

Inheritance Study and Stable Sources of Maydis Leaf Blight (*Cochliobolus heterostrophus*) Resistance in Tropical Maize Germplasm

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Maydis leaf blight (MLB), a serious foliar fungal disease of maize, may cause up to 40% losses in yield. The present studies were undertaken to identify the stable sources of MLB resistance, its inheritance study, and testing of MLB resistance linked markers from diverse background in the Indian adapted tropical maize genotypes. A set of 112 inbred lines were screened under artificially created epiphytotic conditions at three hotspot locations. Analysis across multi-locations revealed significant effects of genotypes and environments, and non-significant effects due to genotypes \times environment interaction on disease incidence. A total of 25 inbred lines with stable resistance were identified across multi-locations. Inheritance of resistance was studied in six F_1 s and two F_2 s of resistant and susceptible parents. The null hypothesis of segregation of resistance and susceptible for mono and digenic ratios in two F_2 populations was rejected by Chi-square test. The non-significant differences among the reciprocal crosses depicted the complete control of nuclear genome for MLB resistance. Partial dominance in F_1 s and normal distribution pattern in F_2 s of resistant and susceptible parents suggested polygenic nature of MLB resistance. Correlation studies in F_2 populations exhibited significant negative correlation between disease score and days to flowering. Five simple sequence repeats (SSRs) markers, found associated to MLB resistance in different studies were unable to differentiate amongst MLB resistance and susceptible parents in our study. This emphasizes the need of fine mapping for MLB resistance in Indian germplasm. The identified stable sources of resistance and information on inheritance study can be used further in strengthening of resistance breeding against MLB.

Keywords: artificially created epiphytotic, inbred lines, partial dominance, reciprocal crosses

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Introduction

Maydis leaf blight (MLB), also named as southern corn leaf blight (SCLB) caused by the ascomycete fungus *Cochliobolus heterostrophus* (Drechs.) Drechs; synonym, *Helminthosporium maydis* Nisikado is a serious fungal disease of maize throughout the world where warm and humid conditions are exist during crop growing season (White 1999). In India, MLB appears in hills, plains and peninsular parts. The currently predominant form of *C. heterostrophus* is Race O, in severity, which can cause yield losses of up to 40% (Byrnes et al. 1989).

Different reports are available in the literature for genetics of MLB resistance. Most of those studies have been conducted using temperate germplasm. The recessive nature of MLB resistance genes was reported in various studies (Van Eijnatten 1961; Craig and Fajemisin 1969; Smith and Hooker 1973; Zaitlin et al. 1993). In few studies, resistance to Race 'O' was reported as quantitatively inherited primarily with partially dominant gene action (Pate and Harvey 1954; Holley and Goodman 1989). Consequently, quantitative trait loci (QTL) for field resistance to MLB have been mapped (Burnette and White 1985; Balint-Kurti et al. 2006a, 2007, 2008; Kump et al. 2011) on chromosome nos 3, 4, 6, 8 and 9 but bin location 3.04 contributes significantly for MLB resistance, has been found repeatedly across various studies. In India, where tropical maize is cultivated, reports on MLB inheritance and mapping are lacking. Several genomic regions for MLB resistance have been found in temperate germplasm (Balint-Kurti et al. 2007). There is need to check those genomic regions in Indian germplasm. Keeping in mind the economic importance of MLB disease in Indian as well as international perspectives, there is an immediate need to identify the stable sources of resistance and examine its inheritance pattern. Therefore, attempts were made in the present study to screen the Indian maize germplasm for MLB resistance and to know its inheritance pattern. Further, linked markers to MLB resistance, mapped mostly in temperate genetic background, were tested in tropical adapted genotypes to confirm similar QTL across the populations and regions.

