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Mapping QTLs for resistance to Northern Leaf Blight in Tropical Maize (*Zea mays* L.)

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A B S T R A C T

Northern Corn Leaf Blight (NCLB or NLB) caused by *Exserohilum turcicum* is a common disease of maize in many parts of the world including India. Resistance to NCLB is complexly inherited and controlled by several quantitative trait loci (QTL) distributed across the genome. Phenotype as well as linked DNA marker based selection for resistance to NCLB is expected to be effective. Hence an investigation was carried out involving a total of 569 $F_{2:3}$ families derived from two crosses viz., PH234 X PHBP3 and PH234 X PH84K, where PH234 is resistant parent and PHBP3 and PH84K are susceptible parents. During rainy 2016 and 2017 seasons, these F_3 progenies were screened for their reaction to NLB. Characterization of QTLs affecting resistance to NLB was undertaken using the genetic linkage map with polymorphic SNP marker loci and the phenotypic data of $F_{2:3}$ families. Total five QTLs conferring resistance to NLB were identified which were spread on chromosomes 2, 3, 4, 7 and 10. While the QTL on chromosome 7 was more consistent, others were less consistent. Phenotypic variation explained by these QTLs varied from 3% to 16%. The detection of more than one QTL supports the hypothesis that quantitative genes control resistance to *Exserohilumturcicum*.

Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops of the world and contributes to food security in most of the developing countries. In India, maize is emerging as the third most important crop after rice and wheat. Its importance lies in the fact that it is not only used for human food and animal feed, but at the same time, it is also widely used for corn starch industry, corn oil production, baby corns etc.

One of the major factors limiting the productivity of maize in the tropical Asian region is the increased incidence of insect pests and diseases. Among the various maize diseases, Northern Corn Leaf Blight (NCLB),

caused by the pathogen *Exserohilum turcicum* is a serious threat to maize cultivation worldwide, reportedly causing yield losses of more than 50% (Raymundo and Hooker 1981 and Perkins and Pederson 1987). NCLB is common in areas that have high humidity combined with moderate temperatures in the northeastern United States, in sub-Saharan Africa and in areas of China, Latin America, and India (Adipala *et al.,* 1995 and Dingerdissen *et al.,* 1996). In India, the disease is prevalent in Karnataka, Andhra Pradesh, Tamil Nadu, Bihar, Himachal Pradesh, Maharashtra and other regions (Harlapur *et al.,* 2000).

Resistance to NCLB is complexly inherited and controlled by several quantitative trait loci (QTL) distributed across the genome. Phenotype as well as linked DNA marker based selection for resistance to NCLB is expected to be effective. Studies on resistance to NCLB point to a complex genetic architecture with many quantitative trait loci (QTL) distributed throughout the genome (Inghelandt *et al.,* 2012 and Poland *et al.,* 2011). Quantitative trait loci (QTL) for NCLB resistance have been identified from several populations (Brewster *et al.,* 1992; Dingerdissen *et al.,* 1996; Schechert *et al.,* 1999; Welz and Geiger, 2000; Welz *et al.,* 1999; Wisser *et al.,* 2008 and Balint-Kurti *et al.,* 2010) and are distributed throughout the genome (Wisser *et al.,* 2006). In the present study, an attempt was made to validate these QTLs and identify novel QTLs using $F_{2:3}$mappingpopulations derived from two half- sib populations.

