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RESEARCH ARTICLE

Genetic analysis of maydis leaf blight resistance in subtropical maize (*Zea mays* L.) germplasm

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Abstract. Knowledge on the genetics of maydis leaf blight (MLB) is crucial to breed the resistant maize cultivars to combat disease epidemics as a sustainable and cost-effective approach. The present investigation was framed to understand the genetics of MLB resistance in subtropical maize. Two contrasting genotypes CM119 (susceptible) and SC-7-2-1-2-6-1 (resistant) were used to generate six genetic populations, namely P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2, and evaluated in three target environments for MLB resistance under artificial epiphytotic condition. The CM119 and SC-7-2-1-2-6-1 showed susceptible and resistant reactions with mean disease reaction of 3.89–3.98 and 1.88–2.00, respectively. The derived generations, namely F_1, F_2, BC_1P_1 and BC_1P_2 showed mean disease reaction of 2.15–2.28, 2.44–2.51, 2.19–2.24 and 2.22–2.28, respectively in the test locations. The segregating generations (F_2: 0.35–0.37; BC_1P_1: 0.24–0.29 and BC_1P_2: 0.17–0.20) showed variation for MLB disease resistance over the parental and first filial generations (P_1: 0.11–0.17; P_2: 0.08–0.13 and F_1: 0.12–0.14). The genetic analysis of MLB resistance revealed the nonallelic interactions of duplicate epistasis type across the test locations. Among the gene interactions, dominance × dominance [l] effect was predominant over additive × additive [i] and additive × dominance [j] effects. The segregation analysis and the prediction of the number of major loci revealed at least two major genes associated with MLB tolerance in subtropical maize. Our investigation paved the foundation for the improvement of subtropical maize germplasm of MLB resistance.

Keywords. gene action; genetic effects; generation mean analysis; inheritance pattern; maize; maydis leaf blight; resistance breeding; *Cochliobolus heterostrophus*.

Introduction

Maydis leaf blight (MLB) disease or southern corn leaf blight (SCLB), caused by *Cochliobolus heterostrophus* (Drech.) [(*Bipolaris maydis* (Nishik. and Miyake) Shoemaker)] is one of the potential threats to global maize production. The MLB on maize distributed across the globe; however, shows higher incidences in regions of high temperature of 20–32°C and high humid weather (> 80%). The

pathogen *C. heterostrophus* possess three physiological races, namely race O, race-T (Hooker 1972; Ullstrup 1972), and race C (Wei 1988). The race-T of a pathogen is more prevalent in the USA. The extensive usage of CMS-T cytoplasm based maize lines to develop commercial maize hybrids in the USA resulted in a major epidemic during 1970 (Ullstrup 1972). Race C, more prevalent in China, is pathogenic to maize inbred lines with CMS-C cytoplasm (Wei 1988). Similarly, the race 'O' predominantly prevalent in the southern Atlantic coast of the USA, India, Africa and Western Europe (Misra 1979; Balint-Kurti *et al.* 2007), which can infect all types of susceptible maize cultivars irrespective of the cytoplasm (Hooker *et al.* 1970; Smith

1975) and can reduce the grain yield up to 41% (Sharma and Rai 2000).

Controlling the disease through protective fungicides is not so effective when there is high disease pressure coupled with the predominance of susceptible cultivars in the regions (Poole and Arnaudin 2014). At this juncture, the exploitation of genetic host resistance could be the best and sustainable means of MLB disease management. However, only a few reports are available on the genetics and gene(s) of MLB resistance in maize for race-O. Moreover, to date, none of the maize cultivars have been reported a complete immune against MLB, nevertheless few inbred lines were reported conferring a significant degree of resistance against MLB (Sharma and Rai 2005; Manjunatha et al. 2019). The non-availability of immune maize inbred lines and genes conferring complete immunity have enforced the breeders to exploit the polygenic and quantitative resistance to MLB.

The choice of an effective breeding procedure for the improvement of quantitative disease resistance depends on the inheritance, genetic effects, nature and magnitude of gene action governing a resistance mechanism. Several reports are available on the inheritance pattern of MLB resistance in maize, namely monogenic recessive (Faluyi and Olorode 1984; Zaitlin et al. 1993; Chang and Peterson 1995; Cai et al. 2003), digenic linked recessive (Craig and Fajemisin 1969), digenic independent recessive (Thompson and Bergquist 1984), multi-genic partial dominant and quantitative (Pate and Harvey 1954; Kumar et al. 2016) and dominant R gene (Rai et al. 2010) inheritance. Recently, quantitative trait loci (QTL) mapping showed a significant contribution of bin location 3.04 and 9.04 in assigning resistance to MLB (Carson et al. 2004; Balint-Kurti and Carson 2006; Balint-Kurti et al. 2006; Kump et al. 2011). Further, there are a few reports available on gene action and genetic effects associated with MLB (Thompson and Bergquist 1984; Burnette and White 1985; Sharma et al. 2003). However, hardly there are any multi-location studies on dissection of genetic effects for MLB resistance in subtropical maize inbred lines.

