Mapping of QTL conferring resistance to Turcicum Leaf Blight using Microsatellites in Maize (*Zea mays* L.)

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Abstract

Turcicum Leaf Blight (TLB), caused by *Exserohilum turcicum* is a foliar disease of maize. This study was conducted to identify quantitative trait loci (QTL) for TLB resistance in maize. A mapping population constituting 185 $F_{2:3}$ populations was developed by crossing two inbred lines *viz.*, CM 212 (susceptible) and V 336 (resistant), and was evaluated in two environments to generate phenotypic data for QTL mapping. A polymorphic survey of 183 pairs of simple sequence repeat (SSR) or microsatellite primers between the two parents helped in identification of 101 polymorphic markers. Data on four disease severity traits *viz.*, Percent Disease Index (PDI), Area Under Disease Progress Curve based on PDI (AUDPC-PDI), Lesion Area (LA), and Area Under Disease Progress Curve based on LA (AUDPC-LA) were generated for QTL mapping. Eight QTL intervals for resistance to TLB were located on chromosomes 2, 3, 4, 5, 7 and 9. Out of the eight QTL; one QTL was reported for LA on chromosome 4 flanking phi019 and bnlg2162 markers at the low disease-pressure environment (E_1), six QTL at high disease-pressure environment (E_2) and one QTL across pooled environments. Out of the six QTL identified at high disease-pressure environment, one QTL for AUDPC-PDI was identified on chromosome 9 flanked by markers phi065 and phi016 while the remaining five QTL for LA were identified on chromosomes 2, 3, 5 and 7. One QTL for PDI was identified across environments analysis on chromosome 3 flanked by markers mmc0071 and bnlg1160. For these QTL, the LOD values ranged from 2.70 to 14.84 and corresponding R^2 (% variation explained) ranged from 12.96 to 18.98 % in the individual environments. All QTL showed overdominance gene action except QTL 4 (dominance) at their respective chromosome.

Abbreviations

AUDPC: area under disease progress curve
DAS, days after sowing
LA, lesion area
LOD, logarithm of odds
MAS, marker-assisted selection; PD percent disease index
QTL, quantitative trait loci
SSR, simple sequence repeat
TLB, Turcicum leaf blight

Introduction

Turcicum Leaf Blight (TLB) commonly known as *Northern Corn Leaf Blight* is caused by the ascomycete fungus *Exserohilum turcicum* (Pass.) K.J. Leonard and Suggs syn. *Setosphaeria turcica* (Renfro and Ullstrup, 1976). TLB infection depends on the level of genetic resistance of the genotype, climatic conditions during the growth cycle and the production system and causes significant losses (28 to 91 percent) to yield and grain quality (Singh *et al.*, 2004, 2014). The disease symptoms primarily appear on the leaves. Plants may be infected at any growth stage, but usually at or after anthesis. Lesions on susceptible plants are 4-20 cm long and 1-5 cm wide, which is elliptical in shape and grayish-green to tan in color. Severity of TLB increases exponentially under highly humid and low temperature conditions (Singh *et al.*, 2004). Environments with heavy dew have also increase TLB severity (Dingerdissen *et al.*, 1996). Resistance to TLB is controlled by qualitative race-specific single genes *Ht1* (Hooker, 1963), *Ht2* (Hooker, 1977), *Ht3* (Hooker, 1981), *HtN* (Gevers, 1975), and *HtP* (Ogliari *et al.*, 2005) and quantitatively race nonspecific multiple genes acting together or separately (Juliana *et al.*, 2005). Quantitative or polygenic resistance was effective in tropical areas (Carson and Van Dyke, 1994) including India (Singh *et al.*, 2004). Compared to most major resistance genes, *Ht* genes are highly environment-dependent particularly with regards to light and temperature (Thakur *et al.*, 1989) and

they tend to confer delayed lesion development rather than complete resistance. However, partial resistance to TLB in contrast appears to be relatively stable over a wide range of temperature and light conditions (Carson and Van Dyke, 1994).

A number of DNA markers have been employed for genetic mapping. However, simple sequence repeats (SSR) markers are proven to be highly reliable and reproducible compared to several other marker types. Screening of molecular markers for parental polymorphism among maize cultivars forms the basis for constructing high-density genetic linkage maps that can facilitate Quantitative Trait Loci (QTL) mapping against TLB. QTL identification in return helps in marker-assisted selection (MAS) for increasing gain from selection. Very little work has been done in the Indian germplasm for precise identification of QTL against TLB. Large numbers of SSR markers have been mapped in maize (Zwonitzer *et al.*, 2010). Several of these markers still need to be incorporated into the genetic map of maize to construct a high-resolution map. Genomic regions associated with quantitative resistance to TLB have been identified in several studies using different populations and environments with a view of eventually improving host resistance (Brewster *et al.*, 1992; Dingerdissen *et al.*, 1996; Schechert *et al.*, 1999; Welz *et al.*, 1999a, 1999b; Welz and Geiger, 2000; Federer *et al.*, 2001; Wisser *et al.*, 2006; Asea *et al.*, 2009, 2012; Balint-Kurti *et al.*, 2010). Keeping the above facts in view, experiments were conducted in the present study to identify SSR markers co-segregating with resistant to TLB disease, identify QTL for TLB in the Indian maize germplasms pool, and validate some of the previously reported QTL for TLB resistance in maize which could be used in further breeding programs.

