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ORIGINAL PAPER

Mapping of resistance to spot blotch disease caused by *Bipolaris sorokiniana* in spring wheat

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Abstract Spot blotch caused by *Bipolaris sorokiniana* is a destructive disease of wheat in warm and humid wheat growing regions of the world. The development of disease resistant cultivars is considered as the most effective control strategy for spot blotch. An intervarietal mapping population in the form of recombinant inbred lines (RILs) was developed from a cross 'Yangmai 6' (a Chinese source of resistance) × 'Sonalika' (a spot blotch susceptible cultivar). The 139 single seed descent (SSD) derived F_6, F_7, F_8 lines of 'Yangmai 6' × 'Sonalika' were evaluated for resistance to spot blotch in three blocks in each of the 3 years. Joint and/or single year analysis by composite interval mapping (CIM) and likelihood of odd ratio (LOD) >2.2, identified four quantitative trait loci (QTL) on the chromosomes 2AL, 2BS, 5BL and 6DL. These QTLs were designated as *QSb.bhu-2A, QSb.bhu-2B, QSb.bhu-5B* and *QSb.bhu-6D*, respectively. A total of 63.10% of phenotypic variation was explained by these QTLs based on the mean over years. Two QTLs on chromosomes 2B and 5B with major effects were consistent over 3 years. All QTL alleles for resistance were derived from the resistant parent 'Yangmai 6'.

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Introduction

Spot blotch caused by *Bipolaris sorokiniana* is a disease causing substantial damage to wheat (*Triticum aestivum* L. em. Thell) in the warm and humid regions of the world such as Eastern India, South East Asia, Latin America, the tarai of Nepal, China and Africa (Raemakers 1991; Saari 1998). For a long time, spot blotch was considered a part of *Helminthosporium* Leaf Blight (HLB) which was understood to be a complex of many pathogenic fungi occurring simultaneously at different growth stages of the wheat. However, recently it has been established that leaf blight observed in the North-Eastern Plain Zone of India is actually spot blotch caused by *B. sorokiniana* (Sacc.) shoem syn. *Drechslera sorokiniana* (Sacc.) Subrm and Jain (syn. *Helminthosporium sativum*, teleomorph *Cochliobolus sativus*) (Chaurasia et al. 2000). Due to wide spread losses, this disease is considered as the most significant disease of wheat in North-Eastern Plain Zone of India (Saari 1998; Joshi et al. 2007b). Although spot blotch has assumed epidemic proportions in the eastern parts of India, more recently it has been reported to spread into the cooler traditional rice–wheat production areas (Villareal et al. 1995; Chand et al. 2003).

The average yield losses due to combined effects of leaf blight pathogens for South Asia and India are reported to be 19.6 and 15.5%, respectively. In the eastern part of the

Indian subcontinent, on-farm studies indicated crop losses of up to 16% in Nepal and 15% in Bangladesh (Saari 1998). Diseased plots yielding 43% less than fungicide protected plots have been found in Mexico (Villareal et al. 1995). Some studies report that the losses due to foliar blight may be as high as 100% under most severe conditions of infection (Mehta 1994). The increasing threat of spot blotch in the last decade has attracted the attention of plant breeders to develop varieties that are resistant to this pathogen.

The genetic basis of resistance to spot blotch appears to be nonspecific and complete resistance or immunity to spot blotch has not been observed among resistant wheat germplasm lines (Chaurasia et al. 1999). Although, inheritance studies on resistance to spot blotch indicate both monogenic (Srivastava et al. 1971) and polygenic (Joshi et al. 2004b) types of resistance, the experience of wheat workers to achieve partial resistance in breeding populations (Chaurasia et al. 1999; Joshi et al. 2004b) suggests a polygenic type of resistance.

Despite the understanding that the use of resistant cultivars is the best way to control the disease, the progress in breeding for resistance to spot blotch has not been to the expected level (Chaurasia et al. 1999; Joshi et al. 2007b). One of the important reasons for this slow progress has been the identification of reliable levels of resistance. The effect of environment on disease expression is substantial. Therefore, proper conclusions can be drawn only when evaluation of resistance is repeated over several years (Chaurasia et al. 1999). Erect leaf posture (Joshi and Chand 2002), leaf tip necrosis (Joshi et al. 2004a, 2007a) and staygreen trait have been demonstrated to have positive effects on resistance to spot blotch and can be used as morphological markers. However, more information with respect to the identification of suitable molecular markers is required.

Reports of tagging and mapping of several disease resistance genes and QTLs are available in wheat (Langridge et al. 2001). However, information on closely linked molecular markers for resistance to the spot blotch pathogen of wheat is lacking with only few reports available so far (Kumar et al. 2004; Sharma et al. 2007). Thus the objective of the present study was to identify the QTLs associated with spot blotch resistance in spring wheat.

