4BL (Zhang et al. 2020).

Days to heading (DH) and plant height (PH) have long been associated with SB resistance and become a linkage drag that breeders want to get rid of (Duveiller and Sharma 2009). Both DH and PH often show negative correlation with SB severity, i.e., late and tall wheat lines tend to have low SB severity, although early and short genotypes with good SB resistance have also been found (Singh et al. 2015). In other diseases, such association has also been reported, like Fusarium head blight (Xu et al. 2020), Septoria tritici blotch (Goudemand et al. 2013), Septoria nodorum blotch (Lu and Lillemo 2014), and tan spot (Kollers et al. 2014). This must mainly ascribe to disease escape, although other underlying mechanism like pleiotropy or tight linkage might have contributed to the association (He et al. 2016).

Since 1990s, the International Maize and Wheat Improvement Center (CIMMYT) increased its efforts on germplasm screening and breeding for SB resistance (Dubin and Rajaram 1996), and elite breeding lines combining good SB resistance and high yielding have been regularly compiled as Helminthosporium Leaf Blight Screening Nursery (HLBSN). The nursery has been distributed regularly to breeders and researchers mainly in South Asia and South America for their breeding and researcher activities (Singh et al. 2015). Genetic studies for several resistance sources have been conducted to better understand the resistance loci and their flanking

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markers to be used in marker-assisted selection (MAS). A synthetic wheat derived line SYN1 with high SB resistance was analyzed by Zhu et al. (2014), and three QTL turned out to be significant for the trait, residing on chromosomes 1B, 3BS, and 5AL. Recently, two more CIMMYT resistance lines, BARTAI and WUYA, were mapped for SB resistance, and the results indicated a polygenic control of the trait in both lines (Singh et al. 2018).

The objectives of the current study were to map QTL for SB resistance in two recombinant inbred line (RIL) populations and to identify molecular markers tightly linked to the QTL for potential use in MAS.

MATERIALS AND METHODS

Plant materials. Two elite CIMMYT breeding lines, CASCABEL (pedigree SOKOLL//W15.92/WEEBILL1) and KATH (WHEATEAR/KRONSTAD F2004), have exhibited excellent SB resistance in our previous experiments. The two lines were crossed to an SB susceptible cultivar CIANO T79 (BUCKY/(SIB)MAYA-74/4/BLUEBIRD//HD-832.5.5/OLESEN/3/CIANO-67/PENJAMO-62) to produce two RIL populations. The populations were generated following the single seed descent method to F_{2.7} generation for field experiments, both having 232 progenies.

Fig. 1. Frequency distribution of area under the disease progression curve (AUDPC) values of spot blotch for the **A, B, and C**, CASCABEL × CIANO T79 and the **D, E, and F**, KATH × CIANO T79 populations in **A and D**, 2013, **B and E**, 2014, and **C and F**, 2015. Phenotypic values of the parents are indicated, with CA standing for CASCABEL, K for KATH, and C for CIANO T79.

TABLE 1. Analysis of variance for spot blotch and its heritability estimates in the CASCABEL × CIANO T79 (CC) and KATH × CIANO T79 (KC) populations^a

Populations	Source	df	Mean square	F value	P value	Heritability
CC	Genotype	225	174,571	23.03	<0.0001	0.89
	Year	2	4,519,550	596.21	<0.0001	
	Genotype × year	450	19,910	2.63	<0.0001	
	Rep (year)	3	207,290	27.35	<0.0001	
	Error	675	7,580			
KC	Genotype	230	142,654	11.43	<0.0001	0.85
	Year	2	7,581,452	607.20	<0.0001	
	Genotype × year	460	21,488	1.72	<0.0001	
	Rep (year)	3	165,091	13.22	<0.0001	
	Error	690	12,486			

^a Six lines of CC and one of KC were not included in ANOVA analysis due to missing data.

TABLE 2. Pearson correlation coefficients between spot blotch and days to heading/plant height in the CASCABEL × CIANO T79 (CC) and KATH × CIANO T79 (KC) populations across years^a

Traits	CC			KC		
	2013	2014	2015	2013	2014	2015
Days to heading	-0.51**	-0.39**	-0.47**	-0.46**	-0.57**	-0.54**
Plant height	-0.15	-0.39**	-0.27**	-0.30**	-0.38**	-0.32**

^a **, $P < 0.0001$.

Field trials and phenotyping. The field experiments were conducted in CIMMYT's Agua Fria experimental station in three consecutive cropping seasons, from 2012–13 to 2014–15, with sowing in November and harvest in March. The two populations were planted in two-row plots of 1 m length on 80 cm raised beds with two replications.