Material and methods

Plant materials

Maize inbred CM 212, derived from population USA/AccNo.2132 (Alm) after 6-7 generations of inbreeding, is an early maturing maize inbred line with high susceptibility to TLB and known for its good combining ability and developing single crosses with high heterosis. The male parent (V 336) derived from the maize population CML145/P63CDHC181 after 6-7 generations of selfing is a highly TLB resistant line in an early maturity group. The $F_{2:3}$ families of 185 selfed F_2 plants of CM 212 × V 336 were raised and evaluated through field trials for resistance against TLB in two environments *viz.*, Agriculture Research Farm, Banaras Hindu University, Varanasi, Uttar Pradesh, India (Varanasi-E_1) and TLB hot spot at the Agricultural Research Station in Nagenahalli, Karnataka, India (Nagenhalli-E_2).

Parental Polymorphism Assay

A set of 500 SSR markers obtained from different sources [Applied Biotechnology Centre, International Maize and Wheat Improvement Center (CIMMYT) and Asian Maize Biotechnology Network (AMBIONET)] were used to screen maize inbreds to identify polymorphic SSR markers. The parent polymorphism survey was conducted at the Molecular Breeding Lab in BHU Varanasi during 2010-2012. Parental polymorphism survey revealed that the parents CM 212 and V 336 were differentiated by 101 SSR polymorphic primers and were suitable candidates to develop mapping population.

Disease Severity Field Evaluation

All $F_{2:3}$ families plants were screened for TLB in two different environments: Varanasi-E1 (83.03 °E longitude and 25.18 °N latitude with an elevation of 123.23 meters above the sea level in the North Gangetic Plain) and Nagenahalli-E_2 (12.20 °N; 76.42 °E with an elevation of 695 meters above sea level and 705 mm/year average rainfall). The field experiments were carried out during *Kharif* (Rainy) season of 2017 at BHU, Varanasi and Nagenahalli, Karnataka. The $F_{2:3}$ families were evaluated together with parental check lines in a randomized complete block design with two replications. Each replication consisted of two-row plots of 3 meter in length and 60 cm in width with plants spaced at 25 cm from each other within a row. The susceptible check was planted every 20^{th} rows to promote disease build up and spread. All the recommended agronomic practices were followed to obtain normal growth in both environments

Disease Development

The spreader-row technique was used for field inoculation at Varanasi location to promote disease build up and spread. Inoculums were produced and maintained separately on susceptible varieties. Plants were inoculated at the 6–7 leaf stage. The inoculums were prepared by growing the fungal mycelium on sorghum grains. After proper fungal growth (7-10 days), the grains were dried in shade at room temperature. A fine powder of these grains was prepared with the help of a mixer–grinder and a pinch of this powder was put in the leaf whorl. Inoculation was done in evening to avoid the maximum day temperature during incubation period. The procedure was same as reported by Carson *et al.* (2004). In Nagenahalli the isolate of pathogen was provided by the Agricultural Research Station, Nagenahalli. Conidia from a specific pathogenic isolate of *Exserohilum turcicum*, collected from diseased plants leaves from the field, were cultured on casein lactose

hydrolysate (CLH) agar medium. Inoculums were prepared, multiplied, and washed from plates into a container for preparation of suspension. Plants were inoculated at 6-7 leaf stage by pouring 1 ml of inoculums on leaf whorl.

Disease Assessments

Four disease traits of TLB *viz.*, Percentage Disease Index (PDI), Area Under Disease Progress Curve based on PDI (AUDPC-PDI), Lesion Area (LA) and Area Under Disease Progress Curve based on LA (AUDPC-LA), were recorded in two different environments (E_1 and E_2). In Varanasi (E_1) data was recorded at three different growth stages of maize *viz.*, Flowering stage 50 days after sowing (50 DAS), Dough stage (60 DAS) and Brown husk stage (70 DAS) whereas, in Nagenahalli (E2) data was recorded at five different growth stages *viz.*, Pre-flowering stages (30 DAS and 40 DAS), Flowering stage (50 DAS), Dough stage (60 DAS) and Brown husk stage (70 DAS). PDI was calculated using a 1-5 scale (Payak and Sharma, 1985). LA was calculated according to the formula given by Leath (1986): A= (L × W) (0.7854). Where, A is the lesion area, L is the lesion length, and W is the lesion width. LA was taken from infected leaves of each entry and mean was calculated by summing and averaging of all infected leaves. AUDPC was estimated using the formula given by Campbell and Madden (1991):

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

Where X_i is the disease index expressed as a proportion at the i^{th} observation, t_i is the time (days after planting) at the i^{th} observations and n is the total number of observations. *AUDPC-PDI* and *AUDPC-LA* were also calculated at the same growth stages in the two environments

Statistical Analysis

Statistical analysis of all four characters [PDI, AUDPC-PDI, LA, and AUDPC-LA] for ANOVA and traits correlation was performed using PROC GLM procedure using SAS (V 9.2) software package (SAS Institute Inc., 2004). ANOVA was calculated for all four disease parameters in each environment as well as pooled across environments, which revealed significant differences for treatment, environment as well as treatment by environment interaction.

Heritability and Traits correlation

Estimates of broad sense heritability (h^2) was calculated from ANOVA produced by ANOVA over environments using PROC GLM procedure of SAS software according to the formula suggested by Burton and de Vane (1953) for each disease character:

$$h^2 = \frac{\sigma^2 g}{\sigma^2 g + \sigma^2 e}$$

Where, $\sigma^2 g$ = genotypic variance and $\sigma^2 e$ = environmental variance. Correlation was estimated for all four disease parameters of each environment as well as across the environments by PROC GLM procedure of SAS software.

