Original Research Article

Screening of NCLB Disease and QTLs Mappingin*Zea Mays*

ABSTRACT

Northern Corn Leaf Blight (NCLB) is a foliar disease, caused bya fungal pathogen (*Setosphaeria turcica*) that damages maize crop due toheavy losses in every year. A study was conducted for NCLB and identified quantitative trait loci (QTLs) in maize. Mapping population $F_{2:3}$ families was developed involving two inbreds*viz* CM 212 (susceptible)& CM 145 (resistant).The $F_{2:3}$ families mapping population was evaluated in two environments (BHU, Varanasi and Nagenahalli, Mandya) for resistance to NCLB. In the polymorphic survey about 360 pairs of simple sequence repeat(SSR) primer used between two parents (CM 212 & CM 145)and identified 54 polymorphic markers. Data was recorded for disease severity traits *viz* Percent Disease Index (PDI), Area Under Disease Progress Curve(AUDPC) based on PDI, Lesion Area, AUDPC based on lesion area in QTL Mapping. The QTLs were identified interestingly, on 4th chromosome *viz*: 4.03/4.05, 4.08/4.1, 4/4.07 and 4.08/4.1 from observed disease severity in both environments.The initially two QTLs (QTL-1 & QTL-2) detected with trait Percent Disease Index and other two (QTL 3 & QTL 4)detected with AUDPC based on PDI.

Keywords: *Zea mays*; *Setosphaeria turcica*; Lesion area; Northern corn leaf blight; QTLs

Introduction

Northern Corn Leaf Blight(NCLB)commonly known as *Turcicum Leaf Blight* is caused by ascomycete fungus *Setosphaeria turcica* (Luttrell). Leonard and Suggs expressed its conditional state *ExserohilumTurcicum,* (Passerini) Leonard and Suggs [1]. The NCLB is depending on the level of genetic resistance of the genotype, climatic conditions during the growth cycle and production system and causes significant damages28 to 91% from total yield [2]. In India, NCLB was first reportedby [3].The disease symptoms primarily appear on the leaves. Plants are infected at any growth stage, but usually identified after anthesis. Susceptible plantlesions are 4-20 cm long and 1-5 cm wide which is elliptical in shape and grayish-green to tan in color. NCLB severity is increases exponentially at highly humid and low temperature conditions [4]. In some places heavy dew is one factor for increasing NCLB disease severity. The genetics of NCLB resistance is controlled by qualitatively race-specific single gene *Ht1*, *Ht2*, *Ht3*,*HtM*, *HtN*, and

*HtP*and quantitatively race nonspecific multiple gene acting together or separately [5]. Ogliari[6] reported certain recessive genes expression in NCLB infection.

Quantitative or polygenic resistance genes are effective in tropical areasincluding in India [4].The NCLB resistance is slow in breeding program [7] due to absence of reliable screening techniques to identify derived resistance in the breeding lines. Therefore screening must be repeated over several years to ensure accuracy. This problem is solvedby molecular markers, which have emerged as potential tools for mapping of genes [8]. DNA markers are now widely used to locate and follow numerousinteracting genes that determine a complex trait. Genetically linkage maps areprovidingdirect method for selecting desirable genes via detecting molecular markers. DNA markers are base on DNA polymorphism and identified quantitative trait loci (QTL), involved in conditioning partial resistance. QTLs identification is help in MAS selection for improving genetic traits in maize crops. Genomic races are associated with quantitative resistance to NCLBand identified different maize varieties in both environments [9,10]. Many QTLs precisely mapped and display some conflicts resistance to pathogens [11].Genomic regions associated with quantitative resistance to NCLB, have been identified in several studies using different populations with a view of eventually improving host resistance [12]. Poland [13] was dissected NCLB resistance in maize using the nested association mapping (NAM) population, which offers the advantage of a higher mapping resolution and a broader allelic sampling than the above mentioned linkage mapping studies. In view of the above facts, the present study was designed to evaluate NCLB disease infection and underlying QTLs in two different states of Uttar Pradesh and Karnatakaas a broad maize population.