Materials and Methods

Plant materials

The study consisted of two experiments. In the first experiment, a set of 112 inbred lines as test entries, and 2 inbred lines as control treatment [one each of resistant (HKI288-2) and susceptible (CM119)] (Table 1) available under all India coordinated research project on maize in India was used for identification of stable sources of resistance. These lines were developed from diverse sources at India and some at CIMMYT, Mexico. In the second one, two resistant (CML 269, P72c1Xbrasil1177-2) and two susceptible (HKI4C4B, ESM113) (Table 2) inbreds identified over the years were selected for studying the inheritance pattern of MLB. These four parents were already known to have differential and stable reaction and were again re-validated for their disease reaction during 2012, 2013 and 2014. Six F₁ crosses [CML 269 (R) × HKI 4C4B (S), P72c1Xbrasil1177-2 (R) × ESM 113 (S), HKI4C4B (S) × CML 269 (R), ESM 113 (S) × P72c1Xbrasil1177-2

Table 1. Disease reactions of inbred lines for MLB at three hotspot locations under artificially created epiphytotics

Disease score	Category	Inbred name
≤2.0	Resistant	HOP II, HKI42050, CM145, P72c1Xbrasil1177-2, G18seqcef74-2-1, HKI1352-5-8-9, V336, CML33, CML269, V390, BML7, CM501, T2STR1107, EC646012, HKI164-7-4-2, HKI193-2, HKIMBR139-2, Tempx Trop(H0)QPM-B-B-B-57, CLQRQCY41, PFSRR3, PFSRR9, JCY2-1-2-1, JCY2-2-4-1-1, JCY2-7-1-2, HKI288-2 (25)*
2.1 to 3.0	Moderately resistant	SCF, 42048-2-2-1, HKIPC7***, AE 40, WSKOTHAIWAXY1-1, Pop.31DMR-88-3#-B*13-B, S99TLWQ-HG-B-B-B-20, V335, HKIC78, HKI141, HKI 1040C2, HKI 1094-WG, CML44, BML13, CM121, CM144, LM15, LM16, CM130, LTP1, CML141, CML154, CML287, CM502, HKI 2-6-2-4, CML161, HKI164-4(1-3), HKI191-1-2-5, HKI193-1, CLQRQCY47B, CML451, PFSRS2, PFSRS3, PFSRR10, PFSR51016-1, JCY3-7-1-2, LM13 (37)*
3.1 to 4.0	Moderately susceptible	Mas madu (sh2 sh2)**, Win Sweet Corn, 951-7**, EW-DMR-G-C7-HS-(SIB)-9, La Posta Seq C7-F10-3-1**, Pop.31DMR-88-3#-B13-B***, P3C45SB-33-##-11, P390AM/CMLC4F230-B-2-1, V334, HKI1040-11-7, HKI1128, HKI1163, V345, CML384, CM500, CM114, CM202, KML225, LTP1, ITNA004, CML165, HKI34(1+2)-1, DMRQPM58-26, SW93D-313-23-PO-49-54-12 (25)*
4.1 to 5.0	Susceptible	WOSC**, SCM PINK**, WSCShrunken × MUS MADHU**, Cuba 380**, DMSC 28**, WINPOP-1***, WINPOP-3***, WINPOP-4***, WINPOP-43***, WINPOP-16***, WINPOP-21***, HKIPC4B***, HYD05R/13-2, CM 115, ESM11-3, S01slyq-B-B-B-13-B, SC24-(C12)-3-2-1-1**, HKIPC322***, HKIPC323***, HKI484-5, HKI586-1WG'33, HKI1040-5, HKI163 ^s , V345, BML6, HKIPC8***, CM119 (27)*

*Total number of inbreds in each category; **represents the sweet corn genotypes; ***represents the popcorn genotypes; CML – lines developed by the CIMMYT; CM – lines developed under AICRP-Maize, India.

(R), CML 269 (R) × P72c1Xbrasil1177-2 (R) and HKI 4C4B (S) × ESM 113 (S)] were performed during 2012 for this purpose. Two F₂s populations of plant size 361 and 352 were generated by selfing individuals' plants from two different F₁ crosses [CML 269 (R) × HKI 4C4B (S), P72c1Xbrasil1177-2 (R) × ESM 113 (S)] performed using off-season facility during 2012.

