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Cytogenetic and pathological investigations in maize × teosinte hybrids: Chromosome behaviour, spore identification, and inheritance of maydis leaf blight resistance

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ABSTRACT

Maize (*Zea mays* L.), a globally important cereal crop, is susceptible to numerous diseases, with Maydis Leaf Blight (MLB) caused by *Bipolaris maydis* posing a significant yield threat. This study investigated cytogenetic behavior, pathogen identification, and inheritance of MLB resistance in a maize × teosinte (*Zea mays* ssp. *mexicana*) crosses. The experiment was conducted in the subtropical Gangetic region under natural MLB incidence. F_2 populations (n=381) derived from an agronomically superior but susceptible maize parent and a resistant teosinte parent were evaluated. Cytological analysis of F_1 pollen at metaphase I revealed irregular pairing, with $9^{II}+2^{I}$ or $8^{II}+4^{I}$ configurations, indicating partial chromosomal homology between parents. Pathogen isolation from symptomatic F_2 leaves on potato dextrose agar confirmed *B. maydis* through colony morphology and characteristic olivaceous brown, spindle-shaped, multi-septate conidia. Disease scoring (0 to 5 scale) showed 58.53% resistant (score 1), 33.07% moderately resistant (score 2), and minimal susceptibility. Chi-square analysis of resistance vs. susceptibility (score ≥2) fit a 9:7 ratio ($p<0.05$), suggesting two-gene complementary epistasis controls MLB resistance. Descriptive statistics indicated low disease incidence (8.39%) and severity (9.41%) in the population. The results confirm teosinte as a valuable genetic resource for MLB resistance breeding in maize. Future studies under artificial epiphytotic conditions are recommended to validate findings and improve selection efficiency.

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INTRODUCTION

Maize, a staple food crop, is a versatile crop that is widely cultivated worldwide. It is a monoecious species with separated male and female organs at the plant level, making its genetic diversity remarkable due to cross pollination. Maize ranks third in the world after wheat and rice in area and production, but ranks first in productivity. The diversity of maize has been captured in various forms, including inbred lines, native land races, and open pollinated populations. Maize is an excellent model for basic research, as it is nicked as Drosophila of the plant kingdom.

Maize suffers from about 110 diseases on a global basis caused by fungi, bacteria, and viruses. The disease spectrum varies in different agro-climatic zones, and about 13.2% of the economic produce of maize is estimated to be lost annually due to diseases alone. Among the several foliar diseases, the one caused by the genus *Helminthosporium* are the most severe.

There are four species in the genus *Helminthosporium* viz., *Exserohilum turcium* (Pass.) Leonard and Suggs (Synonym: *Helminthosporium turcicum* pass.), *Helminthosporium maydis*, *Helminthosporium carbonum* (Ullstrup), *Helminthosporium* leaf spot, and *Helminthosporium rostratum (*Drechsi). Maydis leaf blight (MLB), also known as Southern corn leaf blight, is a fungal disease caused by *Drechslera maydis* or *Bipolaris maydis*, is reported from maize growing regions including India. It has the ability to reduce the yield up to an extent of 70% in susceptible cultivars. MLB may cause significant grain yield losses (Thompson and Bergquest, 1984), up to 70%. Losses in the grain yield due to maydis leaf blight disease ranging from 6.40 to 36% have been reported from different parts of the country. There are different races (O, T, and C) identified in maize host, including sweet corn. Numbers of studies have been conducted on the genetic of MLB resistance in maize.

MLB resistance genes were recessive in nature, and only few studies reported resistance to Race 'O' was quantitatively inherited primarily with partially dominant gene action. QTL for MLB resistance was mapped on chromosome no. 3, 4, 6, 8, and 9, but bin location 3.04 contributed significantly for MLB resistance. In India, MLB inheritance and mapping are missing in maize. Numerous genomic regions for MLB resistance were found in temperate germplasm.

A comparison of the results of MLB resistance QTL suggested that bins 6.01 are the main region for MLB resistance QTL (Thompson and Bergquist, 1984; Zaitlin *et al.*, 1993; Balint-Kurti *et al.*, 2008; Chang and Peterson, 1995).