A mixture of five local *B. sorokiniana* isolates with high pathogenicity were used for field application with a sorghum inoculum method as described in Singh et al. (2015). Briefly, reactivated *B. sorokiniana* isolates were used to inoculate sorghum grains in glass bottles, which were then incubated at room temperature for 6 weeks. For field inoculation at Zadoks' GS29 stage, the infested sorghum grains were distributed in the middle of the double row at a rate of 20 g per plot. Field SB evaluation started at 4 or 5 weeks after inoculation, according to disease development. Saari and Prescott's double-digit scale (00 to 99) was used for disease scoring (Saari and Prescott 1975), where the first digit (D1) indicates the height of disease progress and the second digit (D2) represents the proportion of infected leaf area. SB severity was then calculated with the following formula: Severity (%) = (D1/9) × (D2/9) × 100. The disease evaluation was repeated four times at weekly intervals, and the severity values were used for calculating area under the disease progression curve (AUDPC). Average AUDPC values over two replications in single years and mean values across years were used as the phenotypic data for QTL analysis.

Statistical analyses. The PROC GLM module in SAS program ver. 9.2 was used to conduct analysis of variance (ANOVA), and the results were used to calculate heritability estimates, using the formula $H^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_{g \times y}^2 / y + \sigma_e^2 / ry)$, in which σ_g^2 stands for genetic variance, $\sigma_{g \times y}^2$ for genotype-by-year interaction, σ_e^2 for error variance, y for the number of years, and r for the number of replications. The PROC CORR function was used to derive Pearson correlation coefficients.

Genotyping. Genomic DNA was extracted from young leaves with the CTAB method (CIMMYT 2005). The two populations were genotyped with the DArTseq platform at the Genetic Analysis Service for Agriculture (SAGA) at CIMMYT, Mexico. A few more single nucleotide polymorphism (SNP) markers including those for *Rht-B1* and *Vrn-A1* were genotyped with KASPar technology at CIMMYT (Dreisigacker et al. 2016). Markers with missing data points greater than 20% and minor allele frequency less than 30% were excluded from subsequent analysis. Redundant markers were deleted using the BIN functionality of the ICIMapping ver. 4.1 software (available at www.isbreeding.net).

Linkage and QTL mapping. JoinMap ver.4 (Van Ooijen 2006) was employed to perform linkage analysis, with LOD scores from 5 to 15 for grouping different linkage groups (LG) and the maximum likelihood algorithm for ordering markers within each group. LGs were assigned to chromosomes via BLAST searches of the marker sequences against the IWGSC Chinese Spring (CS) RefSeq ver. 1.0. MapQTL ver. 6.0 (Van Ooijen 2009) was used for QTL analysis. Interval mapping (IM) was first conducted to find potential QTL with a LOD higher than 2.0 for each trait, and then multiple QTL mapping (MQM) for each potential QTL was carried out, using the tightly linked markers to each QTL identified in IM as cofactors. QTL were taken as significant when exceeded the LOD threshold, i.e., 3.5 for the KATH × CIANO T79 population and 3.4 for the CASCABEL × CIANO T79 population (1,000 permutations, $\alpha = 0.05$), in at least one environment. Additionally, if a QTL does not exceed the threshold but shows a LOD value greater than 2.0 in multiple environments, it is reported as a potential QTL. MapChart ver. 2.3 (Voorrips 2002) was used to draw linkage map and LOD curves.

QTL projection onto the IWGSC CS reference genome. To enable the comparison of QTL mapped in different studies, markers flanking and within a QTL confidence interval were projected onto the IWGSC CS RefSeq ver. 1.0 reference genome. Physical positions of the projected markers were obtained from either T3/Wheat (<https://triticeaetoolbox.org/wheat/>) or URGI (<http://wheat-urgi.versailles.inra.fr/Seq-Repository/BLAST>) via BLAST searches.

RESULTS

Phenotyping for SB resistance. SB disease pressure varied among the three cropping seasons, where the 2013 to 2014 (denoted as 2014 hereafter) and 2014 to 2015 (2015) cycles exhibited similar disease pressure that was much higher than the 2012 to 2013 (2013) cycle. As to the two populations, the KATH × CIANO T79 population (denoted as KC hereafter) showed always slightly higher grand mean AUDPC than the CASCABEL × CIANO T79 (CC) population (Fig. 1). Distribution of lines for SB resistance in both populations were continuous and transgressive segregation toward both resistance and susceptibility could be found (Fig. 1). ANOVA indicated that year was the biggest source of variation for SB in both populations; nevertheless, significant effects of genotype were found, as well as that of genotype × year. High heritability estimates of 0.89 and 0.85 for CC and KC were obtained, respectively (Table 1).