Materials and Methods

Plant Material

The $F_{2:3}$ families of 159 maize crop of CM 212 × CM 145 were raised and evaluated in field trial for resistant to NCLB in two diseased environment*viz*, Varanasi (E_1) and hot spot of NCLB at Nagenahalli, Mandya(E_2).Maize inbred CM 212 derived from population A-Theo 21 after 7-8 generation of inbreeding in early duration maize inbred and highly susceptible to NCLB of maize but has very good combining ability and involved in the number of commercial single cross hybrids of early maturity group was used as female parent (Fig. 1). The male parent (CM 145)

was derived from maize population Peru 330 after 6-7 generation of selfing is a highly resistant lines for NCLB in early maturity group. The selected female parent CM 212 flowers were hand emasculated for pollen transfer in the male parent CM 145 during cool hours of the day to get sufficient F_1 seeds. F_1s plants were produce F_2 seed self by covering pollen bag that prevent out crossing through other breeding material. F_2s plants were raised $F_{2:3}$ mapping population of 159 plantsby self crossand seeds of each $F_{2:3}$ families' were increased by single seed decent method in NCLB evaluation.

Experimental field design

All $F_{2:3}$maize crops were screened for NCLB in two different environments: at Agricultural Research Farm, Banaras Hindu University, Varanasi and Nagenahalli, Mandya. Varanasi is located at 83.3^0' E longitude and 25.2^0' N latitude of 128.93 meters above the sea level in north gangetic plain and Nagenahalli,Mandya (University of Agricultural Banglore, Karnataka) is situated atnorth latitude$12^0$13' N; $76^0$19 E; 695 m.s.l. elevation; 705 mm/ rainfall. The field experiments were carried out during June-July *Kharif*(Rainy) season at BHU, Varanasi and Mandya, Karnataka. The $F_{2:3}$were evaluated together with parental and check lines in a randomized block design with two replications. Each replication consisted two rows of each entry and plot was 3 meter in length. Row to row distance was kept at 60 cm and plant to plant distance 25 cm. All the recommended package of practices was followed to obtain the normal growth of the experimental crop of both environments.

Disease Development

The spreader- row technique was used for field inoculation of a susceptible variety (Dhari local) for Varanasi and NAI 219-J for Nagenahalli that had been planted every 20^{th}rows to promote disease build up and spread. Inoculums was produced and maintained separately on susceptible variety. Plants were inoculated at the 6–7 leaf stages. The inoculums were prepared by growing the fungal mycelium on sorghum grains. After proper fungal growth (seven-ten days), the grains were dried in the shade and at room temperature. A fine powder of these grains was prepared with the help of a mixer–grinder and pinches of this powder were put in the leaf whorl. Inoculation was spray in evening to avoid the maximum day temperature during incubation period.

Disease Assessments

Four components of disease parameter as PDI, AUDPC based on PDI, LA and AUDPC based on lesion area were recorded for two different environments. To assess the PDI, PDI displayed by all plants of each rows was recorded at three different growth stages (GS) *viz*., flowering stage 50 days after sowing (50 DAS), Dough (60 DAS) stage and brown husk stage (70 DAS) whereas, Karnataka data was recorded at five different growth stage *viz,* Pre-flowering (30 DAS and 40 DAS) Flowering stage (50 DAS), Dough (60 DAS) stage and Brown husk stage (70 DAS) using the 1-5 scale [14]. AUDPC based on PDI at three growth stages for Varanasi and five growth stages for Mandya(30 DAS, 40 DAS, 50 DAS, 60 DAS and 70 DAS) over time was estimated using the formula.Similarly, LesionArea was recorded at three different growth stages (GS) stages*viz;* Flowering stage (50 DAS), Dough (60 DAS) stage and brown husk stage (70 DAS) and calculated according to the formula given by Leath [15]:A= (L × W) (0.7854).Lesion area was taken from infected leaves of each entry and mean was calculated by mean of all infected leaf and alsoAUDPC estimated by using following formula[16]:

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

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

Heritability and Traits correlation

Estimates of broad sense heritability (h^2) was calculated by ANOVA over environments using PROC GLM procedure of SAS software according to the formula suggested by Burton and de Vane [17] 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 over the environments by PROC GLM procedure of SAS software.

