Mapping of QTL for resistance to spot blotch caused by *Bipolaris sorokiniana* and the stay green trait in wheat (*T. aestivum* L.) lines Ning 8201 and Chirya 3

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INTRODUCTION

Spot blotch disease of wheat caused by *Bipolaris sorokiniana* is an important disease in warm and humid wheat growing regions. This disease is spreading toward cooler traditional wheat growing areas. Losses due to spot blotch may be up to 100% in most severe conditions (Fig 1). The other trait called 'stay green' was also evaluated in the Chirya3 x Sonalika pupulation. Stay green (SG) is referred to as delayed senescence where leaves remain green even after seeds reach maturity.

Fig 1: Symptoms of spot blotch on leaves and leaf sheath

MATERIALS AND METHODS

Evaluation of RI lines for disease and stay green trait

The RILs of Ning8201/Sonalika and Chirya3/Sonalika were evaluated in the field at the Agricultural research farm of Banaras Hindu University, Varanasi, India. The RIL lines of Ning 8201 x Sonalika were evaluated for three years (2003-04, 2004-05 and 2005-06) while the RILs of Chirya 3 x Sonalika were evaluated for two years (2004-05 and 2005-06). Disease severity was recorded at three growth stages (GS) viz., GS 63, GS 69 and GS 77 on Zadoks scale (Zadoks et al 1974) to calculate AUDPC using the following formula (Van der Plank 1963):

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

Where, Yi = disease level at time ti

t(i + 1) - ti = Time (days) between two disease scores

Stay green trait was measured visually to record the difference of leaf and spike greenness scores on a 0-9 scale of Silva et al (2000) in Chirya 3 x Sonalika population