TABLE 3. Phenotypic effects (%) of quantitative trait loci (QTL) for spot blotch resistance in the CASCABEL × CIANO T79 (CC) and KATH × CIANO T79 (KC) populations and their association with phenological traits^a

Population	QTL ^b	Position (cM)	Position (Mb) ^c	Left marker	Right marker	2013	2014	2015	Mean	R source ^d	Traits associated ^e
CC	1B	210.8–215.6	619.6–622.7	1042822	1117717	3.5	2.2	3.4	2.5	C	
	2B	121.5–143.1	52.7–73.3	1228793	1167654		2.8	3.0	2.9	C	
	4A	25.5–35.3	620.8–624.1	3023781	992456	4.6	2.8		2.8	CA	
	4B	72.6–98.9	30.9–622.4	Rht-B1	2279882	6.3			2.4	CA	DH, PH
	5A	44.2–48.6	582.6–587.4	2341646	Vrn-A1	20.8	14.1	22.0	19.4	CA	DH
	5B	73.9–77.7	412.7–431.9	996745	10592866	8.9	13.9	21.4	17.2	CA	
	Accumulated percentage of variation explained					44.1	35.8	49.8	47.2		
KC	1B	149.0–151.8	673.6–674.5	2263671	100193437		4.6		3.1	K	
	2B	66.2–71.2	65.4–68.6	1017820	3947227	4.2	6.7	15.0	8.4	C	
	4B	88.7–91.3	609.5–619.1	4989130	1114716	8.0	3.4	4.3	5.7	C	
	4B.2	94.4–115.4	613.1–652.4	998971	1095421	5.6		4.5	4.8	K	
	4D	0.0–17.5	32.3–336.8	BS00036421	1059032		4.1		2.3	K	PH
	5A	174.6–188.2	570.5–591.2	987242	IWA4449	25.9	13.5	24.9	25.6	K	DH
	Accumulated percentage of variation explained					43.7	32.3	48.7	49.9		

^a The percentage of explained phenotypic variation is shown in the table, QTL are listed if they were over the LOD threshold of 3.5 for CC and 3.4 for KC (in bold) in at least one environment or over the threshold of 2 in multiple environments.

^b Numbers were used to distinguish QTL mapped in a same linkage group.

^c The projected physical positions in the IWGSC Chinese Spring RefSeq ver. 1.0 reference genome are shown.

^d CA, CASCABEL; K, KATH; and C, CIANO T79.

^e PH, plant height; and DH, days to heading.

Correlation coefficients of SB among the 3 years were high in both populations, ranging from 0.73 to 0.81 in CC and from 0.66 to 0.77 in KC. Correlation coefficients of SB with DH and PH were all negative and mostly significant, and those between SB and DH were always higher than those between SB and PH (Table 2).

Genotyping and linkage analysis. The linkage map of CC has been published in Hu et al. (2019). Briefly, 1,798 high-quality markers were used to construct 43 LGs in the population, representing all 21 wheat chromosomes. For KC, 1,249 non-redundant markers of high quality were used for constructing 29 LGs, representing all wheat chromosomes. The LGs covered 3,531

cM, with an average genetic distance between markers of 2.8 cM. A and B subgenome LGs had total genetic length of 1,493 and 1,365 cM, respectively, whereas D subgenome LGs had a total genetic length of only 673 cM.

QTL mapping for the CASCABEL × CIANO T79 population. Across the 3 years, QTL on chromosomes 1B, 5A, and 5B were consistently detected, of which the latter two showed major effects of up to 22.0% for 5A and 21.4% for 5B (Table 3 and Fig. 2), acting in an additive mode (Fig. 3). Additionally, three minor QTL were detected in 1 or 2 of the 3 years, being located on chromosomes 2B, 4A, and 4B. For the reported QTL, CASCABEL

Fig. 2. Quantitative trait loci (QTL) profiles for spot blotch resistance in the CASCABEL × CIANO T79 population. Genetic distances are shown in centimorgans to the left side of the linkage groups. Only framework markers are presented, except for the QTL regions. A threshold of 3.0 is indicated by a dashed vertical line in the LOD graph.

contributed all resistance alleles but the ones on 1B and 2B (Table 3).