PCR Product analysis

Genomic DNA was isolated from 21 days of old seedlings of the F_2 plants by the modified method based on Saghai- Maroof[18]. To identify simple sequence repeat (SSR) markers linked to QTL, 360 SSR markers distributed throughout maize genome were screen during the parental polymorphism survey. Out of 103 SSR markers that showed polymorphism form parental screening, only 54 SSR markers were used for genotyping of $F_{2:3}$selfed plant. The polymerase chain reaction consisted of 1.5 µl 10x PCR Buffer, 0.15 µl dNTPs ,1.2 µl $MgCl_2$, 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 program initial denaturation at 95°C for 5 min, denaturation at 94°C for 1 min, annealing at 57-63°C for 1 min, 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 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 fragment. Gel photograph was taken using Alpha imager gel documentation system by placing the gel under UV lamp.

Map construction and QTL detection

Linkage map was constructed using 54 SSR primers identified as polymorphic during the parental polymorphism survey. For each segregating marker, a Chi-square analysis $\{\chi^2=\Sigma$ (observed-expected) was performed for deviation to expected segregation ratio (1:2:1). Linkage analysisof SSR markers was conducted using the Kosambi[19] mapping function with a minimum log10 odds ratio (LOD) of 2.0 and maximum recombination frequency of 0.4 performed by Map-Maker/EXP 3.0.QTL analysisfor each individual environment and a combined one across all two environments were performed by composite interval mapping using Windows QTL Cartographer 2.5[20]. A QTL was considered significant to LOD (log10 of the likelihood of odds ratio) value that derived from permutation analysis was large than 2. Additive and dominance effects for detected QTLs were estimated using the Zmap procedure of QTL Cartographer. The R^2value of the phenotypic variance explained by marker genotype at the QTL, (coefficient of determination) was taken from the peak QTL position as estimated by QTL Cartographer. Gene action was determined by the ratio of the absolute value of the estimated dominance effect divided by the absolute value of the additive effect; (additive = 0 to 0.20; partial dominance = 0.21 to 0.80; dominance = 0.81 to 1.20; and over dominance > 1.20).

Results

Percent Disease Index and AUDPC

Mean percent disease index was recorded at 70 days after sowing (70 DAS) for NCLB of resistant (CM 145) and susceptible parents ranged from 29.84 (E_1) to 42.69 (E_2) and 55.37 (E_1) to 8497 (E_2), respectively. The mean PDI of $F_{2:3}$ lines ranged from 37.22 (E_1) to 72.81 (E_2) (Table-1). The disease progress curve and frequency distribution based on PDI and AUDPC values in two environments have been presented in Fig 2 (a, b, c, f, g & h). The examination of these figures revealed that in all cases disease was at lowest at flowering stages (30 DAS) and height and Brown Husk (70 DAS). The Figure 2 (a, b, c, f, g & h) also revealed that in general the disease progress was low in Environment 1 (Varanasi) than in Environment 2 (Nagenahalli). The mean PDI value of F2:3 line was near for over to values of susceptible parent (CM-212) (Fig. 2a, 2b, 2d, 2c) where as Mean AUDPC values base on PDI also indicated that almost 100 lines (%) were near to susceptible parent (CM 212) about 38 lines were at the middle of resistant (CM 145) and susceptible parents and only for lines 13 (%) were near the resistant parents. Thus examination from of Table 1 and Figure 2a, b, c, f, g & h were indicated the disease severity was more in environment 2 (Nagenahalli) base on PDI and AUDPC values.