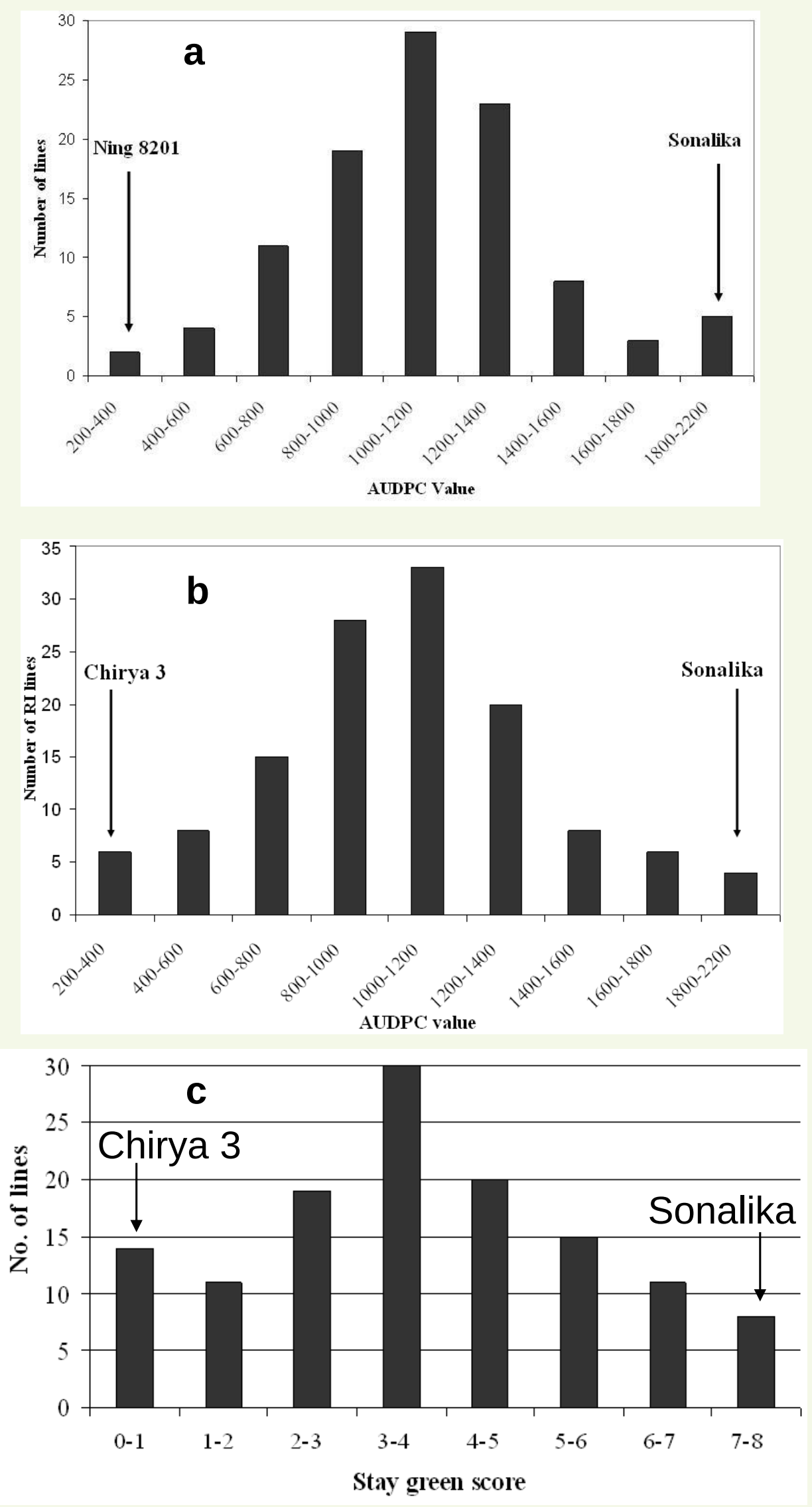

Fig 2**a, 2b,** Distribution of spot blotch AUDPC values for 103 RILs of 'Ning8201' x 'Sonalika' and 128 RILS of Chirya3 x Sonalika.

2c, Distribution of SG values for 128 RILs of 'Chirya3' x 'Sonalika

Microsatellite analysis

DNA exraction and microsatellite analysis was performed as described by Röder et al (1998). Microsatellite fragments were detected on an automated laser fluorescence ALF express sequencer (Amersham Biosciences Europe GmbH, Freiburg, Germany). Fragment sizes were calculated using the computer program Fragment Analyzer Version 1.02.

QTL analysis

WinQTLCartographer v2.5 was used for QTL analysis. We selected approximately 20 microsatellite markers from each chromosome evenly distributed in reference to the ITMI map. Mapmaker v 2.0 was used to analyse all polymorphic markers to create a framework map. Name 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. Similarly *QSG.bhu as* QTL for resistance to stay green detected at Banaras Hindu University

Table 1: Correlation coefficients among the spot blotch disease severity and AUDPC in three years in Ning 8201 x Sonalika population

Traits	%disease year 1	%disease year 2	%disease year 3	AUDPC year 1	AUDPC year 2
%disease year 2	0.49**				
%disease year 3	0.53**	0.62**			
AUDPC year 1	0.93**	0.46**	0.50**		
AUDPC year 2	0.51**	0.85**	0.61**	0.48**	
AUDPC year 3	0.60**	0.64**	0.89**	0.58**	0.61**

**Significant at p<0.001

Table 2: Correlation coefficients among the SG and spot blotch in Chirya3 x Sonalika population

Traits	SG 1st year	SG 2nd year	AUDPC 1st year
SG 2nd year	+0.541**		
AUDPC 1st year	-0.412**	-0.384**	
AUDPC 2nd year	-0.491**	-0.599**	+0.598**

