

QTLs for resistance to spot blotch of wheat caused by *Bipolaris sorokiniana*

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INTRODUCTION

Spot blotch disease of wheat caused by *Bipolaris sorokiniana* is a disease causing substantial damage to wheat (*Triticum aestivum* L. em. Thell) in South Asia (Saari, 1998; Joshi et al., 2007a). Some traits viz., erect leaf posture (Joshi and Chand 2002), leaf tip necrosis (Joshi et al. 2004a) and stay green trait (Joshi et al. 2007b) have been demonstrated to have positive effects on resistance to spot blotch. However, reports on suitable molecular markers are not available. Thus the objective of the present study was to identify QTLs associated with spot blotch resistance in spring wheat.

MATERIALS AND METHODS

One hundred and thirty nine single seed descent (SSD) derived Recombinant Inbred Lines (RILs, F₈) of the cross between two wheat genotypes Yangmai 6 (Resistant) and Sonalika (Susceptible) were investigated. The F₆, F₇ and F_{7,8} generations of RILs of Yangmai 6×Sonalika were evaluated in for spot blotch resistance in RCBD during the crop seasons 2003-04, 2004-05 and 2005-06 at the Agricultural research farm of Banaras Hindu University, Varanasi, India. Spreader rows of A-9-30-1 were also planted to induce disease development. Agronomic practices recommended for normal fertility (120 Kg N: 60 Kg P₂O₅: 40 Kg K₂O) were followed.

A pure culture of the most aggressive isolate of *B. sorokiniana* (isolate No. ICMP 13584, Auckland, New Zealand) (Chaurasia et al., 2000) was used for artificial epiphytotic. Disease severity (%) of each row was recorded at three different growth stages (GS) viz., GS 63 (beginning of anthesis to half complete); GS 69 (anthesis complete) and GS 77 (late milking) on Zadoks scale (Zadoks et al., 1974). Area under disease progress curve (AUDPC) based on disease severity was estimated.

Leaves were harvested from 15 days old seedlings of the RILs (F₈). Genomic DNA was isolated using the CTAB method described by Doyle and Doyle (1990). PCR reactions of gwm (gatersleben wheat microsatellite) and barc (Beltsville Agriculture Research Center) microsatellite markers were performed as described by Röder et al (1998) and Somers et al (2004). Microsatellite fragments were detected on an automated

laser fluorescence ALF express sequencer (Amersham Biosciences Europe GmbH, Freiburg, Germany). An external standard with four fragments (73, 122, 196 and 231bp) was loaded in one lane. The order and distribution of the microsatellite markers were based on the ITMI (International Triticeae Mapping Initiative) population of wheat. Single marker regression analysis was performed using the software *Qgene* developed by Nelson (1997). When a marker was observed significant ($P < 0.001$), all RILs were genotyped with the respective marker and QTL Cartographer version 2.5 (Wang et al. 2005) was used to confirm the QTLs by IM (interval mapping) and CIM (composite interval mapping).

Analysis of variance (ANOVA) for mean AUDPC values of each year was performed using SAS software (version 6.03; SAS Institute Inc., Cary, NC 1997). Heritability (h^2) was estimated following Singh and Ceccarelli (1996). Analysis of variance was performed to test the significance of marker trait association for each marker in all RILs. Phenotypic correlation coefficients of spot blotch disease severity and AUDPC values were calculated using *Qgene* software (Nelson 1997).

Mapmaker v 2.0 (Lander et al., 1987) was used for all polymorphic markers to create a framework map with 129 markers. Later on some more markers were added to enrich the neighbouring regions. Finally, Mapmaker was used to create linkage map of significant markers. The linkage map was constructed using the LOD (Likelihood of odd ratio) of >3 and recombination fraction of <0.4 . QTL analysis was performed with the software QTL Cartographer version 2.5. QTLs were verified by LOD scores compared to as empirical genome-wide significant threshold calculated from 1000 permutations for $P < 0.01$ to control Type-I error. QTLxQTL and QTLxEnvironment interactions were calculated using QTL Network V 1.60 and PlabQTL V 1.2 (<http://www.uni-hohenheim.de/~ipspwww/soft.html>) respectively. The names of the QTLs were assigned following International Rules of Genetic Nomenclature (<http://wheat.pw.usda.gov/ggpages/wgc/98/Intro.htm>): *Q**S**b.bhu* as QTL for resistance to spot blotch disease detected at Banaras Hindu University.