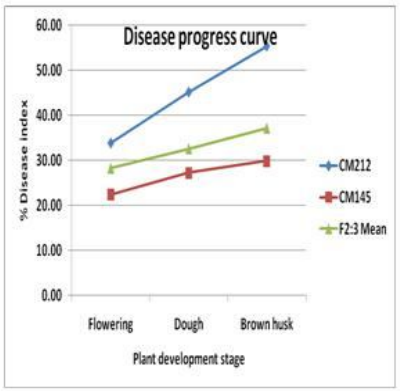
Lesion Area and AUDPC

Mean lesion area was also recorded at 70 days after sowing of 159 F2:3 lines ranged from 11.30 (Varanasi) to 19.48 (Nagenahalli). However, mean lesion area of the resistant (CM 145) and susceptible (CM 212) and 70 DAS ranged from 4.45 (Varanasi) to 6.83 (Nagenahalli) and 13.28 (Varanasi) to 29.68 (Nagenahalli) respectively. Disease progress curve based of lesion areaand Lesion Area have been presented in Fig. 2c, d, I and j. The AUDPC based on lesion area of the $F_{2:3}$ families ranged from 53.03-639.21 area in Varanasi and 88.48-736.46 (Nagenahalli). There values indicated large phenotypic variation in Varanasi as well as Nagenahalli with hgh severity of disease in Nagenahalli. Further examination of disease progress curve based on Lesion Area indicated that mean values of 159 F2:3 families at three stages where lying somewhere between susceptible and resistance lines in both the environments. Further the frequency distribution of 159 lines also indicated majority of lines (150) were near to resistant lines (CM 145) in Environment-1 (Varanasi) where as majority of $F_{2:3}$ 159 lines (150) were between resistant (CM 145) and susceptible (CM 212).

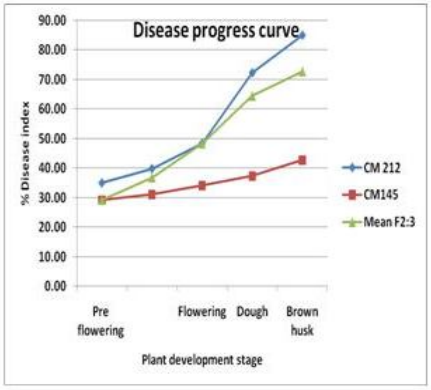
Heritability and Traits Correlation

The examination of Analysis of Variance revealed highly significant differences among treatment and highly significant differences among environment × treatment of AUDPC based on lesion area of NCLB (Table 2).The heritability estimates in broad sense (Table 3) ranged from 0.48 to 0.99 for all four disease parameters in NCLB. The highest value of heritability estimates was recorded for lesion area (0.99) and AUDPC based on lesion area (0.99). However, moderate value of heritability was observed for PDI (0.56). Lowest value of heritability was observed for AUDPC based on PDI (0.48).

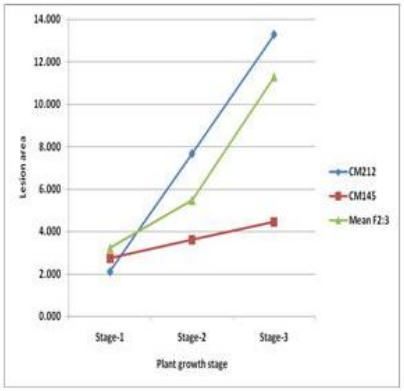
The phenotypic correlation between all four disease parameters for the cross CM 212 × CM 145 has been presented in Table 4.. A significant correlation (0.94, 0.70 and 0.74) was between PDI and AUDPC (PDI) for environment 1, environment 2 and over the environments. Similarly, lesion area was significantly correlated (0.72, 0.69 and 0.78) with AUDPC (LA) in environment 1, environment 2 and over the environments whereas, AUDPC (PDI) was significantly correlated (0.28 and 0.26) with lesion area in environment 2 and over environments. However, no correlation was found between PDI and lesion area in single or over environments. PDI was not significantly correlated with AUDPC (LA) in single or over environments. Similarly, no correlation was observed between AUDPC (PDI) and AUDPC (Lesion area) in single or both environments (Table 4).



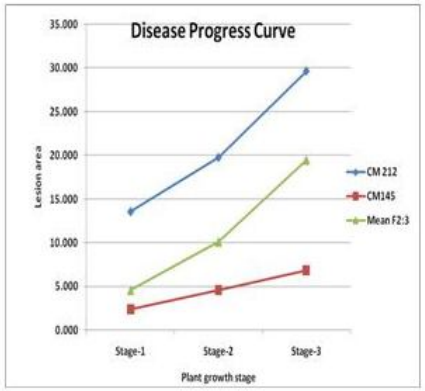
a. PDI (Varanasi)