Materials and methods

Plant materials

A total of 139 single seed descent (SSD) derived recombinant inbred lines (RILs, F_6, F_7, F_8 generations) of the cross 'Yangmai 6' × 'Sonalika' were evaluated in field trials for resistance to spot blotch. The cultivar 'Sonalika' of Indian origin was a popular cultivar in north-eastern India during the 1970s and 1980s and is highly susceptible to spot blotch, whereas 'Yangmai 6' is a Chinese cultivar (of unknown origin) carrying resistance to spot blotch.

Field evaluation for disease severity

The RILs of 'Yangmai 6' × 'Sonalika' cross were evaluated in the field at the Agricultural research farm of Banaras Hindu University, Varanasi, India (North-Eastern Plains Zone, 25.2°N and 83.0°E) during the crop seasons 2003–2004, 2004–2005 and 2005–2006 for F_6, F_7 and F_8 generations, respectively. Each year, the lines were evaluated in three randomized complete blocks. Each line was sown in single row of 3 m under irrigated conditions. Row to row and plant to plant distance was 25 and 5 cm, respectively. Based on the number of days to heading of the RILs observed in the F_5 generation, the RILs were divided in to three groups. Sowing was done at staggered time intervals of 3 days in the F_6, F_7 and F_8 generations to synchronize the days to heading between progeny rows of the three groups, thereby attempting to minimize the days to heading × disease severity interaction. To promote disease build up and spread, one row of the susceptible parent was planted after every 20th row and in alleys along the plots. Spreader rows of the highly susceptible cultivar A-9-30-1 were also planted in the alleyways of the experimental plots 2 weeks prior to sowing the experiment to induce disease development. To achieve the highest possible disease pressure, planting was carried out during the second and fourth week of December which allows the post-anthesis stage to coincide with warm temperature conducive to the disease that occurs in March (Chaurasia et al. 2000).

Agronomic practices recommended for normal fertility (120 kg N: 60 kg P_2O_5: 40 kg K_2O) were followed. The full dose of P_2O_5 and K_2O was applied at the time of sowing. Nitrogen was given as split application; 1/2 at sowing, 1/4 at first irrigation (21 days after sowing), and 1/4 at the time of second irrigation (40 days after sowing).

Creation of artificial epiphytotic conditions in the field

A pure culture of the most aggressive isolate of *B. sorokiniana* (isolate No. ICMP 13584, Auckland, New Zealand) identified at Banaras Hindu University, Varanasi, India (Chairasia et al. 2000) was used for the creation of artificial epiphytotic. The isolate was multiplied on wheat grains and a spore suspension adjusted to 10^4 spores/ml of water was uniformly sprayed at three different growth states (GS) viz., tillering (GS20), flag leaf emergence

(GS37) and anthesis (GS65) on Zadoks scale (Zadoks et al. 1974) during evening hours. The field was irrigated immediately after inoculation and a total of six irrigations were given in the entire crop period to provide a favourable environment for the development of spot blotch disease. Furrow irrigation was used with intervals of approximately 15–20 days between two irrigations. First irrigation was given 21 days after sowing.

Disease assessment

Disease severity (%) displayed by all the leaves of each row was recorded at three different growth stages (GS) viz., GS 63 (growth stage 63, beginning of anthesis to half complete), GS 69 (growth stage 69, anthesis complete) and GS 77 (growth stage 77, late milking) on Zadoks scale (Zadoks et al. 1974). Since the susceptible parent displayed highest disease severity at GS 77, to make better judgment about the level of resistance, disease severity recorded at this stage was used as disease severity of each line. Area under disease progress curve (AUDPC) based on disease severity (GS63, GS69 and GS77) over time has been suggested to be a pragmatic approach for disease assessment (Jeger 2004) and was estimated using the following formula (Roelfs et al. 1992):

$$\text{AUDPC} = \sum_{i=1}^{n} \left[\left\{ \left(Y_i + Y_{(i+1)} \right)/2 \right\} \times \left(t_{(i+1)} - t_i \right) \right]$$

where, Y_i = disease level at time t_i, $t_{(i+1)} - t_i$ = time (days) between two disease scores, n = number of dates on which spot blotch was recorded.