The QTL on 5A was in the *Vrn-A1* region, showing association with DH as expected, and the one on 4B bordered *Rht-B1*, being overlapped with QTL for DH and PH (Table 3 and Fig. 2). When DH and PH were used as covariates in MQM mapping, all but the 5B QTL were markedly affected, in terms of either the confidence interval or phenotypic effects. Notably, the 2B QTL was non-significant in the latter model, where a new QTL on 6D turned out to be significant in 2014 and 2015. As for the 5A QTL, its effects decreased substantially in 2013 and was nonsignificant in the other 2 years (Supplementary Table S1).

QTL mapping for the KATH × CIANO T79 population. Like the CC population, a QTL on 5A in the *Vrn-A1* region was consistently detected with major effects, ranging from 13.5 to 25.9% (Table 3). When DH and PH were used as covariates, however, it became nonsignificant in 2014, but remained to be significant in the other 2 years, with lower percentage of variation explained (Supplementary Table S1).

QTL on 2B and 4B were consistently significant across years, with mostly minor effects of less than 10%, except for the unexpectedly high phenotypic effects of 15% for the former in 2015. Three more minor QTL were detected on 1B, 4B, and 4D, being significant in limited environments (Table 3 and Fig. 4). Of these QTL, the resistance alleles came mostly from KATH, except for 2B and 4B. When DH and PH were used to adjust the QTL mapping, the 4D QTL disappeared, and a minor QTL on 7A became significant in 2014 (Table 3 and Supplementary Table S1).

Projection of the SB QTL onto the CS reference genome. The major QTL on 5A in both CC and KC was not projected due to its clear association with *Vrn-A1* (Figs. 2 and 4). The major QTL on 5B in CC was projected to a physical range of 406.9 to 431.9 Mb, falling within the confidence interval of *QSh.bhu-5B* identified in Yangmai 6 (315.5 to 447.2 Mb) (Kumar et al. 2009), and being overlapped with that in Ning 8201 (315.5 to 418.8 Mb) (Kumar et al. 2010) (Fig. 5). Additionally, the QTL region encompassed an SNP reported by Bainsla et al. (2020) and tightly linked to two SNPs identified by Gurung et al. (2014). The hot spot region for this QTL cluster was then delimited by the SNP marker *w SNP_Ku_c50354_55979952* and the simple sequence repeat (SSR) marker *gwm213*, encompassing a physical range of 404.2 to 418.8 Mb. Other QTL on

5B did not overlap with the one identified in the current study, including the *Tsn1* gene for fungal pathogens with *ToxA* (Fig. 5). Likewise, QTL on other chromosomes were projected onto the CS reference genome too (Supplementary Fig. S1).

DISCUSSION

Field resistance to SB in wheat is a typical quantitative trait, being controlled by multiple QTL with moderate to minor effects (Singh et al. 2018). So far, no major gene conferring immunity has been identified, and even QTL with major effects are rare (Gupta et al. 2018). In the current study, a QTL on 5B exhibited major effects across years in the CC population, which could be of high potentiality to be used in wheat breeding. Its confidence interval agreed well with that of *QSh.bhu-5B* (Kumar et al. 2009, 2010), which was later designated as *Sb2* (Kumar et al. 2015). However, the QTL in the latter study was mapped away from our QTL, as well as from *QSh.bhu-5B* (Fig. 5). This was unexpected since the line YS116, in which *Sb2* was precisely mapped, was a RIL of the Yangmai 6 × Sonalika population (Kumar et al. 2015), thus the position of the resistance gene should remain unchanged. In our QTL projection analysis, only one of the three SNPs identified in Gurung et al. (2014) fell into the QTL interval of *Sb2* reported by Kumar et al. (2015), whereas six markers from three subsequent studies supported the confidence interval of *QSh.bhu-5B* that was mapped in Kumar et al. (2009) and Kumar et al. (2010). Therefore, we propose that the position of *Sb2* should be in the *QSh.bhu-5B* region that coincided or closely linked to the 5B QTL identified in the current study. The different position of *Sb2* in Kumar et al. (2015) might have been caused by events like accidental out-crossing or chromosomal rearrangement during the generation of YS116, or the poor collinearity between CS and Yangmai 6/Ning 8201 in the *Sb2* region. By scrutinizing the *Sb2* QTL profiles, we found that the peaks were closer to the proximal markers *gwm317* and *gwm231* in Kumar et al. (2009) and Kumar et al. (2010), respectively, whereas the peak was tightly linked to the distal marker 1137742 in the current study (Fig. 2). This strongly implies that the underlying gene might be localized in the overlapped region of the three QTL intervals delimited by the SNP marker *w SNP_Ku_c50354_55979952* and the SSR marker *gwm213*, encompassing the significant SNPs in GWAS by Gurung et al. (2014) and Bainsla et al. (2020). Nevertheless, this is still a big genomic region, and fine mapping is needed to further narrow down the region for gene cloning and development of diagnostic markers.