Among the various biometrical techniques, generation mean analysis is one of the powerful methods for detecting the gene action (Hayman 1958) for quantitatively inherited traits, including the disease resistance (Shashikumar et al. 2010; Akbar et al. 2018). The genetic analysis provides the information on main genetic effects (additive and dominance) and digenic interactions (additive × additive [i], additive × dominance [j] and dominance × dominance [l]) responsible for the inheritance of a trait. These estimates of the genetic effects give insight about formulating the breeding strategy for handling the breeding material in the segregating generation. Further, the knowledge on the nature and magnitude of the gene action of MLB resistance could help in designing the breeding programme for the development of MLB resistant subtropical maize cultivars. Therefore, the present investigation was designed to understand the inheritance, nature and magnitude of the gene effects

associated with MLB resistance in subtropical maize inbred lines.

Materials and methods

Development of population

Genetic material consisted of six generations (P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2). Two genotypes CM119 and SC-7-2-1-2-6-1 (plant germplasm registration no. INGR 07025) were selected based on contrasting disease reaction to MLB disease. The F_1 was derived by crossing the inbred lines CM119 × SC-7-2-1-2-6-1 during the rainy season (kharif), 2014 at New Delhi. The seeds of F_1s were raised during rabi season 2014–2015 at Winter Nursery Centre, ICAR-Indian Institute of Maize Research, Hyderabad. The F_1s were self-pollinated to produce F_2 seeds, while BC_1P_1 and BC_1P_2 seeds were developed by backcrossing with parents CM119 and SC-7-2-1-2-6-1, respectively.

Evaluation of genetic material

The six populations, namely P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2 generations were evaluated for their spectrum of resistance against MLB disease under artificial epiphytotic conditions at (i) Research Farm, ICAR-Indian Agricultural Research Institute, New Delhi (216 m above mean sea level (AMSL); 28°36′50″N, 77°12′32″E), (ii) Research Field, Chaudhary Charan Singh Haryana Agricultural University-Regional Research Station, Uchani, Karnal (256 m AMSL; 29°73′N, 76°98′E) and (iii) Research Field, CSK HPKV Hill Agriculture Research and Extension Centre, Dhaulakuan, Himachal Pradesh (468 m AMSL; 30°46′N, 77°48′E) during the rainy season (kharif), 2016. All the agronomic operations and management practices were followed as per the standard procedure (Prakash et al. 2019). However, no fungicides were used to ensure absolute disease expression. Each replicate consisted of an average of 20 plants from each parent (P_1 and P_2), 23 plants of F_1, 258 plants of F_2, 108 plants of BC_1P_1 and 107 plants of BC_1P_2.

Inoculum preparation and mass multiplication

The virulent isolates of *C. heterostrophus* (Delhi, Karnal and Dhaulakuan) were procured from the respective test locations. The purification and mass multiplication of isolates were done at Cereal Laboratory of Plant Pathology Division, IARI, New Delhi. The culture was multiplied on potato dextrose agar (PDA) medium in the BOD incubator at 28 ± 2°C. The multiplied isolates were used to inoculate the susceptible maize genotype CM119 separately to ensure the purity of isolate. Subsequently, the hyphal tips of

C. heterostrophus were placed on PDA. Mass multiplication of *C. heterostrophus* pure culture was undertaken on sorghum seeds (Payak and Sharma 1983).

Pathogen inoculation and recording of disease reaction

The pathogen isolates from the respective locations were used for pathogen inoculation in the test locations. In each test location, the pathogen inoculation was carried out twice with an interval of 10 days between first and second inoculation to ensure proper disease establishment. The first inoculation was done by placing the optimum quantity of the inoculum into the central whorl of 25 days old maize plants (Payak and Sharma 1983). The second inoculation was undertaken on 35 days old maize plants to create high disease pressure. The disease reaction was measured 20–25 days after the second inoculation with standard 1–5 scale; where 0.0–1.0 was for highly resistant, 1.1–2.0 for resistant, 2.1–3.0 for moderately resistant, 3.1–4.0 for moderately susceptible and 4.1–5.0 for susceptible (Payak and Sharma 1983) (table 1).

Biometrical analysis

The scaling test (Hayman and Mather 1955) and joint-scaling test (Cavalli 1952) were performed to test the adequacy of the additive–dominance model. The four scales were calculated using the formulae: $A = 2\overline{BC_1P_1} - \bar{P}_1 - \bar{F}_1$; $B = 2\overline{BC_1P_2} - \bar{P}_2 - \bar{F}_1$; $C = 4\bar{F}_2 - 2\bar{F}_1 - \bar{P}_1 - \bar{P}_2$ and $D = 2\bar{F}_2 - \overline{BC_1P_1} - \overline{BC_1P_2}$. The significance of scales was tested as per Jinks and Jones (1958). Wherever the additive dominance model was inadequate, the six-parameter model of Hayman (1958) was employed to separate the genetic variance into main effects. The genetic effect components encompass mean effect parameter [m], additive [d], dominance [h], additive x additive [i], additive x dominance [j]

Table 1. The disease scale employed to quantify the disease incidence in various generations (Payak and sharma 1983).

Disease reaction	Symptom	Disease scale
Highly resistant	Very slight infection, one or two to few scattered lesions on lower leaves	0.0–1.0
Resistant	Light infection, a moderate number of lesions on lower leaves	1.1–2.0
Moderately resistant	Moderate infection, abundant lesions on lower leaves, a few on middle leaves	2.1–3.0
Moderately susceptible	Heavy infections, abundant lesions on lower and middle leaves, extending to upper leaves	3.1–4.0
Susceptible	Very heavy infection, lesions abundant on almost all leaves; plants prematurely dry or killed by the disease	4.1–5.0

and dominance x dominance [l] types of gene action. The generation variance analysis was performed as per Mather and Jinks (1982):

$$Y = m + \alpha[a] + \beta[d] + \alpha^2[i] + 2\alpha\beta[j] + \beta^2 \ [l],$$

where Y is the generation mean of target trait, m is the mean of all possible homozygous lines which can be derived from a target cross, α and β are the coefficients of genetic parameters, [a] is the additive gene effects, [d] is the dominance gene effects, [i] is the additive x additive gene effects, [j] is the additive x dominance gene effects, and [l] is the dominance x dominance gene effects.