**Significant at p<0.001

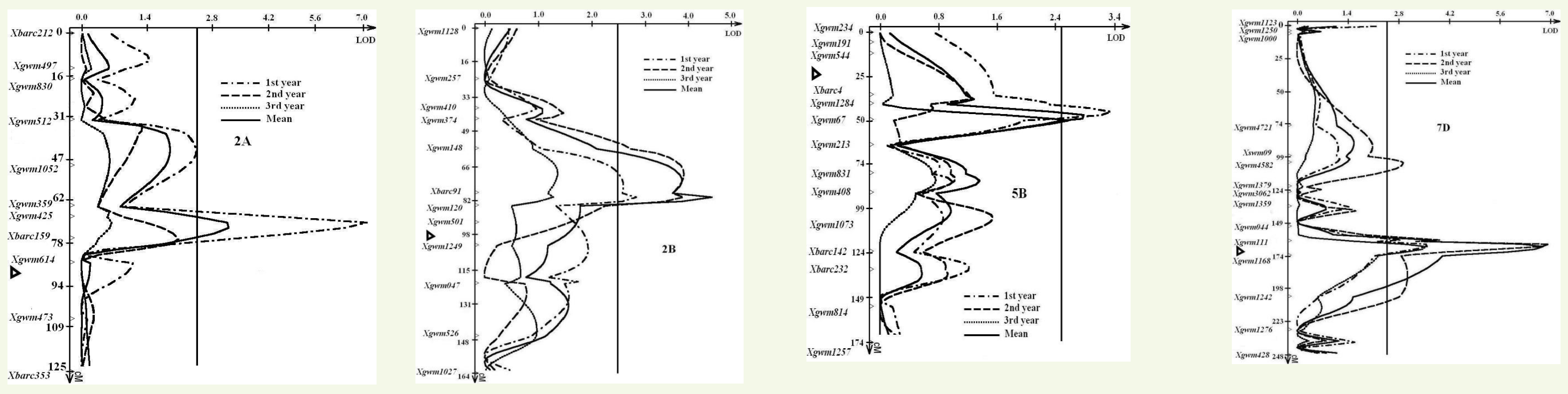

Fig 3: LOD curves obtained by CIM for four QTLs mapped on chromosome 2AS, 2BS, 5BL and 7DS in 'Ning 8201' × 'Sonalika' RI population. The vertical line indicate the threshold LOD value (2.5). Short arms are toward the top and open triangles indicate the probable position of centromeres

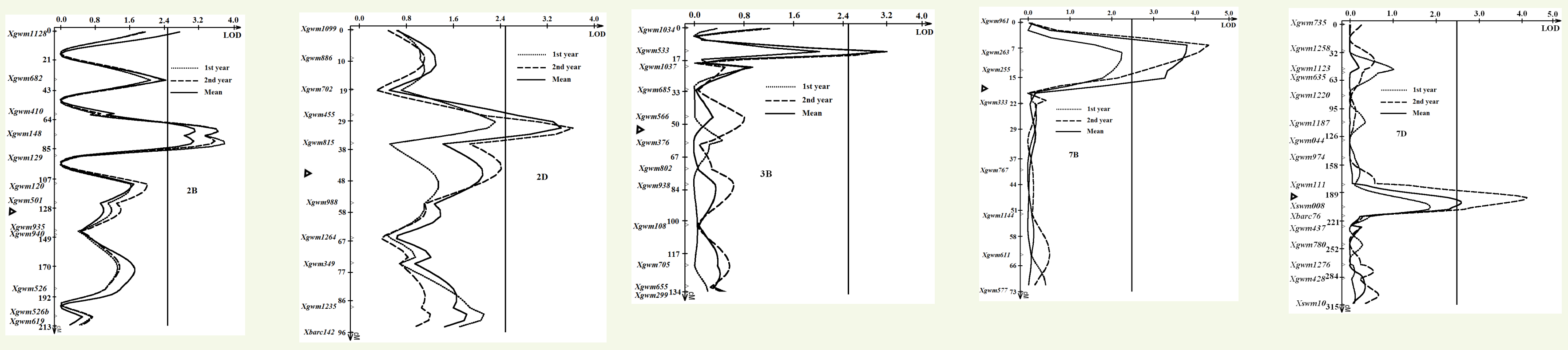

Fig 4: LOD curves obtained by CIM for five QTLs mapped on chromosome 2BS, 2DS, 3BS, 7BS and 7DS in 'Chirya 3' × 'Sonalika' RI population. The vertical line indicate the threshold LOD value (2.5). Short arms are toward the top and open triangles indicate the probable position of centromeres