Field experiments

The first experiment was laid out in an augmented randomized complete block design using 112 test and 2 control treatments [HKI288-2 (R), CM119 (S)] in 16 blocks. The two control treatments (one each of resistant and susceptible) were allocated once in each of the 16 blocks, however test entries were allocated only once in the design at three identified hotspots locations (Delhi, Karnal and Ludhiana) during 2012. Each inbred line was planted in 2 rows of 3 m length. For inheritance study, the parents along with their six F₁s were sown in 4 rows of 3 m length using randomized block design with three replications during 2013 and 2014 at Delhi location. The two F₂ populations of size 361 and 352 plants generated from two different F₁s [CML 269 (R) × HKI 4C4B (S), P72c1Xbras-

Table 2. Descriptive statistics for disease score and days to flowering in genetic materials used in this study

Genotype/population	Descriptive statistics						
	Disease score data				Days to silking		
	Nos.	Min.	Max.	Mean	Min.	Max.	Mean
CML269 (R)	90	1.0	1.5	1.19±0.24	63.0	64.0	63.7±0.6
HKI4C4B (S)	90	4.0	5.0	4.63±0.33	53.0	54.0	53.7±0.6
P72c1Xbrasil1177-2(R)	90	1.0	1.5	1.12±0.21	64.0	65.0	64.7±0.6
ESM113 (S)	90	4.0	5.0	4.46±0.41	53.0	54.0	53.7±0.6
CML269 × HKI4C4B (R×S)	90	1.5	3.0	2.26±0.37	57.0	59.0	58.0±1.0
HKI4C4B × CML269 (S×R)	90	1.5	3.0	2.24±0.36	57.0	58.0	57.7±0.6
P72c1Xbrasil1177-2 × ESM113 (R×S)	90	1.5	3.0	2.15±0.41	59.0	60.0	59.7±0.6
ESM113 × P72c1Xbrasil1177-2 (S×R)	90	1.5	3.0	2.29±0.42	58.0	59.0	58.3±0.6
CML269 × P72c1Xbrasil1177-2 (R×R)	90	1.0	2.0	1.56±0.36	58.0	61.0	59.3±1.5
HKI4C4B × ESM113 (S×S)	90	4.0	5.0	4.51±0.40	49.0	51.0	50.0±1.0
F ₂ -(CML269 × HKI4C4B)	361	1.5	5.0	3.12±0.60	48.0	71.0	58.3±4.2
F ₂ -(P72c1Xbrasil1177-2 × ESM113)	352	1.0	5.0	3.13±0.65	49.0	73.0	61.0±3.6

il1177-2 (R) × ESM 113 (S)] were planted during 2013 to study the segregation pattern of disease resistance. Row to row and plant to plant spacing of 70 cm × 20 cm was kept in both the experiments.

Disease screening under artificially created epiphytotic condition

Artificial inoculation was employed during field screening following the method of Carson et al. (2004) with minor modifications. Cultures of three isolates (Isolate-Delhi, Isolate-Ludhiana and Isolate-Karnal) of *Cochliobolus heterostrophus* Race 'O' were prepared in conical flask containing sorghum grains (nearly 45 g). Flasks with sorghum grains were soaked in water for about 3–4 hours followed by draining off excess water. It was then autoclaved twice, seeded with fungus under aseptic conditions and kept for incubation at 25–27 °C for 15 days. The flasks were shaken once in 2–3 days to facilitate uniform growth on grains. After incubation of about a fortnight the material was dried at room temperature on clean paper sheet under shade. The grains were then ground into fine powder which was used in inoculation.

Experimental materials and border rows were artificially inoculated with Race 'O' inoculum in the leaf whorl of each plant at four-to six-leaf stage, followed by spraying of 10–12 ml of water in each whorl. The field was kept adequately moist by providing irrigation so as to commence the fungal growth. The inoculation was repeated after 10 days of first inoculation to avoid any chance of disease escape. Minimum of 90 plants in parents and F₁ populations and whole plants populations (361 and 352 nos) of both the F₂s in each plot were rated using 1.0–5.0 scale [≤2.0 (resistant) to 5 (susceptible)] (Payak and

Sharma 1983). Disease scoring was done twice for parents, their F_1 s and F_2 s; first at 10 days after initiation of 50% flowering in a population followed by 2nd scoring at 15 days interval, but the set of 112 inbred lines screened at three locations was scored only once (10 days after initiation of 50% flowering in the half of genotypes).