The variance components were calculated to decipher the genetic parameters using following formulae: additive variance (D) = $4VF_2 - 2 \ (VBC_1P_1 + VBC_1P_2)$; dominance variance (H) = $4VF_2 - 1/2VD - VE$ and environmental variance (E) = $1/3 \ (VP_1 + VP_2 + VF_1)$; where F_1 is first filial generation, F_2 is second filial generation, BC_1P_1 and BC_1P_2 are first backcross generations with parents P_1 and P_2, respectively. The narrow sense-heritability was computed from variance components as per Warner (1952) and Mather and Jinks (1982) in F_2 and F_∞ generations, respectively and genetic advance as per Falconer and Mackay (1996) with following formulae.

$$h^2(F_2) = \frac{0.5D}{(0.5D + E)},$$

$$h^2(F_\infty) = \frac{D}{(D + E)},$$

The Castle–Wright equation (Castle 1921; Wright 1952) was employed to estimate the minimum number of resistance loci contributing to MLB resistance:

$$n = \frac{(m_1 - m_2)^2}{8(VF_2 - VF_1)},$$

Where *n* is minimum number of genomic regions associated with MLB resistance, m_1 and m_2 are the means of parent P_1 and P_2, respectively, VF_1 and VF_2 are the variances of F_1 and F_2 generations, respectively.

Results

Generation means and variance

The means with standard error, range and variance for parents, F_1, F_2 and backcross generations (BC_1P_1 and BC_1P_2) across locations are presented in table 2. Inbred line SC-7-2-1-2-6-1(P_2) showed a resistant (score 1.88–2.0) and CM-119 (P_1) was moderately susceptible (score 3.91–3.98) across the testing locations and found significantly different from each

Table 2. Mean, range and variances for MLB disease reaction across the generations and locations.

| Generations	n	Mean $\pm$ SE	Range	Variance
New Delhi				
P_1	20	3.89 ± 0.07^a	3.50–4.50	0.11
P_2	20	1.93 ± 0.07^e	1.50–2.50	0.08
F_1	23	2.28 ± 0.08^{bc}	1.50–3.00	0.14
F_2	258	2.50 ± 0.04^d	1.50–4.00	0.35
BC_1P_1	108	2.19 ± 0.05^b	1.50–3.50	0.24
BC_1P_2	107	2.28 ± 0.04^b	1.50–3.50	0.17
Dhaulakuan				
P_1	20	3.91 ± 0.09^a	3.00–4.50	0.17
P_2	20	2.00 ± 0.08^{ef}	1.50–2.50	0.12
F_1	23	2.15 ± 0.09^{bf}	1.50– 3.00	0.18
F_2	258	2.44 ± 0.04^{cd}	1.50–4.00	0.35
BC_1P_1	108	2.24 ± 0.05^b	1.50–4.00	0.29
BC_1P_2	107	2.23 ± 0.04^b	1.50–3.50	0.18
Karnal				
P_1	20	3.98 ± 0.08^a	3.50–4.50	0.13
P_2	20	1.88 ± 0.08^e	1.50–2.50	0.13
F_1	23	2.23 ± 0.08^b	1.50–3.00	0.15
F_2	258	2.51 ± 0.04^d	1.50–4.00	0.37
BC_1P_1	108	2.20 ± 0.05^b	1.00–3.00	0.24
BC_1P_2	107	2.22 ± 0.04^b	1.50–3.50	0.20
CV (t)		1.97		

The generation means with the same alphabet are on par with each other. P_1, parent 1; P_2, parent 2; F_1, first filial generation; F_2, second filial generation; BC_1P_1, backcross population derived from parent 1; BC_1P_2, backcross population derived from parent 2; CV (t), critical value of t.

other for MLB resistance (figure 1). The disease scale of F_1's was significantly higher (moderately resistant) than resistant parent SC-7-2-1-2-6-1 (P_2) in all the test locations. Further, the mean performance of F_1 for disease tolerance has significantly differed from the parents (P_1 and P_2) in all the locations except Dhaulakuan, where the mean performance of F_1 was on par with the P_2. Similarly, F_1 showed numerically and significantly better resistance than F_2 across the test locations. Interestingly, the mean resistance response of F_1 was on par with BC_1P_1 and BC_1P_2 populations and mean of BC_1P_1 and BC_1P_2 were found nonsignificant among the test locations. Further, across the locations, F_1, F_2, BC_1P_1 and BC_1P_2 showed moderately resistant score (2.1–3.0).

The parental generations showed the least variance across the locations, whereas F_2 generation recorded the maximum variance among the generations owing to segregation of resistance/susceptible loci. The range of variation across the location for F_2 (2.50 ± 0.00) was quite high as compared to backcross derived generations (BC_1P_1: 2.17 ± 0.17; BC_1P_2: 2.00 ± 0.00) and first filial generation (1.50 ± 0.00).