RESULTS

The distribution of spot blotch AUDPC and the test of normality using Shapiro-Wilk test ($W = 0.9892$, $P = 0.3463$) revealed that the RILs data fit a normal distribution. The analysis of variance for AUDPC revealed significant variation for genotypes and genotype \times year interaction. However, the difference among the three groups of genotypes and Group \times Year interaction was non-significant. Broad sense heritability for AUDPC was moderate to high.

High Pearson correlation coefficients were observed between disease severity and AUDPC within years with a range from 0.82 to 0.90 ($P < 0.0001$). Moderate correlations were observed between years ranging from 0.39 to 0.78 for AUDPC ($P < 0.001$ or $P < 0.0001$). The correlation coefficient between days to heading and AUDPC was non-significant (0.087).

Four QTLs were detected for spot blotch AUDPC (Table 1). Individual QTLs explained between 8.04% and 41.10% of phenotypic variance in composite interval mapping. The two most consistent QTLs mapped on the short arm of chromosome 2B and the long arm of chromosome of 5B (Fig. 1), detected in all three years, while other QTLs present at least in two years were located on the long arm of chromosome 2A and the long arm of chromosome 6D. The QTL on 5BL explained the largest part of phenotypic variance in the third year (41.10%). In the second year, maximum phenotypic variation (14.89%) was controlled by the QTL located on chromosome 2BS. QTLs mapped on 2AL, 2BS, 5BL and 6DL (Table 1) accounted for 14.80%, 20.50%, 38.6% and 15.6% of phenotypic variation based on mean over years, respectively

The QTL, *Qsb.bhu-2A* was located on the long arm of chromosome 2A between the markers interval *Xbarc353-Xgwm445* (37.4 cM). The main QTL *Qsb.bhu-5B* was located on the long arm of chromosome 5B between *Xgwm067* and *Xgwm371* (13.2 cM). The other QTLs, *Qsb.bhu-2B* and *Qsb.bhu-6D* were mapped between *Xgwm148-Xgwm374* (15.0 cM) and *Xgwm732-Xgwm1103* (3.2 cM), respectively.

DISCUSSION

The distribution of 139 RILs suggested that spot blotch resistance is polygenic in the 'Yangmai 6' \times 'Sonalika' cross. Earlier studies (Joshi et al. 2004b) also suggest a polygenic control for spot blotch resistance.

Four QTLs were identified for spot blotch resistance on 2A, 2B, 5B and 6D. We found a major QTL in the marker interval *Xgwm067-Xgwm371* (13.2 cM) on chromosome 5B. In the deletion maps, the microsatellite locus, *Xgwm445* flanking *Qsb.bhu-2A* was assigned to the 2AL1-0.78 deletions bin. The loci *Xgwm148* and *Xgwm374* flanking the QTL *Qsb.bhu-2B* have been assigned to the 2BS1-0.53-0.75 and the C-2BS1-0.53 deletion bins, respectively, while the loci *Xgwm067* and

Xgwm371 flanking *Qsb.bhu-5B* were assigned to the C-5BL6-0.29 and 5BL1-0.55-0.75 bins, respectively (<http://wheat.pw.usda.gov/GG2/index.shtml>). In the public database 'GrainGenes' no physical mapping data were available for markers *Xgwm732* and *Xgwm1103* flanking the QTL *Qsb.bhu-6D*.

ACKNOWLEDGEMENTS

Part of this research was supported by a DAAD-Leibniz (German Academic Exchange Service) fellowship to U. Kumar. Authors are thankful to Ram Awadh, Anette Heber and Sonja Allner for excellent technical assistance. The help rendered by Dr. Rajender Parsad, Indian Agricultural Statistical Research Institute, N. Delhi in the analysis of data is gratefully acknowledged.