Testing of identified MLB linked SSRs markers in tropical genotypes

Belcher et al. (2011) reported several genomic regions in a series of mapping studies for MLB resistance in different genetic background other than Indian adapted germplasm. The lines were generated and tested a set of near isogenic lines carry single or combinations of just two or three introgressions in a B73 background. Introgressions of 3B, 6A, and 9B (bins 3.03–3.04, 6.01, and 9.02–9.03) conferred significant levels of MLB resistance in the field. Therefore, we have selected five simple sequence repeats (SSRs) markers, three from bin location 3.04 (umc2000, umc1920 and bnlg602), and one each from 9.04 (umc1571) and 6.06 (umc1520). These five markers were found associated to MLB resistance consistently in various studies and have explained 8–23% of total variation for its resistance (Balint-Kurti et al. 2006b). They were synthesized and tested in four tropical maize genotypes (two each of resistant and susceptible) well identified over the years for their MLB disease reactions and have been used to develop two different recombinant inbred lines (RILs) populations adapted to Indian condition.

DNA was extracted following the method of Saghai-Marouf et al. (1984) with minor modifications. The PCR reaction was carried out in a volume of 15 μ l. This was containing 50 ng of template DNA, 1 μ M each of forward and reverse primers, 0.2 mM dNTPs, 0.5 U *Taq* polymerase and 1.5 mM $MgCl_2$. Reaction was carried with initial denaturation at 94 °C for 4 min followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 30 sec and extension at 72 °C for 1 min. The final extension step was performed at 72 °C for 7 min. The amplified products were resolved on 3.5% metaphor. Sequence information of SSR markers was downloaded from Maize GDB (www.maizegdb.org).

Data analysis

Disease score data was subjected to the analysis of variance (ANOVA) so as to analyze effects due to genotype, environment and genotype \times environment ($G \times E$) interaction. Further, Bartlett's test was used to assess the significance of error means of squares over locations. Contrast analysis was employed to test the significant differences between test entries and check genotypes for MLB reaction. Analysis was done using SAS 9.3 software. To explore further about the nature of $G \times E$, "GGE Biplot" written in 'R' language was used. Stable genotypes for MLB resistance were identified based on their consistent disease score over locations (Table 1) and biplot analysis. Descriptive statistics was calculated for disease score and days to silking using SAS 9.3 software. Chi-square test was used for testing of hypothesis of monogenic (3:1) and/or digenic disease (9:3:3:1) nature of MLB disease response. Correlation studies and distribution patterns of disease score in the F_2 s plants populations was done using SPSS version 16 software. The allelic pattern

of SSRs markers in resistant and susceptible genotypes was used to confirm the similarity of identified genomic regions of MLB resistance (mostly of temperate) in tropical genetic background.

Results

Stable sources of MLB resistance

The 112 inbred lines as test entries along with two control treatment one each of resistance (HKI288-2) and susceptible (CM119) were screened against *Cochliobolus heterostrophus* Race O in an augmented randomized complete block design at Delhi, Karnal and Ludhiana. Average disease score at Delhi, Karnal and Ludhiana was 3.6, 3.4 and 3.8, respectively. The overall average disease score over three locations was 3.6 with range of 1.5 to 5.0. The disease score in susceptible control (CM119) at three locations was between 4.5 and 5.0. Based on the disease score across locations, 112 tests and 2 control genotypes (HKI288-2, CM119) were grouped in four major classes. Twenty-five genotypes had consistently shown disease score ≤ 2.0 (resistant), 37 genotypes had score between 2.1 and 3.0 (moderately resistant), 25 had between 3.1 and 4.0 (moderately susceptible) and 27 exhibited disease score between 4.1 and 5.0 (susceptible) (Table 1). Almost all the genotypes of sweet corn (eight out of eight) and popcorn (eleven out of twelve) were recorded with a score of > 3.0 (Table 1). Contrast analysis for disease score of control versus test genotypes has shown the significant ($P < 0.01$) variations between them. This reveals that the disease screening was consistent and evenly effective at all hotspot locations, and proper favorable conditions were ensured for development of fungus. Pooled analysis of variance showed significant effects of genotypes ($P < 0.001$) and environments ($P < 0.001$). However, G×E interaction for MLB disease response was non-significant. GGE biplot based on disease score in three environments have represented the inbred lines near the origin points in biplot and therefore, almost all of them were giving the stable expression.