Adequacy of the additive–dominance model

The Mather's scaling tests were employed to predict the adequacy of the additive–dominance model of MLB resistance. All the four scales, i.e. *A*, *B*, *C* and *D* were significantly different from zero across the test locations except scale *C* at Karnal (table 3). The result of the scaling test
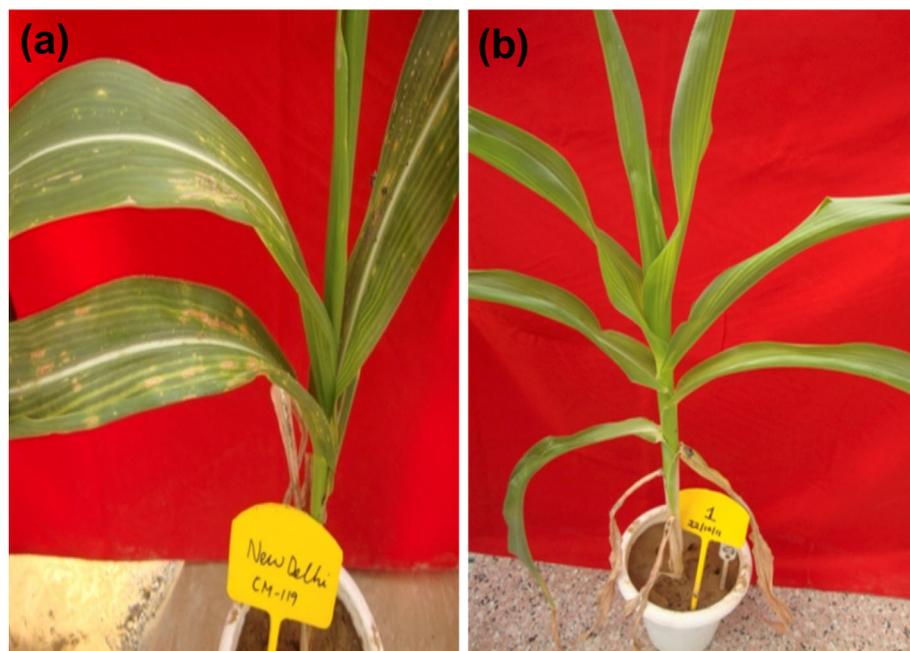
Figure 1. The response of parental maize inbred lines to MLB disease reaction: (a) Inbred CM-119 (P_1) showing moderately susceptible disease reaction (10 days after the second inoculation) with abundant lesions on lower and middle leaves and extending to upper leaves. (b) Inbred line SC-7-2-1-2-6-1(P_2) showing a resistance reaction (10 days after the second inoculation) with light infection characterized by moderate number of lesions on lower leaves.

Table 3. The estimated value of scales A, B, C and D for MLB disease reaction for all the locations.

Scale	Location		
	New Delhi	Dhaulakuan	Karnal
A	1.79 ± 0.10**	1.59 ± 0.12**	1.81 ± 0.11**
B	-0.35 ± 0.09**	-0.31 ± 0.10**	-0.33 ± 0.10**
C	0.39 ± 0.17*	0.47 ± 0.18*	0.27 ± 0.18^{NS}
D	0.53 ± 0.07**	0.41 ± 0.07**	0.61 ± 0.07**

*Significant at $P<0.05$, **significant at $P<0.01$, ^{NS}not significant.

suggested the inadequacy of the additive–dominance model for explaining MLB disease resistance and represents the presence of nonallelic interactions associated with MLB resistance. The joint scaling test also revealed the presence of intergenic interaction and suggested that a simple additive–dominance model was insufficient to explain MLB disease resistance.

Genetic effects

The various genetic effects associated with MLB resistance are presented in table 4. In the present investigation, the six-parameter model of Jinks and Jones (1958) was employed due to the presence of epistatic interactions. The epistatic effects were found more than the main effects at all locations. Among the epistatic interactions, dominance × dominance [l] was found positive and significant across the locations. In contrast, additive × additive [i] and additive × dominance [j] gene interactions were negative and significant across the locations. Additionally, among all the three types of interactions, dominance × dominance [l] was the most prevalent over additive × additive [i] and additive × dominance [j] interactions. Further, dominance gene effect [h] and dominance × dominance [l] possessed opposite sign across the test locations indicating the presence of duplicate gene interaction. Similarly, joint scaling test revealed the predominance of dominance effects [h] over the additive effects [d] (table 4).

Generation variance components and genetic parameters

The generation variance components and genetic parameters are tabulated in table 4. The analysis revealed the predominance of dominance variance ($H = 1.00$ to 1.04) over additive ($D = 0.43$ to 0.61) and environmental ($E = 0.11$ to 0.14) variances. The narrow-sense heritability estimates ranged from 0.59 to 0.72 in F_2, and 0.74 to 0.84 in F_∞

Table 4. Estimates of genetic effects and parameters of MLB resistance across the locations using different genetic models.