RESULTS AND DISCUSSION

Spot blotch

The RILs showed continuous distribution (Fig 2a, 2b) in both the population showed quantitative resistance. The correlation coefficient between the %disease severity and AUDP in all three years of Ning8201 x Sonalika population was high and positive (Table 1). Therefore we used AUDPC values for QTL analysis since AUDPC us consider better parameter over % disease severity. We identified four QTLs for spot blotch in Ning8201 x Sonalika mapping population on the chromosome 2A, 2B, 5B and 7D (Table 3, Fig 3) while five QTLs were identified in the Chirya 3 x Sonalika population on the chromosome 2B, 2D, 3B, 7B and 7D (Table 4, Fig 4). The QTLs were located in the physical map using the deletion map of Sourdill et al (2004). The QTLs and their chromosomal locations were determined using the information from three different populations. The closely linked marker (*Xgwm111*) to the QTL on chromosome 7DS amplified the same size of fragment (137bp) in both the resistant parent viz., 'Ning8201' and 'Chirya 3' harbouring this QTL. Other closely linked marker (*Xgwm148*) to the QTL on chromosome 2BS amplified the same size of fragment (163bp) in three different cultivars resistant to spot blotch with the respective QTL (Table 5). Both markers may be useful as diagnostic markers across the cultivars and in breeding programs for implementation of the respective QTLs

Stay green

The stay green was recorded additionally in the Chirya 3 x Sonalika mapping population. The RI lines showed continuous distribution (Fig 2c). We found significant and negative correlation between the stay green and AUDP value in Chirya 3 x Sonalika population while significant and positive correlation was observed between the AUDPC value of both the year (Table 2).

We identified three QTLs on the short arm of chromosome 1A, 3B and 7D (Fig 5). The QTL explained up to 38.7% of phenotypic variation (Table 6). The QTLs on chromosome 3B and 7D were detected in the region were the QTL for spot blotch is detected.

It indicates that stay green may be either directly or indirectly associated with spot blotch. Alternatively, QTL for both traits may be coincidentally linked in the genetic map

Table 3. QTLs detected by CIM and the QTLxQTL and QxE interactions in Ning8201 x Sonalika population

QTLs	Marker Interval	Interval Size (cM)	Chrom	2003-04		2004-05		2005-06		Mean over years		Q× E
				LOD	R^2	LOD	R^2	LOD	R^2	LOD	R^2	
QSb.bhu-2A	*Xgwm425-Xbarc159*	8.0	2AS	3.2	22.7	-	-	-	-	2.9	15.2	ns
QSb.bhu-2B	*Xgwm148-Xbarc91*	21.2	2BS	2.8	15.1	3.7	18.3	-	-	4.3	23.7	*
QSb.bhu-5B	*Xgwm067-Xgwm213*	9.0	5BL	2.7	16.9	-	-	2.6	11.1	2.5	10.7	**
QSb.bhu-7D	*Xgwm111-Xgwm1168*	3.0	7DS	3.6	25.3	6.9	51.3	3.5	30.4	6.6	39.2	*
Total R^2 by joint analysis				4.6	43.2	6.9	50.8	3.1	36.1	11.2	62.9	
QTL × QTL Interactions												
2A × 2B		-	-	-	-	3.8	10.1	-	-			
2A × 5B		-	-	5.1	11.8	-	-	-	-			ns
2B × 5B		-	-	3.8	6.4	5.4	15.1	4.6	16.7			ns
2B × 7D		-	-	5.0	15.4	-	-	-	-			ns