MATERIALS AND METHODS

The study was conducted at an agricultural research farm located in the subtropical Gangetic region at 28.98° N latitude, 77.70° E longitude, 225 m above mean sea level. The climate during the crop growth period was warm and humid, with an average maximum temperature of 29.1°C, relative humidity of 70.17%, and mean monthly rainfall of 121.75 mm, favorable for Maydis leaf blight (MLB) development. Experimental material comprised a cross between maize (*Zea mays* L.) and teosinte (*Zea mays* ssp. *mexicana*), where maize was agronomically superior but susceptible, and teosinte was resistant. F2 populations were developed for evaluation. Cytological studies were performed on F1 plants using tassels collected before emergence, fixed in Carnoy's solution (3 ethanol:1 glacial acetic acid), and processed using the acetocarmine squash technique to observe meiotic stages such as pachytene, diakinesis, and metaphase. For pathogen isolation, symptomatic leaves were surface sterilized, plated on potato dextrose agar containing streptomycin, and incubated at 25°C. Single-spore cultures were prepared, and Bipolaris maydis was confirmed microscopically by identifying olivaceous brown, spindle-shaped, 5–11 septate conidia (15–20 × 70–160 µm) with bipolar germination. Disease scoring was carried out using a 0–5 scale (Payak and Sharma, 1983, modified), classifying plants from highly resistant (0) to highly susceptible (5). Data were analyzed using descriptive statistics, and segregation patterns in F2 were tested against Mendelian ratios using the chi-square test. Disease incidence (%) was calculated as the proportion of infected plants, while percent severity index (PSI) was computed based on numerical ratings and leaf area affected.

Disease incidence

Disease incidence was assessed as proportion of plants showing symptoms in the field. The plants stand showing MLB symptoms were counted and expressed as a percentage of the total number of stands per plot using the formula (Nwanosike *et al.*, 2015).

DI(%)={(Number of diseased plants)/(Total number of plants observed)}×100
Where, DI = disease incidence

RESULTS AND DISCUSSION

The results obtained from the cytological, pathological, and genetic evaluation of the maize × teosinte cross have provided valuable insights into chromosome behavior during meiosis, disease identification, and the inheritance pattern of resistance to Maydis Leaf Blight (MLB) and Southern Corn Leaf Blight (SCLB). Sample is collected just before tassel emergence from the whorl, preserved in freshly mixed fixative. After 1 day at room temperature, store at 4°C or -10°C, either in original fixative or in 70% ethanol. For younger stages, two to four pre-emergent tassels were collected and grouped roughly according to developmental stage after microscopic examination of microspores from several anthers. Partially or fully starch-filled young pollen were obtained using newly emerged tassels.

The slide of metaphase stage was prepared form the F_1 plant pollen to observe the behavior of chromosomes between maize and teosinte (Fig. 1). In the maize × teosinte cross, there were 20 chromosome at metaphase with nine bivalent and two univalent ($9^{II} + 2^{I}$) (Fig. 2) or eight bivalent and four univalent ($8^{II} + 4^{I}$) were observed. It indicated that one or two chromosome between maize and teosinte had not have the homology. *Zea mays* pairs with members of at least one basic genome tetraploid tripsacum and in hybrid involving octaploid tripsacum all ten chromosome of the basic maize genome frequently compete successfully in synapsis with tripsacum chromosome. The hybrid combined with 36 tripsacum and 10 maize chromosomes are female fertile. Similarly, perennial teosinte (4n) was less fertile when pollinated with 4n *Zea mays* then when selfed. In the 4n intergeneric hybrid, nearly every chromosome number from 29 to 44 was found (Shaver, 1962). The meiotic behavior of the chromosome in various teosinte maize hybrids indicates that homologous chromosomes usually pairs and that the unpaired univalent chromosomes are distributed at random to the daughter nuclei. The division of these univalent chromosomes occurs in either the first or the second division (Longley, 1934).