Microsatellite Analysis

Genomic DNA was isolated from 21-24 days old seedlings of the $F_{2:3}$ plants using a modified method based on Saghai-Maroof *et al.* (1984). To identify SSR markers linked to QTL, 500 SSR markers distributed throughout the maize genome were screened during the parental polymorphism survey. These SSR primers were received from Applied Biotechnology Center, CIMMYT, Mexico and the Asian Maize Biotechnology Network (AMBIONET). Out of 101 SSR markers that showed polymorphism during parental screening, only 91 were used for genotyping of the $F_{2:3}$ selfed families. The polymerase chain reaction mixture consisted of 1.5 µl 10x PCR Buffer, 0.15 µl dNTPs ,1.2 µl MgC_{l2}, 3 µl each of Forwarded Primer and Reverse Primer, 0.1 µl Taq Polymerase and 2 µl template DNA in a final volume of 15 µl. The thermo cycling assays were carried out following the conditions: initial denaturation at 95°C for 5 min, denaturation at 94°C for 1 min, annealing at 57-63°C for 1 min (depending on annealing temperature of each primer), extension at 72°C for 2 min, final extension at 72°C for 7 min. These steps were repeated for 35 cycle of amplification of DNAs. Amplification products were resolved by electrophoresis using 2.5% agarose gel in 1X TAE buffer at 80-100 volt for 1-2 hrs. A 100 bp DNA ladder was used to estimate the size of amplified DNA fragments. Gel photographs were taken using Alpha imager gel documentation system by placing the gel under a UV lamp (Agilent Technologies, USA).

Map Construction and QTL Detection

For each segregating marker, a Chi-square analysis $\{\chi^2 = \Sigma$ (Observed-Expected)/ Expected$\}$ was performed to test for deviation from the expected segregation ratio (0.375:0.25:0.375). Linkage analysis of SSR markers was conducted using the Kosambi (1944) mapping function performed by QTL IciMapping Software V4.1. QTL analysis for each trait at each location was performed by using IciMapping V4.1 with the inclusive

Table 1 - Range and mean value of Percent Disease Index (PDI), AUDPC-PDI, Lesion Area (LA) and AUDPC-LA and coefficients of variation for 185 $F_{2:3}$ lines from the cross of CM 212 × V 336 from individual environments and across environments.

	Percent disease index (PDI)			AUDPC-PDI			Lesion Area (LA)			AUDPC-LA		
Genotype	E_1*	E_2**	Pooled	E_1*	E_2**	Pooled	E_1*	E_2**	Pooled	E_1*	E_2**	Pooled
CM 212	58.60	83.51	71.60	892.10	2241.68	1566.89	17.66	33.26	25.46	189.68	395	292.34
V 336	32.45	38.22	35.34	557.85	1361.33	959.59	5.17	5.97	5.57	66.55	80.06	73.30
$F_{2:3}$ range	32.84 53.15	54.97 83.64	46.86 61.50	583.25 833.10	1641.69 2406.05	1150.86 1512.02	4.08 24.84	5.25 36.77	5.37 27.34	59.75 313.05	67.06 450.05	72.73 301.38
$F_{2:3}$ mean	38.50	72.20	55.50	657.80	1912.69	1285.25	10.64	19.72	15.18	110.49	222.18	166.34
CV#	7.44	6.38	7.46	6.04	5.55	6.30	16.29	14.55	15.80	14.05	15.60	16.12

CV was estimated from 185 entries with parents in RBD; *Agricultural Research Farm, BHU, Varanasi; ** Agricultural Research Station, Nagenahalli, Karnataka

composite interval mapping (ICIM) method (Li *et al.*, 2008). The additive and dominant effects (ICIM-ADD) mapping method was used to identify QTL by stepwise regression, with 1000 permutations and walk speed of 2 cM. A QTL was considered signifi¬cant when the LOD (log10 of the likelihood of odds ratio) value derived from permutation analysis was large than 2.5. Additive and dominance effects for detected QTL were estimated using the Zmap QTL procedure of QTL Cartographer. The R^2 value, the percentage of the phenotypic variance explained by marker genotype at the QTL was taken from the peak QTL position as estimated by ICIM software. Gene action was determined by the ratio of the absolute value of the estimated dominance effect divided by the absolute value of the estimated additive effect (d)/(a) following Stuber *et al.* (1987); (additive = 0 to 0.20; partial dominance = 0.21 to 0.80; dominance = 0.81 to 1.20; and over dominance > 1.20).

Results

Phenotypic Analysis

Percent Disease Index and AUDPC-PDI

Mean PDI at 70 DAS for TLB in the resistant (V 336) and susceptible (CM 212) parents ranged from 32.45 (E_1) to 38.22 (E_2) and 58.60 (E_1) to 83.51 (E_2), respectively. The mean PDI of $F_{2:3}$ families ranged from 38.50 (E_1) to 72.20 (E2) (Table 1). The diseases progress curve based on PDI of $F_{2:3}$ mapping population indicated continuous increasing in disease severity from flowering to brown husk stages in both environments (Fig. 1). The mean disease progress curve remained between susceptible (CM 212) and resistant (V 336) parents in both environments. The resistant and susceptible parental lines exhibited contrasting phenotypes for TLB in both the environments. The AUDPC-PDI of $F_{2:3}$ mapping population ranged from 583.25-833.10 in E_1 to 1641.69-2406.05 in E_2, indicating large phenotypic variation in the population in single environment as well as across environments. We also observed that the disease was less severe at the flowering stage (30 DAS) highest at the Brown husk (70 DAS) stage in both the environments, indicating continuous disease development across different plant growth stages. The ANOVA revealed significant differences among genotypes, environments and genotype x environment for all the four traits (Table 2). Keeping in view the differences in disease pressure in the two environments, it was decided to analyse data of the two environments separately as well as perform the analysis on the pooled data. The broad sense heritability estimates (Table

Table 2 - Pooled analysis of variance of four disease traits (PDI, AUDPC-PDI, LA and AUDPC-LA) involving 185 $F_{2:3}$ lines and parents of the cross CM 212 × V 336 across two environments.