Genetic measure	Location		
	New Delhi	Dhaulakuan	Karnal
Genetic effect			
Six-parameter model			
[m]	2.50 ± 0.03**	2.44 ± 0.03**	2.51 ± 0.03**
[d]	-0.09 ± 0.04*	0.01 ± 0.05^{NS}	-0.02 ± 0.05^{NS}
[h]	-1.68 ± 0.15**	-1.62 ± 0.16**	-1.91 ± 0.16**
[i]	-1.05 ± 0.14**	-0.81 ± 0.14**	-1.22 ± 0.14**
[j]	-2.14 ± 0.11**	-1.90 ± 0.13**	-2.15 ± 0.12**
[l]	2.50 ± 0.24**	2.10 ± 0.27**	2.70 ± 0.25**
Joint scaling test			
[m]	2.77 ± 0.03**	2.80 ± 0.04**	2.80 ± 0.03**
[d]	-0.57 ± 0.03**	-0.55 ± 0.03**	-0.59 ± 0.03**
[h]	-0.71 ± 0.06**	-0.83 ± 0.07**	-0.82 ± 0.07**
χ^2	438.99**	261.61**	415.93**
Generation variance component			
E	0.11	0.15	0.14
D	0.58	0.43	0.61
H	1.00	1.01	1.04
Genetic parameter			
Epistasis	Duplicate	Duplicate	Duplicate
H/D	1.72	2.34	1.70
h^2 (F_2)	0.72	0.59	0.69
h^2 (F_∞)	0.84	0.74	0.82
No. of effective loci	2.30	2.71	2.55

*,**Significant at $P < 0.01$ and $P < 0.05$, respectively. ^{NS}Not significant; [m], mean effect; [d], additive effect; [h], dominance effect; [i], additive × additive effect; [j], additive × dominance effect; [l], dominance × dominance effect; E, environmental variance; D, additive variance; H, dominance variance; F, interaction variance; h^2 (F_2), narrow sense heritability in F_2 population; h^2 (F_∞), narrow sense heritability in F_∞ population.

generations. The estimation of the minimum number of MLB resistance loci revealed the presence of two to three loci across the test locations. H/D is a good indicator of dominance and ranged from 1.70 (Karnal) to 2.34 (Dhaulakuan).

The inheritance pattern of MLB resistance

The inheritance pattern of MLB was observed by testing goodness of fit for the segregation ratio of F_2 generation using the chi-square test in all the test locations (table 5). Visual scoring categorized 258 plants into resistant and susceptible based on disease reaction at New Delhi (resistant, 234; susceptible, 24), Dhaulakuan (resistant, 245; susceptible, 13) and Karnal (resistant, 238; susceptible, 20). The proportion of resistant and susceptible plants was equivalent to 9.75:1, 18.85:1 and 11.9:1 at New Delhi, Dhaulakuan and Karnal, respectively which was tested against a 3:1 ratio. The significant chi-square values for a 3:1 ratio revealed that the segregation pattern does not follow monogenic inheritance. Similarly, the observed values were tested against digenic (15:1) and trigenic (63:1) inheritance. The nonsignificant and significant chi-square values were observed for 15:1 and 63:1 ratios, respectively. Therefore, the nonsignificant chi-square values for 15:1 ratio showed that the observed and expected frequencies were comparable to the segregation pattern of 15:1. Hence, the segregation pattern of MLB resistance followed a digenic inheritance pattern.

However, when these generalized groups were further divided, it was observed that the highly resistant and moderately resistant plants among resistant plants were found at New Delhi, Dhaulakuan and Karnal in the ratio of 0:46, 0:57

and 0:56, respectively. Further, among susceptible plants, susceptible and highly susceptible plants were found in the ratio of 24:0, 13:0 and 20:0 at New Delhi, Dhaulakuan and Karnal, respectively. Subgroups categorization revealed that not even a single plant exited in the extreme classes, i.e., highly resistant and highly susceptible. The proportion of plants was observed in the ratio of 0:1.91:7.83:1:0; 0:4.38:14.46:1:0 and 0:2.8:9.1:1:0 at New Delhi, Dhaulakuan and Karnal, respectively which was tested against 1:4:6:4:1 ratio of digenic inheritance. The significant chi-square values reflected that the data were not best to fit into 1:4:6:4:1 ratio. Further, testing of observed values against 11:5 ratio, which is the combined form of 1:4:6:4:1 (1+4+6=11; 4+1=T5) also revealed the significant chi-square values. Therefore, in conclusion, two genes confer resistance against maydis leaf blight disease, however other ratios, namely 3:1, 63:1, 11:5 and 1:4:6:4:1 showed the significant chi-square values which reflected that these were not the best fitting ratios (table 5).

Discussion

Maize serves as an important source of food and nutrition in developed and developing countries (Mallikarjuna et al. 2014; Agrawal et al. 2018). MLB disease, caused by the fungus *C. heterostrophus*, is one of the potential threats to corn production. The development of resistant cultivars is the most sustainable and cost-effective approach in the management of diseases in crops which necessitates the knowledge on the genetics of disease resistance. The biometrical approach generation mean analysis provides useful genetic information for designing of breeding strategies to

Table 5. Segregation ratio of F_2 for MLB resistance in various test locations.

