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Recurrent Selection for Maydis Leaf Blight Resistance and Grain Yield Improvement in Maize

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Abstract: Leaf blight of maize (*Zea mays* L.), caused by *Bipolaris maydis* (Nisik. and Miyake), is one of the major factors limiting maize production in the plain areas of Pakistan, particularly in the North West Frontier Province (NWFP). The objectives of this study were to estimate selection differential, expected and observed response to selection and heritability for maydis leaf blight resistance and grain yield and to determine progress from selection in a maize population. Two cycles of S_1 recurrent selection were conducted in broad based maize population, Sarhad White (SW). One hundred S_1 lines were compared with their respective original population (C_0) as a check. The experimental material was evaluated under artificial epiphytotics during 2003 and 2004, at NWFP Agricultural University, Peshawar. Selection differentials, expected responses and heritability estimates were determined from the replicated S_1 lines performance of the first cycle. The observed progress was estimated from the replicated S_1 lines of the second cycle that were generated after recombination of selected S_1 lines of the first cycle. Highly significant variations between the cycles were observed for MLB and grain yield. Moderate and high heritability estimates, desirable selection differentials and close correspondence of expected and observed response were manifested for MLB and grain yield. The positive percent deviation of inoculated from uninoculated trials in both cycles (C_1 = 71% and C_2 = 41%) for leaf blight indicated successful development of blight disease after artificial inoculation, while the negative percent deviation of inoculated form uninoculated trials (C_1 = -21 and C_2 = -6%) for grain yield indicated the impact of blight disease on grain yield. Blight disease was significantly reduced from 2.9 to 2.3, whereas, grain yield was significantly increased from 2041 kg ha^{-1} cycle^{-1} to 2527 kg ha^{-1} cycle^{-1} or 19% cycle^{-1}. This yield improvement in SW was probably the result of concomitant reduction in the blight disease severity by -26%. These findings suggested that S_1 recurrent selection was quite effective in improving disease resistance and grain yield. Nevertheless, some additional cycles of selection may be required to further improving grain yield and resistance level to maydis leaf blight in SW maize population.

Key words: Recurrent selection, simultaneous improvement, selection differential, S_1 lines, heritability, maydis leaf blight

INTRODUCTION

Maize or corn (*Zea mays* L.), is queen of cereals due to its high yield efficiency. Maize ranks first in both production (720 million metric tons) and productivity (4906 kg ha^{-1}) in the world among cereal crops (FAO, 2004). In Pakistan, during 2004, it was cultivated over 0.89 million hectares with total production of 2.7 million metric tons and productivity of 3097 kg ha^{-1} (FAO, 2004). Maize is the second most important crop after wheat in Pakistan's North West Frontier Province (NWFP). It is both the primary crop in a majority of the farming systems and staple food of the rural population in much of the Province, especially mountainous areas. In NWFP, maize is often grown as a dual purpose crop, producing grain as well as fodder (Kiramat *et al.*, 2003). It is also used in industry for making starch, oil, polishes, etc. (Aziz *et al.*, 1992). During 2004, maize was grown over 0.5 million hectares in NWFP with production of 0.86 million tons and productivity of 1716 kg ha^{-1} (Minfal, 2004).

Despite its high yield potential, one of the major limiting factors to maize grain yield is its sensitivity to several diseases. As many as 65 pathogens infect maize (Rahul and Singh, 2002). Maydis Leaf Blight (MLB) is also one of the serious diseases of maize. The extent and severity of this disease varies from season to season. In warm (20-32°C) and moderately humid environment of the world, maydis blight is potentially damaging and may

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cause significant yield losses (Bekele and Sumner, 1983; Thompson and Bergquest, 1984). The blight spreads from the basal leaves to the developing ear and then flag leaf of maize plant (CIMMYT, 1985). MLB is also prevalent in the maize growing regions of NWFP and accounts for about 20% or sometimes even more yield losses to the crop in Pakistan (Hafiz, 1986).

Two physiological races, T and O, (anamorph: *Bipolaris maydis*, syn. *Helminthosporium maydis*), (Teleomorph: *Cochliobolus heterostrophus*), cause leaf blight disease in maze (Smith *et al*., 1970). Lesions produced by the T strain are oval and larger than those produced by the O strain. A major difference is that the T strain affects husks and leaf sheaths, while the O strain normally does not.