Source of variation	Df	Mean Sum of Square			
		PDI	AUDPC-PDI	LA	AUDPC-LA
Treatment	186	47.20**	20648.00**	72.21**	5307.69**
Environment	1	206155.73**	293534461.30**	15367.58**	2323510.78**
Replication	1	14.19	97972.90	183.95	2998.08
Treatment*Environment	186	38.43**	17319.20**	64.40**	4844.79**
Error	373	17.12	6551.30	5.75	719.72
CV%		7.46	6.30	15.80	16.12
h^2 (Broad Sense)		0.53	0.51	0.85	0.77

* Significant at 0.05 probability level;**significant at 0.01 probability level.a

Table 3 - Correlation of four disease traits (PDI, AUDPC-PDI, LA and AUDPC-LA) involving 185 $F_{2:3}$ lines with parents on the basis of individual as well as across environments.

Characters	Environments.	PDI	AUDPC-PDI	LA
AUDPC-PDI	Env-1	0.8322 **		
	Env-2	0.8164 **		
	Pooled (Env-1 & Env-2)	0.8098 **		
LA	Env-1	0.1198	0.1007	
	Env-2	0.0517	0.0332	
	Pooled (Env-1 & Env-2)	0.1537 **	0.0995	
AUDPC-LA	Env-1	0.1129	0.1252	0.8438 **
	Env-2	0.1146	0.0995	0.8227 **
	Pooled (Env-1 & Env-2)	0.2079 **	0.1638 **	0.8199 **

* Significant at 0.05 probability level;**significant at 0.01 probability level.

2) resulted moderate for PDI (0.53) and AUDPC-PDI (0.51). The phenotypic correlation among four disease traits is presented in Table 3. Furthermore, there were positive and highly significant correlations between the two traits whether considered individually in E_1 or E_2 or across the two environments. A significant correlation (0.83, 0.82 and 0.81) was observed between PDI and AUDPC-PDI for the E_1, E_2 and across environments, respectively (Table 3).

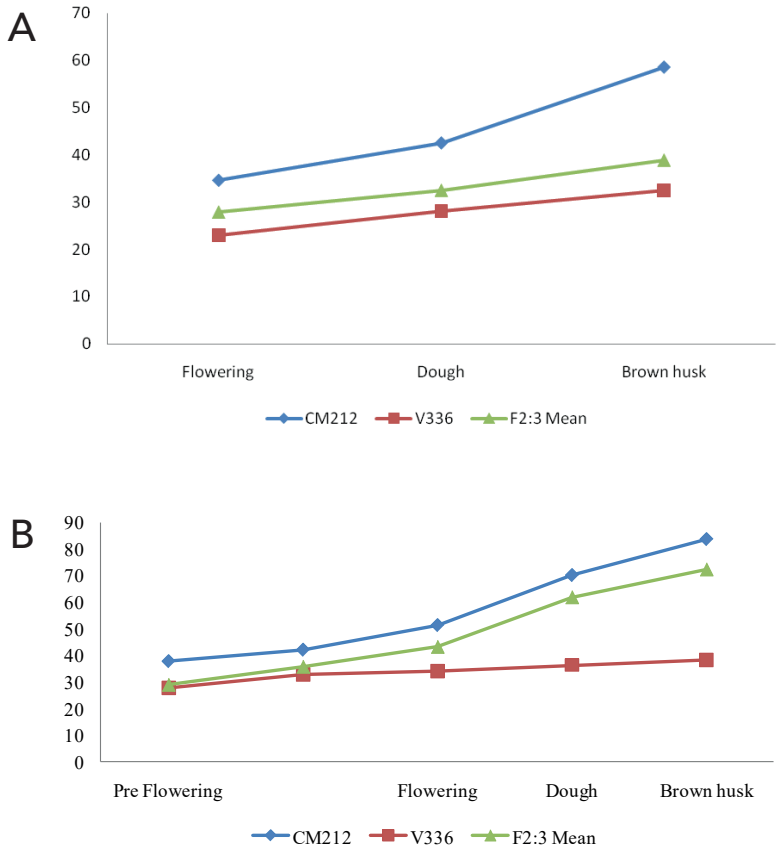

Fig. 1 - Disease progress curve based on mean PDI of P_1, P_2 and $F_{2:3}$ lines of cross CM 212 × V 336 at E_1- Varanasi, Uttar Pradesh (A) and E_2- Nagenahalli, Karnataka (B).