Materials and Methods

Parent material and phenotyping of $F_{2:3}$ mapping population for NLB

The mapping populations for the study were derived two crosses after initial evaluation of parental lines. The two crosses were PH234 X PHBP3 and PH234 X PH84Khaving 271 and 298 F_3progenies respectively. Among the parental lines, PH234 is tolerant and PHBP3 and PH84K are susceptible ones.F_3 mapping populations were evaluated during rainy 2016 and 2017 in NLB hotspot near Hassan, Karnataka (lat.13^0 09' N and long. 76 ^{0} 02' E). The experiments consisting of F_3progenies along with parental lines were planted in Randomized complete design with two replications. Row length of 3 meter with row to row distance 0.6 meter and plant to plant distance 20 cm was uniformly adopted. Appropriate susceptible checks for northern corn leaf blight were sown after every 10th row to increase the disease pressure as well as to serve as spreader rows. To ensure uniform disease infestation, artificial inoculation was done by following the procedure detailed by Shekhar and Kumar (2012). Artificial inoculation was made 20 days after sowing between 3.00 to 6.00 PM and inoculation was repeated one week after first inoculation. The northern corn leaf blight severity was recorded at dough stage i.e., 85 days after sowing by visualizing the leaf area using 1-9 scale (Ribeiro *et al.,* 2016, www.pioneer.com), according to lesion spot development in the middle to upper part of leaves on a scale from 1 (Susceptible) to 9 (Resistant) thereby providing for a total of nine classes or categories where score 9: 0-5% leaf loss, score 8: 6-10% leaf loss, score 7: 11-24% leaf loss, score 6: 25-44% leaf loss, score 5: 45-55% leaf loss, score 4: 56-66% leaf loss, score 3: 67-77% leaf loss, score 2: 78-88% leaf loss and score 1: 89-100 leaf loss.

SNP genotyping protocol

Genotyping was performed on the individual samples by Pioneer Hi-Bred International (Johnston, IA), using a 90-markermultiplex assay on the Illumina (San Diego) Bead Array platform (Jones *et al.,* 2009).

QTL analysis and mapping

The analysis of QTLs controlling the northern corn leaf blight resistance was performed using the means of $F_{2:3}$ family replicates for score data within each season and within families as well as across seasons and across families. The phenotypic data (Rainy 2016 and Rainy 2017) and genotypic data of 90 SNP markers across 10 chromosomes (which were imputed to 3315 markers) were subjected for constructing linkage map in order to identify the QTLs associated with the trait using proprietary software of Dupont Pioneer. LOD scores equal and more than 3.0 were used as threshold for significance testing of the existence of a QTL effect.

Results and Discussion

A total of 569 $F_{2:3}$progeny families derived from two crosses were evaluated for their reactions to NLB during the rainy seasons of 2016 and 2017 at Hassan, Karnataka. Frequency distribution of$F_{2:3}$progenies showed continuous variation in both populations and environments with clear transgressive segregation indicating quantitative resistance to NLB (Fig. 1).The ANOVA revealed significant differences among $F_{2:3}$families in both the populations and seasons for disease incidence. However, since Levene's Test for Homogeneity reveled significant difference in NLB incidence over two seasons, across year pooled analysis was not done. This difference in NLB severity across seasons emphasizes the effect of environmental conditions on the occurrence of this disease.

Genetic linkage map

A total of 873 out of 3315 imputed SNP markers which showed expected 1:2:1 ratio as tested by chi-square test were used to construct a linkage map form 271 $F_{2:3}$families from population 1 (Table 1) and 1102 out of 3315 markers were used for 298 $F_{2:3}$families from population 2 (Table 2). The per cent polymorphism between the two parents used for developing mapping population as revealed by SNP markers were 23.3 and 33.24 in populations 1 and 2 respectively.

Table.1 Summary of complete marker data per chromosome and polymorphic markers for the population PH234 X PHBP3 (Pop 1)

	Complete markers data per Chromosome		Polymorphic SNP markers		
Chromosome	**SNPs**	**Length**	**SNPs**	**Length**	**Parental polymorphism (%)=23.33**
Ch1	569	322	155	318.51	
Ch2	428	257	110	253.45	
Ch3	299	251	70	246.42	
Ch4	276	248	67	246.31	
Ch5	346	228	101	223.55	
Ch6	287	184	82	180.79	
Ch7	268	214	69	205.52	
Ch8	331	213	94	206.56	
Ch9	257	185	62	174.92	
Ch10	254	164	63	154.88	
Total	**3315**	**2266**	**873**	**2210.91**	

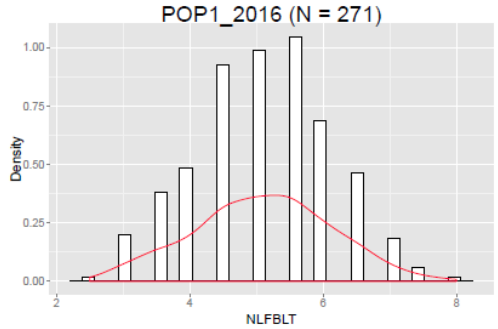
Table.2 Summary of complete marker data per chromosome and polymorphic markers for the population PH234 X PH84K (Pop 2)