There are different ways in which the losses caused by this disease could be reduced. Eradication of crop debris after harvesting can play an effective role in controlling the disease. Fungicidal control is also available but chemical treatments are expensive, often ineffective and sanitation practices in crops like maize are difficult to apply. Therefore, host plant resistance and tolerance in most cases becomes the cheapest and most effective way to control leaf blight diseases in maize (Muriithi and Mutinda, 2001).

Most maize breeding programs are designed to improve disease resistance and grain yield. Recurrent selection methods have been widely used by maize breeders for population improvement. Maize breeders using recurrent selection method have two goals in mind, (i) improvement of population mean performance for the desirable attributes and (ii) maintenance of genetic variation for continued selection. Recurrent S_1 selection is a cyclical breeding system involving three seasons: development of progenies, which are evaluated in the replicated trials. The remnant S_1 seed of such selected families is then recombined in the third season as a result of which one cycle is completed in three seasons. Hence, the units of selection and recombination are S_1 progenies. Due to its efficiency in selecting for additive effects, S_1 selection has proven very effective in population improvement (Moll and Smith, 1981; West *et al*., 1980) and in reducing inbreeding depression (Ceballos *et al*., 1991; Weyhrich *et al*., 1998).

With this overall aim in view, field experiments under artificial inoculated conditions for maydis leaf blight were carried out using S_1 recurrent selection method, to improve maydis leaf blight resistance and grain yield in maize population by estimating selection differential, observed and expected response to selection from S_1 progenies and heritability.

MATERIALS AND METHODS

This study was conducted during the years 2003 and 2004 at NWFP Agricultural University Peshawar, Pakistan. Peshawar is located at 34°N (latitude) and 74°E (longitude) with 530 m above sea level. Two cycles of S_1 recurrent selection were conducted for resistance to Maydis Leaf Blight (MLB) disease caused by *Helminthosporium maydis*.

Recurrent S_1 method was conducted in a broad based maize population 'Sarhad White', which was provided by the Cereal Crops Research Institute (CCRI), Pirsabak, Nowshera, Pakistan. Population Sarhad White (SW) has been evolved from composite of (Vikram (B57×B37)× Akbar). It is a white dent flint genotype with medium tall stature, semi-dense tassel with profuse branching, horizontal leaves and late maturity (115-130 days).

For S_1 line production, the population was grown over an area of 600 m^2 in spring 2002 (March-June). Plant spacing was 75 cm between and 25 cm within the rows which provided about 56000 plants ha^{-1} (CIMMYT, 1985; Ceballos *et al*., 1991; Aziz *et al*., 1992). Two seeds per hill were planted by hand and were later thinned at 3 to 5 leaf stage to single healthy plant per hill. Fertilizers were applied in the form of Urea and Diammonium Phosphate (DAP) at the rate of 250 and 125 kg ha^{-1}, respectively. Entire DAP was applied at the sowing time, while half dose of urea was applied pre plant and the remaining half was side dressed when the plants were at 30 cm tall. The crop was irrigated weekly and mechanical weeding was done twice during the crop season to control weeds.

Approximately 300 healthy and disease free plants were selfed. In selfing ear shoots of the selected plants were covered with shoot bags prior to silks emergence to avoid contamination by foreign pollen. Ear shoots were cut 2-3 cm at the top to allow uniform growth and emergence of silks for effective pollination. Tassels of selected plants were also covered with tassel bags when 50% pollen shed was noted on a particular plant one day before pollination. On the next morning, uniform silk brush of the bagged ears were self pollinated with fresh pollen from their own plants collected in the tassel bags (Poehlman, 1977; Welsh, 1981). At physiological maturity, the selfed ears were individually harvested, threshed and numbered separately. Ears containing enough healthy seeds were selected for the replicated evaluation trial in the following summer season (July-October) during 2003. Same cultural practices were followed for S_1 lines evaluation as discussed earlier. Half of the seeds of each S_1 line were adjusted in two replications with two rows per entry (Hallauer and Martinson, 1975). Each row was 5 m long with row to row distance of 75 cm, while plant to plant distance was maintained at 25 cm.