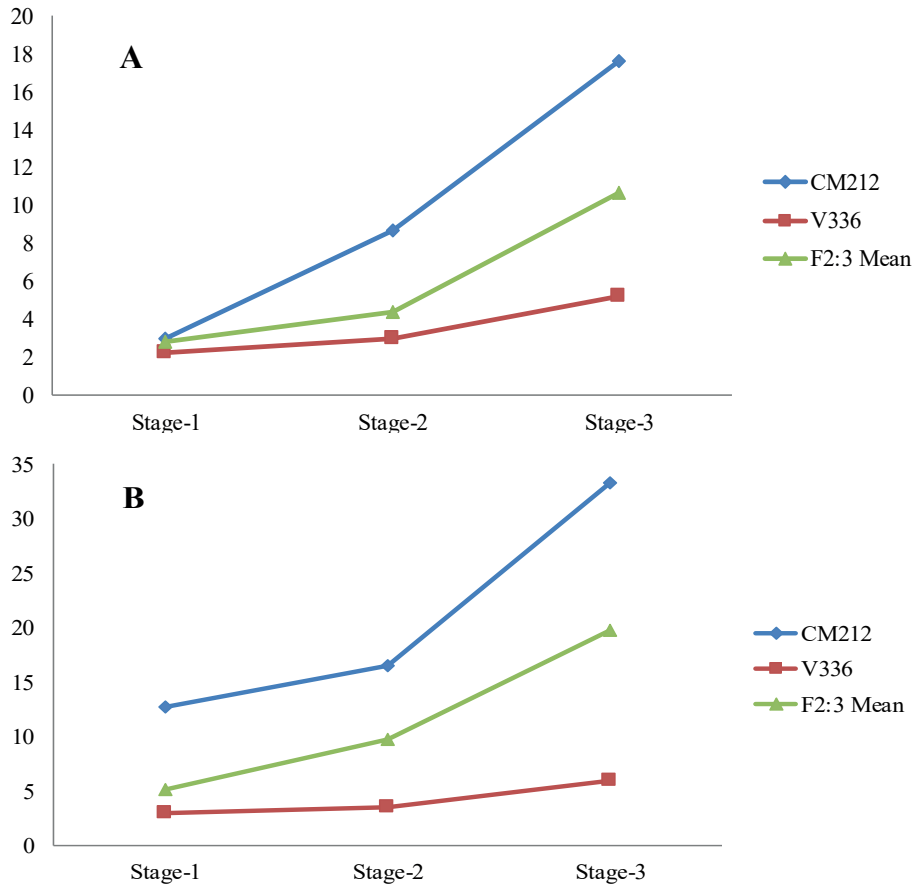

Fig. 2 - Disease progress curve based on mean lesion area of P_1, P_2 and $F_{2:3}$ lines of cross CM 212 × V 336 at E_1- Varanasi, Uttar Pradesh (A) and E2- Nagenahalli, Karnataka (B).

LA and AUDPC-LA

Mean LA at 70 DAS of the 185 $F_{2:3}$ families ranged from 10.64 (E_1) to 19.72 (E_2). The disease progress curve for LA indicated that 185 $F_{2:3}$ families ranged from 4.08 to 24.84 in E_1, and from 5.25 to 36.77 in E_2, with values lying between the resistant and susceptible parents (Fig. 2). The AUDPC-LA of the $F_{2:3}$ families ranged from 59.75-313.05 in E_1 and 67.06-450.05 in E_2. These values indicated phenotypic variation in both environments with high degree of severity in E_2. The ANOVA exhibited highly significant differences among genotypes, environments as well as among environment × genotype for both the traits (Table 2). The broad sense heritability estimates (Table 2) were high for both traits (0.85 and 0.77, respectively). Further, the phenotypic correlation between all four disease parameters is presented in Table 3. LA was significantly correlated (0.84, 0.82 and 0.82) with AUDPC-LA in E_1, E_2 and across the environments, respectively.

SSR Linkage Map and QTL Analysis

We analyzed 500 SSR markers covering the whole genome for polymorphism between CM 212 and V 336 inbred lines. The QTL analysis was performed for E_1 and E_2 separately as well as for the pooled environments for the four traits. We identified polymorphic 101 (21.63%) markers. The construction of genetic map with these markers covered about 2757.01 cM with 91 markers distributed across the maize genome. The average distance between adjacent marker loci was about 30.30 cM. Ten markers remained ungrouped as they were genetically unlinked. Eight QTL intervals for resistance to TLB were located on chromosome 2, 3, 4, 5, 7 and 9 (Table 4). Out of the eight QTL identified, one was reported for LA on chromosome 4 flanked with phi019 and bnlg2162 markers for TLB resistance at BHU, Varanasi (Fig. 3), six QTL were reported at Nagenahalli, Karnataka, and one QTL was reported across environments. Out of the six QTL identified at E_2, one for AUDPC-PDI was reported on chromosome 9 flanked with phi065 and phi016 markers (Fig. 3) and five

QTL for LA were reported on chromosome 2, 3, 5 and 7 (Fig. 4). One QTL (pooled) was detected for PDI on chromosome 3 flanked with mmc0071 and bnlg1160 markers (Fig. 3) in the pooled analysis. For these QTL the LOD values ranged from 2.70 to 14.84 and corresponding R^2 ranged from 12.96 to 18.98 in the individual environments (Table 4). The gene action of all QTL showed overdominance except QTL 4 (dominance) at their respective chromosome (Table 4)

Table 4 - QTL identified for Lesion area (LA), Area under disease progress curve based on PDI (AUDPC-PDI) and Percent disease index (PDI) in $F_{2:3}$ lines of cross CM 212 × V 336 of two different environments of BHU, Varanasi and Nagenahalli, Karnataka.