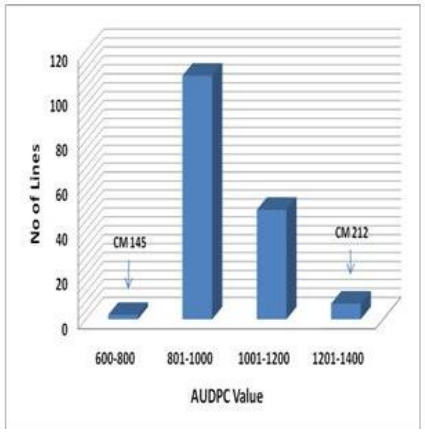
b. PDI (Nagenahalli)



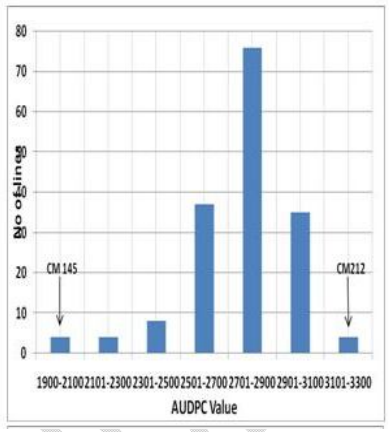
c. Lesion Area (Varanasi)



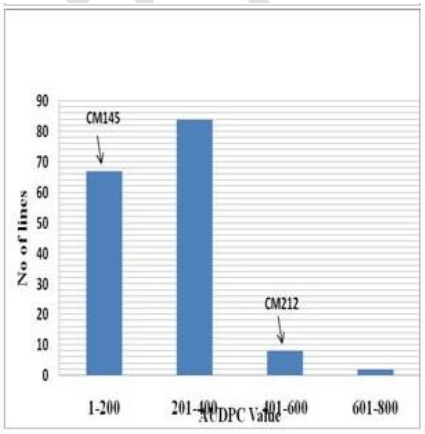
d. Lesion Area (Nagenahalli)



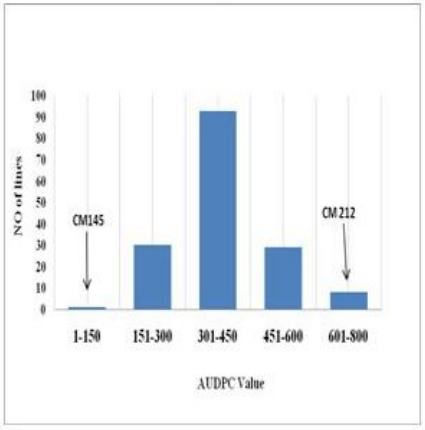
e. AUDPC-PDI (Varanasi)



f. AUDPC-PDI (Nagenahalli)



g AUDPC-Lesion Area (Varanasi)



h. AUDPC-Lesion Area (Nagenahalli)

Fig 1. Disease Progress Curve (a, b, c, d) of two traits *viz* Percent Disease Index (PDI) and Lesion Area (LA) and Frequency Distribution (e, f, g, h) of Area Under Disease Progress Curve (AUDPC) base on PDI as well as lesion area of the mapping population (159 F2:3 families) of cross CM 212 × CM 145 have been present above in two environment Varanasi (E_1) and Nagenahalli (E_2)