Maydis leaf blight inoculation procedure: One hundred S_1 lines of SW population in each cycle along with their respective checks were tested in split-plot arrangements with two replications (Ceballos *et al.*, 1991; Bekele and Sumner, 1983). One row in each of the two row plot of each S_1 line along with check (original population) in each replication was artificially infested with inoculum, prepared from ground leaves infected with maydis leaf blight, by dropping into the whorl when plants were about 30 cm tall. The inoculum was prepared from the plants heavily infected with maydis leaf blight of previous fall crop season (Miles *et al.*, 1980). The other row served as control (Muriithi and Mutinda, 2001). To get an accurate rating of disease severity, data on damage were taken late in the growing season before the leaves began turning brown (CIMMYT, 1985).

A scale of 0-5 was used to estimate disease severity following the CIMMYT procedure, i.e., 0 for no lesion and 5 for heavily blighted leaves. Ratings of 0.0-1.4 were considered resistant, 1.5-2.4 moderate resistant and 2.5-5.0 susceptible (Muriithi and Mutinda, 2001). The severity data estimates were arranged for all inoculated and uninoculated plants in the respective rows per line (CIMMYT, 1985; Muriithi and Mutinda, 2001; Poneleit *et al.*, 1972). As the disease reaction was rather uncertain to fall exactly in each of mentioned classes, an arbitrary gradation of 10 classes scale i.e., 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 was used to measure more accurately the disease severity (Jinkins and Robert, 1961).

In addition to disease severity, the data were also recorded on grain yield. After black layer formation at hilum, ears from each row were hand harvested to obtain yield and grain moisture data (Aziz *et al.*, 1992). Grain moisture determinations were made on samples of five shelled cobs selected randomly from each row using grain moisture tester (Eaton, Model No. 8500, Troy, Micigan, USA) (Webel and Lonnquist, 1967). Grain yield was obtained by adjusting field ear weight to 120 g kg^{-1} moisture assuming 80% shelling (Carangal *et al.*, 1971). After evaluation, the 30 lines selected on the basis of yield superiority, early maturity and tolerance to maydis blight in were planted in isolation to let random mate by open pollination (Widstrom *et al.*, 1992). Rawlings (1980) suggested that an effective population size of 30 would be reasonably adequate for most genetic systems to achieve a balance between short and long-term goals. Hallauer (1992), after reviewing literature on effective population sizes, suggested that approximately 25 to 35 progenies should be inter-mated in the recombination phase for maize recurrent selection programs. The recombination phase was carried out during the winter months (October to March) at the National Sugar Crops Research Institute, Thatta (Sindh). Thatta is a frost free zone, located at 25°N and 68°E with 13 m about sea level and an additional generation of maize can be easily raised. Being a frost free zone, the region provides opportunity to grow a maize crop successfully for recombination during the winter season (October-March). Off type plants were roughed out before flowering along with diseased plants during recombination.

Statistical analysis: Analyses of variance (ANOVA) were performed separately for S_1 lines of the first cycle of each population to calculate selection differential (S), expected response (R_e) and heritability (h^2). Horner (1969) indicated that the genetic variance among S_1 progeny means is equal to the total additive variance plus a portion of the dominance variance. Heritability estimates for each parameter in the first cycle were computed using format of Snedecor and Cochran (1980).

Selection differential was calculated by subtracting mean of 30% selected S_1 lines from the population mean based on all S_1 lines of first cycle on each level of inoculum ($S = \mu_{S1} - \mu_1$) for each character. Predicted gain was determined by multiplying heritability by the selection differential ($R_e = h^2 \times S$), observed in the first cycle, while observed response to selection was computed by subtracting mean of 30% selected S_1 lines of second cycle from the mean of unselected selfed lines of second cycle, ($R_o = \mu_{S2} - \mu_2$), (Khalil *et al.*, 1995; Falconer and Mackay, 1996; Abedon and Tracy, 1998). Percent deviation of disease severity in each cycle was calculated by taking the difference of inoculated from uninoculated into hundred. Similarly, the percent gain $cycle^{-1}$ of inoculated from uninoculated for MLB and grain yield were calculated by the formula (Keeling, 1982):

