Bangladesh J. Bot. **51**(2): 199-206, 2022 (June) DOI: https://doi.org/10.3329/bjb.v51i2.60415

QTLS MAPPING FOR TURCICUM LEAF BLIGHT RESISTANCE IN MAIZE (*ZEA MAYS* L.)

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Keywords: Quantitative trait loci, Turcicum leaf blight, Linkage map, Lesion area, Percent diseases index

Abstract

Turcicum leaf blight (TLB) is a prevalent maize disease found throughout the world, including India. To identify quantitative trait loci (QTLs) for TLB resistance with the help of appropriate mapping populations, namely $F_{2:3}$ families and $F_{2:6}$ families or recombinant inbred lines (RILs) evaluated in two environments were investigated. The cross CM 212 × V 336 was selected to generate the $F_{2:3}$ families and the established $F_{2:3}$ mapping population of cross CM 212 x CM 145 was further advanced to generate the $F_{2:6}$ families for construction of linkage map and mapping of QTLs. The present investigation identified a total of 23 QTLs for TLB resistance in maize. Out of these QTLs, Nine QTLs were found in Linkage Group 4 (LG 4), followed by four QTLs in LG 2, two QTLs in each of LG 1, 3, 5 and 9 and one QTL in each of LG 6 and 7. On the other hand, the LOD values for these QTLs ranged from 2.64 to 14.84 in individual environments and over environments for both mapping populations, while the associated R^2 values ranged from 10.80 to 18.98. The majority of the QTLs had over dominance at their respective chromosomes due to gene action.

Introduction

Turcicum leaf blight (TLB) caused by *Setosphaeria turcica*, anamorph *Exserohilum turcicum* Leonard and Suggs, is the most persistent and devastating disease of maize among the different foliar diseases. The most observed symptoms are long elliptic tan lesions that develop first on the lower leaves and progress upward. Severity of the disease 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 per cent) to yield and grain quality (Singh *et al.* 2004, 2014, Jakhar *et al.* 2017).

TLB can be effectively controlled by cultivating resistant types (Dingerdissen *et al.* 1996), hence disease resistance breeding should be a prime concern. Because there are very few genetic resources with TLB resistance are available for necessitating introgression of resistance genes in to different genetic backgrounds. The use of molecular or DNA markers can improve the efficiency of conventional plant breeding by selecting appropriate marker, linked to the trait of interest that are difficult to evaluate and that are largely affected by the environment (Tanksley *et al.* 1989, Young and Tanksley 1989). The evaluation of microsatellite tools for polymorphism among maize cultivars is the first step in making a large genetic linkage map that will help in the invention of novel Quantitative Trait Loci (QTL) that cause TLB resistance. QTL mapping is a powerful tool for understanding genetically complicated forms of plant disease resistance. The roles of individual resistance loci can be explained, the race specificity of partial resistance genes can be determined, and relationships between resistance genes, plant growth, and the environment can be studied using gene mapping. It provides a framework for marker assisted selection (MAS) of complex disease resistance traits and the positional cloning of partial resistance genes. Genomic region associated with several QTLs underlying quantitative resistance to TLB was identified in

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the recent two decades (Welz and Geiger 2000, Wisser *et al.* 2006, Asea *et al.* 2009, Balint-Kurti *et al.* 2010, Asea *et al.* 2012, Xia *et al.* 2020, Ranganatha *et al.* 2021). Not much work had been done in the Indian germplasms for identification of QTLs against TLB. Only few QTLs conferring resistance to TLB pathogens had been validated (Abalo *et al.* 2009, Asea *et al.* 2012). QTL mapping is a highly effective method for identifying markers that can be used for MAS in a large-scale breeding programme.

Materials and Methods

The mapping populations of two parental combinations were selected because the inbreds V 336 and CM 145 identified as the resistant parent had displayed highly resistance (HR) reactions, whereas CM 212 was chosen as susceptible parent because it displayed highly susceptible (HS) reactions to TLB disease (Table 1). Therefore, a total of 185 $F_{2:3}$ families were derived from a wide cross between CM 212 and V 336, while a total of 155 $F_{2:6}$ families or RILs were derived from progenies of established mapping population (CM 212 x CM 145; $F_{2:3}$ families) (Fig. 1).