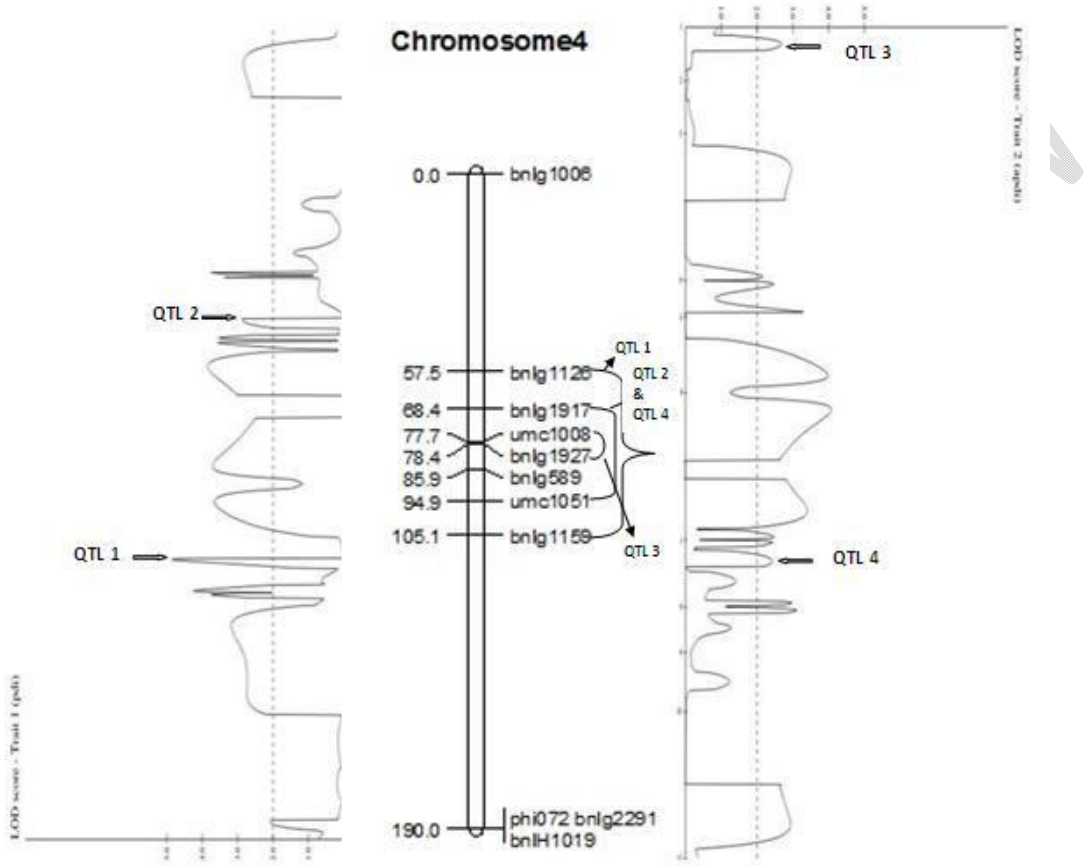


Fig. 2: Linkage map of chromosome 4 including SSR markers associated with disease characters in the $F_{2:3}$families of cross 'CM 212' × 'CM 145'. The horizontal line indicates the threshold LOD value (2.0) for determining significant QTLs. Designed LOD curve showing of QTLs for disease characters on chromosome 4 based on PDI & AUDPC character; flanking markers (bnlg1126-bnlg1159) and (umc 1051-bnlg1917) at a distance of 80.0 and 50 cM with 52.7% and 53.0% phenotypic variance for PDI and flanking markers (bnlg1927-umc1038) and (umc 1051-bnlg1917) at a distance of 40.0 and 50 cM with 56.2%and 50.2% phenotypic variance for AUDPC.

Table 1: Range and mean value of NCLB PDI, AUDPC (PDI), Lesion area and coefficients of variation for 159 $F_{2:3}$ families from the cross of CM 212 × CM 145 from individual environments and across environments.

	Percent disease index (PDI)			AUDPC (PDI)			Lesion Area			AUDPC (Lesion Area)		
Genotype	**E_1***	**E_2****	**Pooled**	**E_1***	**E_2****	**Pooled**	**E_1***	**E_2****	**Pooled**	**E_1***	**E_2****	**Pooled**
'CM 212'	55.37	84.97	70.17	1346.91	3029.88	2188.4	13.28	29.68	21.48	276.33	745.91	511.12
'CM 145'	29.84	42.69	36.26	798.66	1920.17	1359.41	4.45	6.83	5.64	129.49	165.07	147.28
$F_{2:3}$ range	31.5-58.1	55.79-85.9	45.67-65.27	862.5-1313.18	2151.32-3197.87	1542.94-2179.78	1.51-24.41	3.64-40.76	4.22-27.68	53.03-639.21	88.48-736.46	101.00-562.45
$F_{2:3}$ mean	37.22	72.81	55.02	981.45	2761.5	1871.48	11.3	19.48	15.4	229.45	398.28	313.87
CV#	6.29	3.87	7.45	5.93	4.12	6.83	3.14	1.77	2.27	3.29	3.06	3.23

CV was estimated from 159 entries including parents in RBD; *Agricultural Research Farm, BHU, Varanasi *Kharif* 2012;
****** Zonal Agricultural Research Farm, Mandya, Karnataka *Kharif* 2012

Table 2: Pooled analysis of variance of four disease traits (PDI, AUDPC, Lesion Area and AUDPC (Lesion area) involving 159 $F_{2:3}$ families with parents over the environment.