DNA isolation

Leaves were harvested from 15 days old seedlings of the RILs (F_8). Genomic DNA was isolated using the CTAB method described by Doyle and Doyle (1990) with modifications. Briefly, 200–300 mg of frozen leaf tissue per sample were ground in a 'Retsch Mixer Mill MM300' (fabricated by Retsch GmbH, 42781 Haan, Germany) for 30 s twice at 25 times/s and incubated in CTAB extraction buffer [2.0% (w/v) CTAB, 100 mM Tris-HCL pH 8.0, 20 mM EDTA pH 8.0, 1.4 M NaCl, 1% $Na_2S_2O_3$ and 0.2% β-merceptoethanol] for 30–45 min. Chloroform:isoamyl alcohol (24:1) extraction step was done twice. DNA was precipitated with acetate mix (3 M sodiumacetate pH 5.5 + 10 M ammoniumacetate) and isopropanol. The precipitated DNA was dissolved in 1× TE buffer (10 mM Tris-HCl pH 8.0 and 1 mM EDTA pH 8.0). DNA samples were stored at −20°C after overnight incubation at 4°C. DNA was diluted in H_2O to a concentration of 5–10 ng μl^{-1} before use for microsatellite analysis.

Microsatellite analysis

PCR reactions of Gatersleben wheat microsatellite (gwm) and Beltsville Agriculture Research Center (barc) microsatellite markers were performed as described by Röder et al. (1998) and Somers et al. (2004). DNA amplification was carried out in a 96 well thermocycler (Applied Biosystems, Foster City, USA) each containing 50–100 ng template DNA, 250 nM of each primer (one primer was labeled with Cy-5), 200 µM of each deoxynucleotide, 1.5 mM $MgCl_2$, 1× PCR buffer and 1 U of *Taq* DNA Polymerase. The following PCR profile was followed: initial denaturation at 94°C for 3 min, followed by 45 cycles of 94°C for 1 min, 50°C (55 or 60°C) for 1 min, 72°C for 2 min with a final extension step of 10 min at 72°C. The choice of 50, 55 or 60°C for annealing was according to the information provided for the primers (Röder et al. 1998; Somers et al. 2004). Microsatellite fragments were detected on an automated laser fluorescence ALF express sequencer (Amersham Biosciences Europe GmbH, Freiburg, Germany) using a short gel cassette. An external standard with four fragments (73, 122, 196, 231 bp) was loaded in one lane. Fragment sizes were calculated using the computer program Fragment Analyzer Version 1.02 by comparison with the internal and external size standards.

Map construction and QTL detection

The parents 'Yangmai 6' and 'Sonalika' were screened for microsatellite polymorphism. We selected approximately 20 microsatellite markers from each chromosome evenly distributed in the reference ITMI map (Ganal and Röder 2007; Röder et al. 1998). Once the polymorphism was identified between the parents, 74 selected individuals of the RILs (37 most resistant and 37 most susceptible) were screened with the polymorphic microsatellite primer pairs. Initially single marker regression analysis was performed using the software *Qgene* developed by Nelson (1997) to test each marker for the significance of disease severity based on 37 resistant lines and 37 susceptible lines. 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 interval mapping (IM) and composite interval mapping (CIM).

Mapmaker v 2.0 (Lander et al. 1987) was used to analyse all polymorphic markers to create a framework map with 129 markers for initial QTL detection with 74 individuals. Later on more markers were added to enrich the neighbouring regions of significant markers found in initial analysis. Finally, Mapmaker was used to create linkage map of significant markers on chromosomes 2A, 2B, 5B

and 6B. The linkage map was constructed using the Likelihood of odd ratio (LOD) of >3 and recombination fraction of <0.4. The commands 'Order' and 'Rip' were used to assign the order of markers on the map. To include additional marker on the map 'Try' and 'Compare' commands were used.

QTL analysis was performed with the software QTL Cartographer version 2.5. Firstly data were analysed to identify markers associated with spot blotch resistance using single marker analysis using all linked and unlinked loci data at a statistical threshold of $P < 0.01$. Secondly, the data was analysed by composite interval mapping (CIM) using a reduced set of marker loci containing significant loci detected by single marker analysis. The parameters settings for CIM were model 6, forward and backward stepwise regression with threshold of $P < 0.05$ to select cofactors, window size 10 and 2 cM walking speed along chromosomes. 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. LOD scores and coefficients of determination were estimated by CIM for each QTL. QTLs were considered to have a significant effect when LOD statistics exceeded a threshold of 2.2. Adjusted mean for AUDPC values of each year were calculated before pooling the data from all 3 years. QTL × QTL and QTL × Environments interactions were calculated using the program 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 according to the International Rules of Genetic Nomenclature (http://wheat.pw.usda.gov/ggpages/wgc/98/Intro.htm): *QSb.bhu* as QTL for resistance to spot blotch disease detected at Banaras Hindu University.

