Discovering chromosomal regions related to spot blotch disease in wheat (*Triticum aestivum* L.) by QTL analysis

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Abstract

Spot blotch (SB) is a major constraint to wheat (*Triticum aestivum* L.) production in South Asia's warmer plains. SB, also known as leaf blight, is caused by *Bipolaris sorokiniana* and causes significant yield losses in India's Eastern Gangetic Plain Zone. The aim of this study was to map SB resistance via composite interval mapping (CIM) in the PBW343/IC252874 population, which comprised of 165 doubled haploid lines (DHLs), across two years in India. The phenotypic analysis of these lines revealed a constant variance in disease severity, implying that SB resistance is most likely polygenic. DHLs' phenotypic data was also used to map QTLs using SSR markers. The presence of quantitative inheritance with transgressive segregation for SB resistance in the population was also revealed. The QTLs were discovered on 12 chromosomes i.e. 1B, 1D, 2A, 2B, 2D, 3B, 4A, 4D, 5A, 5B, 6A, and 7A. A new QTL was detected on chromosome 4D being linked to SB in both years. We have also found two consistent QTLs on the chromosomes, 2B and 5B with the average PVE of 17.9% and 19.9% respectively. These findings reveal new genomic areas linked to spot blotch disease, which could be used in disease resistance breeding strategies in wheat advancement with further validation.

Introduction

For nearly three-quarters of the world's population, wheat (*Triticum aestivum* L.) is their primary source of nutrition (FAO, 2018). It provides edible grain which forms staple food for billions of people worldwide (Verma et al. 2020). It is necessary to expedite wheat improvement in order to fulfil estimated world food demand by 2050. The latest United

Nations projections indicate that world population will reach 10 billion in the year 2056 (Prakash et al. 2019). During this heavily populated region of the world, which encompasses India, Pakistan, Nepal, and Bangladesh, the Green Revolution played a key role in ensuring food security (Joshi et al., 2007b). Wheat yields, on the other hand, are under threat from a range of disease kinds and pests, as well as from increased climate unpredictability, all of which threaten crop growth and yields (Gurung et al., 2012). Climate resilient, disease-resistant, and high-agronomic-value wheat has the potential to greatly boost wheat yields and meet future food needs (Mondal et al., 2016). This devastating wheat disease, which affects 9 million hectares of crops farmed by primarily smallholder farmers in the Eastern Gangetic Plains (Fig. 1), is the most common in the region (CIMMYT, 2013). Shrestha et al. (1998) reported a yield loss of up to 23.8%, Mehta (1998) asserted that spot blotch resulted in high yield losses ranging from 20 percent to 100 percent. For example, Siddique et al. (2006) revealed that productivity losses in Bangladesh could be as high as 22 percent. It was observed by Duveiller et al. 2005, that the combined effect of spot blotch and tan spot HLB complex resulted in a yield drop of around 30 percent on average. In order to combat the devastating effects of spot blotch, researchers are concentrating their efforts on developing disease-resistant strains. Several regions of India's North Eastern Plains Zone (NEPZ), Nepal's Tarai region, and Bangladesh's North Western region have been ravaged by the illness. Spot blotch is estimated to cause yield losses ranging between 15.5 and 19.6 percent, with losses exceeding 100 percent in the most severe cases of infection.

There have been ranges of management measures proposed and applied to prevent the disease including timely sowing, appropriate fertilisation, chemical control, crop rotation, and tillage system (Wolf et al., 1998). Although host resistance has long been recognised as a vital component of the disease management system, it has only recently been recognised as such. Natural SB resistance is widespread and is determined by a