Source of variation	**df**	**Mean Sum of Square**			
		PDI	**AUDPC (PDI)**	**LA**	**AUDPC (LA)**
Treatment	160	43.8775**	52513.1**	85.96594**	28090.107**
Environment	1	216553.9556**	668950065.2**	10802.03688**	4645744.053**
Replication	1	1525.3706	2352089.1	10.39012	17800.160
Treatment*Environment	160	36.5099**	49950.2**	53.60911**	17231.768**
Error	321	5265.7929	5903563.6	39.06648	33123.90
CV%		7.451317	6.828542	2.269582	3.234539

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

SSR linkage map and QTL analysis

We analyzed 360 microsatellite markers covering the whole genome for polymorphic between NCLB susceptible (CM 212) and NCLB resistant (CM 145). We identified 54 (15%) marker which were polymorphic. We are reporting genotyping and mapping of 159 $F_{2:3}$ families with only 54 polymorphic primers. The construction of genetic map of this population covered about 208.49 cM with 18 SSR markers distributed over maize genome was classified in linkage groups. The average distance between adjacent marker loci was about 11.58 cM.Thirty six markers remain ungrouped genetically unlinked. All markers were located to the linkage groups using the map maker group. Four QTLs intervals for resistance to NCLB were identified on chromosome 4 (Fig.2) in the single environment of Nagenhalli (E_2). The LOD values ranged from 2.43 to 4.85 and corresponding R^2 ranged from 50.2 to 56.2 in the individual environments (Table 4). The data were subjected to combined analysis across the environments for the four disease severity traits *viz*; PDI, AUDPC, LA and AUDPC and individual analysis of each environment for four traits were also performed).QTL1, QTL2, QTL3, QTL4 identified for trait Percent Disease Index and AUDPC based on PDI in environment 2(Nagenhalli) only. The four of composite analysis across the environment and rest of six analyses in individual environment revealed no QTLs as LOD values recorded very less than threshold 2.0. Interestingly QTL2 identified by trait PDI at marker interval (umc 1051-bnlg 1917) with map distance 50.00 was identified to QTL 4 with same bin location (4.08/4.1) marker interval (umc 1051-bnlg 1917) and map position 50.00 (Table 5) but by trait AUDPC values based on PDI. The both QTLs (2 &4) expressed 2.85 & 2.43 LOD values and 53.0 & 50.2 R^2 indicating about diversity with respect to trait location. In this study we are reporting three QTLs (QTL 2 and QTL4) in all present on chromosome 4 with significantly large phenotypic variation (50.2 to 56.2 R^2 values). The gene action of all QTLs showed over dominance at their respective chromosome bin locations (Table -5).

Table 3: Heritability of four disease traits (PDI, AUDPC, Lesion Area and AUDPC (Lesion area) involving 159 $F_{2:3}$ families with parents over the environments

Characters	Heritability
PDI	0.56
AUDPC (PDI)	0.48
LA	0.99
AUDPC (LA)	0.99

Table 4: Correlationof four disease traits (PDI, AUDPC, Lesion Area and AUDPC (Lesion area) involving 159 $F_{2:3}$ families with parents on the basis of individual as well as over environments.

Characters	Environments.	PDI	AUDPC(PDI)	LA
AUDPC(PDI)	Env-1	0.94**		
	Env-2	0.70**		
	Pooled (Env-1 & Env-2)	0.74**		
LA	Env-1	0.07	0.06	
	Env-2	0.08	0.28**	
	Pooled (Env-1 & Env-2)	0.08	0.26**	
AUPDC(LA)	Env-1	0.12	0.13	0.72**
	Env-2	0.00	0.15	0.69**
	Pooled (Env-1 & Env-2)	0.11	0.26	0.78**

**significant at 0.01 probability level.

Table 5: QTL identified for Percent disease index (PDI) and Area under disease progress curve in $F_{2:3}$ populations of cross CM 212 × CM 145.

