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Dissection of wheat spot blotch disease resistance QTLs in to single Mendelian genes

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Abstract

Two QTLs imparting spot blotch resistance and located on chromosomes 2BS and 5BL were dissected in to a single Mendelian gene using 341 single seed descent (SSD) derived F_3 and F_4 lines of YS102 × Sonalika and 335 F_3, F_4 and F_5 lines of YS116 × Sonalika cross. The resistant parental lines YS102 and YS116 were selected on the basis of phenotypic and genotyping data of base mapping population (Yangmai 6 × Sonalika in F_{12} generation) used for QTL mapping. Both the populations were tested for spot blotch resistance under artificial epiphytotic conditions in the field. Disease severity (%) and AUDPC values of each line were calculated. The parent YS116 was known to possess a single major QTL on chromosome 5BL, while YS102 possess QTL on chromosome 2BS. The test for goodness of fit (1:2:1 in F_3, 3:2:3 in F_4 and 7:2:7 in F_5 generations) confirmed segregation at one locus in both the crosses. These SSD derived lines from both the crosses may further be used for fine mapping and map based cloning of the QTLs of spot blotch resistance OTLs.

Key words: *Bipolaris sorokiniana*, SSD, *Triticum aestivum*, QTLs, disease severity, AUDPC

Introduction

Bipolaris sorokiniana (Sacc.) Shoemaker, syn. *Helminthosporium sativus*, teleomorph *Cochliobolus sativus* (Ito and Kuribayashi) Drechs. ex Dastur. is an ascomycetous fungus with a wide geographic distribution (Sivanesan et al. 1990). Spot blotch is one of the most prominent diseases of wheat (*Triticum aestivum* L.) in warmer and humid regions of the world which causes significant yield loss (Saari 1998). Spot blotch affects around 9 mha of North-Eastern Plains Zone of India (Saari 1998; Joshi et al. 2007a, 2007) and now, it has been reported to spread into the North Western Plains Zone (NWPZ) which is a cooler traditional rice-wheat production area covering around 10 mha of wheat (Chand et al. 2003; Villareal et al. 1995). Yield loss due to spot blotch reported to 15% to 100% depends on environmental factors like humidity and temperature (Saari et al.1998; Mehta et al.1994). Joshi et al. (2004) observed that the genotypes with leaf tip necrosis (LTN) showed less spot blotch symptoms while spot blotch resistance positively associated with stay green trait (Joshi et al. 2007a). Several Quantitative trait loci (QTLs) for spot blotch resistance have been mapped by many researchers. Kumar et al. (2009, 2010, 2005) identified spot blotch resistance QTLs on chromosome 2A, 2B, 2D, 5B, 6D, while Lillemo et al. (2013) detected QTLs on chromosome 5B, 7A and a major QTL on 7D. Sharma et al. (2007) identified three chromosomal regions (5B, 6A and 6D) associated with spot blotch resistance. More recently, Zhu et al. (2014) reported molecular markers for spot blotch resistance on 1B, 3B and 5A chromosomes in a synthetic-derived wheat line of CIMMYT.

In previous study (Kumar et al. 2009, 2010), spot blotch resistance QTLs, *QSb.bhu*-2B and *QSb.bhu*-5B were mapped using microsatellite markers by using a cross between Yangmai 6 (resistant) and Sonalika (susceptible). In the present study, we dissected the QTLs into a single Mendelian gene, which may be further used in fine mapping of the QTLs and map based cloning.