The field experiments were carried out by using four disease traits *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 Lesion area (AUDPC-LA) during *Kharif* (Rainy Season) of 2017 at Varanasi-E_1 (25.2° N, 83.3° E) and Nagenahalli-E_2 (12° N, 76° E). PDI was calculated using the 1-5 scale (Payak and Sharma 1985), LA was calculated according to the formula given by Leath and Pederson (1986), and AUDPC was estimated using the formula given by Campbell and Madden (1990).

Table 1. List of three maize inbred lines along with their pedigree details, place of origin, characteristics features and disease reactions.

Inbred lines	Pedigree details	Place of origin	Characteristics features	Disease reactions
V 336	CML 145,P63CDHC181-3-2-1-4#2-BBBB#F-BBBBB#	Almora	Early maturity, large tassel with light purple glume, flint-dent, orange grains	HR
CM 145	Pop 31	Almora	Early maturity, tall plant and cob height, with good yield and straight leaf altitude	HR
CM 212	USA/AccNo.2132(Alm)-3-2-f-#B-#	Almora	Early maturity, medium plant and cob height, with good yield and straight leaf altitude	HS

Statistical analysis of all four characters (PDI, AUDPC-PDI, LA and AUDPC-LA) for ANOVA and traits correlation were performed by PROC GLM procedure using SAS (V 9.2) software package.

DNA extracted from seedlings that were 21-24 days old using a modified method based on Saghai-Maroof *et al.* (1994). The genetic linkage map was constructed using primers identified polymorphic in different cross combination during the polymorphism survey. For each segregating marker, a Chi- square (χ^2) analysis was performed to test for deviation from the expected segregation ratio. Linkage analysis of SSR markers was conducted using the Kosambi (1944) mapping function performed by QTL IciMapping Software. Quantitative trait loci (QTL) analysis for each trait at each location was performed by using IciMapping V4.1 with the inclusive composite interval mapping (ICIM) method (Li *et al.* 2018).