Location	Tested ratio	Observed		Expected		χ^2 value
		R	S	R	S	
New Delhi	Monogenic	234	24	193.5	64.5	33.91**
Dhaulakuan	(3:1)	245	13	193.5	64.5	54.83**
Karnal		238	20	193.5	64.5	40.94**
New Delhi	Digenic	234	24	241.88	16.12	4.11[NS]
Dhaulakuan	(15:1)	245	13	241.88	16.12	0.64[NS]
Karnal		238	20	241.88	16.12	1.00[NS]
New Delhi	Trigenic	234	24	253.97	4.03	100.53**
Dhaulakuan	(63:1)	245	13	253.97	4.03	20.30**
Karnal		238	20	253.97	4.03	64.30**
New Delhi	Digenic	234	24	177.38	80.62	57.84**
Dhaulakuan	(11:5)	245	13	177.38	80.62	82.50**
Karnal		238	20	177.38	80.62	66.30**
		HR:R:MR:S:HS		HR:R:MR:S:HS		
New Delhi	Digenic	0:46:188:24:0		16.12:64.50:96.75:64.5:16.12		149.05**
Dhaulakuan	(1:4:6:4:1)	0:57:188:13:0		16.12:64.50:96.75:64.5:16.12		160.30**
Karnal		0:56:182:20:0		16.12:64.50:96.75:64.5:16.12		139.20**

The disease scale of 0.0–3.0 (high to moderate resistance) and 3.1–5.0 (moderate to highly susceptible) was considered to categorize resistance and susceptible reactions, respectively.
**Significant at $P < 0.01$; [NS]not significant.

take advantage of gene interactions, and effects that exist in the succession breeding generations. The F_1 generation showed a moderate level of MLB resistance (2.15–2.18), although the resistance level is lower than the resistant parent, P_2 (table 2). The lower mean performance of F_1 generations (2.15–2.28) over mid parental values (2.91–2.95) and nonsignificant differences among the backcross and F_1 generations indicating the importance of dominance gene action on MLB resistance, in the direction of the better parent (P_2). Therefore, the dominance nature of MLB resistance would be suitable for the exploitation of heterosis for MLB resistance in commercial maize hybrids production. The nonsegregating parental (P_1 and P_2) and first filial (F_1) generations showed minimal phenotypic variation, with narrow range of disease scale (1.00–1.50) as compared to a broader range of variances (1.50–2.50) in segregating generations (F_2, BC_1P_1 and BC_1P_2) (table 1). The higher narrow range of variation in F_1 and parents indicates the minimal influence of environment on MLB inheritance across the three test locations. The larger phenotypic variance associated with segregating generations is associated with segregation of loci and modifiers associated with MLB resistance and susceptibility.

Further, the larger variation in segregating generations was following the theoretical components of F_2 ($VF_2 = VA + VD + VE$) and backcross ($VBC = \frac{1}{2}VA + VD + VE$) generation variances (Sullenberger *et al.* 2018). The genetic variance components revealed the predominance of additive (0.43–0.61) and dominance variance (1.00–1.04) over the environmental variances (0.11–0.15) across the test locations. The preponderance of genetic variances over the environment resulted in the higher narrow-sense heritability (h^2) of 0.59 to 0.72 in F_2 and 0.74 to 0.82 in F_∞ generations. Therefore, the directional phenotypic selection for the MLB resistance could be employed to exploit the moderately high h^2 of MLB resistance.

Genetic analysis of target traits to determine the genetic effects is essential for initiating the planned and scientific breeding programmes. Determination of individual gene effects for quantitative characters is not easy; on the other hand, it could be summed up as cumulative effects of all the genes (Wright 1952). Generation mean analysis allows partitioning of total variability into additive and nonadditive components, which is helpful in selecting the mating design, defining time of selection and breeding methodology. The inadequacy of the additive–dominance model in the study enforced to opt six-parameter model. The joint scaling test showed significant and negative additive effects [d] across the locations, and the six-parameter model showed nonsignificant additive effects in Dhaulakuan and Karnal, although small significant negative additive effect were found in New Delhi. On the other hand, the dominance effects [h] were significant and negative across the test locations in both joint scaling test and six-parameter model analysis. The negative additive and dominance effects of MLB resistance provide an opportunity to enhance the MLB resistance

through hybrid breeding. Further, the dispersal of resistance alleles in parents and genetic recombination during the crossing process could be the main reason for negative dominance effects (Lyimo *et al.* 2011). Additionally, the significant and negative values of additive × additive [i] and additive × dominance [j] suggest that the genes fixed in CM119 and SC-7-2-1-2-6-1 are acting against each other to enhance the MLB disease resistance. Among the interaction components, the negative and significant values of fixable additive × additive [i] and nonfixable additive × dominance [j] gene effects across the locations could be exploited to enhance the MLB resistance through line development and hybrid breeding, respectively. In the duplicate epistasis interaction, the internal cancellation of oppositely signed dominance and dominance × dominance gene effects could reduce heterosis for MLB resistance. Further, the presence of duplicate epistasis indicated the likelihood of getting transgressive segregates for MLB resistance in the succeeding generations (Sharma *et al.* 2017). Additionally, values of *H/D* ratio across the locations indicate the predominance of dominance over additive gene action.

The inheritance pattern of disease reaction is crucial to decide the mating design and breeding strategy for target trait improvement. The generation mean and segregation ratio analysis results showed that two genes govern the inheritance of MLB resistance with duplicate epistasis. Further, the digenic inheritance was supported by minimum number of gene governing MLB resistance by Castle–Wright equation (table 4). Similarly, the digenic inheritance of MLB resistance was observed in temperate maize (Craig and Fajemisin 1969; Thompson and Bergquist 1984). Further, genomics studies identified two to three major bin location for MLB (Balint-Kurti *et al.* 2006, 2007; Belcher *et al.* 2012). On the other hand, a few studies showed polygenic inheritance with partial dominance of MLB resistance in maize (Kump *et al.* 2011; Kumar *et al.* 2016). Additionally, our results from generation mean analysis showed the presence of significant interactions and epistasis. Therefore, the inheritance of MLB resistance could be associated with a few major genes coupled with minor QTLs. Further, the nature of resistance inheritance could be population-specific and specific population demands, appropriate breeding methods for the improvement of MLB resistance in maize (Hettiarachchi *et al.* 2009).