combination of factors including genetics and environment (Singh et al., 2010). In early marker-trait association studies, a number of genes, including the simple satellite repeat (SSR) genes gwm67, gwm570, and gwm469, were found to be associated with SB resistance (Andrie et al., 2007), but newer QTL mapping studies have provided more detailed information on the chromosome locations and effects of SB resistance QTL. In the Chinese resistant cultivar "Yangmai#6," Kumar et al. 2009 found four quantitative trait loci (QTL) on chromosomes 2AL, 2BS, 5BL, and 6DL. Same research group discovered SB resistance QTL on chromosomes 2AS, 2BS, 5BL, and 7DS in the cultivar "Ning 8201," and also on chromosomes 2AS and 2DS on chromosomes 2BS, 3BS, 7BS, and 7DS in the cultivar "Chirya#3" (Rudd et al., 2017). In a mapping population derived from the cross Avocet/Saar, Lillemo et al. 2013 discovered that the pleiotropic multi-fungal resistance gene Lr34, located on chromosome 7DL, had a stronger effect on SB resistance than the gene Lr46, located on chromosome 1BL, in a mapping population derived from the cross Avocet Saar, with the former gene designated Sb1. Using the SYN1 CIMMYT synthetic wheat derived line, Zhu et al. 2014 found QTL for SB resistance in the SYN1 CIMMYT on chromosomes 1B, 3BS, and 5AL. Kumar et al. 2010 discovered a big QTL on chromosome 5BL, which they named Sb2 (Kumar et al., 2015). Sb3 is the name of a QTL on chromosome 3BS that has been fine-mapped and found to be important (Lu et al., 2016). Adhikari et al. 2012 employed association mapping to discover several QTL on multiple chromosomes in the Brazilian SB resistant cultivar "BH 1146," while Singh et al. 2016 identified two significant QTL on chromosomes 7BL and 7DL in the Brazilian SB resistant cultivar "BH 1146." As a result, the current study was conducted to find and locate QTLs associated with spot blotch in the PBW343/IC252874 population.

Plant Materials And Experimental Set Up

Resistance was investigated in two-year trials at the Rajendra Prasad Central Agricultural University's research farm in Pusa, Bihar, India (25° 57' 08" N; 85° 40' 13" E). A total of 165 DH lines, including the parents PBW343 and IC252874 were sown during the 2017-2018 and 2018-2019 growth seasons. The third week of December was chosen for seeding to coincide with the post-anthesis period and the warmer temperatures favorable to spot blotch. Three replications of 165 doubled haploid lines and their parents were sowed in an alpha lattice design in each experiment. Two 2 m long beds with four rows each made up the experimental plots. Because it displayed very sensitive reactions (IR = 7–9 on a 0–9 scale) and a 99 score (double-digit score) of SB severity at various stages of crop in the field.

Irrigation and management practises

Throughout the two years, the required agronomic methods for irrigated and normal fertility (125 kg N; 55 kg P2O5 and 35 kg K2O ha⁻¹) conditions were followed. At the time of seeding, full doses of K2O and P2O5 were administered. Split application of nitrogen was used, with 55 kilogramme N ha⁻¹ applied at sowing, 25 kg N ha⁻¹ applied at first irrigation (20 days after sowing), and 35 kg N ha⁻¹ used at second irrigation (42 days after sowing). To ensure optimum soil moisture, irrigation was applied to the crop at five critical growth stages (at crown root initiation Zadok, GS 21; tiller completion Zadok, GS 29; late jointing Zadok, GS 36; flowering Zadok, GS 61; and milk stage Zadok, GS 75). Weeds were manually plucked out.

Disease Scoring for spot blotch resistance

Single digit scoring

After the completion of heading in all genotypes, we noted symptoms in the flag leaf (F) and penultimate leaf (F-1) for single digit scoring. In each plot of three replications, single digit scoring was performed using the CIMMYT standard diagram (Muzeeb – Kaazi et al., 1996).

(%) Disease severity = (sum of numeric rating/ total numbers of plant observed) ×100

Double digit scoring

SB severity (Fig 4) was measured at the GS63, GS69, and GS77 stages of plant development, using Saari and Prescott's double-digit (00–99) severity scale, with the first (D1) and second (D2) digits representing disease progression vertically from the ground and percentage of leaf area infected with SB, respectively.