Statistical analysis

Analysis of variance (ANOVA) for AUDPC for all 3 years was performed using PROC GLM of SAS software (version 6.03; SAS Institute Inc., Cary, NC 1997). Heritability (h^2) was estimated from the analysis of variance following Nyquist (1991) as $h^2 = 1 - [\text{MS(Genotype} \times \text{Environment)}]/\text{MS(Genotype)}$. Phenotypic correlation coefficients of spot blotch disease severity and AUDPC values were calculated using *Qgene* software (Nelson 1997).

Results

Phenotypic variation

Mean spot blotch severity (%) of the resistant ('Yangmai 6') and the susceptible ('Sonalika') parents at GS 77 (Zadoks scale, Zadoks et al. 1974) ranged from 20 (2003–2004) to 24% (2005–2006) and 85 (2004–2005) to 88% (2005–2006), respectively (Table 1). The disease severity of F_1 ranged from 42 (2003–2004) to 46% (2005–2006).

The continuous distribution of spot blotch AUDPC as presented in Fig. 1 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 parental lines exhibited contrasting phenotypes for spot blotch mean AUDPC in all the 3 years. No line was more susceptible than 'Sonalika' but few lines (<5) showed a lower AUDPC

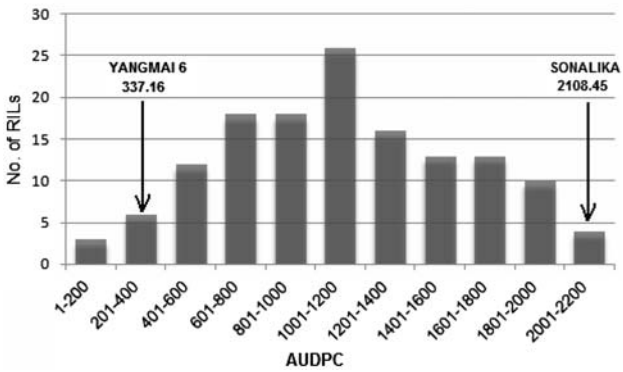

Fig. 1 Distribution of spot blotch AUDPC values averaged over 3 years for 139 RILs derived from a cross between 'Yangmai 6' × 'Sonalika'

Table 1 Range of spot blotch AUDPC and severities and days to heading (DH), and mean values for spot blotch AUDPC and disease severity (%) for the parental lines, F_1 and the RILs

Genotype	Spot blotch AUDPC			Disease severity (%)		
	2003–2004	2004–2005	2005–2006	2003–2004	2004–2005	2005–2006
'Yangmai 6'	330.0 ± 77.0	346.8 ± 78.1	334.6 ± 77.5	20.4 ± 4.1	22.7 ± 4.6	24.0 ± 3.7
'Sonalika'	2189.3 ± 200.9	2036.2 ± 191.4	2099.7 ± 196.1	88.3 ± 4.5	85.4 ± 4.1	88.6 ± 2.3
F_1	978.7 ± 103.2	970.0 ± 95.7	972.5 ± 97.1	42.8 ± 3.4	45.3 ± 3.1	46.6 ± 4.0
RILs mean	898.6 ± 111.2	867.1 ± 135.8	913.5 ± 108.6	52.1 ± 4.8	55.7 ± 5.4	57.9 ± 5.4
RILs range	287–2173	134–1856	176–2200	5.3–86.6	5.0–91.6	6.6–90.0
DH range	75–80	76–81	74–78			

value than the resistant parent 'Yangmai 6'. The spot blotch AUDPC of the RILs ranged from 134 (2004–2005) to 2200 (2005–2006) showing large phenotypic variation in the population (Table 1).

The analysis of variance for AUDPC values revealed a significant variation for genotypes and genotype-by-year interaction (Table 2). In each year, variation due to genotypes was highly significant. Broad sense heritability based on AUDPC values of 3 years was estimated to be 0.68. The data from different years were used separately for QTL mapping.

Trait correlations

High Pearson correlation coefficients were observed between disease severity and AUDPC value 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 $P < 0.0001$). Disease severities as well as AUDPC in the third year were more highly correlated to second year values rather than to the first year values. However, there was a significant correlation ($P < 0.001$) between first year and second year as well as first year and third year data (Table 3). The correlation coefficient between days to heading and AUDPC was non-significant when calculated across 3 years (0.083). For each of the 3 years also correlation coefficient values were non-significant (0.050, 0.064 and 0.171 in first, second and third year, respectively) (Table 3). Likewise, the correlations between disease severity and days to heading were also non-significant for each of the years (0.040, 0.056 and 0.082) and across years (0.058) (Table 3). This suggested that disease severity/AUDPC and days to heading behaved as independent variables, possibly because the synchronization due to time intervals in sowing was quite successful in solving this problem.