Initially, the origin of *Sb2* was traced back to Chinese varieties Yangmai 6 (Kumar et al. 2009) and Ning 8201 (Kumar et al. 2010), now it appears that the gene exists also in CIMMYT (CASCABEL) and Afghan gene pool (Bainsla et al. 2020). *Sb2* might have an even wider distribution since it was significant in a GWAS study on a global collection of 528 spring wheat accessions (Gurung et al. 2014).

Tsn1 is another important gene on the 5BL region, conferring susceptibility reaction against fungal diseases where *ToxA* is involved, like tan spot, *Septoria nodorum* blotch and SB (Faris et al. 2010; McDonald et al. 2018). Based on its role on SB resistance and the location on 5BL, Friesen et al. (2018) suggested that *Tsn1* is the susceptible allele of *Sb2*. However, in our QTL projection analysis, *Tsn1* was clearly different from *Sb2* (Fig. 5; since CS does not have the *Tsn1* gene, the mapping interval of the gene by Lu et al. (2006) was used). Additionally, we have genotyped *Tsn1* in the CC population, which turned out to be monomorphic (data not shown). This could have two interpretations, (i) *Tsn1* and *Sb2* are different genes or (ii) the 5B QTL found in the current study is not *Sb2* but a new QTL, and we incline the former one. The 10 SNPs significantly associated with SB resistance in Ahirwar et al. (2018) must correspond to *Tsn1* instead of *Sb2*, based on their projected region on the CS genome. This is in accordance with the fact that *ToxA* presents in the Indian population of *B. sorokiniana* (Navathe et al. 2020), so that the *Tsn1*–*ToxA* interaction took place,

Fig. 3. Phenotypic effects of different combinations of the 5A and 5B quantitative trait loci for spot blotch resistance in the CASCABEL × CIANO T79 population. + and – denote the resistant and susceptible alleles, respectively. The grand mean AUDPC values across the three cropping seasons are used here, and the different letters following the group mean values indicate significant difference at $\alpha = 0.01$.

resulting in the significance of *Tsn1* for SB susceptibility in the population.

An alternative conclusion regarding the multiple QTL mapped on the 5B region could be that they represent a cluster of SB resistance genes, considering the different *B. sorokiniana* isolates used in the abovementioned studies. Nevertheless, this chromosomal region is very important for SB resistance, which warrants further investigation.

Apart from the 5B QTL, another major QTL on 5A was found in both populations, exhibiting even higher phenotypic effects; nevertheless, it might not be a real SB resistance QTL, considering its association with *Vrn-A1* that controls DH in the two populations. This QTL was detected in the two populations reported in our previous study (Singh et al. 2018), having major effects that decreased significantly when adjusted for DH and PH. In other studies, markers closely linked to *Vrn-A1* could also be identified

for SB resistance (Bainsla et al. 2020; Gurung et al. 2014; Zhu et al. 2014). Despite this, the possibility that a SB resistance gene resides in the *Vrn-A1* region cannot be excluded, since the 5A QTL remained significant after adjusting for DH in some environments (Supplementary Table S1). Similarly, a few minor QTL might have been caused by PH, like the 4D QTL in the KC population. Such QTL can be easily picked up due to their association with PH and/or DH, and when the disease data are adjusted for the two phenological traits, such QTL generally disappear or become less significant. This applies not only to SB, but also to other diseases like Fusarium head blight (He et al. 2019) and Septoria nodorum blotch (Lu and Lillemo 2014), highlighting the importance of scoring DH and PH in genetic studies conducted in field conditions.

The 2B QTL in the two populations were projected to the same region on the 2BS chromosome, and because their resistance alleles were both contributed by CIANO T79, they must be the same QTL.

Fig. 4. Quantitative trait loci (QTL) profiles for spot blotch resistance in the KATH × CIANO T79 population. Genetic distances are shown in centimorgans to the left side of the linkage groups. Only framework markers are presented, except for the QTL regions. A threshold of 3.0 is indicated by a dashed vertical line in the LOD graph.