Using the algorithm provided, the severity of disease was determined for each DHL (Duveiller et al., 2005).

Disease severity = $(D_1/9) \times (D_2/9) \times 100$

Where,

D_1 = 1st digit (vertical disease progress)

D_2 = 2nd digit (severity of infection)

Estimating Area under Disease Progress Curve (AUDPC)

The area under the disease progress curve (AUDPC) value for each genotype was calculated using the disease severity. The area under the disease progression curve was developed to determine disease progression. AUDPC was calculated independently for flag leaf (F), penultimate leaf (F-1), and double digit scoring (Das et al. 1992).

$n-1$

$AUDPC = \sum_{i=1}^{n-1} [(t_{i+1} - t_i) (y_i + y_{i+1}) / 2]$

Where,

i

Y_i = Disease severity in the i th date

t_i = Date on which the disease was scored

n = number of dates on which disease was recorded

Genotyping

The wheat Coleoptiles were used to extract DNA (<http://www.triticarte.com.au/content/DNA-preparation.html>). A consensus map was used to do this (Somers et al. 2004). In Thermal Cycler22, a 3 minute denaturation at 94°C was followed by 30 second cycles at 50/65°C, 30 second cycles at 72°C, and a final 2 minute cycle at 72°C (Sharma et al. 2016). On 541 SSRs, the parents' SSR markers were evaluated for polymorphism. Between

parental genotypes, were polymorphic (Fig 5)). The 130 SSRs (gwm, wmc, swm, barc, and cfd) were utilised to create wheat chromosome linkage maps. The visual evaluation of polymorphic SSR profiles was done by coding PBW343 alleles as "A" and IC252874 alleles as "B." The letters "H" and "NA" stand for heterozygote and missing bands, respectively. On 3 percent agarose gels, allele bands were seen.

Linkage map construction and QTL mapping

Using the software MapDisto 2.1.7.1, the genotyped data from the DH population was utilised to create twelve linkage maps. For the conversion of recombination frequency to genetic distance, the Kosambi mapping function and interval position type were utilised. QTL Cartographer v2.5 was used to conduct the analyses (Wang et al., 2010). In the composite interval mapping (CIM) approach, the software Windows QTL Cartographer 2.5 was used to perform forward regression using five backdrop markers, a window size of 10.0 cM, and a walking speed of 2 cM. Model 6 was used to set the trait for Composite interval mapping (CIM), which was utilised to determine plausible QTL sites and a 1,000 permutation test threshold of $P = 0.05$. Two or more linked markers associated with a characteristic with $LOD > 3.0$ were identified as putative QTLs. Suggestion QTLs were classified as QTLs with two or more connected markers found at a LOD of 2.0 to 3.0. (McIntyre et al. 2010). The LOD value was set at a minimum of 2.0 in order to account for both suggestive and minor QTL.

Statistical analysis

The disease severity scores across different years were used to obtain best linear unbiased estimates (BLUEs) and predictions (BLUPs) by fitting linear mixed effects models in R v4.0.3 (R Core Team, 2019) using where Y_{ik} is the trait of interest, μ is the mean effect, $Year_i$ is the effect of the i th year, $Line_k$ is the effect of the k th line, and ϵ_{ik} is the error associated with the k th line. The genotypes were treated as fixed effects in the BLUEs model, while all of the effects in the BLUPs model were treated as random effects. When genotypes are treated as random effects, the impact of screening time and other environmental factors on SB severity is reduced (Tomar et al., 2021). To explore the distribution across the DHLs, the disease severity scores generated by fitting the BLUPs and BLUEs models were displayed using ggplot2 v3.3.3 (Wickham, 2016) and ggpubr v0.4.0 (Kassambara, 2020) in R v4.0.3.