Polygenic nature of MLB resistance and marker survey

Mode and nature of inheritance are the preliminary requirements for successful breeding programme, therefore, efforts were made to study the inheritance of MLB resistance in Indian germplasm which is still unknown. For inheritance study, the parents along with their six F_1 s were screened in randomized block design with three replications during 2013 and 2014 at Delhi. The two F_2 populations of size 361 and 352 plants generated from two different F_1 s [CML269 (R) × HKI4C4B (S), P72c1Xbrasil1177-2 (R) × ESM 113 (S)] were planted during 2013 to study the inheritance of MLB resistance. The average disease score under artificially created epiphytotics for resistant parents (CML269 and P72c1Xbrasil1177-2) was 1.19 and 1.12, and for susceptible parents (ESM113 and HKI4C4B), it was 4.46 and 4.63, respectively (Table 2). The range of disease score in resistant and susceptible parents was observed to 1.0 to 1.5 and 4.0 to 5.0, respectively,

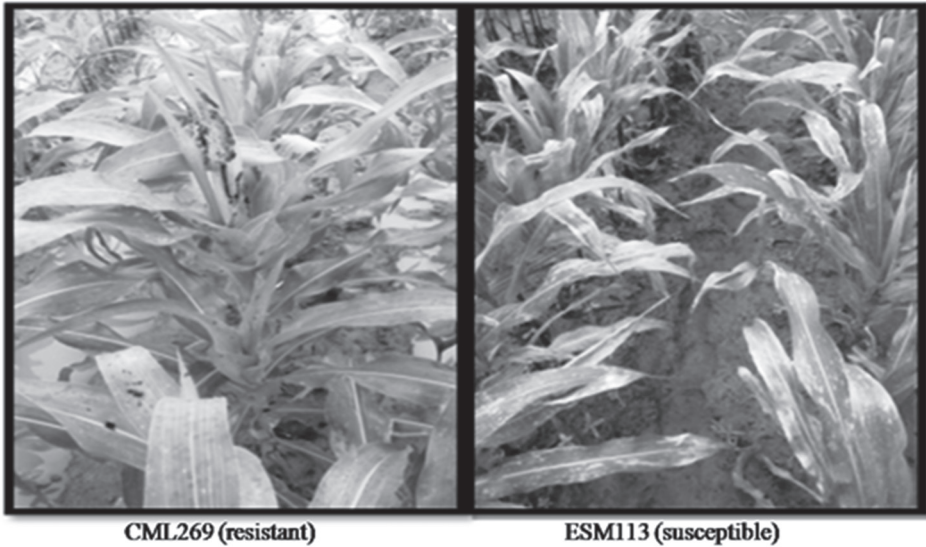
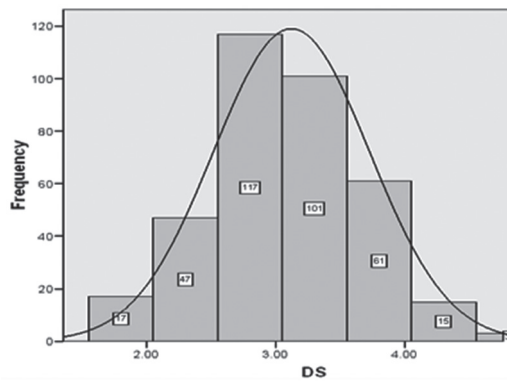


