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# Accumulation of desirable alleles for southern leaf blight (SLB) in maize (Zea mays L.) under the epiphytotic of *Helminthosporium maydis*

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# Abstract

To accumulate desirable alleles for southern leaf blight (SLB) resistance and for grain yield, 76 inbred lines were evaluated under the epiphytotic of *Helminthosporium maydis*. Highly significant differences (P<0.01) were observed among the lines for SLB severity, area under disease progress curve (AUDPC) and grain yield. The broad sense heritability ( $h_{BS}$ ) obtained for SLB was 0.59, whereas it was 0.91 and 0.61 for AUDPC and grain yield, respectively. Selection differential (S) was positive for grain yield (835 kg ha<sup>-1</sup>) and was negative for SLB severity (-0.61) and AUDPC (-164.7), indicating a declining trend for these two disease parameters. On the other hand regression analysis also showed a negative effect for disease severity on the total grain yield and R<sup>2</sup> value was 0.1933. A negative correlation was observed for yield and SLB showing the importance of increase resistance to boost up the production of maize crop. The cycle of recurrent selection (RS) showed a reliable gain from selection while the expected response showed the extent of improvement in the next generation for SLB, AUDPC and other related parameters. On the basis of frequency distribution curve, the desirable lines for SLB resistance have been shown in different categories. This study revealed the effectiveness of RS for enhancing resistance against SLB and improvement of grain yield in maize crop. Using these parameters sufficient evidence were found for required variability in maize germplasm that can be manipulated in desirable direction for the welfare of humanity.

Keywords: maize, Area under disease progress curve (AUDPC), grain yield, recurrent selection, Southern leaf blight, disease resistance.

Abbreviations: SLB\_Southern leaf blight, AUDPC\_Area under disease progress curve,  $h_{BS}$ \_Broad sense heritability,  $R^2$ \_Coefficient of determination, RS\_Recurrent selection, S\_Selection differential,  $R_e$ \_Response to selection, CV\_Coefficient of variation, SW\_Sarhad White.

# Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops of the world grown in the irrigated and rainfed areas. It belongs to family *Gramineae* and genus *Zea*. Maize grows best in deep well aerated, warm, loam soil, rich in organic matter and with a high nitrogen, phosphorus and potassium content. Optimum crop performance is achieved under moderately high summer temperature with warm nights and adequate rainfall that is evenly distributed during the growing season. Maize is the world's most extensively grown crop with an annual worldwide production of 822 and 817 million tons in 2008 and 2009, respectively (http://faostat.fao.org). It has been estimated that more than half of the increased demand in the world food in term of cereals as a whole will be produced from maize farmers and consumers (Yan et al., 2011). By 2050, the predicted 9 billion people in the world

will require almost 70% more food than today's population and a large proportion of the increased demand will definitely come from developing countries (FAO 2009). Maize is vital model plant for molecular studies like cytogenetics, genetics, genomics and functional genomics (Ali and Yan, 2012). Thus, there is a remarkable innovation stream for maize breeders to utilize in their attempts to significantly increase maize production in an environmentally sensitive way (Yan et al., 2011). Several factors effects the total production of maize, like favorable weather conditions, cultivation of hybrids, balance use of inputs and the use of adopted objective techniques of yield estimation. Despite of the fast growing achievements in the field of maize breeding, still in the developing countries yield, as compared to the developed countries, is very low.