Result

Phenotypic Evaluation of DHLs

The analysis of variance for AUDPC values revealed a significant variation for genotypes and genotype-by-year interaction (Table 1). Large variation in disease severity was observed across the different growth stages with disease pressure increasing from GS63, GS69 and GS77 (Table 2). Across the environments, overall disease pressures were the lowest in E1 and highest in E2. Within the same year, both populations responded similarly as observed from their comparable disease severity/AUDPC score ranges. To enhance the accuracy and map stable QTLs across the environments, linear mixed-effects models were used to obtain fitted values of disease severity, accounting for G X E effect. These values are termed as BLUPs (genotypes as random effects) and BLUEs (genotypes as fixed effects) from here onward. The BLUPs showed lower variance than the BLUEs which meant BLUPs were able to reduce the environmental variance across the years to a larger extent.

Mean spot blotch severity (%) of the resistant parent (PBW343) and the susceptible parent (IC252874) parents at GS 77 (Zadoks scale, Zadoks et al. 1974) ranged from 80% (2017– 2018) to 87% (2018–2019) (Table 1). The disease severity of DHLs ranged from 52% (2017–2018) to 59% (2018–2019). The continuous distribution of spot blotch AUDPC and the test of normality using Shapiro– Wilk test ($W = 0.973$, $P = 0.327$) revealed that the DHLs data fit a normal distribution. The parental lines exhibited contrasting phenotypes for spot blotch mean AUDPC for all the two years. The spot blotch AUDPC of the DHLs showing large phenotypic variation in the population.

The resistant parent PBW343 was found to be immune to SB showing highly resistant disease severity score of 00, on the double-digit scale, across all the growth stages studied whereas susceptible parent IC252874 showed high susceptibility across the growth stages and AUDPCs when compared with range of disease scores of respective data sets. At GS63, disease score BLUE of the susceptible parent IC252874 were 40.0 while for the DHLs, it ranged from 0.29 to 41.00. The disease score, BLUP of IC252874 was 24.20 with DHLs showing a range from 08.23 to 26.71. At GS69, disease score BLUEs of the susceptible parent, IC252874 was 71.43 while for the DHLs, it ranged from 09.61 to 71.32. The disease score BLUP of IC252874 was 56.21 with DHLs showing a range from 23.72 to 56.48. At GS77, disease score BLUE of susceptible parent IC252874 were 87.51 while for the DHLs, it ranged from 34.00 to 79.36. The disease score BLUPs IC25287 was 81.34, respectively, with DHLs showing a range from 40.13 to 76.14. The AUDPC values showed a similar trend, where the disease score BLUEs of the resistant parent PBW343 and susceptible parent IC252874 were 200.13 and 1400.39, respectively, while for the DHLs, it ranged from 323.45 to 1217.32. The disease score BLUPs of PBW343 and IC252874 were 221.34 and 1323.27 respectively, with DHLs showing a range from 245.61 to 1235.59. The genotypes, DH10, DH125, DH80 and DH134 were found highly resistant across all the growth stages. Overall, less than 5% of lines were categorized under highly resistant category while 25 and 30% of genotypes showed moderate to high susceptibility, respectively. The rest of the lines fell under resistant to moderately resistant category. The data from different years were used separately for QTL mapping.

Linkage map and QTL detection

The anchored markers helped to form 12 linkage groups representing wheat chromosomes in the DH mapping population. A total no. of eighteen QTLs was detected for spot blotch AUDPC over the two years (Table 3). We found the QTLs for spot blotch resistance on chromosomes 1B, 1D, 2A, 2B, 2D, 3B, 4A, 4D, 5A, 5B, 6A, and 7A over the course of two years (Fig 3). The LOD values ranged from 2.61 to 15.39 (Fig 6) and the corresponding R^2 ranged from 7.43 to 21.12 in the individual years. Individual QTLs explained between 11.31 and 39.15 % of phenotypic variance in the composite interval mapping. Using composite interval mapping, two most consistent QTLs mapped on the chromosome 2B and 5B flanked by the marker, Wmc109-gwm312 and Cfd71-wmc173 respectively and the alleles for reduced disease severity were derived from the resistant parent PBW343. On the other hand, QTLs present in at least single year were located on the chromosome 1D, 3B, 2D, 4A and 4D. The QTL on 2A explained the largest part of phenotypic variance in the second year (22.13%). In the first year, maximum phenotypic variation (18.75%) was controlled by the QTL located on chromosome 5B. Genetic maps consisting of 10 loci on chromosome 1B, 5 loci on 1D, 12 loci on 2A, 7 loci on 2B and 13 loci on chromosome 2D, 14 loci on chromosome 3B, 15 loci on chromosome 4A, 8 loci on chromosome 4D, 12 loci on chromosome 5A, 8 loci on chromosome 5B, 13 loci on chromosome 6A and 7A were developed.