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Materials and methods

Plant materials

Two single seed descent (SSD) derived lines originating from the cross YS102 × Sonalika (F_3 and F_4 generations) and YS116 × Sonalika (F_3, F_4 and F_5 generations) were used. The parents YS116 and YS102 were selected based on the phenotypic (similar to Sonalika for height and maturity) and genotyping (presence or absence of single QTL) data. The F_1s are selfed to obtain F_2 seed. A total of 341 individual plants derived from YS102 × Sonalika and 335 individuals from YS116 × Sonalika from F_2 populations were selected and threshed separately. The populations were advanced to F_3, F_4 and F_5 generations using SSD method. Well known highly susceptible parent Sonalika was used as common in crosses with resistant parents YS102 and YS116. The resistant parents, YS102 and YS116 were selected based on phenotypic and genotypic information of base population Yangmai 6 × Sonalika used for spot blotch resistance QTL mapping (Kumar et al. 2005, 2009, 2010). YS102 carries spot blotch resistance QTL, *QSb.bhu-2B* while YS116 carries the QTL *QSb. bhu-5B* (Table 1).

Table 1. Markers from the QTL region used to select the parents to develop SSD lines from Yangmai 6 × Sonalika population

Chromosome	2A	2A	2A	2A	2B	2B	2B	2B	2B	5B	5B	5B	5B	6D	6D	6D	6D
Marker	*Xgwm 71*	*Xgwm 445*	*Xgwm 312*	*Xgwm 294*	*Xgwm 148*	*Xgwm 374*	*Xgwm 120*	*Xgwm 501*	*Xgwm 935*	*Xgwm 66*	*Xgwm 540*	*Xgwm 67*	*Xgwm 371*	*Xgwm 408*	*Xgwm 1241*	*Xgwm 732*	*Xgwm 1103*	*Xgwm 715*
Yangmai 6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sonalika	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
YS102	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-
YS116	+	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-

Field evaluation for disease severity

SSD derived lines along with parents and Yangmai 6 were evaluated in the field under artificial epiphytotic conditions at the research farm of Borlaug Institute for South Asia (BISA) Samastipur, Bihar in the year 2012-2013, 2013-2014 and 2014-2015, respectively. Following Kumar et al. (2009), one row of the susceptible parent Sonalika was planted after every 20th row to promote disease build up and spread. Planting was done in the fourth week of December which allowed the post-anthesis stage to coincide with warmer temperature conducive to the disease that occurs in March (Chaurasia et al. 2000). Agronomic practices recommended for commercial cultivation and fertilization (120kg N: 60kg P_2O_5: 40kg K_2O) were practiced.

Creation of artificial epiphytotic conditions in the field

For artificial epiphytotic, a pure culture of the most aggressive isolate of *Bipolaris sorokiniana* (isolate No. ICMP 13584, Auckland, New Zealand) (Chaurasia et al. 2000) HDBHU, NCBI KJ412455) was used. The inoculum was kindly provided by Prof. Ramesh Chand, Banaras Hindu University, Varanasi. The spore suspension adjusted to 10^4 spores/ml in water was used for inoculation at three different growth stages (GS) *viz*., tillering (GS20), flag leaf emergence (GS37) and anthesis (GS65) on Zadoks scale (Chaurasia et al. 2000; Zadoks et al. 1974). Spore suspension was uniformly sprayed during evening hours and the field was irrigated immediately to provide a favorable environment for the development of spot blotch.

AUDPC calculation

Disease severity was recorded at three different growth stages *viz*., GS63 (beginning of anthesis to half completion), GS69 (anthesis complete) and GS77 (late milking) on Zadok's scale (Zadoks et al. 1974). Lines were evaluated visually on 0 (no symptoms) to 100 (completely susceptible) scale. AUDPC based on disease severity over time was estimated using the following formula (Roelfs et al. 1992):

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

Where, Y_i = disease level at time t_i, $t_{(i+1)} - t_i$ = time (days) between two disease scores

Estimation of the number of genes

To confirm the number of gene(s), the SSD derived lines of YS116 × Sonalika (F_3, F_4 and F_5) and YS102 × Sonalika (F_3 and F_4) were grouped separately into three classes (Singh and Rajaram 1992). These classes were: (i) homozygous, resistant parent type, (ii) homozygous, susceptible parent type and (iii) segregating. The observed and expected distributions of F_3, F_4 and F_5 lines in disease severity categories were tested by χ^2 analyses.