QTL	Trait	Bin	Marker-Interval	Map Position (cM)	LOD	R^2	Gene effects			Gene action
							D	A	d/a	
Environment 1										
QTL1	LA	4.11/4.08	phi019-bnlg2162	23	2.7003	18.725	5.5041	-0.37	-14.88	OD
Environment 2										
QTL2	AUDPC- PDI	9.03/9.04	phi065-phi016	177	3.6231	18.983	19.326	2.706	7.14	OD
QTL3	LA	2.03/2.07	bnlg1621-umc1560	46	12.151	13.234	10.011	-0.14	-71.51	OD
QTL4	LA	2.05/2.02	bnlg2328-bnlg1017	194	12.265	13.245	-4.869	-5.14	0.95	D
QTL5	LA	3.08/3.09	bnlg1350-mmc0001	327	10.959	13.226	10.035	-0.16	-62.72	OD
QTL6	LA	5.01/5.03	bnlg1836-phi113	222	14.837	13.246	10.109	0.024	421.21	OD
QTL7	LA	7.02/7.02	bnlg1094-bnlg1792	203	11.633	12.955	9.258	0.112	82.66	OD
Pooled										
QTL8	PDI	3.05/3.06	mmc0071-bnlg1160	409	2.8646	16.924	-1.5083	-0.22	6.86	OD

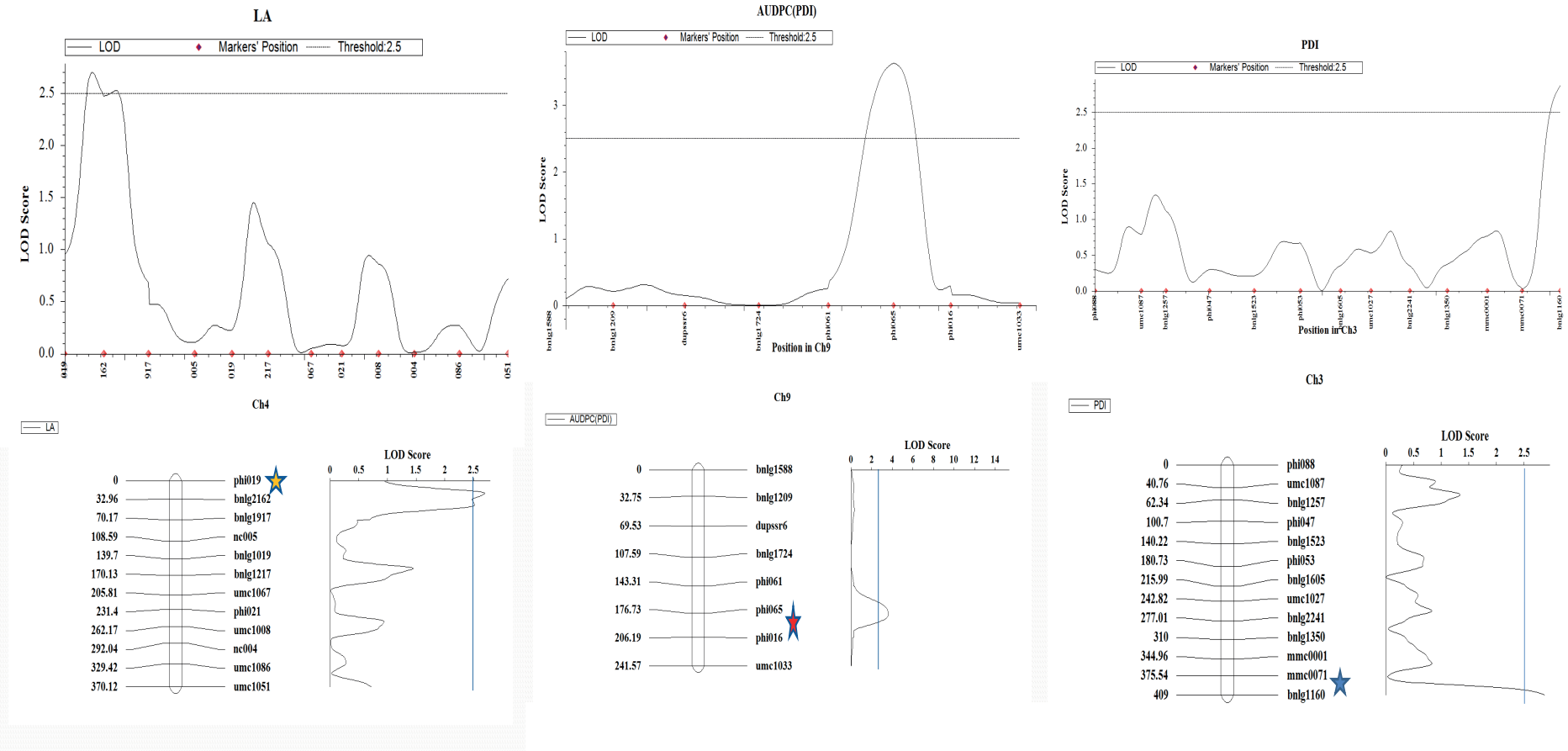

Fig. 3 - Linkage map of chromosome 4, 9 and 3 including SSR markers associated with disease traits in the $F_{2:3}$ families of the cross CM 212 × V 336 and LOD curve of QTL 1, QTL 2 and QTL 8 with flanking markers phi019-bnlg2162, phi065-phi016 and mmc0071-bnlg1160 were identified for disease trait Lesion area in E_1 (A); for disease trait AUDPC-PDI in E2 (B); for disease trait PDI in pooled environments (C), respectively. The horizontal line indicates the threshold LOD value (2.5) for determining significant QTL