Discussion

Spot blotch is one of the major constraints to the global wheat production, especially in areas with hot and humid climate (Tomar et al., 2021). To counter the constraints from foliar diseases like SB, there is a need for constantly identifying and introgressing new sources of resistance. The DHLs used in the present study showed wide range of variation for different traits and has already been reported to possess various QTLs for heat tolerance (Pankaj et al., 2022; Pankaj et al., 2022). In the present study, during phenotypic evaluation of disease severity for SB, four DH viz. DH10, DH125, DH80 and DH134 were identified to be highly resistant against SB. Because no wheat cultivar presently grown in North-Eastern plains of India possesses resistance to SB, these lines become an important resource for transfer of SB resistance. The phenotypic evaluation of the segregating population showed a wide range of SB severity scores from highly resistant to highly susceptible, which indicated that more than one locus for resistance was segregating in the population. The continuous distribution of spot blotch AUDPC and the test of normality using revealed that the DHLs data fit a normal distribution (Fig. 7). The distribution of 165 DHLs for spot blotch AUDPC suggested that spot blotch resistance is polygenic and not controlled by a single gene in the PBW343 and IC252874 cross. Earlier studies on the inheritance of resistance to spot blotch (Joshi et al. 2004b) also suggested a polygenic control. The resistant parent, PBW343 have two genes for disease resistance and resistance was found to be dominant over susceptibility. It revealed that, for spot blotch resistance, duplicate and complementary gene effects are contributing significantly along with additive gene effects (Goel et al., 2005). Singh et al., 2019 unravelled the genetics and map the resistance to Tan spot and *Septoria nodorum* blotch in the PBW343/Kenya Nyangumi derived recombinant inbred line (RIL) population. Therefore, PBW343 could be the ideal donor or resistant parent in deciphering disease resistance. To achieve the highest possible disease pressure, sowing was carried out during the second week of December which allows the post-anthesis stage to coincide with warm temperature conducive to the disease. Similarly, Kumar et al., 2009 sown seeds in the third or fourth week of December led the post-anthesis stages to coincide with relatively higher temperature that favoured disease development. It has been reported that spot blotch disease becomes more severe when the mean temperature exceeds 26°C (Chaurasia et al. 2000). The phenotypic evaluation of the segregating population showed a wide range of SB severity scores from highly resistant to highly susceptible, which indicated that more than one locus for resistance was segregating in the population. AUDPC was calculated using the disease severity (%) data, recorded at three growth stages (GS63, GS69, GS77). Kaur et al., 2021 also conducted disease scoring at three different growth stages (GS) on Zadoks' scale (Zadoks et al., 1974), which are GS55 (flowering stage or FS), GS75 (medium milk/dough stage or DS), and GS87 (hard dough stage or HDS).

In this study, DHLs were classified into four groups based on days to heading and disease severity was recorded at specific growth stages when days to headings were synchronized by differential sowing of the DHLs. Hence, the problem of variation in earliness was overcome. Similarly, RILs were classified into three groups based on days to heading and disease severity was recorded at specific growth stages when days to headings were synchronized by differential sowing of the RILs (Kumar et al., 2009).