$$\text{Percent gain cyle}^{-1} = \frac{C_2 - C_1 \times 100}{C_1}$$

Combined ANOVA for two cycles was conducted according to Abedon and Tracy (1998), using General Linear Model (GLM) in SAS program (SAS, 1996). Cycle means of combined analyses for both characters were compared using Least Significant Difference (LSD) test at 5% probability level (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Maydis leaf blight resistance: Mean squares of combined analysis of variance regarding MLB disease score revealed highly significant ($p \leq 0.01$) variations among S_1 lines and their interaction with cycle (Table 1). These results suggested that these variations could be useful for effective selection against MLB resistance. Highly significant differences ($p \leq 0.01$) were observed between the cycles. The two interactions i.e., (Inoculum×S_1) and (Cycle×Inoculum×S_1) were non significant.

The heritability, calculated from the analyzed data of C_1, was 0.56 regarding MLB resistance (Table 2). Negative value of selection differential (-0.97) indicated resistance for leaf blight in the selected individuals for the next cycle. Observed response, estimated from C_2, was higher (-0.69) than the expected response (-0.54) which showed improvement of maydis leaf blight resistance in the second cycle (Table 2).

Deviation of inoculated from control for disease severity was lower than the check (original population = C_0) in both the cycles (Table 3). Due to selfing of visually resistant and healthy plants, lower deviation of inoculated plants from uninoculated plants was observed in the second cycle (C_2). This indicated improvement and stability in S_1 lines against blight disease.

The mean score of maydis leaf blight in the second cycle was significantly lower (2.3) than the first cycle (2.9).

Table 1: Mean squares (MS) for maydis leaf blight disease (MLB), (0-5) and grain yield (kg ha^{-1}) of combined analysis of two cycles of S_1 recurrent selection pursued in Sarhad White maize population

Source	df	MLB disease (0-5)	Grain yield (kg ha^{-1})
Cycle (C)	1	72.60**	47339369.50**
Rep (C)	2	0.42	43472.73
Inoculum (In)	1	276.13**	20713622.48*
C×In.	1	27.38*	5587155.92*
Error a	2	0.58	324142.40
Selfed lines (S_1)	99	1.54**	1843676.77**
C×S_1	99	1.71**	1993349.38**
In.×S_1	99	0.24^{NS}	182922.37**
C×In.×S_1	99	0.26^{NS}	173541.00**
Error b	396	0.20	77013.43
CV%		17.91	12.14

*, **: Significant at p = 0.05 and 0.01, respectively, NS: Non Significant

Table 2: Broad sense heritability (h^2) in C_1, Selection differential (S), expected (R_e) and observed (R_o) response to selection of disease severity and grain yield for Sarhad White after two cycles of S_1 line recurrent selection for maydis leaf blight resistance

Trait	h^2	S	R_e	R_o
Disease rate (0-5)	0.56	-0.97	-0.54	-0.69
G. yield (kg ha^{-1})	0.82	915.39	750.76	697.90

The percent gain per cycle for maydis leaf blight was -26, which revealed 26% reduction in the disease severity from C_1 to C_2 (Table 4). After 4 cycles of full-sib recurrent selection, Ceballos *et al.* (1991) reported significant decrease in Northern Corn Leaf Blight (NCLB) by an average of 19% cycle^{-1} without reducing in grain yield. Similarly, Jinahyon and Russell (1969) were able to reduce mean score (on a 0.5 to 5.0 scale) for stalk rot from 3.7 to 1.7 for three cycles of recurrent S_1 line selection. Significant decrease in common rust (*Puccinia sorghi* Schw.) from 37.0 to 8.2% in Min 11 and 57.0 to 9.9% in NECDR maize populations were reported by Abedon and Tracy (1998), after conducting three cycles of full-sib recurrent selection in three sweet corn populations. Smith and Cordova (1987) reported significant improvement in *Exserohilum turcicum* infection form 4.5 to 3.7 and from 3.8 to 2.9 across two locations using three cycles of S_1 recurrent selection.