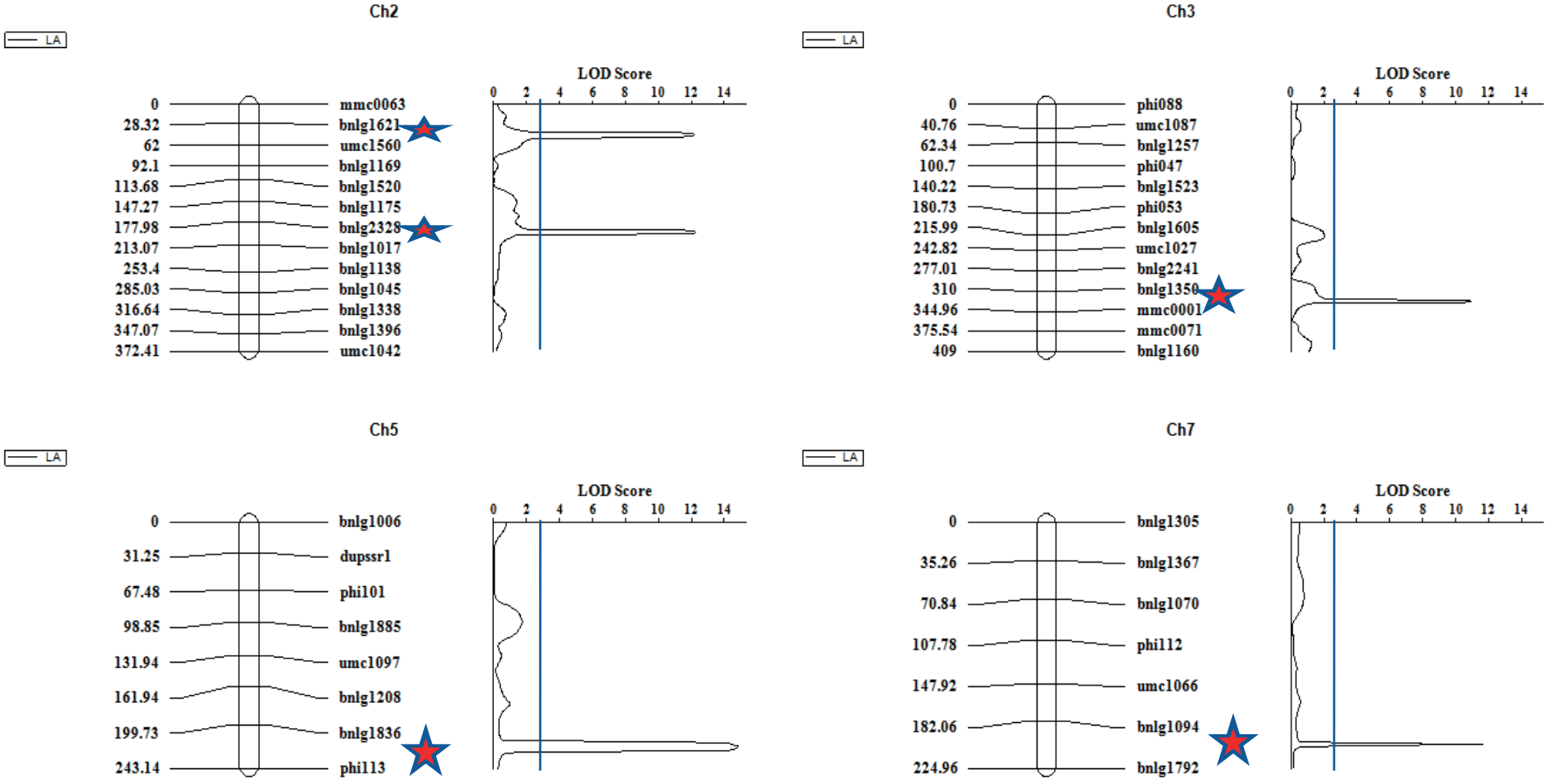

Fig. 4 - Linkage map of chromosome 2, 3, 5 and 7 including SSR markers associated with disease traits in the $F_{2:3}$ families of cross CM 212 × V 336 and LOD curve of five QTL (QTL 3, QTL 4, QTL 5, QTL 6 and QTL 7) with flanking markers bnlg1621-umc1560, bnlg2328-bnlg1017, bnlg1350- mmc0001, bnlg1836-phi113 and bnlg1094-bnlg1792 were identified for disease trait Lesion area (LA) in E_2, respectively. The horizontal line indicates the threshold LOD value (2.5) for determining significant QTL