Following two years of disease recording at different growth stages, an accurate evaluation of the population for resistance to spot blotch was obtained under field conditions. The results suggest that accuracy and reproducibility of experimental conditions and of the scoring method used for spot blotch evaluation is reliable. Most of the recent studies on spot blotch (Joshi et al. 2007a) are also based on AUDPC. We deployed

approximately 10-20 microsatellite markers covering wheat chromosomes. The ratio of polymorphic markers of nearly 30% was consistent with results of Prasad et al. (1999) and Roy et al. (1999). The order and orientation of the mapped microsatellite markers in our study was in agreement with those in the map of the ITMI population (Ganal and Röder 2007). There were five loci that did not segregate in 1:1 mendelian ratio showed segregation distortion. These loci were randomly distributed throughout the genome. However, the linkage map was not affected by the distortion and we did not include these loci in the linkage map. Since only 35% of the markers were polymorphic, gaps in certain region were not covered. Across two years, we identified eighteen QTLs for spot blotch resistance on chromosomes 1B, 1D, 2A, 2B, 2D, 3B, 4A, 4D, 5A, 5B, 6A and 7A. This study supports the finding of Kaur et al., 2021, discovered five QTLs, Q.Sb.pau-2A, Q.Sb.pau-2B, Q.Sb.pau-3B, Q.Sb.pau-5B, and Q.Sb.pau-6A, linked to SB resistance were mapped across chromosomes 2A, 2B, 3B, 5B, and 6A. Similar results were obtained by Tomar et al., 2021 where Physical map of candidate QTLs for spot blotch were mapped on 1A, 1B, 1D, 2A, 2D, 4A, 5B, and 6D chromosomes. Kaur et al., 2021 reported the QTL, Q.Sb.pau-5B, linked to SNP S5B_703858864, was validated on this BC2F1 population. Therefore, marker on the nearby position could prove be a potential diagnostic marker for SB resistance. In a review report, Gupta et al., 2018 has provided a linkage map covering important chromosomes associated with spot blotch disease (Fig. 8).

The published data of Sourdille et al. 2004, about deletion mapping of more than 700 microsatellite markers on specific chromosome segments enabled us to check the physical location of markers linked to the detected QTLs. The order and orientation of markers on the maps developed in our mapping population were also in agreement with the IWGSC physical maps (<http://www.wheatgenome.org>). Therefore, it could be possible to assign the QTL on the physical map. Most of the alleles for reduced disease severity were derived from the resistant parent PBW343 for the QTLs except *Qsb_rpcau_2D*, *Qsb_rpcau_2B*, *Qsb_rpcau_2A*, *Qsb_rpcau_4D* detected in the PBW343/IC252874 mapping population. Two most consistent QTLs mapped on the chromosome 2B and 5B flanked by the marker, Wmc109-gwm312 (18.4cM) and Cfd71-wmc173 (9.4cM) respectively. The average phenotypic variances for both the QTL were 17.9% and 19.9%. QTLs on 2B and 2D are similar to the ones reported in the Mexican conditions as deduced either by their physical positions or by the contribution of the SB resistance allele (He et al., 2020). Similarly, Kumar et al., 2009 reported two consistent QTLs mapped on the short arm of chromosome 2B and the long arm of chromosome of 5B detected in all 3 years. Tomar et al., 2021 also reported two significant chromosomal regions/QTLs on 2B and 5B that were consistent between the locations viz. Pusa, India, and Jamalpur, Bangladesh. We found a total no. of two QTLs on chromosome 1B. However, the flanking markers were different in both the years. Gurung et al. 2014 also reported a QTL on 1BL for SB in an association mapping panel. Comparing the QTL positions of both studies by a BLAST of the marker sequences to the IWGSC RefV1.0 genome sequence of Chinese Spring, indicated the QTL to be the different. The results showed that the QTL in Gurung et al. 2014 was in the proximal region of chromosome 1BL, whereas the QTL of this study was in the far distal region of chromosome 1BL. In a mapping population derived from the cross Avocet × Saar, Lillemo et al. 2013 demonstrated the major effect of pleiotropic multi-fungal resistance gene *Lr46* located on chromosome 1BL on SB resistance, the former gene being named as *Sb1*. In our case, chromosome, 3B and 5A were also associated with SB. Similarly, Zhu et al. 2014 identified QTL for SB resistance on chromosomes 1B, 3BS and 5AL. A QTL, *Qsb_rpcau_6A* was found on chromosome 6A in a single year only. The QTL is flanked by gwm570-wmc553 and accounted for the phenotypic variance of 12.52%. In a review report of Gupta et al., 2017, the marker, gwm570 was associated with SB resistance in a F2 population. Several simple satellite repeat (SSR) markers gwm67, gwm570, and gwm469, were linked to SB resistance in early marker-trait association studies. (Sharma et al., 2007).