One of the major factors for yield loss is its sensitivity to several diseases and the reason is insignificant work in the field of resistance breeding. The current scheme of study has been designed to give some basic information for developing numerous disease resistant hybrids and varieties harboring resistance genes. Almost 65 pathogens infect maize crop (Rahul and Singh, 2002). The most important diseases causing drastic reduction in maize yield are Southern leaf blight (SLB), northern leaf blight (NLB), gray leaf spots (GLS) and different types of rust. SLB or Maydis leaf blight (MLB) is one of the most important disease of maize caused by fungus Cochliobolus heterostophus (Bipolaris maydis) and constitutes a major threat to maize production worldwide (Kump et al., 2011). This fungus reduces crop stand and yield substantially under epiphytotic conditions. Several factors such as susceptible varieties, crop growth stage and planting time contribute to high disease intensities and ultimate yield losses (Ali et al., 2011a). The extent and severity of SLB varies from season to season and infection initiated late in the season caused considerable losses in grain yield of maize. These losses were 9.7% and 11.7% in 1975 and 1976, respectively (Gregory et al., 1978). It is reported that rainfall, relative humidity and temperature are critical factors in spreading of Bipolaris maydis (Peet and Marchetti, 1972). In many warm (20°-32°C) and moderately humid environment of the world, this disease has been potentially damaging the crop and causing significant losses. SLB spreads from the basal leaves to the developing ear and then to the flag leaf of the maize plant. Two races, race O and race T, are responsible for causing this disease in Pakistan, while race C has been reported only in China (Wei et al., 1988). In 1970's an epidemic was caused by race T in maize with Texas male sterile cytoplasm in most maize-growing areas of the USA but maize with normal cytoplasm was resistant to the pathogen. According to Hull (1952), "recurrent selection was meant to include re-selection generation after generation, with interbreeding among selected progenies to provide genetic recombination". The purpose of RS is to gradually increase the frequency of favorable alleles in a population while maintaining genetic variability. To increase maize yield per unit area, it is imperative for plant breeders to develop maize varieties and hybrids that are high yielding, widely adaptable, early maturing, disease resistant, responsive to improved production practices and adjustable in the existing cropping patterns (Ali et al., 2011b). As up till now no known genes confer complete immunity to this disease therefore; maize breeders rely on polygenic, quantitative resistance to SLB (Kump et al., 2011). In view of the economic importance of maize crop, the present study was conducted to assess the effect of SLB on grain yield and to increase the frequency of favorable alleles for resistance to SLB and grain yield in maize, using S1 line RS breeding procedure. Some other objectives of the study were to estimate broad sense heritability (h<sup>2</sup><sub>BS</sub>), selection differential (S) and expected response to selection (Re) as well as the correlation response of SLB on grain yield. Furthermore, to figure out the most desirable genotypes harboring disease resistance alleles that may be used in future breeding programs to develop resistant hybrids and varieties.

## Results

# Disease Severity of SLB

The analysis of variance for SLB scores revealed highly significant differences ( $P \le 0.01$ ) among the  $S_1$  lines. Broad sense heritability ( $h_{BS}$ ) calculated from variance component

was 0.59 (Table 1). The mean value of disease severity for the selected  $S_1$  lines mean (0.69) was less than the for overall population mean (1.30), which resulted a negative value of the selection differential (-0.61). R<sub>e</sub> or gain from selection value was also negative (-0.36, Table 1). Regression coefficient (b) of MLB for  $S_1$  lines is presented in Figure 1, while the frequency distribution for MLB based on the last reading of disease severity recorded for S<sub>1</sub> lines is given in Figure 2. The two checks Jalal and Poineer-3025 were outstanding for resistance to SLB, as they showed no symptoms of disease and exhibited zero value for MLB (Table 2). The top four S<sub>1</sub> lines showing slight lesions of the disease under discussion were among the selected  $S_1$  lines having minimum value of 0.50, each are presented in Table 2. Maximum disease score (1.5) was observed for the selected line E-67, which was the same disease score as observed for the original base population (Table 2).

#### Area under disease progress curve (AUDPC)

The analysis of variance for AUDPC showed highly significant differences ( $P \le 0.01$ ) among the S<sub>1</sub> lines (Table 1). Among the selected S<sub>1</sub> lines minimum AUDPC (263) was recorded for E-22 while maximum (595) was recorded for E-67 and SW (Table 2). The h<sub>BS</sub> calculated from variance components was 0.91. Negative value of S (-164.71) and R<sub>e</sub> (-149.6) were obtained for AUDPC. Co-efficient of variation (18 %) was observed for AUDPC during analysis (Table 1). The increase and decrease regarding AUDPC for different lines and check has been shown in Figure 3. This figure showed a reasonable amount of variation in the maize lines and gave a chance to select different lines for further improvement of maize crop against the most dangerous disease in the world.