Previously reported QTL on 2BS do not overlap with this one, thus it must be a new QTL (Supplementary Fig. S1). In each of the two populations, a QTL on 1B was mapped; but they were in different regions. The one in CC has not been reported previously, whereas the one in KC mapped closely to the 1B QTL identified in CIMMYT lines BARTAI and WUYA, implying a common QTL in CIMMYT breeding lines (Supplementary Fig. S1). Based on source of resistance and chromosome location, the three QTL on 4B must be different from each other; but the 4B.2 QTL in KC might be the same as reported in BARTAI and WUYA (Singh et al. 2018). The confidence interval of the 4B QTL in CC harbors that of the newly mapped *Sb4* gene (Zhang et al. 2020), suggesting their potential association. The 4A QTL identified in CC must be new (Supplementary Fig. S1).

Apart from *Sb2*, the results of the current study were similar to those of our previous study that SB resistance in field trials is controlled mostly by multiple QTL with minor effects, and is strongly influenced by phenological traits especially DH (Singh et al. 2018). Both in Mexico and India, SB resistance, or more precisely, SB escape, is associated with late heading, which is not preferred in breeding (Duveiller and Sharma 2009). Nevertheless, SB resistant lines with early heading have been identified in both populations used in this study (data not shown). This could be achieved via two ways. First, to use the late flowering allele of *Vrn-A1* that is associated with SB resistance, and select relatively early lines among progenies, since there are many other QTL controlling earliness of wheat. Second, to use the early flowering allele of *Vrn-A1* and employ *Sb2* or other SB resistance genes/QTL to count balance the susceptibility effects brought by the former. The advantage of breeding for SB resistance is that the three *Sb* genes are all present in locally adapted lines and thus no need to introduce them from exotic sources that often bring unwanted traits, and the pleiotropic effects of *Sb1* (syn. *Lr34/Yr18/Sr57/Pm38/Ltn1*) on rusts are especially beneficial for breeders in SB prone regions.

Fig. 5. Comparison of reported quantitative trait loci (QTL) related to spot blotch resistance on chromosome 5BL, with their physical positions projected to the IWGSC Chinese Spring (CS) RefSeq v1.0. Molecular markers are indicated to the left of the chromosomes, whereas their physical positions are shown to the right. Markers in the QTL regions reported in the current study appear in bold and are highlighted in red. For each QTL, the resistance source (only available for biparental mapping studies) and the corresponding citation are denoted.