Kaur et al., 2021 discovered a QTL, Q**Sb.pau-6A** and it was mapped 53 Mb from QTL SNP_3021829 (Bainsla et al., 2020) mapped in the same genomic region. A gene for Ubiquitin family protein was found flanking the QTL. Ubiquitin-related proteins impart plant resistance by degrading flagellin-sensing 2 (FLS2) receptor, which binds the microbe associated molecular pattern (MAMP), flagellin (Trujillo and Shirasu, 2010; Lu et al., 2011). Ubiquitin, which is a part of the ubiquitin–proteasome system (UPS), controls various pathways including response to biotic and abiotic stresses (Sadanandom et al., 2012), and acts as one of the major systems in plant immunity (Üstün et al., 2016). The QTL mapping achieved in this study should therefore serve as a starting point for developing a more detailed map and initiating a marker assisted selection approach. Successful MAS and cloning of the key resistance QTL will critically depend on the introduction of novel flanking markers and high-resolution mapping populations in the future. Thus, it is necessary to investigate additional marker systems, such as genotyping by sequencing, in order to develop markers for marker-assisted transfer of additional QTLs found in this work.

Declarations

Competing interests:

The authors declare no competing interests.

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Tables

Table1. Analysis of variance for spot blotch scores in DHL population evaluated in India over two crop seasons was performed (207-18-19)

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Genotype	165	1428.3	6.2	3.54	<0.0001
Year	2	953.7	440.7	13.16	<0.05
Genotype x Year	330	698.4	1.6	1.47	<0.0001
Rep within Year	3	103.5	32.6	32.5	<0.0001
Pooled Error	495	495.1	1.0		

Table2. Phenotypic evaluation of the disease severity of spot blotch disease in Resistant/Susceptible parents and DHLs

Env	Stage	RP	SP	DHLs					
		PBW343	IC252874	Range	Median	Mean	SD	CV	Mean
BLUEs	GS63	13.25	40.0	0.29 - 41.00	13.17	11.41	7.19	0.97	13.69
	GS69	58.10	71.43	09.61 - 71.32	39.34	37.26	12.6	1.65	42
	GS77	75.14	87.51	34.00 - 79.36	68.05	71.24	9.53	0.47	70.15
	AUDPC	200.13	1400.39	323.45 - 1217.32	821.27	821.32	212.01	1.57	820.53
BLUPs	GS63	12.59	24.20	08.23 - 26.71	11.26	11.65	2.56	0.61	13.79
	GS69	49.62	56.21	23.72 - 56.48	37.48	39.21	6.53	1.5	42.07
	GS77	72.38	81.34	40.13 to 76.14	67.21	68.02	6.2	0.42	70.04
	AUDPC	221.34	1323.27	245.61 - 1235.59	817.38	823.27	133.29	0.42	820.84

RP- Resistant parent; SP - Susceptible parents; DHLs- double haploid lines; CV- coefficient of variation; SD- standard deviation; Env- environment; BLUEs- best linear unbiased estimates; BLUPs- best linear unbiased predictions, AUDPC - area under disease progression curve.