The marker intervals cited are those flanking the peak of the LOD scan *, **Significant at the 0.05 and 0.01 probability level respectively
‡LOD = logarithm of odd ratio

Table 4 Quantitative trait loci (QTLs) detected in 'Chirya 3' × 'Sonalika' population

QTLs	Marker Interval	Interval Size (cM)	Chrom	2004-05		2005-06		Mean over years		Q× E
				LOD‡	R^2	LOD	R^2	LOD	R^2	
QSb.bhu-2B	*Xgwm148-Xgwm129*	15	2BS	3.6	18.8	3.7	16.2	3.1	13.1	*
QSb.bhu-2D	*Xgwm455-Xgwm815*	9	2DS	2.2	6.5	2.3	10.2	2.5	10.7	*
QSb.bhu-3B	*Xgwm533-Xgwm1037*	8	3BS	2.0	6.4	3.0	9.5	3.1	9.7	ns
QSb.bhu-7B	*Xgwm263-Xgwm255*	5	7BS	2.3	7.5	4.1	13.1	3.8	10.2	**
QSb.bhu-7D	*Xgwm111-Xswm008*	25	7DS	4.8	10.4	4.1	17.1	2.6	11.9	ns
Total R^2 by joint analysis				6.4	25.2	8.1	29.7	13.9	43.4	
QTL × QTL Interactions										
2B × 2D		-	-	2.1	6.8	-	-			ns
2B × 7B		-	-	-	-	3.5	9.3			ns
3B × 7B		-	-	3.2	8.0	1.8	5.9			ns
7B × 7D		-	-	-	-	3.6	8.9			ns

The marker intervals cited are those flanking the peak of the LOD scan. ns=non-significant; ‡LOD logarithm of odd ratio
*, ** Significant at the 0.05 and 0.01 probability level respectively *R2* represents the percentage of phenotypic variance explained

Table 5 Assignment of QTLs for spot blotch resistance in three mapping populations to deletion bins and the significant marker and fragment size (base pair) in resistant parents

QTL	*'Yangmai 6' × 'Sonalika'	*'Ning 8201' × 'Sonalika'	*'Chirya 3' × 'Sonalika'
QSb.bhu-2A	C2AL1-0.78 (*Xgwm445*-192bp)	C2AS5-0.78 (*Xgwm425*-136bp)	-
QSb.bhu-2B	C2BS1-0.53-0.75 (*Xgwm148*-163bp)	2BS1-0.53-0.75 (*Xgwm148*-163bp)	2BS1-0.53-0.75 (*Xgwm148*-163bp)
QSb.bhu-2D	-	-	2DS5-0.47-1.00 (*Xgwm455*-155bp)
QSb.bhu-3B	-	-	3BS9-0.57-0.78 (*Xgwm533*-95bp)
QSb.bhu-5B	C5BL6-0.29(*Xgwm067*-78bp)	C5BL6-0.29(*Xgwm067*- 81bp)	-
QSb.bhu-6D	6DLŦ(*Xgwm732*-135bp)	-	-
QSb.bhu-7B	-	-	7BSŦ(*Xgwm263*-134bp)
QSb.bhu-7D	-	7DS5-0.36-0.61(*Xgwm111*-137bp)	7DS5-0.36-0.61(*Xgwm111*-137bp)

The data of Yangmai 6/Sonalika taken from Kumar et al (2009); * Resistant parent; Ŧ Linked markers were not mapped to the deletion bin

Table 6: Effects of quantitative loci (QTLs) that enhance stay green in 'Chirya 3' × 'Sonalika' recombinant inbred (RI) population