Microsatellite polymorphism and marker segregation

We tested 473 genomic microsatellite markers covering the genome for polymorphisms between the parents 'Yangmai 6' and 'Sonalika'. Out of these, 151 (31.9%) simple sequence repeat (SSR) markers were polymorphic and used for genotyping 74 RILs (F_8) initially. Later on, all the RILs (139 lines) were genotyped with the significant markers. The rate of polymorphism was highest in the B genome (41.2%) as compared to genomes A (30.3%) and D (29.6%). We observed around 1.33% loci in heterozygous state which may represent unfixed loci in the RILs. The population was in the advanced stage (F_8) and the alleles were expected to segregate in equal proportions. Most marker loci segregated in the expected 1:1 ratio ($P < 0.05$) with few exceptions, that is 12 marker loci did not fit the 1:1 ratio.

Table 2 Analysis of variance for spot blotch AUDPC over 3 years

Source	*df*	MS	*F* value	*P* value
Year	2	1.9×10^7	1514.19	<0.01
Block (year)	6	1.7×10^5	13.50	<0.01
Genotype	138	7.7×10^5	61.30	<0.01
Genotype × Year	276	2.4×10^5	19.68	<0.01
Error	828	12681.90		

The degrees of freedom (*df*) and mean sum of squares (MS) are shown for each analysis

QTL detection and mapping

Four QTLs were detected for spot blotch AUDPC. The LOD values ranged from 2.4 to 12.8 and the corresponding R^2 ranged from 8.72 to 41.10 in the individual years (Table 4**).** Individual QTLs explained between 8.72 and 41.10% of phenotypic variance in the 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. 2), were detected in all 3 years, while other QTLs present in at least 2 years were located on the long arm of chromosome 2A and the long arm of chromosome 6D (Fig. 2). 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, whereas the QTLs on 6DL was not significant. The QTLs mapped on 2AL, 2BS, 5BL and 6DL accounted for 14.80, 20.50, 38.6 and 22.5% of phenotypic variation based on mean over years, respectively. The mean values and the variation in AUDPC of different QTL classes are presented in Fig. 3.

Genetic maps consisting of 16 loci on chromosome 2A, 12 loci on 2B, 14 loci on 5B, and 6 loci on chromosome 6D were developed. Using composite interval mapping the QTL, *QSb.bhu-2A* was located on the long arm of chromosome 2A between the markers interval *Xbarc353-Xgwm445* (37.4 cM) and the main QTL *QSb.bhu-5B* was located on the long arm of chromosome 5B between the markers interval *Xgwm067-Xgwm371* (13.2 cM). Other QTLs *QSb.bhu-2B* and *QSb.bhu-6D* were mapped between the markers *Xgwm148-Xgwm374* (15.0 cM) and *Xbarc175-Xgwm732* (30.1 cM), respectively. For four QTLs detected in the 'Yangmai 6' × 'Sonalika' mapping population, the alleles for reduced disease severity were derived from the resistant parent 'Yangmai 6'. The QTL × QTL and QTL × environment interactions were investigated. There were 12 possible digenic epistatic effects between these four QTLs. Only three combinations (2AL × 2BS,

Table 3 Correlation coefficients among the spot blotch severities and days to heading with AUDPC in 3 years of testing of 139 RILs of the cross 'Yangmai 6 × Sonalika'

Traits	%disease 1st year	%disease 2nd year	%disease 3rd year	AUDPC 1st year	AUDPC 2nd year	Days to heading
%disease 1st year						0.04 (NS)
%disease 2nd year	0.39**					0.05 (NS)
%disease 3rd year	0.49**	0.71**				0.05 (NS)
%disease across year						0.05 (NS)
AUDPC 1st year	0.82**	0.41**	0.48**			0.05 (NS)
AUDPC 2nd year	0.39**	0.84**	0.77**	0.38**		0.06 (NS)
AUDPC 3rd year	0.52**	0.69**	0.90**	0.50**	0.77**	0.17 (NS)
AUDPC across years						0.08 (NS)

NS non-significant

** Correlation was significant at the 0.001 probability level

Table 4 Effects of quantitative loci (QTLs) that reduce spot blotch severity in 'Yangmai 6 × Sonalika' recombinant inbred (RI) population detected by composite interval mapping (CIM)