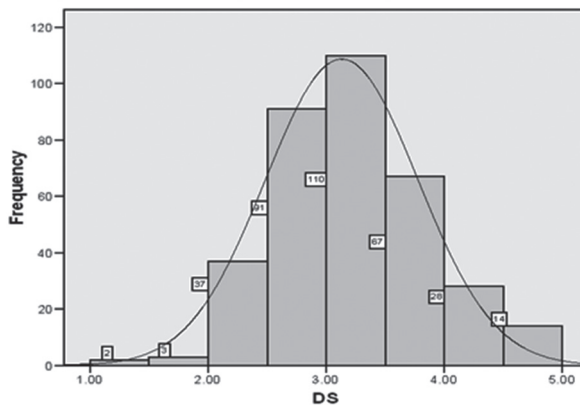
Figure 1. Resistant (CML269) and susceptible (ESM113) inbred lines used as parents in one of the crosses for study of inheritance of MLB resistance. The heavy infestation of MLB was observed in susceptible lines compared to the resistant one

Table 3. Details of Chi-square test for testing null hypothesis of segregation of MLB resistance and susceptible for mono and digenic ratios in two different F₂ populations

F ₂ Populations	Ratio	DF	Expected	Observed	Chi-square value	Sig.
F ₂ -[CML269(R) × HKI4C4B (S)]	3	1	270.75	181	29.75	<i>P</i> <0.001
	1		90.25	180	89.25	
			361	361	119	
	9	3	203.1	17	170.5	<i>P</i> <0.001
	3		67.7	165	139.3	
	3		67.7	161	129.3	
	1		22.6	18	0.9	
			361.0	361	440.1	
F ₂ -[P72c1Xbrasil1177-2(R)× ESM113 (S)]	3	1	264	172	32.1	<i>P</i> <0.001
	1		88	180	96.2	
			352	352	128.2	
	9	3	198	16	167.3	<i>P</i> <0.001
	3		66	156	122.7	
	3		66	158	128.2	
	1		22	22	0.0	
			352	352	418.3	



F₂ population-I (size-361)



F₂ population-II (size-352)

Figure 2. Distribution in F₂ populations for maydis leaf blight score. Population-I was derived from cross CML269 (R) × HKI4C4B (S) and population-II from P72c1Xbrasil1177-2 (R) × ESM113 (S). The continuous distribution for disease score in both F₂ populations has indicated the polygenic control for MLB resistance in tropical maize

indicating that screening was done under high disease pressure (artificially created MLB epiphytotic) (Fig. 1). In F₁ cross CML269 (R) × HKI4C4B (S), average disease score was 2.26 with range of 1.5 to 3.0. Similarly in its reciprocal cross, the average disease score was 2.24 with range of 1.5 to 3.0. In second F₁ cross P72c1Xbrasil1177-2 (R) × ESM (S) and its reciprocal, the average disease score was 2.15 and 2.29, respectively, with a range of 1.5 to 3.0. Further an average disease score of 1.56 with range of 1.0 to 2.0 in R × R crosses was observed in contrast to 4.51 with range of 4.0 to 5.0 in S × S crosses (Table 2). The null hypothesis (H₀) of segregation of 3 (resistance): 1 (susceptible) for monogenic and 9 (resistance): 3 (moderately resistance): 3 (moderately susceptible): 1 (susceptible) for digenic ratios in two different F₂ populations of size 361 and 352

was rejected by Chi-square test at $P < 0.001$ (Table 3). Further, continuous variation for disease ratings was perceptible in F_2 populations (Fig. 2).

The disease score in each plant of different F_2 populations was correlated with days to flowering. Pearson correlation studies on two F_2 populations for disease score and days to flowering exhibited significant ($P < 0.001$) negative correlation (0.42 and 0.56) amongst them.

Further, none of the five SSR markers could differentiate the resistant parents from susceptible ones. The approximately size of amplicon was ranges from 50 bp (umc1920) to 150 bp (bnlg602; umc1520) for different markers.