In conclusion, presently, there are hardly any subtropical maize inbred lines showing complete immunity for MLB across the environments. Therefore, maize inbred line SC-7-2-1-2-6-1 could be effectively used as a source of MLB resistance in subtropical maize breeding. Further, SC-7-2-1-2-6-1 inbred may be exploited as a source to generate the maize transgressive segregates by crossing with other inbred lines showing the variable level of MLB resistance and agronomically superior phenotype (Sharma *et al.* 2003; Kumar *et al.* 2016). Additionally, despite quantitative nature and partial dominance, the digenic inheritance of MLB resistance in subtropical maize could be exploited for the

rapid conversion of maize inbred lines using stringent phenotypic selection.

References

Agrawal P. K., Mallikarjuna M. G. and Gupta H. S. 2018 Genetics and applied genomics of quality protein maize for food and nutritional security. In *Biotechnologies of crop improvement*, vol. 3, pp. 151–178. Springer, Cham.

Akbar W., Aslam M., Maqbool M. A., Ali M. and Arshad M. 2018 Inheritance pattern of mungbean yellow mosaic disease resistance and gene action for different traits in mungbean (*Vigna radiata* (L.) Wilczek) under protected and unprotected field conditions. *Plant Breed.* **137**, 763–772.

Balint-Kurti P. J. and Carson M. L. 2006 Analysis of quantitative trait loci for resistance to southern leaf blight in juvenile maize. *Phytopathology* **96**, 221–225.

Balint-Kurti P. J., Krakowsky M. D., Jines M. P., Robertson L. A., Molnár T. L., Goodman M. M. and Holland J. B. 2006 Identification of quantitative trait loci for resistance to southern leaf blight and days to anthesis in a maize recombinant inbred line population. *Phytopathology* **96**, 1067–1071.

Balint-Kurti P. J., Zwonitzer J. C., Wisser R. J., Carson M. L., Oropeza-Rosas M. A., Holland J. B. and Szalma S. J. 2007 Precise mapping of quantitative trait loci for resistance to southern leaf blight, caused by *Cochliobolus heterostrophus* race O, and flowering time using advanced intercross maize lines. *Genetics* **176**, 645–657.

Belcher A. R., Zwonitzer J. C., Cruz J. S., Krakowsky M. D., Chung C. L., Nelson R. *et al.* 2012 Analysis of quantitative disease resistance to southern leaf blight and multiple disease resistance in maize, using near-isogenic lines. *Theor. Appl. Genet.* **124**, 433–445.

Burnette D. C. and White D. G. 1985 Inheritance of resistance to *Bipolaris maydis* race O in crosses derived from nine resistant inbred lines of maize. *Phytopathology* **75**, 1195–1200.

Cai H. W., Gao Z. S., Yuyama N. and Ogawa N. 2003 Identification of AFLP markers closely linked to the *rhm* gene for resistance to Southern corn leaf bight in maize by using bulked segregant analysis. *Mol. Genet. Genomics* **269**, 299–303.

Carson M. L., Stuber C. W. and Senior M. L. 2004 Identification and mapping of quantitative trait loci conditioning resistance to southern leaf blight of maize caused by *Cochliobolus heterostrophus* race O. *Phytopathology* **94**, 862–867.

Castle W. E. 1921 An improved method of estimating the number of genetic factors concerned in cases of blending inheritance. *Science* **54**, 223.

Cavalli L. L. 1952 A joint-scale test. In *An analysis of linkage in quantitative inheritance* (ed. E. C. R. Rieve and C. H. Waddington), pp. 135–144. HMSO, London.

Chang R. Y. and Peterson P. A. 1995 Genetic control of resistance to *Bipolaris maydis*: One gene or two genes? *J. Hered.* **86**, 94–97.

Craig J. and Fajemisin J. M. 1969 Inheritance of chlorotic lesion resistance to *Helminthosporium maydis* in maize. *Plant Dis. Rep.* **53**, 742–743.

Falconer D. and Mackay T. F. C. 1996 *Introduction to quantitative genetics*. Pearson Education, India.

Faluyi J. O. and Olorode O. 1984 Inheritance of resistance to Helminthosporium maydis blight in maize (*Zea mays* L.). *Theor. Appl. Genet.* **67**, 341–344 .

Hayman B. I. 1958 The separation of epistatic from additive and dominance variation in generation means. *Heredity* **12**, 371–390.

Hayman B. I. and Mather K. 1955 The description of genic interactions in continuous variation. *Biometrics* **11**, 69–82.

Hettiarachchi K., Prasanna B. M., Rajan A., Singh O. N., Gowda K. T. P., Pant S. K. and Kumar S. 2009 Generation mean analysis of Turcicum leaf blight resistance in maize. *Indian J. Genet.* **69**, 102–108.

Hooker A. L. 1972 Southern leaf blight of corn—Present status and future prospects. *J. Environ. Qual.* **1**, 244–249.

Hooker A. L., Smith D. R., Lim S. M. and Beckett J. B. 1970 Reaction of corn seedlings with male-sterile cytoplasm to *Helminthosporium maydis*. *Plant Dis. Report.* **54**, 708–712.

Jinks J. L. and Jones R. M. 1958 Estimation of the components of heterosis. *Genetics* **43**, 223–234.