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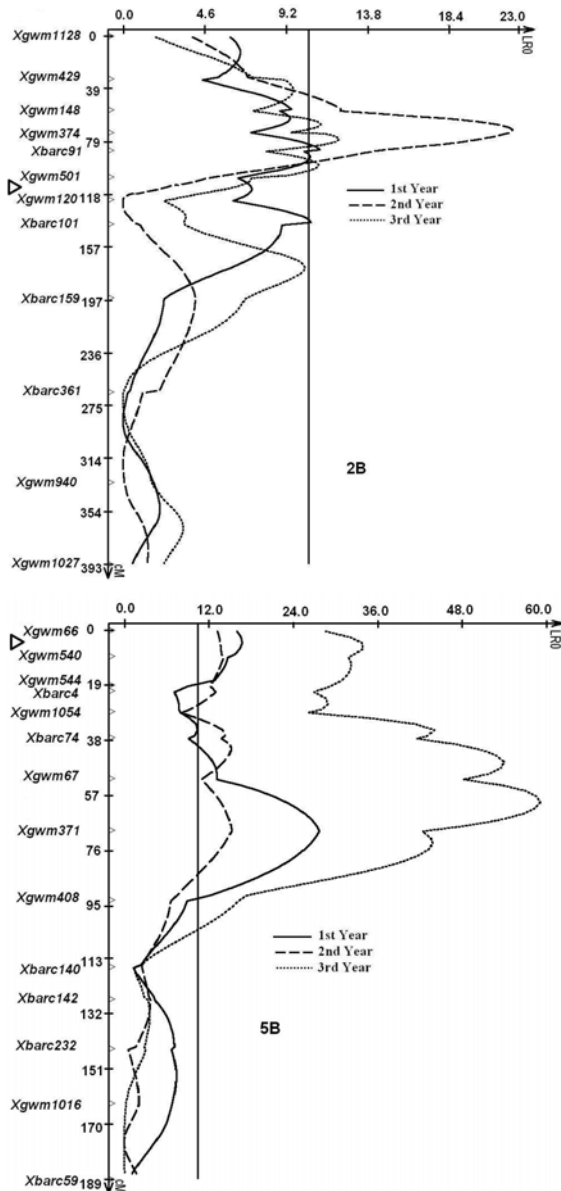


Fig. 1. LOD curves obtained by composite interval mapping for four quantitative trait loci mapped on chromosome 2BS and 5BL indicated by arrow that reduce spot blotch severity in ‘Yangmai 6’ x ‘Sonalika’ recombinant inbred population. The vertical line indicate the threshold LOD value (2.2) determining significant QTLs ($LR = LOD \times 4.6$). Short arms are toward the top and open triangles indicate the probable position of centromeres.

Table 1. Effects of quantitative loci (QTLs) that reduce spot blotch Severity in ‘Yangmai 6 x Sonalika’ recombinant inbred (RI) population detected by composite interval mapping (CIM). The marker intervals cited are those flanking the peak of the LOD scan. R^2 represents the percentage of phenotypic variance explained for each QTL

QTLs	Marker Interval	Interval Size (cM)	Chrom	R^2 mean across years		Q x E
				LOD	R^2	
<i>Qsb.bhu-2A</i>	<i>Xbarc353</i> – <i>Xgwm445</i>	37.4	2AL	3.5	14.8	*
<i>Qsb.bhu-2B</i>	<i>Xgwm148</i> – <i>Xgwm374</i>	15.0	2BS	5.8	20.5	**
<i>Qsb.bhu-5B</i>	<i>Xgwm067</i> – <i>Xgwm371</i>	13.2	5BL	12.9	44.6	**
<i>Qsb.bhu-6D</i>	<i>Xgwm732</i> – <i>Xgwm1103</i>	3.0	6DL	4.1	15.6	*
R^2				19.4	60.2	
QTLxQTL						
2A x 2B	-	-	-	-	-	Ns
2B x 5B	-	-	-	-	-	Ns
5B x 6D	-	-	-	-	-	Ns