Grain yield: Realized progress with any breeding scheme, depends largely upon the effective identification of superior genotypes and the accuracy with which the experiments are conducted (Alam, 1999). Walters *et al.* (1991) reported that effective recurrent selection for a quantitative trait will increase the frequency of favorable alleles in a maize population. As a result, fewer deleterious alleles will be exposed for the quantitative traits than those breeding schemes involving inbreeding.

Mean square values regarding grain yield were highly significant ($p \leq 0.01$) for cycles, S_1 lines and their interaction with cycles (Table 1). Analysis of variance for inoculum and its interaction with cycle was significant ($p \leq 0.05$).

Heritability value estimated for grain yield was 82%, whereas selection differential was 915.39 kg ha^{-1} estimated from the first cycle data. Expected and observed response to selection agreed well (Table 2). The expected response (750.76 kg ha^{-1}) estimated from first cycle was higher than the observed response (697.90 kg ha^{-1}). High heritability and close correspondence of expected and observed response to selection indicated that considerable progress may be expected from visual selection for grain yield. These results agree with those of Carangal *et al.* (1971), who reported very close correspondence of expected and observed responses (446 vs. 421 kg ha^{-1}, respectively) after two cycles of recurrent S_1 selection in a high yielding maize synthetic.

The percent deviation of inoculated from uninoculated for grain yield was higher (-21%) in S_1 lines of first cycle, followed by the check (-14%) of first cycle (Table 3). However, S_1 lines of second cycle showed more stability by recording low deviation (-6%). It is also

Table 3: Mean comparison of uninoculated (Uninoc.) and inoculated (In) S_1 lines with the check (original population = C_0) in C_1 and C_2 for disease severity and grain yield (kg ha^{-1})

	Selfed Lines			Check (C_0)		
	(C_1)			2003		
Trait	In	Uninoc.	Deviation (%)	In	Uninoc.	Deviation (%)
Disease rate (0-5)	3.7	2.1	71	3.2	1.5	117
G. yield (kg ha^{-1})	1796.0	2285.0	-21	2076	2437	-14
	Selfed Lines			Check (C_0)		
	(C_2)			2004		
Trait	In	Uninoc.	Deviation (%)	In	Uninoc.	Deviation (%)
Disease rate (0-5)	2.7	1.9	41	3.5	2.3	52
G. yield (kg ha^{-1})	2450	2605	-6	2227	2543	-12

Table 4: Cycles means and the percent gain cycle^{-1} for maydis leaf blight and grain yield (kg ha^{-1}) of Sarhad White population evaluated at Agricultural University Peshawar, using S_1 line recurrent selection during 2003 and 2004

Trait	LSD	C_1	C_2	Gain (%)
Disease rate (0-5)	0.4	2.9A	2.3B	-26
G. yield (kg ha^{-1})	63.4	2041B	2527A	19

Means followed by different letter for each trait differ significantly at p>0.05

confirmed by the higher mean yield of second cycle (2527 kg ha^{-1}) from the first cycle (2041 kg ha^{-1}). The gain cycle^{-1} was 19% (Table 4). It could be argued that improvement in grain yield and more stable performance showed by S_1 lines on both levels of inoculum in the second cycle were associated with resistance to MLB. Ceballos *et al.* (1991) reported similar association of disease resistance and higher grain yield.

Highly significant increase in grain yield (507 kg cycle^{-1}) in four populations was reported by De Leon *et al.* (1993), after conducting three cycles of S_1 line recurrent selection for Downy mildew resistance. Ceballos *et al.* (1991) reported 19% gain cycle^{-1} in early and 7% gain cycle^{-1} in intermediate disease pressure trials for grain yield in maize populations. They used S_1 full-sib recurrent selection for resistance to *Exserohilum turcicum.* Similarly, Weyhrich *et al.* (1998) observed significant increase in grain yield in the BS 11 maize population. They reported 110 to 220 kg ha^{-1} gains per cycle after completing four cycles of S_1 progeny selection with 10-S_1 and 30-S_1 selection intensity in the BS 11 maize population.

High heritabilities, desirable selection differentials, close correspondence of expected and observed responses and significant improvement in MLB disease resistance and grain yield lead to suggest that recurrent S_1 selection was quite effective in improving the maize population used in this study. However, some additional cycles of selection will be required to further improve grain yield and resistance level to maydis leaf blight in SW maize population.

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