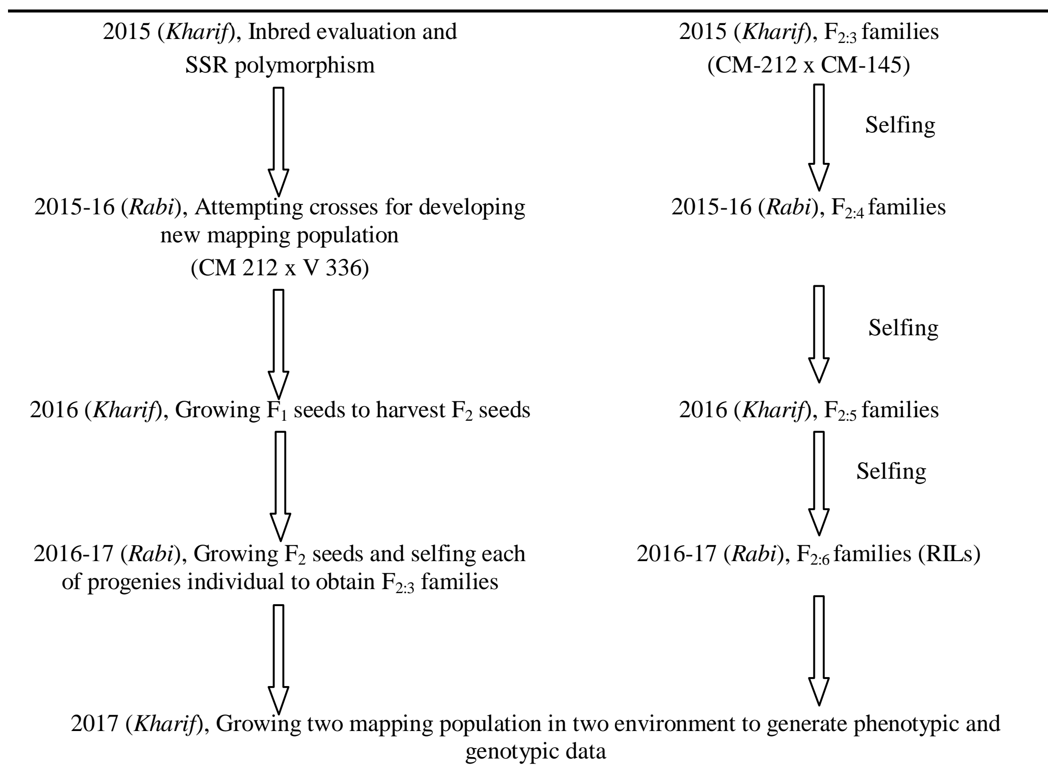


Fig. 1. Steps involved in development of appropriate mapping populations.

Results and Discussion

Phenotypic analysis was done with two crosses (CM 212 x V 336 and CM 212 x CM 145) and with four TLB traits *viz.* PDI, AUDPC-PDI, LA, AUDPC-LA in two different environments (Varanasi-E_1 and Nagenahalli-E_2). Moderate level of disease occurred at Varanasi, whereas in Nagenahalli, the level of disease incidence was quite high. Disease severity was low at Pre flowering stage and was maximum at brown husk stage in both the environment. The diseases graph kept on rising from pre flowering state to post flowering to dough stage to finally brown husk stage in both the environments. But the average disease incidence was over all higher in E_2. The average disease severity of mapping population in Environment 1 (E_1) was below susceptible parent whereas in Environment 2 (E_2), it was near to susceptible parent clearly indicating higher severity of disease in E_2 as compared to E_1. Similar trends were observed for both the crosses for all the four traits. In India, Nagenahalli (E_2) is considered a TLB hotspot. Singh *et al.* (2014) observed a higher natural occurrence of TLB in E_2 when compared to the rest of the country.

The ANOVA revealed significant differences among treatments, environments and treatment x environment for both the traits. Keeping in view the differences in disease pressure in two environments, it was decided to analyse data of the two environments separately as well as the pooled data.

For genotyping, a total of 91 polymorphic SSR or microsatellite markers were detected for the cross CM 212 x V 336 and 83 for the cross CM 212 x CM 145. The Chi-square (χ^2) test was used to determine the expected segregation ratio among genotypic data of SSR markers in the $F_{2:3}$

and RILs mapping populations. When compared to table values at the 1% probability levels, 10 out of 101 SSR marker loci for cross CM 212 x V 336 and 20 out of 103 SSR marker loci for cross CM 212 x CM 145 exhibited non-significant values.