QTLs	Marker interval	Interval size (cM)	Chrom	2003–2004		2004–2005		2005–2006		Mean over years	
				LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2 Q × E
QSb.bhu-2A	*Xbarc353-Xgwm445*	37.4	2AL	–	–	3.2	13.79	2.8	15.58	3.8	14.80*
QSb.bhu-2B	*Xgwm148-Xgwm374*	15.0	2BS	2.4	8.72	4.8	14.89	2.6	9.71	5.8	20.50**
QSb.bhu-5B	*Xgwm067-Xgwm371*	13.2	5BL	6.0	18.42	3.4	11.76	12.8	41.10	11.5	38.62**
QSb.bhu-6D	*Xbarc175-Xgwm732*	30.1	6DL	2.5	14.61	–	–	2.5	19.05	3.3	22.50*
Total R^2 by joint analysis				10.4	31.20	7.67	32.52	15.4	58.7	19.2	63.10
QTL × QTL Interactions											
2A × 2B		–	–	–	–	3.7**	–	–	–		ns
2B × 5B		–	–	–	–	3.5**	–	3.4**	–		ns
5B × 6D		–	–	2.9**	–	–		–	–		ns

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. Significant QTL × QTL and QTL × environments interactions (Q × E) are also presented

LOD logarithm of odd ratio

*, ** Significant at the 0.05 and 0.01 probability level, respectively

2BS × 5BL, 5BL × 6DL) showed significant and additive QTL × QTL interactions (Table 4). The identified QTLs located on the long arm of chromosome 2A, short arm of chromosome 2B, long arm of chromosome 5B and long arm of chromosome 6D showed significant QTL × environment interactions.

Discussion

The distribution of 139 RILs for spot blotch AUDPC (Fig. 1) suggested that spot blotch resistance is polygenic and not controlled by a single gene in the 'Yangmai 6' × 'Sonalika' cross. Earlier studies on the inheritance of resistance to spot blotch (Adlakha et al. 1984; Joshi et al. 2004b) also suggested a polygenic control. Planting of 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). To minimize environmental effects, the epiphytotic conditions were created by artificial inoculation. AUDPC was calculated using the disease severity (%) data, recorded at three growth stages (GS63, GS69, GS77). In this study, 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. Hence, the problem of variation in earliness was overcome. Therefore, the correlation coefficient values between disease severity/AUDPC and days to heading were non-significant within each experiment and across mean of experiments. Following 3 years of disease recording at different growth stages, an accurate evaluation of the population for resistance to spot blotch was obtained under field conditions. Although genotype-by-environment interactions