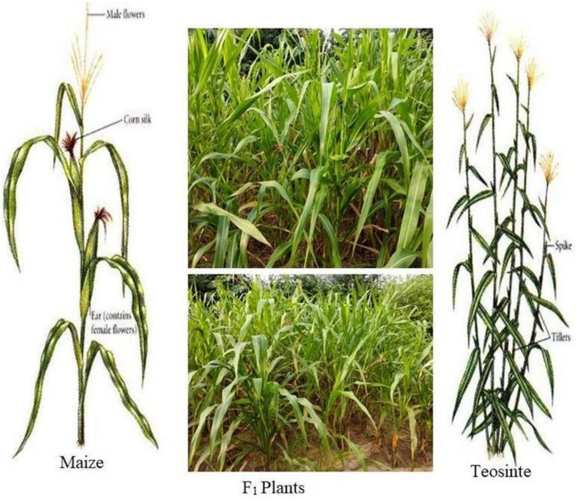
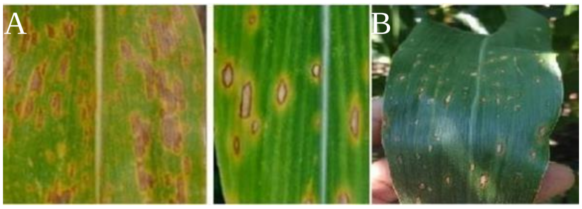


Fig. 1. Field view of F_1 plants of cross between maize and teosinte

Fig. 2. Cytology of meiosis (metaphase of F_1 plant pollen grain)

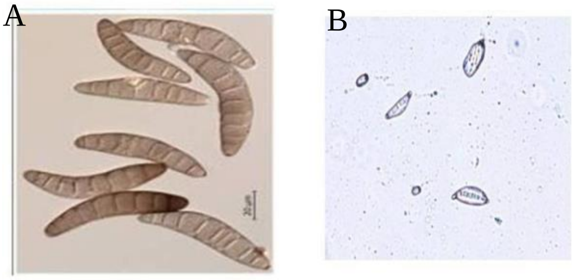
Identification of maydis leaf blight (MLB) spores on F_2 plants

The $F_{1:2}$ seeds were planted in isolation. There are 381 F_2 plants were survived in the field. In the present study, the artificial inoculum of the MLB spores was not carried out while disease appeared naturally. Symptoms of disease appeared on leaves. The identification of the *Helminthosporium maydis* fungus and its pathogenicity was studied taking the leaf samples from the F_2 maize plants. Based on the shape and size of the lesions, the following typical symptom types were noted in the infected maize leaves collected from the field. Fusiform or elliptical lesions, longitudinally elongated lesions, restricted by veins, developing into long strips of lesions, long and narrow linear lesions, punctiform or minute necrotic lesions (Fig. 3).



A. Large lesion on middle leaf, B. Several small lesions on leaf

Fig. 3. Symptoms of MLB found on the F_2 plant of maize × teosinte



A. Dark gray spore (Sun *et al.*, 2020), B. Spore identified under microscope

Fig. 4. Identification of MLB spores on F_2 leaves

In general, more than one symptom was observed in one sample site or even in the entire field. Distinct colony morphology on potato dextrose agar (PDA) was observed for different plants after 7 days. The isolates from F_2 plants produced dark gray to black colonies with abundant sporulation. Similarly, the colonies produced by other isolates were blackish gray with marginally pigmented zones, abundant sporulation and irregular margins. The isolates from some plants produced gray to grayish dark colonies with sparse gray aerial mycelia, and the colonies showed a cottony appearance. The colonies exhibited white to slightly gray mycelia with a fluffy cottony appearance. The colonies produced by isolates were slightly gray to dark gray, with abundant aerial mycelia. The slide was prepared and a conidial morphology was observed under the microscope. The conidia shapes was curved or slightly straight indicated the presence of *Helminthosporium maydis* on the leaves of F_2 plants (Fig. 4). Sun *et al.* (2020) observed the typical symptoms caused by *B. maydis* were elongated strip lesions, or fusiform, elliptical lesions.

Statistical analysis

The descriptive statistical analysis of the disease scoring data of MLB/SCLB was carried out.