#### **Reproductive traits**

The resultant product of the entire yield components represented as grain yield (kg ha<sup>-1</sup>), and all the index parameters for grain yield showed highly significant differences ( $P \le 0.01$ ) among the S<sub>1</sub> lines (Table 1). The total yield of the selected lines and their disease severity data has been compared and shown in Figure 4. The h<sub>BS</sub> was moderately high for grain yield (0.61) while lowest heritability was obtained for grain weight (0.36) followed by kernel row number (0.48, Table 1. Regarding grain yield, mean value of the population was 3248.38 kg ha<sup>-1</sup>, which was less than the selected  $S_1$  lines means (4084 kg ha<sup>-1</sup>). The selection differential was 835 kg ha-1 and the value of expected response to selection was 508.12 kg ha<sup>-1</sup>. The S and  $R_e$  concerning all the traits are given in table 1. Among  $S_1$ lines maximum grain yield was obtained for the selected E-64 (5.502 ton  $ha^{-1}$ ) followed by E-65 (5.028 ton  $ha^{-1}$ ). Minimum grain yield (3.050 ton  $ha^{-1}$ ) was obtained by S<sub>1</sub> line E-51. The original population of Sarhad White (SW) showed yield of 4.331 ton ha<sup>-1</sup>. During this study hybrid P-3025 out classed the experimental material in terms of production (8.127 ton ha<sup>-1</sup>) and having maximum kernel row number and grain weight. The data pertaining to 300-grain weight showed that the 300-grain weight has a remarkable role in increasing the grain yield in relation with other yield related components. Maximum 300-grain weight was observed for P-3025 (99 g) and its yield was highest among all the genotypes. The Coefficient of variation (CV) showed the reliability of the experiment and is summarized in Table 1 for all the selected traits.

**Table 1.** Mean squares (MS), heritability  $(h_{BS}^2)$ , selection differential (S), expected response (R<sub>e</sub>) and coefficient of variation (CV) for MLB AUDPC, 300-grain weight (g, WT), Kernel row cob<sup>-1</sup> (KR) and grain yield in maize.

Traits	MS	h <sup>2</sup> <sub>BS</sub>	S	R <sub>e</sub>	CV
MLB	0.87**	0.59	-0.61	-0.36	26
AUDPC	86056**	0.91	-164.7	-149.6	18
Yield	1979255**	0.61	835	508.12	21
WT	283**	0.36	5.93	2.12	16
KR	469**	0.48	1.02	0.49	13

Note: SLB, AUDPC (scale-0-5), 300-grain weight (WT) (g), Kernel row  $cob^{-1}$  (KR) and grain yield (ton ha<sup>-1</sup>) of maize S<sub>1</sub> lines.



**Fig 1.** This figure shows the regression co-efficient (b) of southern leaf blight for disease severity in relation with the toatal grain yield for all the  $S_1$  lines with a disease severity scale of 0-5.

## Discussion

#### Disease resistance

Plants have developed a stunning array of structural, chemical, and gene-based defenses designed to identify invading organisms and stop them prior of causing extensive damage. Disease resistance is the major aim of the breeding community and to provide such genotype having a resistant genetic background is the primary goal of plant breeding. The resistant material will drastically reduce the inputs of the farming community and will ultimately increase the grain yield. The variability observed in this experiment for all the traits showed that maize possess remarkable genetic diversity for almost all traits of economic importance including disease resistance (Ali and Yan, 2012). Maize exhibits great potential for disease resistance genes and identification of the source of resistance with accumulation of all the resistant genes are extremely important to figure out desirable genotypes for a successful breeding program. The inbred lines regardless of the nature of their cytoplasm were significantly different in their responses to H. maydis (Bekele and Sumner, 1983). Negative value of the selection differential indicated a decreasing trend in SLB disease severity but it has been stated that the severity of disease varies extremely from one environment to the other (Welz and Geiger, 2000). This could be the result of selection for resistant lines against the disease. It has been reported that recurrent selection for SLB resistance significantly reduced the disease severity and plenty of resistant alleles can be collected in improved version of genotypes after several cycles (Shah et al., 2006). Negative value of both, response to selection and selection differential, indicated a declining trend in SLB disease severity. This could be the result of selection for lines resistant against the disease. Moreover, it was obvious that the traits under investigation were highly heritable and can produce more repeatable phenotypes at different locations. The high heritability and negative value of selection differential and response to selection indicated that additive