Results and discussion

The analysis of variance indicated significant variation for spot blotch within the populations (Table 2). The SSD lines showed near continuous distribution in F_3 as tested with Shapiro-Wilk test using "Shapiro.Wilk" package of R-program while the lines are skewed toward parental type and discontinuous distribution was observed in advanced generations (Fig. 1a, 1b). Mean spot blotch severity (%) of the resistant parent YS102 and YS116 at GS77 on Zadok's scale (Zadoks et al. 1974) were 28.9 ± 4.8 and 30.5 ± 5.5 respectably respectively whereas the percent disease severity shown by susceptible parent ('Sonalika') was up to 88.6% (± 4.4). The parents used to develop mapping populations did not differ with each other for plant height and days to maturity. The same was true in mapping populations except few transgressive segregants. Although the study of Joshi et al. (2002) revealed that days to maturity and plant height are not associated with spot blotch resistance however, the

Table 2. Analysis of variance for spot blotch AUDPC for YS102 × Sonalika and YS116 × Sonalika SSD derived lines

Source	DF	SS	MS	Variance	F	P value
YS102 × Sonalika						
Years (Y)	1	1×10^{12}	1×10^{12}	1×10^{9}	408.01	= 0.01
Replications (Within year)	2	1×10^{12}	1×10^{11}	2×10^{10}	293.62	= 0.01
Genotype (G)	340	6×10^{12}	1×10^{11}	3×10^{9}	5.80	= 0.01
G × E	340	9×10^{11}	2×10^{9}	0.0001	0.87	= 0.01
Error	680	2×10^{12}	1×10^{9}			
YS116 × Sonalika						
Years (Y)	2	5×10^{10}	2×10^{9}	4×10^{6}	51.12	= 0.01
Replications (Within year)	2	1×10^{6}	3×10^{5}	7×10^{3}	18.09	= 0.01
Genotype (G)	334	1×10^{6}	3×10^{5}	5×10^{4}	14.19	= 0.01
G × E	667	5×10^{7}	1×10^{5}	3×10^{4}	1.23	= 0.01
Error	863	1×10^{8}				

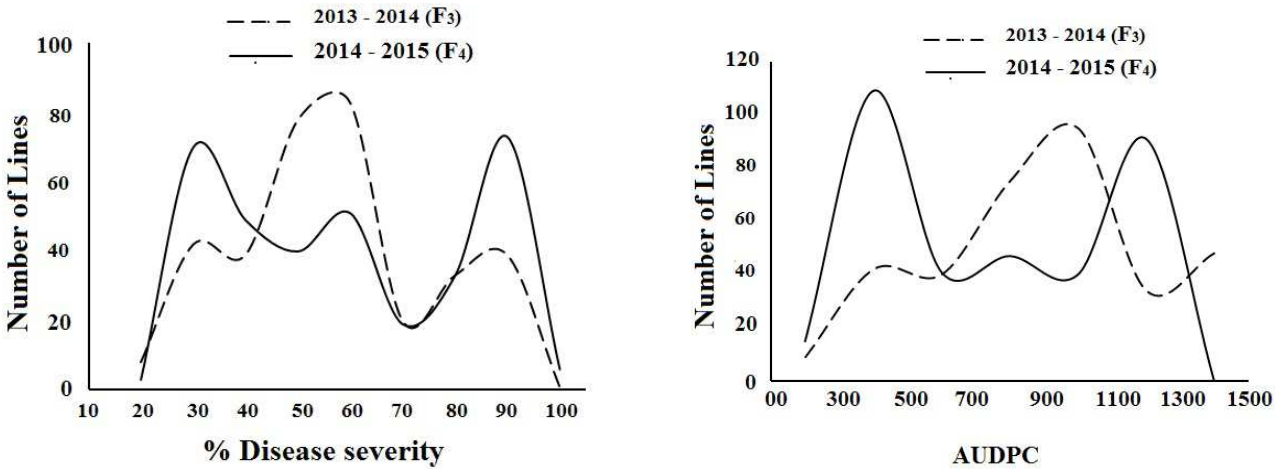

Fig. 1(a, b). Distribution of spot blotch disease severity (%) at GS77 and AUDPC in F_3 and F_4 generations from the cross YS102 × Sonalika