QTLs	Marker Interval	Interval Size (cM)	Chrom	2004-05		2005-06		Q×E
				LOD‡	R^2	LOD	R^2	
QSg.bhu-1A	*Xgwm691-Xgwm752*	11.4	1AS	6.2	24.8	2.8	11.0	*
QSg.bhu-3B	*Xgwm533-Xgwm1037*	8.0	3BS	4.8	17.9	-	-	*
QSg.bhu-7D	*Xgwm111-Xswm008*	28.0	7DS	-	-	4.3	22.2	*
Total R^2 by joint analysis				7.9	38.7	5.4	27.6	
QTL × QTL interactions								
1A × 3B		-	-	8.4**	-	-	-	ns
1A × 7D		-	-	-	-	5.1**	-	ns

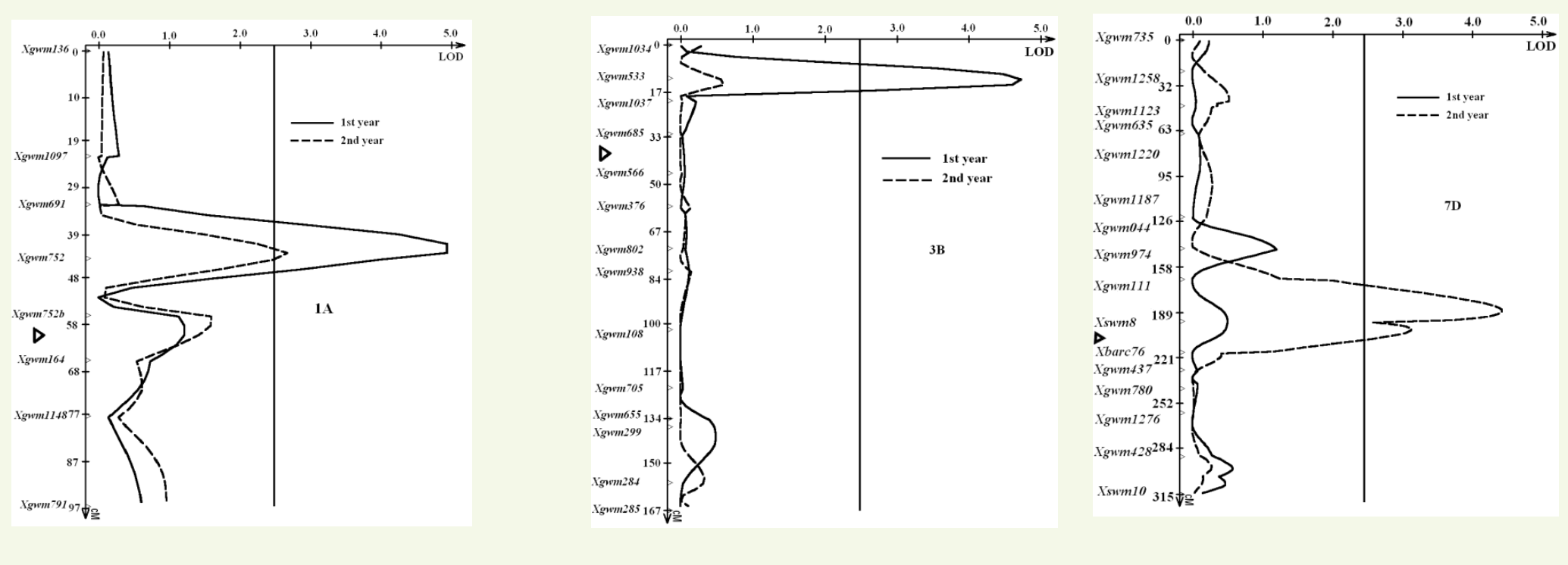

Fig 5: LOD curves obtained by CIM for QTLs mapped on chromosomes 1A, 3B and 7D for stay green trait in 'Chirya 3' × 'Sonalika' RI population. The vertical line indicate the threshold LOD value (2.5). Short arms are toward the top and open triangles indicate the probable position of centromere

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