Discussion

The sufficient genetic variation was observed among lines for their reaction to MLB. Very high disease score observed in susceptible check has indicated the presence of adequate inoculum load in evaluation experiment for effective screening of entries. High disease scores for sweet corn and popcorn revealing vulnerability of specialty corns for MLB. The non-significant effects due to $G \times E$ interaction shows that three isolates of MLB (Isolate-Delhi, Isolate-Ludhiana and Isolate-Karnal) expressed uniform virulence at testing locations according to the in-built defense mechanism of genotype. Based on suitability of agro-climatic conditions for maize cultivation in India, the whole country has been divided into five major zones where the disease screenings against MLB are being done for entries received under all India coordinated research project (AICRP) trials. Three hotspot locations, viz. Delhi, Ludhiana and Karnal, used for inbred evaluation against MLB disease have been grouped in same zone (Zone II). Since $G \times E$ interaction is non-significant, therefore MLB disease score received from these locations may be analyzed together. These results corroborate with the findings of Balint-Kurti et al. (2007). They found significant variance due to genotypes in contrast to non-significant interaction due to years for MLB variation. Further, stability of genotypes for disease reaction across locations was also confirmed by GGE biplot where almost all genotypes were placed toward the center. The identified stable lines can further be used in breeding programme targeting MLB resistance.

The average disease score of F_1 s between $R \times S$, and their ranges clearly indicated the partial dominant nature of MLB resistance over the susceptibility in such maize germplasm background. Moreover, non-significant difference between reciprocal crosses of two F_1 s revealed that resistance and susceptibility to MLB disease in such genetic backgrounds is controlled by nuclear genome. Rejection of null hypothesis for mono and digenic controls of resistance, partial dominant expression of resistance over susceptibility in the F_1 s and continuous distribution of F_2 s plants in two different populations has indicated the polygenic control of MLB resistance. Quantitative genetic resistance to MLB has also been observed in various earlier studies (Zwonitzer et al. 2010; Kump et al. 2010). Plants have evolved qualitative as well as quantitative resistance to combat the attack of pathogens. Qualitative resistance confers a high level of resistance but it is race specific and non-durable (Van Inghelandt et al. 2012). Therefore, there are chances of breakdown of resistance with the appearance of newly evolved virulent races. In contrast,

quantitative resistance is non-race specific and more durable. Owing to these properties, quantitative inheritance is preferred over qualitative one in resistance breeding. Now, most of disease resistances deployed in new varieties is of mostly quantitative types (Van Inghelandt et al. 2012). In MLB disease, the several genomic regions for resistance have been located on chromosomes 3, 6 and 9 (Belcher et al. 2011) supporting the polygenic nature of MLB resistance.

Days to flowering has been considered as significant factor for foliar diseases like MLB, northern corn leaf blight (NCLB) and grey leaf spot (GLS) (Belcher et al. 2011). In this study, significant negative correlation ($r = 0.42$ and 0.56 ; $P < 0.001$) was observed between days to flowering and disease score. Late flowering maize lines tend to be more resistant to NCLB, MLB and GLS. Several other studies have also found significant negative correlation amongst many of foliar diseases with days to flowering and maturity (Bubeck et al. 1993; Wissner et al. 2006). In one of the studies; 48, 45 and 52% variation of resistance to NCLB, MLB and GLS diseases, respectively, was ascribed to days to flowering (Wissner et al. 2011). Whether negative correlation between disease score and days to flowering is due to pleiotropic effect of the genes and/or tightly linkage of genomic regions on the same chromosomal segments warrants further studies.

Further, none of the five SSR markers selected from previous reported genomic regions (bin location 3.04, 9.04 and 6.06) could differentiate the resistant parents from susceptible ones, which implies the availability of different genomic regions for MLB resistance in such tropical genotypes. Multiple genomic regions for MLB resistance in maize (bin 3.04, 6.06 and 9.03–9.04) have been reported consistently in a series of mapping studies in mostly temperate genetic backgrounds (Belcher et al. 2011). Therefore, there is need to map for MLB resistance using tropical maize germplasm, which may strengthen the resistance breeding further. Since these five markers were tested in four tropical adapted diverse parents of two RILs populations, therefore their association to MLB resistance can be further tested in another tropical genetic background.

The results of this study established that the differential reaction to MLB does exist in the tropical maize germplasm. Twenty-five lines of stable sources of resistance across locations were identified, which may be useful for MLB resistance breeding programme. Inheritance study showed partial dominance of MLB resistance over the susceptibility along with role of nuclear genome in determining its resistance. The quantitative nature of resistance to MLB was also indicated which have important bearing while adoption of strategies for development of resistant cultivars in maize.

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