transgressive segregants for height and maturity are excluded from the analysis to avoid any possible confounding effect. Since Yangmai 6 has been used for spot blotch QTL mapping using as resistant parent and the RILs (YS102 and YS116) from Yangmai 6 × Sonalika were used in current investigation, Yangmai 6 was used as standard check to compare the level of resistance in parental lines of YS102 × Sonalika and YS116 × Sonalika. The mean spot blotch severity (%) for Yangmai 6 was 20.9 which indicated that the RIL YS102 and YS116 (28.9 ± 4.8 and 30.5 ± 5.5) are moderately resistant (Table 3). The test for goodness of fit (1:2:1 in F_3, 3:2:3 and 7:2:7 in the F_4 and F_5 generations, respectably) suggested segregation for one locus (Table 4). The F_3 and F_4 generations (YS102 × Sonalika) fit well in 1:2:1 and 3:2:3 ratio, respectively. As expected, we observed continuous distribution in F_3 (W = 0.97, P value < 0.01) while discontinuous in F_4 (W = 0.93, P value < 0.01). The lines derived from the cross YS116 × Sonalika showed a ratio of 1:2:1, 3:2:3 and 7:2:7 in the F_3, F_4 and F_5 generations, respectively. The cross YS116 × Sonalika displayed continuous distribution in F_3 (W = 0.96, P value < 0.01) while discontinuous in F_4 (W = 0.93, P value < 0.01) and F_5 generations (W = 0.83 P value < 0.01). The SSD derived lines fits single gene segregation model (1:2:1 in F_3, 3:2:3 in F_4 and 7:2:7 in F_5) as confirmed with χ^2 test in the F_3, F_4 and F_5 generations, where resistance in YS116 and YS102 has partial dominance. The inheritance to spot blotch is partial or incomplete which is well confirmed in various studies (Bhushan et al. 2002; Joshi et al. 2004; Kumar et al. 2007; Ragiba and Prabhu 2009). A similar approach was used by Joshi et al. (2004) to confirm the number of spot blotch resistance genes in different segregating populations. Therefore, it is concluded that we are able to dissect the QTL in to single Mendelian gene. Although, AUDPC is considered as most pragmatic

Table 3. Range and mean values for spot blotch AUDPC and disease severity at GS77 for the parental lines and mapping population of the cross YS102 × Sonalika and YS116 × Sonalika

Genotype	Spot blotch AUDPC			Disease severity (%)		
	2012 - 2013	2013 - 2014	2014 - 2015	2012 - 2013	2013 - 2014	2014 - 2015
Yangmai 6	330.0 ± 77.0	346.8 ± 78.1	260.0 ± 84.0	20.4 ± 4.1	22.7 ± 4.6	19.8± 3.3
Sonalika	1870.0 ± 157	1808.7 ± 86.4	1357.5 ± 89.0	85 ± 7.2	88.6 ± 6.4	87.5± 6.6
YS102 × Sonalika						
YS102	—	380.0 ± 28.2	320.0±29.0	—	28.0±2.8	29.9 ±5.5
Range	—	480 - 1653	270 - 1420	—	20 - 100	20 - 100
Mean	—	939.3 ± 313	605.9±216	—	68.0±7	59.4±6
YS116 × Sonalika						
YS116	483.6 ± 28.2	466.3 ± 29.0	345.0 ± 59.0	29.0 ± 4.3	30.0 ± 3.8	31.0 ± 3.5
Range	300 - 1905	340 - 1850	290 - 1397	20 - 90	20 - 100	25 - 93
Mean	903 ± 300	916.± 313	606 ± 216	55 ± 6	59 ± 7	57 ± 6