Kumar B., Hooda K. S., Gogoi R., Kumar V., Kumar S., Abhishek A. *et al*. 2016 Inheritance study and stable sources of maydis leaf blight (*Cochliobolus heterostrophus*) resistance in tropical maize germplasm. *Cereal Res. Commun.* **44**, 424–434.

Kump K. L., Bradbury P. J., Wisser R. J., Buckler E. S., Belcher A. R., Oropeza-Rosas M. A. *et al.* 2011 Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population. *Nat. Genet.* **43**, 163–168.

Lyimo H. J. F., Pratt R. C. and Mnyuku R. S. O. W. 2011 Heritability and gene effect estimates for components of partial resistance to grey leaf spot of maize by generation mean analysis. *Plant Breed.* **130**, 633–639.

Mallikarjuna M. G., Nepolean T., Hossain F., Manjaiah K. M., Singh A. M. and Gupta H. S. 2014 Genetic variability and correlation of kernel micronutrients among exotic quality protein maize inbreds and their utility in breeding programme. *Indian J. Genet. Plant Breed.* **74**, 166–173.

Manjunatha C., Gogoi R., Singh B., Jeevan B., Rai S. N. and Singh P. K. 2019 Phenotypic and physiological characterization of maize inbred lines resistant and susceptible to maydis leaf blight. *Indian Phytopathol.* **72**, 217–224.

Mather K. and Jinks J. L. 1982 *Introduction to biometrical genetics*, 3rd edition. Chapman and Hall, London.

Misra A. P. 1979 Variability, physiological specialization and genetics of pathogenicity in grarninicolous *Helminthosporia* affecting cereal crops. *Indian Phytopathol.* **32**, 1–22.

Pate J. B. and Harvey P. H. 1954 Studies on the inheritance of resistance in corn to *Helminthosporium maydis* leaf spot 1. *Agron. J.* **46**, 442–445.

Payak M. M. and Sharma R. 1983 Disease rating scales in maize in India. In *Techniques of scoring for resistance to diseases of maize in India*, pp. 1–4. All India Co-ordinated Maize Improvement Project, New Delhi.

Poole N. F. and Arnaudin M. E. 2014 The role of fungicides for effective disease management in cereal crops. *Can. J. Plant Pathol.* **36**, 1–11.

Prakash R. N., Zunjare R. U., Muthusamy V., Chand G., Kamboj M. C., Bhat J. S. and Hossain F. 2019 Genetic analysis of prolificacy in 'Sikkim Primitive'–a prolific maize (*Zea mays*) landrace of northeastern Himalaya. *Plant Breed.* **138**, 781–789.

Rai D., Kumar A., Kumar M and Prasad R. 2010 Field screening of maize genotypes against maydis leaf blight caused by *Helminthosporiun maydis* Nisicado and Miyake. *Int. J. Plant Prot.* **2**, 265–266.

Sharma P., Punia M. S., Kamboj M. C. and Mehra R. 2017 Generation mean analysis of maydis leaf blight resistance in maize. *Indian Phytopathol.* **70**, 302–306.

Sharma R. and Rai S. N. 2000 Assessment and quantitative analysis of losses due to *Dreschlera maydis* in maize. In *Proceedings of*

international conference on integrated plant disease management for sustainable agriculture, pp. 246–248. Indian Phytopathological Society, New Delhi.

Sharma R. and Rai S. N. 2005 Evaluation of maize inbred lines and hybrids for resistance to maydis leaf blight. *Indian Phytopathol.* **58**, 339–340.

Sharma R. C., Rai S. N., Mukherjee B. K. and Gupta N. P. 2003 Assessing potential of resistance source for the enhancement of resistance to Maydis leaf blight (*Bipolaris maydis*) in maize (*Zea mays* L.). *Indian J. Genet. Plant Breed.* **63**, 33–36.

Shashikumar K. T., Pitchaimuthu M. and Rawal R. D. 2010 Generation mean analysis of resistance to downy mildew in adult muskmelon plants. *Euphytica* **173**, 121–127.

Smith D. R. 1975 Expression of monogenic chlorotic-lesion resistance to *Helminthosporium maydis* in corn. *Phytopathology* **65**, 1160–1165.

Sullenberger M. T, Jia M., Gao S. and Foolad M. R. 2018 Genetic analysis of late blight resistance in *Solanum pimpinellifolium*

accession PI 270441: Heritability and response to selection. *Plant Breed.* **137**, 89–96.

Thompson D. L. and Bergquist R. R. 1984 Inheritance of mature plant resistance to *Helminthosporium maydis* Race O in Maize 1. *Crop Sci.* **24**, 807–811.

Ullstrup A. J. 1972 The impacts of the Southern corn leaf blight epidemics of 1970–1971. *Annu. Rev. Phytopathol.* **10**, 37–50.

Warner J. N. 1952 A method for estimating heritability. *Agron. J.* **44**, 427–430.

Wei J.-K. 1988 Pathological and physiological identification of race C of *Bipolaris maydis* in China. *Phytopathology* **78**, 550.

Wright S. 1952 The genetics of quantitative variability. In *Quantitative genetics* (ed. E. C. R. Reever and C. H. Waddington), pp. 4–41. H. M. Stat Office, London.

Zaitlin D., DeMars S. and Ma Y. 1993 Linkage of rhm, a recessive gene for resistance to southern corn leaf blight, to RFLP marker loci in maize (*Zea mays*) seedlings. *Genome* **36**, 555–564.

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