genes control the disease. Sheih and Lu (1993) reported that additive genetic effects accounted for a major part of the total variation in resistance among the genotypes. Another possibility might be that the  $S_1$  lines have high concentration of proteins, lignins, phenolics and callose, providing extra source of resistance to maydis leaf blight. Fig 1 depicts regression co-efficient (b) of SLB for  $S_1$  lines while the frequency distribution for SLB based on the last reading of disease severity recorded for the genotypes are given in Figure 2. The two checks Jalal and hybrid P-3025 were performed remarkably well for resistance against SLB as they showed no symptoms of disease and exhibited zero value for AUDPC. Five entries (E-14, E-14, E-31, E-4 and E-65) S<sub>1</sub> lines showing slight lesions of the disease under discussion were among the selected  $S_1$  lines, having a value of 0.50 each showing the effectiveness of recurrent selection as a breeding procedure for improving maize against SLB with the high potential of yield. The AUDPC estimates the area under the actual infection curve. It is expressed in %-days (accumulation of daily percent infection values) and interpreted directly without transformation. The higher the AUDPC, the more susceptible is the clone or variety. The AUDPC is calculated from all the three ratings at different time thus leading to a more accurate phenotypic evaluation. The loss of active leaf area results in less photosynthetic available region during the grain filling stage which eventually results in producing smaller kernels. This reduction may eventually contribute to the overall yield losses. The advantage of using AUDPC was that it was simple to calculate, uses multiple evaluations, and did not rely on transformations, which are often based on assumptions about the distribution of the data points (Campbell and Madden, 1990). The  $h_{BS}^2$  calculated from variance components was 0.91 for AUDPC showed that this trait was highly affected by genetic makeup of the plants with a mild influence of the environment. It is, therefore, suggested that consistent selection of resistant lines and their recombination will regularly decrease the frequency of deleterious alleles responsible for susceptibility to this

Genotypes	SLB	AUDPC	Grain yield	KR	WT
E-1	0.5	280	3.871	12	78
E-10	0.5	280	3.060	12	60
E-14	0.5	280	4.148	12	74
E-22	0.5	263	4.687	11	92
E-31	0.5	280	4.330	13	90
E-44	0.5	280	4.611	15	83
E-51	1	385	3.050	12	79
E-55	1	490	3.673	11	65
E-64	1	315	5.502	13	73
E- 65	0.5	280	5.028	12	81
E-67	1.5	595	4.149	12	79
E-71	0.5	280	3.212	10	69
E-72	0.5	280	3.770	14	93
S. white	1.5	595	4.331	14	89
Pahari	0.5	280	3.854	14	74
Azam	0.5	175	4.231	13	83
Jalal	0	0	4.864	15	94
P-3025	0	0	8.127	17	99

 Table 2. Mean values of 13 selected S1 lines out of the total 76 evaluated lines and 5 checks of maize genotypes for selected traits studied during the year 2006-2007.

Southern leaf blight (SLB), Area under disease progress curve (AUDPC), grain yield (ton ha-1), kernel rows cob-1 (KR) and 300 grain weight (WT) of selected maize S<sub>1</sub> lines



Fig 2. Frequency distribution for maydis leaf blight (MLB) based on last reading of disease severity for the inbred lines.

disease in each cycle. But keeping one important thing in mind for accurate phenotyping is that the readings for all the parameters related to disease resistance, should be continued until the susceptible cultivars reach high levels of disease, nearly 100 %, so that it should be clear that how much loss it can cause. For this purpose data collection in the current study was based on regular interval and multiple times to depict a clear idea about the resistant lines. The present investigation corroborate with previous findings that inbreds, regardless of the nature of their cytoplasm, were significantly different in their responses to H. maydis (Bekele and Sumner, 1983). The fact that most characterized resistance genes are inherited as single genetic loci allows plant breeders to assemble several resistant genes in a single cultivar of economic importance. The resistance lines identified in this study can be used further in breeding programs to increase resistance against SLB.