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LITERATURE CITED

- Ahirwar, R. N., Mishra, V. K., Chand, R., Budhlakoti, N., Mishra, D. C., Kumar, S., Singh, S., and Joshi, A. K. 2018. Genome-wide association mapping of spot blotch resistance in wheat association mapping initiative (WAMI) panel of spring wheat (*Triticum aestivum* L.). *PLoS One* 13: e0208196.
- Ayana, G. T., Ali, S., Sidhu, J. S., Gonzalez Hernandez, J. L., Turnipseed, B., and Sehgal, S. K. 2018. Genome-wide association study for spot blotch resistance in hard winter wheat. *Front. Plant Sci.* 9:926.
- Bainsla, N. K., Phuke, R. M., He, X., Gupta, V., Bishnoi, S. K., Sharma, R. K., Ataci, N., Dreisigacker, S., Juliana, P., and Singh, P. K. 2020. Genome-wide association study for spot blotch resistance in Afghan wheat germplasm. *Plant Pathol.* 69:1161-1171.
- CIMMYT. 2005. Laboratory Protocols: CIMMYT Applied Molecular Genetics Laboratory, 3rd Ed. CIMMYT, D.F., Mexico.
- Dreisigacker, S., Sukumaran, S., Guzmán, C., He, X., Lan, C., Bonnett, D., and Crossa, J. 2016. Molecular marker-based selection tools in spring bread wheat improvement: CIMMYT experience and prospects. Pages 421-474 in: *Molecular Breeding for Sustainable Crop Improvement*. V. Rajpal, S. Rao, and S. Raina, eds. Springer.
- Dubin, H., Arun, B., Begum, S., Bhatta, M., Dhari, R., Goel, L., Joshi, A., Khanna, B., Malaker, P., and Pokhrel, D. 1998. Results of the South Asia regional Helminthosporium leaf blight and yield experiments, 1993-94. Pages 182-187 in: *Helminthosporium Blights of wheat: Spot blotch and tan spot*. E. Duveiller, H. J. Dubin, J. Reeves, and A. McNab, eds. CIMMYT, El Batán, Mexico.
- Dubin, H., and Rajaram, S. 1996. Breeding disease-resistant wheats for tropical highlands and lowlands. *Annu. Rev. Phytopathol.* 34:503-526.
- Duveiller, E. M., and Sharma, R. C. 2009. Genetic improvement and crop management strategies to minimize yield losses in warm non-traditional wheat growing areas due to spot blotch pathogen *Cochliobolus sativus*. *J. Phytopathol.* 157:521-534.
- Faris, J. D., Zhang, Z., Lu, H., Lu, S., Reddy, L., Cloutier, S., Fellers, J. P., Meinhardt, S. W., Rasmussen, J. B., Xu, S. S., Oliver, R. P., Simons, K. J., and Friesen, T. L. 2010. A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. *Proc. Natl. Acad. Sci. USA* 107:13544-13549.
- Friesen, T. L., Holmes, D. J., Bowden, R. L., and Faris, J. D. 2018. ToxA is present in the U.S. *Bipolaris sorokiniana* population and is a significant virulence factor on wheat harboring *Tsn1*. *Plant Dis.* 102:2446-2452.
- Goudemand, E., Laurent, V., Duchalais, L., Tabib Ghaffary, S. M., Kema, G. H. J., Lonnet, P., Margalé, E., and Robert, O. 2013. Association mapping and meta-analysis: Two complementary approaches for the detection of reliable Septoria tritici blotch quantitative resistance in bread wheat (*Triticum aestivum* L.). *Mol. Breed.* 32:563-584.
- Gupta, P. K., Chand, R., Vasistha, N. K., Pandey, S. P., Kumar, U., Mishra, V. K., and Joshi, A. K. 2018. Spot blotch disease of wheat: The current status of research on genetics and breeding. *Plant Pathol.* 67:508-531.
- Gurung, S., Mamidi, S., Bonman, J. M., Xiong, M., Brown-Guedira, G., and Adhikari, T. B. 2014. Genome-wide association study reveals novel quantitative trait loci associated with resistance to multiple leaf spot diseases of spring wheat. *PLoS One* 9:e108179.
- He, X., Dreisigacker, S., Singh, R. P., and Singh, P. K. 2019. Genetics for low correlation between Fusarium head blight disease and deoxynivalenol (DON) content in a bread wheat mapping population. *Theor. Appl. Genet.* 132:2401-2411.
- He, X., Lillemo, M., Shi, J., Wu, J., Bjørnstad, Å., Belova, T., Dreisigacker, S., Duveiller, E., and Singh, P. 2016. QTL characterization of Fusarium head blight resistance in CIMMYT bread wheat line Soru#1. *PLoS One* 11:e0158052.
- Hu, W., He, X., Dreisigacker, S., Sansaloni, C. P., Juliana, P., and Singh, P. K. 2019. A wheat chromosome 5AL region confers seedling resistance to both tan spot and Septoria nodorum blotch in two mapping populations. *Crop J.* 7:809-818.
- Jamil, M., Ali, A., Gul, A., Ghafoor, A., Ibrahim, A. M. H., and Mujeeb-Kazi, A. 2018. Genome-wide association studies for spot blotch (*Cochliobolus sativus*) resistance in bread wheat using genotyping-by-sequencing. *Phytopathology* 108:1307-1314.
- Kollers, S., Rodemann, B., Ling, J., Korzun, V., Ebmeyer, E., Argillier, O., Hinz, M., Plieske, J., Kulosa, D., Ganai, M. W., and Röder, M. S. 2014. Genome-wide association mapping of tan spot resistance (*Pyrenophora tritici-repentis*) in European winter wheat. *Mol. Breed.* 34:363-371.
- Kumar, S., Röder, M. S., Tripathi, S. B., Kumar, S., Chand, R., Joshi, A. K., and Kumar, U. 2015. Mendelization and fine mapping of a bread wheat spot blotch disease resistance QTL. *Mol. Breed.* 35:218.