The mean, standard error, standard deviation, sample variance, Kurtosis, Skewness, range were calculated (Table 1). The mean disease score was observed 1.43±0.03 with the variance 0.37. Disease incidence for MLB disease was observed 8.39 and disease severity was observed 9.41 for F_2 plants derived from maize × teosinte.

Table 1. Descriptive statistics for disease score of MLB in F_2 Populations

Sl	F_2 Population	Maize × teosinte
	Disease score data	MLB/SCLB
1	Mean	1.434210526
2	Standard error	0.03132886
3	Standard deviation	0.610711906
4	Sample variance	0.372969032
5	Kurtosis	1.629555686
6	Skewness	1.301425214
7	Range	3
8	Minimum	1
9	Maximum	4
10	Count	381
11	Disease incidence	8.39
12	Disease severity	9.41

Screening of F_2 population and its phenotyping for SCLB

The maize and teosinte were taken for this study. One of the parental varieties *viz.*, maize was moderately susceptible to Southern Corn Leaf Blight (SCLB) while teosinte, the wild progenitor of maize was resistance to SCLB. These two lines were space planted during Kharif season. The F_1 was developed using maize as the female parent and teosinte as the male parent. Simultaneously, both the parental lines were maintained through selfing as well as by intermating in isolation to avoid contamination. The F_1 hybrids and parents were harvested carefully and the seeds of all F_1 cobs were mixed. The F_1 seeds were planted in rows and allow to controlled selfing (Fig. 1). The F_1 plants showed resistance to SCLB indicated that the resistance is dominant. The F_1 plants were intermated to generate selfed F_2 seeds. A total 35 F_1 plants were selfed carefully and harvested.

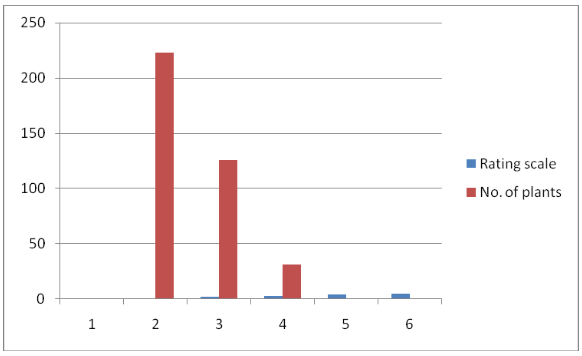


Fig. 5. Disease frequency graph of MLB/SCLB in PVT x Teosinte cross

The bulk $F_{1:2}$ seeds space planted to raise about 400 F_2 plants for phenotypic characterization. Out of 400 F_2 plants, only 381 plants could be survived and phenotyped (Fig. 5). The data on SCLB was recorded on the basis of the disease rating score given by sharma and payak, 1990.

Table 2. Disease rating of various diseases in PVT x Teosinte cross in F2 plants

Disease	PVT ×TEOSINTE	MLB/SCLB
score	Disease reaction	No. of plants observed
0	Highly resistant	0
1	Resistant	223
2	Moderately resistant	126
3	Moderately susceptible	31
4	Susceptible	1
5	Highly susceptible	0
Total		381

The F_2 Plants showed variation in disease rating. Out of 381 F_2 plants screened for MLB disease, 223 plants were observed 1 rating of disease score, 126 plants showed 2 rating of disease score, 31 plants showed 3 rating of disease. However, 1 plant was observed 4 rating of disease score (Table 2). Surprisingly, none of the plants showed 5 rating of disease score.

Analyses of disease score of various genotypes have shown significant variation between them. Almost 75% of F_2 genotypes in maize × teosinte were recorded with a score of <3.0.

This reveals that the disease screening was consistent and effective in the location. The disease score data of 381 F_2 seeds showed skewed distribution. Further, Chi-square analysis was carried out in the F_2 plants disease score data. The 0 and 1 rating was considered as resistance and 2 to 5 rating was considered as different classes of susceptible or as susceptible. The chi- square analysis was tested for all possible ratios for goodness of fit. In F_2 out of 381 plants, 223 showed resistant reaction and 158 plants were susceptible which indicated a good fit to 9 : 7 ratio with a probability of <0.05 (Table 3). It indicated that the inheritance of resistance to MLB controlled by two gene with epistasis of complementary gene action.