Discussion

QTL identification and mapping is important to study genetically complex forms of plant disease resistance. QTL mapping facilitates studies of interactions between resistance genes, pathogens, and environment. In this study we are reporting eight TLB resistant QTL in maize. Moderate to high incidence of disease for all the four traits (PDI, AUDPC-PDI, LA, and AUDPC-LA) was observed in both environments. However, the disease incidence was high in E_2 as compared to E_1, despite similar epiphytotic conditions were provided in both the environments. Nagenahalli (E_2) is considered as hot spot for TLB in India. High natural incidence of TLB in E_2 was also reported earlier by several workers in E_2 as compared to rest of the country (Singh *et al.*, 2014). Long duration of high humidity and slightly cooler temperature (15-25 °C) are important parameters for ideal TLB disease development. In the present study both environments had adequate levels of humidity. In E_1 (Varanasi) during July, although the humidity remains high but average temperature ranges between 32 and 35 °C, which does not favor natural incidence of the disease. Whereas in E_2 (Nagenahalli) the average temperature remains around 20 to 25°C, which is quite optimal for TLB. Balint-Kurti *et al.* (2010) also mapped QTL for TLB in two environments. Balint-Kurti *et al.* (2010) observed that disease pressure was lower in Clayton (NC) than in Aurora (NY). They attributed this to slightly high temperature during the growing season in Clayton (NC), while in Aurora (NY), the cooler temperatures were optimal for TLB. So, the trend in the present study supports adoption of different inoculation techniques owing to local practices for creating epiphytotic conditions for TLB. Balint-Kurti *et al.* (2010) demonstrated that environments, including seasonal fluctuations coupled with type of inoculation methods affect the diseases occurrence and identification of QTL for diseases resistance. Mapping of QTL in $F_{2:3}$ families have also been reported by several workers (Schechert *et al.*, 1999). The heavy disease pressure maintained in field plots with artificial epiphytotic conditions combined with replicated disease evaluations in the two different environments ensured that our assay was sufficiently sensitive to detect QTL effects on TLB resistance. The moderate estimates of heritability indicated that resistance to pathogens was heritable and early generation selection could result in improved germplasm under high disease pressure evaluations. Moderate to high heritability (0.58 to 0.90) has also been reported by Asea *et al.* (2012) for TLB in maize. This also suggests that reasonable progress in selection is possible for TLB in maize. Broad-sense heritability estimates based on variance components analysis of $F_{2:3}$ families used for QTL analysis were 0.70 for number of lesions and disease severity (Freymark *et al.*, 1994). Comparatively high to medium estimates of heritability of LA and PDI indicated better expression of TLB in segregating generations and appropriate disease traits to study TLB. AUDPC is the most appropriate trait for QTL analysis and have been reported by earlier workers (Leath and Pederson 1986; Welz and Geiger 2000). High correlation coefficients were observed between PDI and AUDPC-PDI and between LA and AUDPC-LA within individual environments and across environments with a range from 0.81 to 0.84 ($P<0.0001$). However, the correlation coefficient between PDI and LA; PDI and AUDPC-LA; AUDPC-PDI and AUDPC-LA were non-significant and low, when calculated for individual and across environments. Such correlations have been observed previously while considering different disease traits for mapping QTL for TLB. Balint-Kurti *et al.* (2010) reported moderate Pearson correlation coefficient 0.49 to 0.67. Due to environmental variations in the two environments, we choose to analyze each environment with respect to four disease traits separately as well as pooled analysis over environments. We initially observed eight QTL, located on Chromosome arm 2, 3, 4, 5, 7 and 9 and all QTL identified were effective and were environment-specific. Balint-Kurti *et al.* (2010) reported many QTL for TLB resistance, out of which 6 were present on Chromosome arm 4 at bins 4.06/4.08. We are reporting one QTL on chromosome 4, located between bins 4.11 to 4.08. Environment specific TLB resistant QTL have been observed in a number of previous studies (Asea *et al.*, 2009, 2012). Balint-Kurti *et al.* (2010) reported two WMD QTL at bins 2.00/2.01 and 4.08 from the overall analysis. They further reported that QTL in bin 4.08 was detected in all three environments analyzed separately. Likewise, only one IP QTL in bin 2.02 was detected in all three environments. Balint-Kurti *et al.* (2010) reported three QTL present on linkage group 6 at Bins 6.05/6.07. Further, we are reporting one QTL associated with AUDPC-PDI on Chromosome 9 (QTL 2) at Bins 9.03/9.04, where as Balint-Kurti *et al.* (2010) reported it at Bin, 1.06. The four TLB disease traits were helpful in mapping eight QTL. Six QTL were associated with LA, one with AUDPC-PDI and one with PDI. The Bins of eight QTL present on Chromosome 2, 3, 4, 5, 7 and 9 were quite similar or close to the locations observed by Balint-Kurti *et al.* (2010). In maize, resistance to TLB is a complex quantitatively inherited trait. QTL for TLB from other studies were compared to disease resistance and reported by Dingerdissen *et al.* (1996) and Welz and Geiger (2000). They reported AUDPC is more appropriate trait for QTL analysis in maize. Dingerdissen *et al.* (1996) identified QTL for AUDPC on chromosome 1 and on 2S, 3L, 5S, 6L, 7L,

8L and 9S whereas; Welz and Geiger (2000) discovered QTL for AUDPC were located on chromosome 1 to 9 in three different mapping populations. All three populations carried QTL in identical genomic regions on chromosomes 3 (bin 3.06/07), 5 (bin 5.04) and 8 (bin 8.05/06). In our study, QTL for AUDPC has been identified in 9.03/9.04 with 18.98% phenotypic variance in individual environments. Gene action was mostly over dominant or recessive.

Conclusions

The mapping of QTL has been one of the major goals for lo¬cating markers that can be broadly used for MAS in a breeding program. However, the use of MAS is lagging behind owing to the lack of consistency of QTL across environments. As a result, we record eight QTL; one at E_1 (Varanasi), six at E_2 (Nagenahalli), and one at pooled environments, indicating that resistance alleles could be transferred to susceptible lines at the same location. To extend resistance to TLB, it would also be helpful for MAS to initiate a pyramiding programme for multiple genes.

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