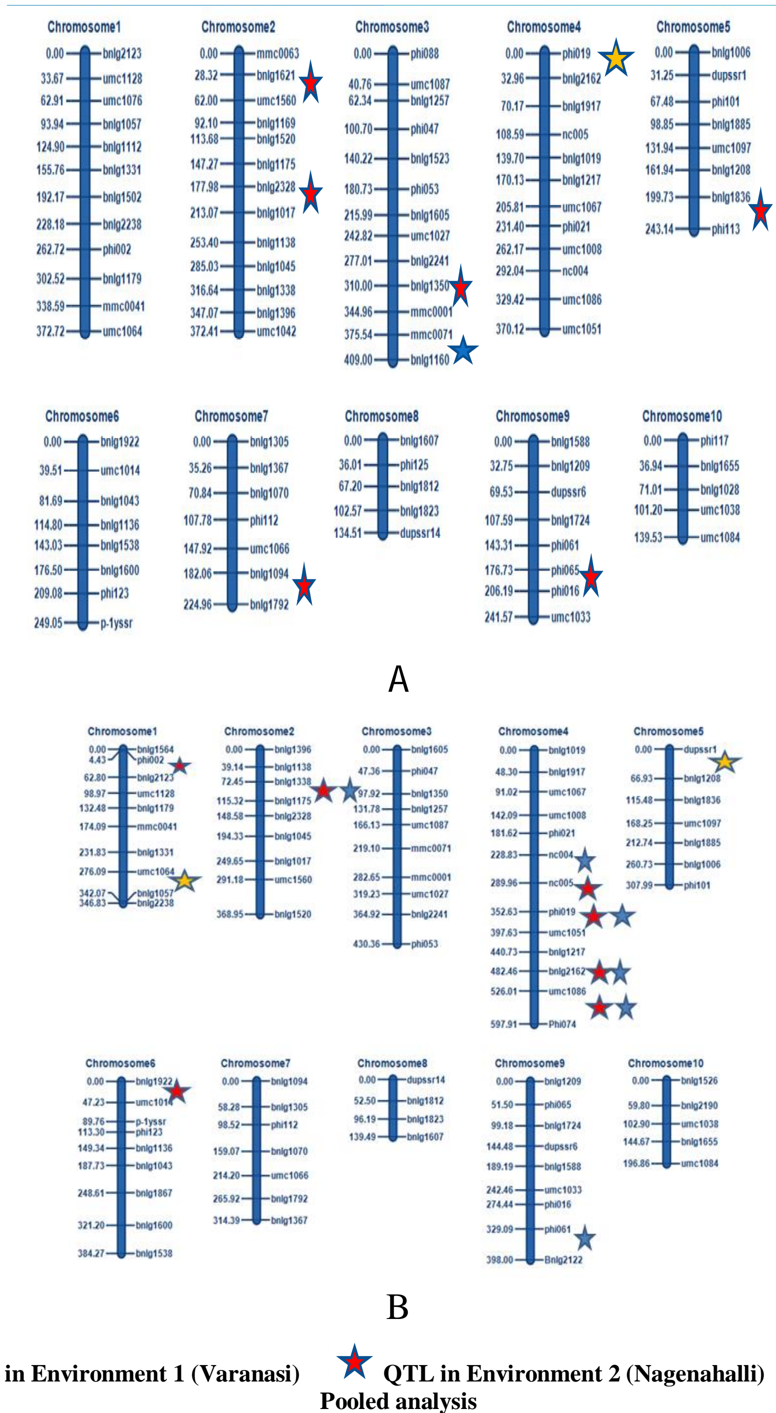
For the cross CM 212 x V 336, a total of 91 microsatellite markers were mapped on 10 linkage groups (LGs) with a total map length of 2757.01 cM and an average distance of 30.30 cM, and 83 microsatellite markers were mapped on 10 linkage groups with a total map length of 3485.05 cM and an average distance of 41.99 cM for the cross CM 212 x CM 145 (Table 2).

Table 2. Polymorphic SSR primers assigned to each chromosome and their average distances considering 185 $F_{2:3}$ families of CM 212 x V 336 and 155 RILs of CM 212 x CM 145.

Linkage groups (LGs)	No. of SSR markers		Length (cM)		Average distance (cM)	
	$F_{2:3}$	RILs	$F_{2:3}$	RILs	$F_{2:3}$	RILs
LG 1	12	10	372.72	346.83	31.06	34.68
LG 2	13	9	372.41	368.95	28.65	40.99
LG 3	13	10	409.00	430.36	31.46	43.04
LG 4	12	13	370.12	597.91	30.84	45.99
LG 5	8	7	243.14	307.99	30.39	44.00
LG 6	8	9	249.05	384.27	31.13	42.70
LG 7	7	7	224.96	314.39	32.14	44.91
LG 8	5	4	134.51	139.49	26.90	34.87
LG 9	8	9	241.57	398.00	30.20	44.22
LG 10	5	5	139.53	196.86	27.91	39.37
Whole Genome	91	83	2757.01	3485.05	30.30	41.99

Individual linkage group map lengths varied from 134.51 cM (LG 8) to 409.00 cM (LG 3) for the cross CM 212 x V 336 and 139.49 cM (LG 8) to 597.91 cM (LG 4) for the cross CM 212 x CM 145. The number of markers mapped per linkage group ranged from 5 (LG 8 and LG 10) to 13 (LG 2 and LG 3) for the cross CM 212 x V 336 and 4 (LG 8) to 13 (LG 4) for the cross CM 212 x CM 145 (Table 2). Zwonitzer *et al.* (2010) used 871 SSR and SNP markers to create a linkage map with a total length of 1,697.3 cM.