	Complete markers data per Chromosome		Polymorphic SNP markers		
Chromosome	**SNPs**	**Length(cM)**	**SNPs**	**Length(cM)**	**Parental polymorphism (%)=33.24**
Ch1	569	322	185	317.23	
Ch2	428	257	138	252.75	
Ch3	299	251	98	246.42	
Ch4	276	248	82	246.31	
Ch5	346	228	113	226.8	
Ch6	287	184	112	180.79	
Ch7	268	214	95	205.29	
Ch8	331	213	107	210.31	
Ch9	257	185	82	181.19	
Ch10	254	164	90	159.23	
Total	**3315**	**2266**	**1102**	**2226.32**	

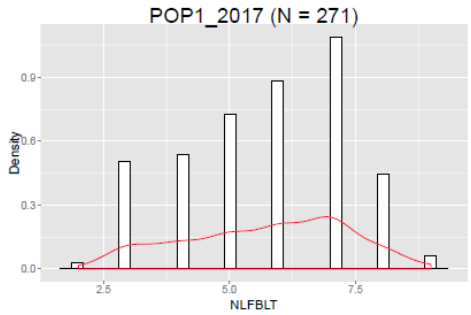
Table.3 QTLs detected for NLB resistance in individual populations for both the seasons

Population	**Kharif 2016**					**Kharif 2017**				
	Chr.	**Pos.**	**LOD**	**N**	**R^2 (%)**	**Chr.**	**Pos.**	**LOD**	**N**	**R^2 (%)**
PH234 X PHBP3 (Pop1)	7	85.2	9.3	271	12	7	79	2.1	271	5
						2	175	6.6	271	9
						10	118	5.4	271	6
PH234 X PH84K (Pop2)	3	104	13.2	298	16	3	102	11.2	298	12
	4	178	6.7	298	7	4	154	6.2	298	6
						7	94	9.7	298	10
Pooled Analysis (Pop1 & Pop2)	7	87	15	569	9	3	115.4	3.4	569	3
						7	94	5.4	569	16

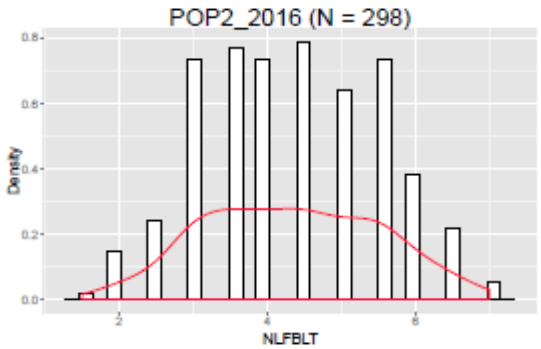
Fig.1 Frequency distribution of F_3 progenies of the cross PH234 X PHBP3 (Pop 1) and PH234 X PH84K (Pop 2)