Table3. Quantitative trait loci (QTL) detected for spot blotch disease in both years 2017-2018 and 2018-2019

Year	Trait	QTL Name	Marker interval	Additive effect	LOD score	PVE %	Positive allele
Env1(2017-18)	Spot Blotch(SB)	<i>Qsb_rpcau_2A</i>	<i>Barc212-wmc382</i>	-0.287	2.61	15.32	PBW343
		<i>Qsb_rpcau_5B</i>	<i>Cfd71-wmc173</i>	-0.153	11.16	18.75	PBW343
		<i>Qsb_rpcau_1B</i>	<i>gwm131-gwm498</i>	-0.203	4.37	12.18	PBW343
		<i>Qsb_rpcau_5A</i>	<i>Gwm304-barc141</i>	-0.094	7.30	9.81	PBW343
		<i>Qsb_rpcau_1D</i>	<i>cfa2129-cfd48</i>	-0.152	8.04	8.04	PBW343
		<i>Qsb_rpcau_2D</i>	<i>wmc503-gwm261</i>	0.130	7.42	12.01	IC252874
		<i>Qsb_rpcau_2B</i>	<i>Wmc109-gwm312</i>	2.621	6.04	10.32	IC252874
Env2(2018-19)	Spot Blotch(SB)	<i>Qsb_rpcau_4A</i>	<i>Cfd257-wmc718</i>	1.402	8.21	10.26	PBW343
		<i>Qsb_rpcau_4D</i>	<i>Wmc48-cfd106</i>	-0.076	4.53	7.43	PBW343
		<i>Qsb_rpcau_2B</i>	<i>Wmc109-gwm312</i>	-1.803	6.87	15.34	IC252874
		<i>Qsb_rpcau_2A</i>	<i>Gwm359-wmc728</i>	1.945	5.09	8.04	IC252874
		<i>Qsb_rpcau_5B</i>	<i>Cfd71-wmc173</i>	2.841	15.39	21.12	PBW343
		<i>Qsb_rpcau_1B</i>	<i>wmc611-cfd59</i>	-2.722	6.71	12.72	PBW343
		<i>Qsb_rpcau_3B</i>	<i>Barc68-gwm72</i>	0.382	4.2	11.53	PBW343
		<i>Qsb_rpcau_5A</i>	<i>Wmc492-barc40</i>	2.351	7.25	16.25	PBW343
		<i>Qsb_rpcau_6A</i>	<i>Gwm570-wmc553</i>	0.426	8.04	12.52	PBW343
		<i>Qsb_rpcau_2A</i>	<i>Gwm614-gwm497</i>	2.681	9.67	22.13	PBW343
		<i>Qsb_rpcau_4D</i>	<i>Gwm213-gwm608</i>	-0.125	10.36	14.63	IC252874

Figures

Figure 1

The India wide distribution of the spot blotch pathogen *Bipolaris sorokiniana*

Figure 2

Frequency distribution of spot blotch disease severities

Figure 3

The PBW343/IC252874 linkage map for spot blotch resistance. QTL was discovered in both the years 2017-18-19. The Kosambi mapping function was used to calculate marker positions, which are listed in cM position from the top of each linkage group. C1-1B, C2-1D, C3-2A, C4-2D, C5-3B, C6-4A, C7-4D, C8-5A, C9-6A, C10-7A, C11-2B, C12-5B

Figure 4

Disease severity/symptoms for spot blotch on wheat leaves

Figure 5

Amplification profiles of parental lines, P1 and P2 showing polymorphism

Figure 6

Representative QTL profiles of SB score shown in the genetic positions in centimorgan on the lower side. A LOD threshold of 2.5 is depicted by the vertical dashed line on LOD graph.

Figure 7

Distribution of spot blotch AUDPC values averaged over 2 years for 165 DHLs derived from a cross between PBW343/IC252874

Figure 8

An integrated wheat genetic map displaying the locations of various QTL and genes for spot blotch resistance/sensitivity on wheat chromosomes.