During the present investigation, a total of 23 QTLs for TLB resistance in maize were identified. Out of these, nine QTLs were found in Linkage Group 4 (LG 4), followed by four QTLs in LG 2, two QTLs in each of LG 1, 3, 5 and 9 and one QTL in each of LG 6 and 7 (Fig. 2). On the other hand, a total of eight QTLs were detected in $F_{2:3}$ mapping population of the cross CM 212 x V 336, while fifteen QTLs were reported in RILs of the cross CM 212 x CM 145. Balint-Kurti *et al.* (2010) reported many QTLs for NCLB resistance, out of which 6 were present on linkage group 4 at bins 4.06/4.08. For Northern corn leaf blight in maize, Dingerdissen *et al.* (1996), Schechert *et al.* (1999) and Welz *et al.* (1999) reported the existence of most QTLs on the 3rd, 5th, and 8th linkage groups.



QTL in Environment 1 (Varanasi) **QTL in Environment 2 (Nagenahalli)** **QTL in Pooled analysis**

Fig. 2. Genetic linkage map and position of the QTLs associated with Turcicum leaf blight resistance mapped from $F_{2:3}$ families of the cross CM 212 x CM 145 **(A)** and RILs mapping population of the cross CM 212 x V 336 (**B**).

At Varanasi (E_1), three QTLs were detected for TLB resistance. One QTL for Lesion area (LA) was found in $F_{2:3}$ mapping population of the cross CM 212 x V 336 and two QTLs for PDI were found in $F_{2:6}$ families or RILs of the cross CM 212 x CM 145 (Table 3).

Table 3. QTLs identified for Per cent disease index (PDI), AUDPC-PDI, Lesion area (LA) and AUDPC-LA in $F_{2:3}$ families of cross CM 212 × V 336 and RILs mapping population of cross CM 212 × CM 145.

Mapping population	Disease trait	Bin location	Left marker	Right marker	LOD value	R^2 (%)	Genetic effects		Gene action
							Add	Dom	
Environment 1									
$F_{2:3}$	LA	4.11/4.08	phi019	bnlg2162	2.70	18.73	5.50	-0.38	OD
RILs	PDI	1.11/1.06	umc1064	bnlg1057	4.00	12.02	5.09	-0.16	OD
	PDI	5.02/5.04	dupssr1	bnlg1208	2.75	12.10	3.94	0.12	OD
Environment 2									
$F_{2:3}$	AUDPC-PDI	9.03/9.04	phi065	phi016	3.62	18.98	19.33	2.71	OD
	LA	2.03/2.07	bnlg1621	umc1560	12.15	13.23	10.01	-0.14	OD
	LA	2.05/2.02	bnlg2328	bnlg1017	12.27	13.25	-4.87	-5.14	D
	LA	3.08/3.09	bnlg1350	mmc0001	10.96	13.23	10.04	-0.16	OD
	LA	5.01/5.03	bnlg1836	phi113	14.84	13.25	10.11	0.02	OD
	LA	7.02/7.02	bnlg1094	bnlg1792	11.63	12.96	9.26	0.11	OD
RILs	PDI	4.05/4.11	nc005	phi019	2.81	15.15	-9.01	-0.38	OD
	PDI	4.11/4.08	phi019	umc1051	2.94	15.25	-9.03	-0.41	OD
	AUDPC-PDI	2.01/2.04	bnlg1338	bnlg1175	7.12	11.16	262.32	10.56	OD
	AUDPC-PDI	4.08/4.08	bnlg2162	umc1086	2.91	11.17	244.97	17.42	OD
	AUDPC-PDI	4.08/4.04	umc1086	phi074	5.19	11.21	240.05	11.04	OD
	AUDPC-PDI	6.05/6.04	bnlg1922	umc1014	7.77	11.06	282.83	3.47	OD
	LA	1.08/1.11	phi002	bnlg2123	2.64	17.74	-4.23	-0.31	OD
Pooled Analysis									
$F_{2:3}$	PDI	3.05/3.06	mmc0071	bnlg1160	2.86	16.92	-1.51	-0.22	OD
RILs	PDI	4.11/4.08	phi019	umc1051	2.84	16.49	-3.39	-0.53	OD
	AUDPC-PDI	2.01/2.04	bnlg1338	bnlg1175	5.41	10.91	136.72	6.97	OD
	AUDPC-PDI	4.08/4.08	bnlg2162	umc1086	2.75	10.80	101.55	10.18	OD
	AUDPC-PDI	4.08/4.04	umc1086	phi074	4.50	10.95	113.12	6.99	OD
	LA	9.03/9.01	phi061	bnlg2122	2.88	11.32	-4.03	0.36	OD
	AUDPC-LA	4.03/4.05	nc004	nc005	2.66	14.81	-42.20	-7.60	OD