QTLs	Trait	Bin	Marker-Interval	Map Position (cM)	LOD	R^2	Gene effects			Gene action
							D	A	d/a	
QTL 1	PDI	4.03/4.05	bnlg1126 -bnlg1159	80.0	4.85	52.7	10.23	4.65	2.2	OD
QTL 2	PDI	4.08/4.1	umc1051-bnlg1917	50.0	2.85	53.0	-5.06	2.98	- 1.69	OD
QTL 3	AUDPC	4/4.07	bnlg1927-umc1008	40.0	2.68	56.2	-77.24	-44.3	1.74	OD
QTL 4	AUDPC	4.08/4.1	umc1051-bnlg 1917	50.0	2.43	50.2	-90.19	-60.7	1.48	OD

Discussions

The development of reliable QTLs mapping for expression of NCLB disease was crucial to the success this study. The heavy disease pressure is required to assess accurately the potential of plant genotypes to resist the onset and progress of NCLB and determine magnitude effect of the genetic

factors that contribute resistance. The two parents (CM 212 and CM 145) used to develop the $F_{2:3}$ populations exhibited the most extreme phenotypes to NCLB reaction. The parent CM 145 had been identified earlier as being highly resistant and the inbred CM 212 was highly susceptible to *E. turcicum*pathogen [21]. In the present investigation, percent disease index and Lesion area was recorded ranged from 29.84-58.10 and 1.51-24.41 among the $F_{2:3}$ families indicated moderately resistance, whereas AUDPC (PDI) and AUDPC (LA) exhibited as partial resistance among $F_{2:3}$ families which were quite less to resistant and susceptible parents. The disease progress curve and frequency distribution curve which based on mean PDI and Lesion area and AUDPC values indicated the disease skewed towards resistance to partial resistance among $F_{2:3}$ families but mostly F_3 lines fell with the range of parents.

High Pearson correlation coefficients were observed between PDI and AUDPC (PDI) and LA and AUDPC (LA) value within individual environments and over environments with a range from 0.69 to 0.94 ($P<0.0001$). Moderate correlations were observed between second and over environments ranging from 0.26 to 0.28 between AUDPC (PDI) and lesion area ($P<0.0001$). This result is similar to Kumar [22] who observed high significant correlation 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 $P<0.0001$). The correlation coefficient between days to heading and AUDPC was non-significant when calculated across 3 years (0.083).

The maize resistance to NCLB is a complex quantitatively inherited trait [22] and comparison of QTL position with previous study that based on bin positions, and identified 33 regions.The maize genome was associated with partial resistance describing from 5.9 to 18% of the total phenotypic variability and identified two QTL associated with anthocyanin production on chromosomes 10:6 and 5:03. Recently [23] used IBM population, an advanced intercross recombinant inbred line population derived from a cross between the lines Mo17 and B 73, evaluated in three environments for two traits related to NLB resistance, weighted mean disease (WMD) and incubation period (IP), and for days to anthesis (DTA). In our study two QTLs for percent disease index in bin 4.03/4.05 and 4.08/4.1 were detected in single environment analyzed separately.Welz HG and HH Geiger [24] 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 Chromosome-3 (bin 3.06/07), chromosome-5 (bin 5.04) and chromosome 8 (bin8.05/06). In our study QTL for AUDPC has been identified in 4/4.07 with 56.2% phenotypic variance in individual

environment. Gene action was mostly partially dominant or recessive. This result is accordance to Souza [25] who observed over dominance gene action for mostly region for SCMV disease in maize.

Conclusions

In the present investigation four QTLs identified on 4^{th} chromosome *viz*: 4.03/4.05, 4.08/4.1, 4/4.07 and 4.08/4.1 from observed disease severity in both environment. The identified QTLs found significant interaction in both environments (BHU Varanasi and Mandya, Karnataka) from pooled together. It will be helpful to opening pyramiding of multiple genes control for maize resistance to NCLB.This study indicated role of genotype x environment interaction in low disease appearance to first environment (E_1) and high disease appearance to high environment.

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