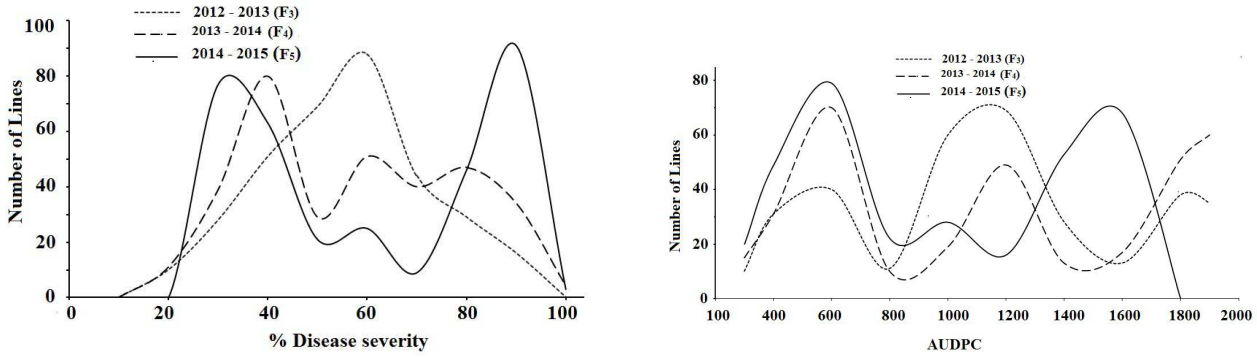

Fig. 2(a, b). Distribution of spot blotch disease severity (%) at GS77 and AUDPC in F_3, F_4 and F_5 generations from the cross YS116 × Sonalika

Table 4. Goodness of fit of ratios observed and hypothesized class frequencies the cross 'YS102 × Sonalika' and 'YS116 × Sonalika'

Generation	% Disease severity							AUDPC						Shapiro-Wilk test	
	Resistant (YS116 type)	Segregating	Susceptible (Sonalika type)	Hypothetical	χ^2 value	P value	Gene number	Resistant (YS116 type)	Segregating	Susceptible (Sonalika type)	Hypothetical	χ^2 value	P value	Gene number	W	P value
YS102 × Sonalika																
F_3	91	168	82	1:2:1	0.5	0.47	1	89	159	93	1:2:1	1.6	0.2	1	0.97	< 0.01
F_4	124	88	129	3:2:3	0.3	0.58	1	122	90	129	3:2:3	0.9	0.3	1	0.93	< 0.01
YS116 × Sonalika																
F_3	89	157	89	1:2:1	1.3	0.25	1	92	155	88	1:2:1	1.9	0.16	1	0.96	< 0.01
F_4	130	80	125	3:2:3	0.3	0.58	1	126	81	128	3:2:3	0.9	0.34	1	0.93	< 0.01
F5	140	46	149	7:2:7	0.7	0.4	1	146	49	140	7:2:7	0.9	0.34	1	0.83	< 0.01

approach to assess spot blotch resistance in wheat (Jeger et al. 2004). In present study, both AUDPC and per cent disease severity at GS77 to categorise the population in three classes (resistant parent type, susceptible type and intermediate) were used and χ^2 analysis was performed (Table 4). Similar results were observed with both, AUDPC and % disease severity analysis recorded at GS77. Further, high correlation (year 1, r = 0.76, P < 0.001; year 2, r = 0.91, P < 0.001; year 3, r = 0.92, P < 0.001) between the AUDPC and % disease severity recorded at GS77 in YS116 × Sonalika revealed that either of these two method is good for mapping studies. Similar results were obtained from YS102 × Sonalika where high correlation (year 1, r = 0.83, P < 0.001; year 2, r = 0.84, P < 0.001) between AUDPC and % disease severity is calculated. Since the reported QTLs are considered as the major QTL (Kumar et al. 2009, 2010), the developed and characterized populations may be used for further fine mapping and map based cloning of spot blotch resistance genes.

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