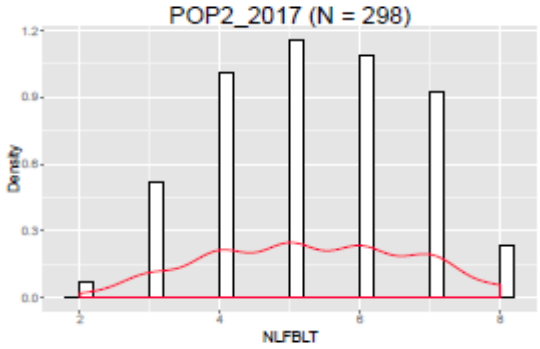
Mean= 5.09, Std. Dev= 1.03, N=271



Mean= 5.7, Std.Dev= 1.6, N=271

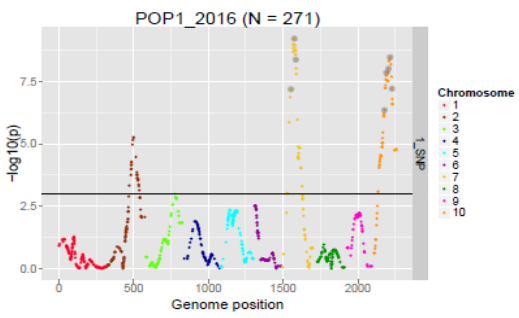


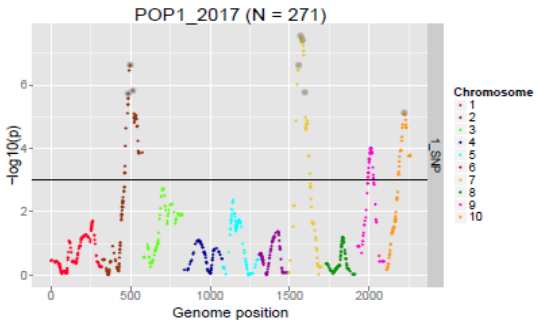
Mean= 4.33, Std. Dev= 1.17, N=298

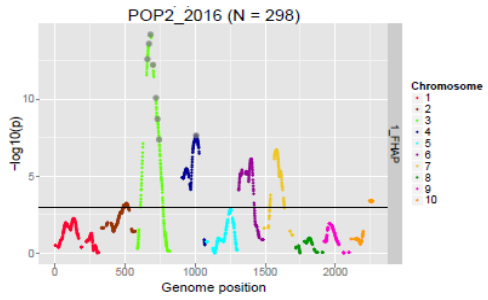


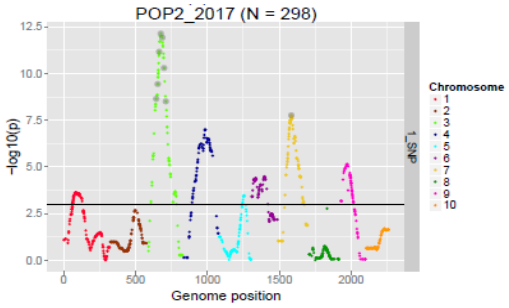
Mean= 5.28, Std. Dev= 1.43, N=298

Fig.2 LOD peaks (-log 10p) for NLB for detected QTLs in two populations during rainy 2016 and rainy 2017. Horizontal solid line indicates threshold LOD score









QTL analysis for NLB resistance in $F_{2:3}$ mapping population

QTLs were considered to be significant when the LOD scores exceed the threshold 3.0. Major and minor QTLs were classified with percentage of phenotypic variation (R^2) more than 10.0 as major QTL and QTL with R^2 less than 10.0 as minor QTL. Three QTL regions associated with NLB resistance were largely distributed over three chromosomes (chromosomes 3, 4 and 7) (Table 3 and Fig. 2). In population 1, one significant QTL was found on chromosome 7 when 2016 data was analyzed with LOD score of 9.3 and a phenotypic variation of 12% (Table 3). However, by using 2017 data, a non-significant QTL was found on the same chromosome in the same vicinity with 5% phenotypic variation while, two more significant QTLs were identified; one each on chromosome 2 and 10 with phenotypic variation of 9% and 6% respectively. In population 2, two significant QTLs were found using 2016 data, one each on chromosome 3 and 4 with LOD scores of 13.2 and 6.7 respectively with R^2 values of 16% and 7% respectively. Whereas, using 2017 data of population 2, three significant QTLs were identified, each one on chromosomes 3, 4 and 7 with LOD scores of 11.2, 6.2, 9.7 respectively explaining a phenotypic variation of 12%, 6% and 10% respectively. In the year-wise pooled analysis of two populations with 569 $F_{2:3}$families, one significant QTL with LOD of 15 and phenotypic variance of 9 % was found on chromosome 7 in 2016. Similar result was observed using 2017 data also with significant QTL on the same chromosome (chr.7) with LOD of 5.4 and phenotypic variation 16%. The lack of commonality between the QTLs identified in different populations could be attributed to combination of various factors *viz.*, the type and size of the mapping population used, segregation of different sets of QTLs in different crosses, detection of QTLs in a segregating population only if both parental lines contributed different alleles of the QTL and epistatic interaction between QTLs in different mapping populations (Beavis and Keim, 1996 and Bohn *et al.*, 1997). Beavis *et al.*, (1991) recorded that a comparison of data for QTL localization in various segregating populations for disease resistance reveals only a few QTLs that are common across populations. This is particularly relevant because of the fact that different climatic and growing conditions at each environment might affect the expression of QTL involved in developmental, morphological and biochemical characters affecting resistance against specific pathogen. However, reasonably consistent major QTL identified in this study on chromosome 7 could be good resource for breeding NLB resistance into tropical maize lines using MAS.

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