Where, OD- Overdominance, D- Dominance.

At Nagenahalli, 13 QTLs for TLB resistance were identified. Six QTLs (one QTL for AUDPC-PDI and five QTLs for LA) came from $F_{2:3}$ mapping population the cross CM 212 x V 336 and seven QTLs (two QTLs for PDI, four QTLs for AUDPC-PDI and one QTL for LA) came from RILs of the cross CM 212 x CM 145 (Table 3). Mapping of QTLs in $F_{2:6}$ families have also been reported by several workers (Freymark *et al.* 1994, Schechert *et al.* 1999, Welz *et al.* 1999).

the present analysis was adequately sensitive to detect QTL effects on TLB resistance due to the high disease pressure maintained in field plots with artificial epiphytotic conditions combined with replicated disease evaluations in two independent environments.

At pooled analysis, seven QTLs positions were identified. One QTL for PDI from $F_{2:3}$ mapping population of the cross CM 212 x V 336 and six QTLs (one QTL for PDI, three QTLs for AUDPC-PDI and one QTL for each LA and AUDPC-LA from RILs of the cross CM 212 x CM 145 (Table 3). The present study was adequately sensitive to detect QTL effects on TLB resistance due to the high disease pressure maintained in field plots with artificial epiphytotic conditions combined with replicated disease evaluations in two independent environments Asea *et al.* (2012).

The traits AUDPC-PDI and LA were the most useful for explaining TLB resistance in maize, with each trait identifying eight QTLs. The other effective traits *viz*., PDI (six QTLs) and AUDPC-LA (one QTL) were also useful for TLB resistance. Dingerdissen *et al.* (1996) found AUDPC QTLs on chromosome 1 and on the 2S, 3L, 5S, 6L, 7L, 8L, and 9S, but Welz and Geiger (2000) discovered AUDPC QTLs on chromosome 1 to 9 in three different mapping populations.

Balint-Kurti *et al.* (2010) mapped QTLs for TLB resistance in two different environments and discovered that disease pressure in Clayton (NC) was lower than in Aurora (NY). As a result of local methods for producing epiphytotic conditions for TLB, the current study's trend supports the use of various inoculation approaches. A majority of previous investigations have revealed TLB resistant QTLs that are specific to the environment (Asea *et al*. 2009, 2012).

In the individual environments and over the environments for both mapping populations, the LOD values for these QTLs ranged from 2.64 to 14.84, and the corresponding R^2 values ranged from 10.80 to 18.98. The gene action for most of the QTLs showed over dominance at their respective chromosome (Table 3).

One of the primary focuses of QTL mapping is to find markers that may be exploited for MAS in a breeding programme in a large scale. The lack of consistency of QTLs across environments has been a key argument against using MAS. It would also be beneficial to use MAS to start a pyramiding strategy for many genes that may control diverse resistance mechanisms.

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(Manuscript Received on 6 August 2020; revised on 17 May 2022)