# Economic yield

Many economically significant characters in maize are quantitatively inherited, such as seed yield, seed protein, quality traits, and resistance to stresses, including numerous diseases (Wisser et al., 2006). Classical breeding methods employing phenotype-based selection have been used to make significant selection gains for quantitative trait improvement over decades (Fu et al., 2009). The genotypes used in this experiment also showed enough level of variability and through RS the desirable genotypes were inter-mated to fix for the desirable genes in a single improved version. Once a completely resistant genotype is obtained through RS then the genes can be transferred to several cultivars by crossing through different breeding procedures. The broad sense heritability of high magnitude regarding grain yield was observed and it showed that this trait could be improved in the following generations. Mean of the population was smaller than that of the selected  $S_1$  lines and a high level of the selection differential has been observed for several traits of economic importance. This showed the ability of diverse germplasm in any breeding program, the more the variability for a trait the more desirable lines can be selected for future breeding to enhance the genetic architecture of maize against several diseases. This approach can easily be followed for enhancing the resistance of maize germplasm against several other diseases and including more cycles of RS and more diverse population can solve the most abstruse phenomenon of disease resistance. Higher expected response to selection was a little higher than the results of

Table 3. Basic description of the genetic materials used in the experiment.

Genotypes	Type	Origin	Kernel type	Stature	Maturity	Pedigree
SW	OPV	Pakistan	Semi-dent	Tall	Medium	[Vikram (B11 x B37)] x Akbar
Phari	OPV	Pakistan	Semi-dent	Medium	Tall	Shaheen x PS-7930
						Pirsabak 7930 x Zia x pirsabak-
Azam	OPV	Pakistan	Flint	Medium	Medium	7930
Jalal	OPV	Pakistan	Flint	Tall	Long	Azam x CHSW
P-3025	Hybrid	Pakistan	Flint	Tall	Long	Poineer



Fig 3. Area under disease progress curve for all the selected genotypes along with the checks.



Fig 4. Comparison of total grain yield, number of rows per cob (KR) and southern leaf blight (SLB) of the selected lines along with checks based on average data of disease severity for the inbred lines.

Carangal et al. (1971) who observed expected response (446 kg ha<sup>-1</sup>) of recurrent  $S_1$  selection in high yielding maize synthetic. The expected response shows the improvement of breeding material in the coming generation, so resistant lines including in the recombination phase will provide more variability in the coming generation to recombine the desirable alleles in few lines for targeted breeding program. The reduction in yield resulted from the loss of active photosynthetic leaf area caused by Southern leaf blight. The higher the lesion on the leaf the more photosynthetic area is destroyed by the pathogen causing maximum yield losses.

The interaction of SLB with several yield related parameters showed that the plants lost their photosynthetic active area and especially the ability of grain filling as the grain weight was low for the lines having high level of susceptibility. Grain yield reduction was related to destruction of photosynthetic tissues and to an earlier and more severe development of disease (Kim et al., 1974). Furthermore, the kernel row numbers drastically influenced the total grain yield and the lines having high level of resistance performed well for that trait.

## Materials and methods

## Site of study

The experiment was conducted at Agricultural Research Farm, Khyber Pakhtunkhwa Agricultural University Peshawar, Pakistan. The soil type of the experimental station is silt loam/alkaline with pH of 8.2-8.3 and the elevation is 365.5m. Standard cultural practices including irrigation, fertilizer application, hoeing and thinning were carried out throughout the growing seasons. One cycle of RS was completed during this study using three generation (phases) in a single year.

## Preparation of inoculum

Maydis infected leaves were collected from plants at harvest during fall of 2005. These infected leaves were sun dried and kept in oven for 24 hours at 60°C. After 24 hours, the leaves were ground with the help of a grinder to make inoculum in powdered form (Ali et al., 2011a). This powder was preserved for artificial inoculation in the following seasons under control environment.

## Preparation of genetic materials ( $S_1$ line production phase)

Improvement in an open pollinated commercial, broad genetic base maize variety has been initiated in Plant Breeding and Genetics Department of Agriculture University, Peshawar using  $S_1$  recurrent selection breeding procedure. Sarhad White (SW) is a late maturity, medium tall stature, semi dense tassel with profuse branching open pollinated variety having semi-dent white kernel grain. One hundred and fifty five half-sib families from improved version of Sarhad White (SW-C<sub>2</sub>) were selected and planted during 2006 (February-June). About 400 S<sub>1</sub> were generated using manual self-pollination procedure. Out of these 400 lines, 76 lines were selected on the basis of their performance for disease resistance to SLB and seed setting at harvest for further evaluation.