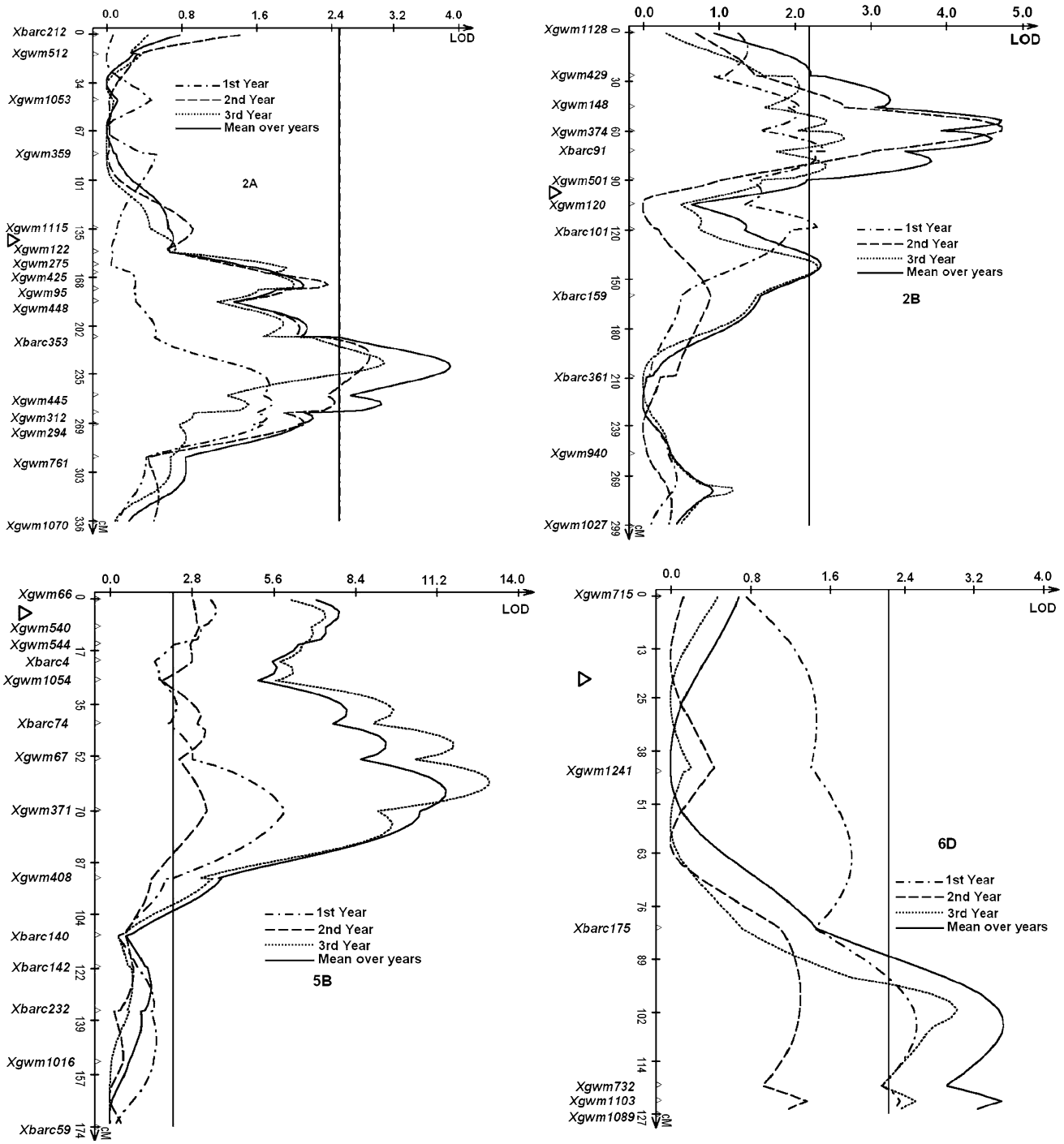

Fig. 2 LOD curves obtained by composite interval mapping for four quantative trait loci mapped on chromosome 2AL, 2BS, 5BL and 6DL that reduce spot blotch severity in 'Yangmai 6' × 'Sonalika' recombinant inbred population. The *vertical line* indicate the threshold LOD value (2.2) determining significant QTLs. *Short arms* are toward the top and *open triangles* indicate the probable position of centromeres

for spot blotch severities were significant, the absence of significance of correlations between days to heading and disease severity/AUDPC and the presence of significant correlations ($P < 0.001$) among the disease severity and AUDPC in different environments (Table 3) reflects the accuracy and reproducibility of experimental conditions and of the scoring method used for spot blotch evaluation. Due to high correlations between the disease severity and AUDPC values, we used AUDPC values in QTL analysis since AUDPC is considered to be more appropriate (Jeger 2004).