- Kumar, U., Joshi, A. K., Kumar, S., Chand, R., and Röder, M. S. 2009. Mapping of resistance to spot blotch disease caused by *Bipolaris sorokiniana* in spring wheat. *Theor. Appl. Genet.* 118:783-792.
- Kumar, U., Joshi, A. K., Kumar, S., Chand, R., and Röder, M. S. 2010. Quantitative trait loci for resistance to spot blotch caused by *Bipolaris sorokiniana* in wheat (*T. aestivum* L.) lines 'Ning 8201' and 'Chirya 3'. *Mol. Breed.* 26:477-491.
- Lillemo, M., Joshi, A. K., Prasad, R., Chand, R., and Singh, R. P. 2013. QTL for spot blotch resistance in bread wheat line Saar co-locate to the biotrophic disease resistance loci *Lr34* and *Lr46*. *Theor. Appl. Genet.* 126:711-719.
- Lu, H. J., Fellers, J. P., Friesen, T. L., Meinhardt, S. W., and Faris, J. D. 2006. Genomic analysis and marker development for the *Tsn1* locus in wheat using bin-mapped ESTs and flanking BAC contigs. *Theor. Appl. Genet.* 112:1132-1142.
- Lu, P., Liang, Y., Li, D., Wang, Z., Li, W., Wang, G., Wang, Y., Zhou, S., Wu, Q., Xie, J., Zhang, D., Chen, Y., Li, M., Zhang, Y., Sun, Q., Han, C., and Liu, Z. 2016. Fine genetic mapping of spot blotch resistance gene *Sb3* in wheat (*Triticum aestivum*). *Theor. Appl. Genet.* 129:577-589.
- Lu, Q., and Lillemo, M. 2014. Molecular mapping of adult plant resistance to *Parastagonospora nodorum* leaf blotch in bread wheat lines 'Shanghai-3/Catbird' and 'Naxos'. *Theor. Appl. Genet.* 127:2635-2644.
- McDonald, M. C., Ahren, D., Simpfendorfer, S., Milgate, A., and Solomon, P. S. 2018. The discovery of the virulence gene *ToxA* in the wheat and barley pathogen *Bipolaris sorokiniana*. *Mol. Plant Pathol.* 19:432-439.
- Navathe, S., Yadav, P. S., Chand, R., Mishra, V. K., Vasistha, N. K., Meher, P. K., Joshi, A. K., and Gupta, P. K. 2020. *ToxA-Tsn1* interaction for spot blotch susceptibility in Indian wheat: An example of inverse gene-for-gene relationship. *Plant Dis.* 104:71-81.
- Saari, E. 1998. Leaf blight disease and associated soil-borne fungal pathogens of wheat in South and South East Asia. Pages 37-51 in: *Helminthosporium Blights of Wheat: Spot Blotch and Tan Spot*. E. Duveiller, H. J. Dubin, J. Reeves, and A. McNab, eds. CIMMYT, Mexico, D.F.
- Saari, E. E., and Prescott, J. M. 1975. A scale for appraising the foliar intensity of wheat disease. *Plant Dis. Rep.* 59:377-380.
- Sharma, R. C., Duveiller, E., and Jacquemin, J. M. 2007. Microsatellite markers associated with spot blotch resistance in spring wheat. *J. Phytopathol.* 155:316-319.
- Singh, P., He, X., Sansaloni, C., Juliana, P., Dreisigacker, S., Duveiller, E., Kumar, U., Joshi, A., and Singh, R. 2018. Resistance to spot blotch in two mapping populations of common wheat is controlled by multiple QTL of minor effects. *Int. J. Mol. Sci.* 19:4054.
- Singh, P. K., Zhang, Y., He, X., Singh, R. P., Chand, R., Mishra, V. K., Malaker, P. K., Reza, M. A., Rahman, M. M., Islam, R., Chowdhury, A. K., Bhattacharya, P. M., Kalappanavar, I. K., Crossa, J., and Joshi, A. K. 2015. Development and characterization of the 4th CSISA-spot blotch nursery of bread wheat. *Eur. J. Plant Pathol.* 143:595-605.
- Van Ooijen, J. 2006. JoinMap 4, Software for the calculation of genetic linkage maps in experimental populations. Kyazma BV, Wageningen, Netherlands.
- Van Ooijen, J. 2009. MapQTL 6, Software for the mapping of quantitative trait loci in experimental populations of diploid species. Kyazma BV, Wageningen, Netherlands.
- Voorrips, R. E. 2002. MapChart: Software for the graphical presentation of linkage maps and QTLs. *J. Hered.* 93:77-78.
- Xu, K., He, X., Dreisigacker, S., He, Z., and Singh, P. K. 2020. Anther extrusion and its association with Fusarium head blight in CIMMYT wheat germplasm. *Agronomy (Basel)* 10:47.
- Zhang, P., Guo, G., Wu, Q., Chen, Y., Xie, J., Lu, P., Li, B., Dong, L., Li, M., Wang, R., Yuan, C., Zhang, H., Zhu, K., Li, W., and Liu, Z. 2020. Identification and fine mapping of spot blotch (*Bipolaris sorokiniana*) resistance gene *Sb4* in wheat. *Theor. Appl. Genet.* 133:2451-2459.
- Zhu, Z., Bonnett, D., Ellis, M., Singh, P., Heslot, N., Dreisigacker, S., Gao, C., and Mujeeb-Kazi, A. 2014. Mapping resistance to spot blotch in a CIMMYT synthetic-derived bread wheat. *Mol. Breed.* 34:1215-1228.