## Field evaluation $(S_1$ line evaluation phase)

A total of 76  $S_1$  lines selected in June 2006 along with 5 check entries (four commercial open pollinated maize varieties i.e. Sarhad White, Pahari, Azam, Jalal and a multinational hybrid, Poineer-3025) were planted for screening during July 2006 in a square lattice design with two replications. The detailed information of all the genotypes used in this study is provided in Table 3.

#### **Procedure** of inoculation

The experimental material was artificially inoculated at four to six-leaf stage (Carson et al., 2004). The inoculum in powdered form was dropped manually in the whorls of each seedling (Miles et al., 1980).

## Disease scoring

Data for disease severity was recorded at each seven days interval (Forbes et al., 1993). Whole plots were visually rated four times during the cycle for percent SLB severity using CIMMYT scale (0-5), 0 was considered for no lesion while 5 was considered for the lines completely destroyed by the pathogen. Lines with score of 0.0-1.4 were considered

resistant, 1.5-2.4 moderately resistant, and 2.5-5.0 susceptible (Muriithi and Mutinda, 2001).

# Area under disease progress curve (AUDPC)

The average data of each score at weekly interval was converted to percent leaf area for computation of Area under disease progress curve (AUDPC) according to the formula suggested by Ceballos et al. (1991) and Forbes et al. (1993) AUDPC =  $\Sigma_{i=1}^{n-1} [(t_{i+1} - t_i) (y_i + y_{i+1})/2]$  Or alternately

AUDPC =  $\sum_{i=1}^{n=1} \frac{y_i + y_{i+1}}{2} (t_{i+1} - t_i)$ 

Where,

t = time in days of each reading

y = percentage of affected foliage at each reading and,

n = number of readings.

#### Grain Yield

The grain yield of each plot was calculated in tones after harvesting and adjusting fresh ear weight to  $150 \text{ g kg}^{-1}$  grain moisture using the following equation:

Croin Viold Va ha <sup>-1</sup>	$(100 - MC) \times FEW \times Shelling Co - efficient$	×10,000
Giani Tielu Kg na =	(100 - 15)×Plot Area	

Where,

MC = moisture content (%) in grains at harvest FEW=Fresh ear weight (kg) at harvest Shelling Co-efficient = Shelling % age/100

#### Kernel rows cob<sup>1</sup>

Number of grain rows per cob was counted for 5 randomly selected cobs after harvest and the average data was used for the analysis of variance.

# 300-grain weight

Grains were collected randomly from each harvested plot and 300 grains were counted from the lot of each plot. With the help of electrical balance, weight was measured and subjected to analysis. All the data were subjected to ANOVA appropriate for Lattice Square Design using computer program "MSTATC". Correlation analysis was performed using the same package. Microsoft Excel was used for calculation of AUDPC and graphs formation. The following formulae were used to estimate  $h^2_{BS}$ , S and R<sub>e</sub>.

- Broad sense heritability  $(h_{BS}^2) = \sigma_G^2 / (\sigma_E^2 + \sigma_G^2)$
- Selection differential (S) =  $\mu_{S1} \mu$  $\mu_{S1}$  = Mean of the selected S<sub>1</sub> lines
- $\mu$  = Population means (comprising all S<sub>1</sub> lines)

Expected response ( $R_e$ ) = S × h<sup>2</sup> was calculated as the product of selection differential and heritability.

#### Selection and recombination (S<sub>1</sub> line recombination phase)

Based upon field evaluation for SLB, AUDPC and grain yield, a total of 13 promising  $S_1$  lines were selected from the 76 lines selected lines under investigation for future breeding and evaluation (i.e. next RS cycle). These selected  $S_1$  lines were recombined during the off season in the following month (November, 2006- February, 2007) in the glass house of Department of Plant Breeding and Genetics, NWFP Agricultural University, Peshawar to constitute the improved version (next RS cycle) for further improvement program.

## Conclusions

Results of this study showed the importance of resistance genotypes in minimizing yield losses from SLB. Selected  $S_1$ lines showed an acceptance level of disease resistance and yield potential. Furthermore, it appears that the RS was quite effective in enhancing resistance to SLB and increasing grain yield in maize. Genetically improved genotypes (P-3025 and Jalal) should be used in crosses with local susceptible varieties to increase resistance to SLB and to increase grain yield and even to develop an extra gene pool for future breeding. High level of disease resistance exhibited by the selected lines could be exploited in future breeding programs for controlling this important disease of maize.

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