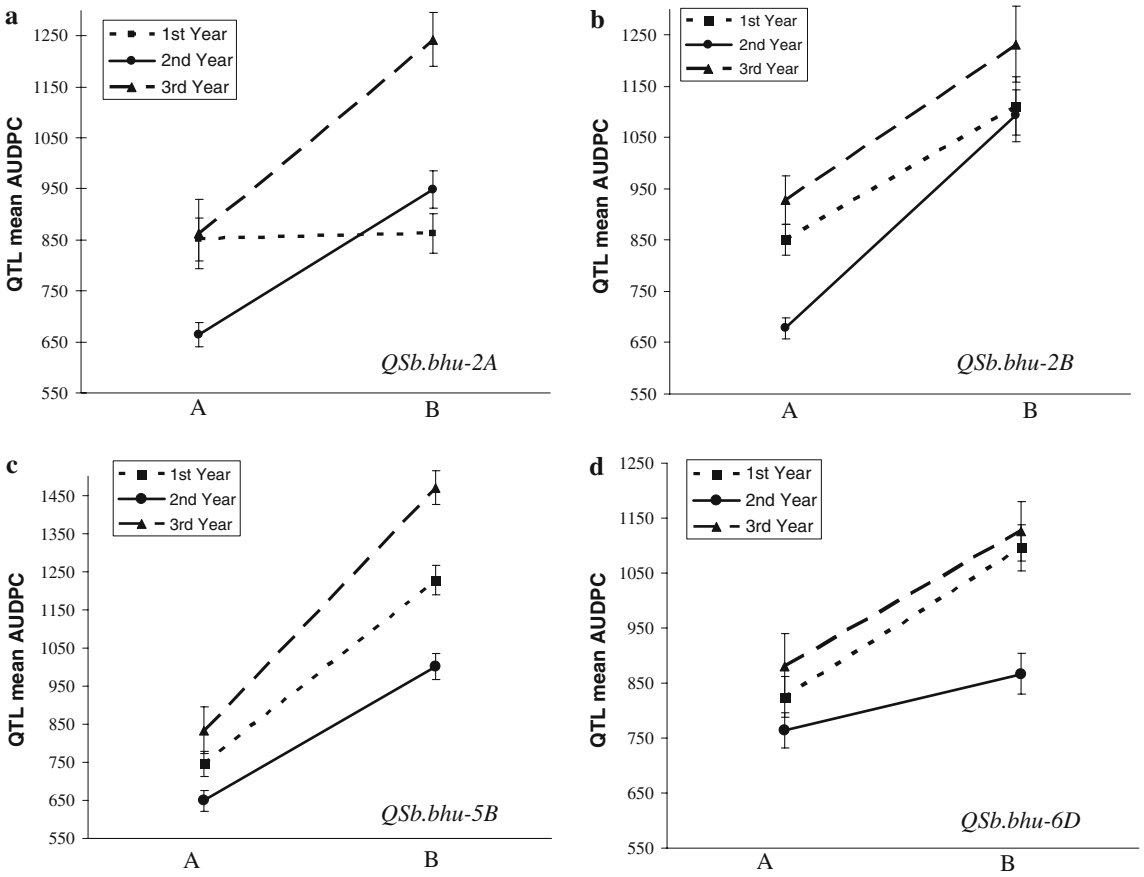

Fig. 3 Mean values and the standard variation in AUDPC of different QTL classes (**a**–**d**). *A* and *B* are the mean phenotypic value of alleles derived from resistant parent and susceptible parent, respectively

Most of the recent studies on spot blotch (Saari 1998; Pandey et al. 2005; Joshi et al. 2007a, b) are also based on AUDPC.

We tested approximately 20 microsatellite markers from each chromosome providing coverage of the whole genome. 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 12 loci that did not segregate in 1:1 ratio showed segregation distortion. These loci were randomly distributed throughout the genome. However, the linkage map was not affected by the distortion and we included these loci in the linkage map.

Since only 31.9% of the markers were polymorphic, gaps in certain region were not covered. However, it is unlikely that some major QTLs remained undetected because the identified QTLs explained a large part of phenotypic variance ranging from 31.2 (2003–2004) to 58.7% (2005–2006) by joint analysis and 63.1% based on mean over years.

Across 3 years, we identified four QTLs for spot blotch resistance on 2A, 2B, 5B and 6D. This study supports the finding of Joshi et al. (2004b) who reported that the resistance in 'Yangmai 6' was governed by at least three major and a few minor genes. 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 three of the detected QTLs. The deletion map of Sourdille et al. (2004) was in reference to ITMI map. The order and orientation of markers on the maps developed in our mapping population were also in agreement with the ITMI maps. Therefore, it could be possible to assign the QTL on the physical map. In the deletion maps developed for wheat, the microsatellite locus *Xgwm445* flanking the QTL *QSb.bhu-2A* were 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. Similarly the loci *Xbarc175* flanking the

QTL *QSb.bhu-6D* was assigned to the 6DL1-0.47-0.68 deletion bin (http://wheat.pw.usda.gov/GG2/index.shtml).

Many plant resistance genes appear to be organized as complex clusters. For example, the *Xa21* resistance gene family of rice and the *Cf*-2 family of tomato are assembled as single, locally restricted clusters of homologous genes (Dixon et al. 1998). The microsatellite marker *Xgwm148* found in our study flanking the QTL located on the short arm of chromosome 2B was also reported to be associated with the powdery mildew resistance gene *Pm26* (Rong et al. 2000). These two genes/QTLs may belong to same gene family clustered together or may be located very close to each other on the short arm of chromosome 2B.

The alleles for reduced disease severity were derived from the resistant parent 'Yangmai 6' for all the QTLs detected in the 'Yangmai 6' × 'Sonalika' mapping population. Additive QTL × QTL interactions resulted in enhanced level of resistance. (Table 4). Marker assisted selection (MAS) simultaneously for these QTLs would be more effective than selection for any one of them since all QTLs showed significant additive QTL × QTL interactions, even though not in all the years. Of the four QTLs detected, two QTLs on chromosome 2BS and 5BL were significant in all years and seem to be essential for resistance expression in 'Yangmai 6'. The other QTLs mapped on 2AL and 6DL were significant in at least 2 years. We observed QTL × environment effect on the expression of all QTLs. This effect could be due to variation in the infection level in the field as indicated by ANOVA. However, although these significant effects were moderate in general when compared to the main effect over 3 years (Fig. 3 a–d). QTL mapping achieved in this study should therefore provide preliminary information to generate a finer map and to initiate a marker assisted selection strategy. Successful MAS and cloning of the major resistance QTL in the future will crucially depend on the generation of new flanking markers and the development of high-resolution mapping populations.

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