Kumar *et al.* (2016) 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. Karimishahri and Sharma (2017) studied 15 hybrids and observed that the mean effects were highly significant for the eight resistant crosses the *a* and *d* effects were significant for only 4 crosses of the *a* and *d* effects.

Though each of the three digenic epistatic effects *dd* was playing a greater role followed by *ad* effects in the resistance crosses. In V-335 x V-13 cross, *dd* effect was significant and negative. The relatively high magnitude and positive effects of *dd* in most of the crosses indicated that these interactions was enhancing susceptibility, while considering the CM-128 x V-335, CM-128 x V-13, CM – 128 x V-128, CM-128 x V-17 and V-327 x V- 335 crosses were observed that *ad* and *dd* effects were highly significant and positive value.

Table 3. Chi-square test for testing null hypothesis of segregation of MLB resistance and susceptible for various ratio in F$_2$ Populations of maize × teosinte cross

			F$_2$ plants		
Ratio	D.F	Observed	Expected	Chi-square value	Significance
3	1	223	285.75	13.78	$p<0.05$
1		158	95.25	41.34	
Total		381	381	55.12	
9	3	223	214.3125	0.35	$p<0.05$
3		126	71.4375	41.67	
3		31	71.4375	22.89	
1		1	23.8125	21.85	
Total		381	381	86.76	
9	1	223	214.3125	0.35	$p<0.05$
7		158	166.6875	0.45	
Total		381	381	0.80	
9	2	223	214.3125	0.35	$p<0.05$
3		126	71.4375	41.67	
4		32	95.25	42.00	
Total		381	381	84.02	
12	2	349	285.75	14.00	$p<0.05$
3		31	71.4375	22.89	
1		1	23.8125	21.85	
Total		381	381	58.74	
9	2	223	214.3125	0.35	$p<0.05$
6		157	142.8750	1.40	
1		1	23.8125	21.85	
Total		381	381	23.60	
15	1	380	357.1875	1.46	$p<0.05$
1		1	23.8125	21.85	
Total		381	381	23.31	
1	2	223	95.25	171.34	$p<0.05$
2		157	190.50	5.89	
1		1	95.25	93.26	
Total		381	381	270.49	

The inheritance of resistance in maize (*Zea mays*) to Southern corn leaf blight caused by *Bipolaris maydis* race O was studied in 1981 and 1982. In 12 families derived from crosses of nine resistant inbreds and three susceptible inbreds, additive genetic effects were highly significant, accounting for 49.0 to 96.9% of the total variation. Significant dominance genetic effects were detected in all but one family, accounted for only 2.5 to 46.9% of the total variation. Pedigree and recurrent selection methods should be effective in breeding for improved resistance to Southern corn leaf blight (Burnette and White, 1985). To identify new resistance sources and establish durability of known resistance sources, 35 short-duration maize inbred lines were evaluated against MLB under natural conditions. Nineteen inbred lines exhibited resistance against MLB and 10 inbred lines found resistant to both TLB and MLB (Chandrashekara, 2014). Twelve maize genotypes were tested against Southern corn leaf blight under laboratory and field conditions. According to disease severity scale (0–5) inbreds SP-3 and NCML-73 were found highly resistant; Local-W moderately resistance (Mubeen *et al.,* 2017). In present investigation, limited availability of the F$_{1:2}$ seeds for that reason the F$_2$ plants were evaluated under natural disease condition. The above result may be confirmed to evaluate same set of seed under artificial epiphytic condition in future.

CONCLUSION

The study highlights that resistance to Maydis Leaf Blight (MLB) in a maize × teosinte cross is governed by two genes showing complementary epistasis, with segregation fitting a 9:7 (resistant: susceptible) ratio. Cytogenetic observations of F$_1$ pollen at metaphase I revealed irregular chromosome pairing, suggesting partial genomic homology between maize and teosinte. The identification of *Helminthosporium maydis* in F$_2$ plants confirmed natural infection under field conditions. These findings indicate that teosinte can serve as a valuable source of MLB resistance in maize improvement programs. Future evaluation under artificial epiphytic conditions is recommended to validate and enhance selection efficiency.

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