GENETIC ANALYSIS OF MATURITY AND MORPHOLOGICAL TRAITS UNDER MAYDIS LEAF BLIGHT (MLB) EPIPHYTOTICS IN MAIZE (*Zea mays* L.)

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ABSTRACT

The difficulty of choosing desirable breeding program has restricted progress in maize breeding. In 2004, the Department of Plant Breeding and Genetics initiated a progress oriented breeding program for improving adapted varieties. This study was aimed to enhance disease resistance via selection of desirable plants based on maturity and morphological traits under disease stress condition. Maydis leaf blight (MLB) caused by *Helmintosporium maydis* is a major disease of maize (*Zea mays* L.). This research was conducted at Agricultural University Peshawar, Pakistan during 2006-07 crop seasons to estimate the efficiency of S_1 recurrent selection for improving morphological traits, maturity characteristics and yield. The S_1 lines were evaluated under epiphytotics of *H. maydis*. Statistical analysis of the data revealed the presence of highly significant variations for maturity traits and morphological attributes in the genetic material. The broad sense heritability (h^2_{BS}) estimates for 50% tasseling was 0.65, for 50% silking was 0.64 and 0.62 for 50% pollen shedding. Means of the selected S_1 lines was greater than the population mean resulting (0.33 and 0.54) selection differential for mid tasseling and pollen shedding. On the other hand negative value (-0.15) was observed for mid silking showing a decline trend. Expected response to selection for days to 50% tasseling, silking and pollen shedding were 0.21, -0.10 and 0.35 days, respectively. The h^2_{BS} was 0.53 for plant height and 0.48 for ear height. Selection differential (S) was 3.86 and 2.33 for plant for plant height (2.04) and ear height of (1.13). Our results suggest that including selection under carefully managed breeding program, early maturity with stay green character and improved morphological attributes effectively enhance MLB resistance in maize.

Keywords: maize, disease resistance, maydis leaf blight, heritability, selection differential, maturity.

INTRODUCTION

Maize (*Zea mays* L.) is the world leading cereal crop. Maize belongs to tribe maydaea and grass family, Poaceae. It is indigenous to America and was domesticated about 8,000 years ago. Maize does not survive in its wild form probably because of the highly cross pollinated nature (Ram and Singh, 2003). It is a short day plant with monoecious nature.

Maize improvement involves formation, evaluation, selection, and recombination of genetically variable families or inbred lines. The product of S and h^2_{BS} resulting a response to selection because cultivars must combine many desirable traits, the process can be complicated and lengthy (Pixely *et al*., 2006). Realized progress with any breeding scheme, however, depends largely upon the abilities of the breeder to identify superior genotypes and the accuracy with which the experiments are conducted (Ali *et al*., 2011). Almost 65 pathogens infect maize crop (Rahul and Singh, 2002). Maydis leaf blight (also known as southern maize leaf blight) is caused by the ascomycete fungi *Bipolaris maydis* and is reported from most maize growing regions of the world. Maydis leaf blight is most serious in warm and wet temperate and tropical areas, where yield losses close to 70% have been reported due to the disease. Several races of *B. maydis* are pathogenic to maize. Symptoms and severity of *B. maydis* depends on the pathogen race and host germplasm. There are 3 physiological races of *B. maydis*: Race T, Race O and Race C. Race T and Race C are pathogenic only to maize germplasm with cytoplasm male-sterile T and cytoplasm male-sterile C, respectively.

Symptoms of Maydis leaf blight vary according to the causal race and host germplasm. Race O produces lesions that are initially small and diamond-shaped. These lesions elongate as they mature, although growth of lesions is restricted by leaf veins. Final lesions are rectangular (2-6 × 3-22 mm), restricted by leaf veins, and tan in color. Lesions caused by isolates of Race O are restricted to leaves. Symptoms of Maydis leaf blight caused by Race T are oval and slightly larger (6-12 × 6-27 mm) than those caused by Race O. Lesion borders are usually characterized by dark, brown borders. Race T causes lesions on all above ground parts of the plant (including stems, sheaths and ears) and can also cause ear rots. Seedlings from seeds infected with Race T often wilt and die within 3 to 4 weeks. Under severe disease pressure, usually when infection occurs prior to silking, lesions may coalesce, blighting the entire leaf. In these circumstances, sugars may be diverted from the stalk for grain filling, thus predisposing the plant to lodging.

This fungus attacks maize crop particularly in the plains of the North West Frontier Province of Pakistan because of the environmental condition suites the pathogen. It 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. The extent and severity of this disease varies from season to season. 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 the spread of *H. maydis* (Peet and Maschtti, 1972). In many warm (20°-32°C) and moderately humid environments of the world, this disease have been potentially damaging the crop and causes significant losses (Thompson and Bergquest, 1984). In Pakistan two races are responsible for causing the disease, race 0 and race T. While race C was reported in China only. The mode of action of race C is that it infects maize plant with C cytoplasm (Wei *et al*., 1988). The lesions produced by race T are oval and larger than those produced by the race C. One of the most prominent differences in both races is that race T infects husks while race O does not infect husk and sheath normally (CIMMYT, 2004). In 1970s an epidemic was caused by race T in corn with Texas Male Sterile cytoplasm in most corn-growing areas of the USA (Ullstrup, 1972). Maize with normal cytoplasm was resistant to the pathogen (Hyre, 1970). During infection the resistant host plant defends itself against potential pathogens by means of physical and chemical factors. These factors may already be present in the host, or may be produced in response to the infection (Singh and Bhatnagar, 1983). The physical characteristics are mechanical barriers that prevent the entry and spread of the pathogen. Recurrent selection has been proposed as a method for the improvement of maize crop yield against this disease. According to Hull (1952), "recurrent selection was meant to include re-selection generation after generation, with interbreeding of selects to provide genetic recombination". The purpose of recurrent selection is to gradually increase the frequency of favorable alleles in a population while maintaining genetic variability. This should provide effective selection over a long period of time.

To increase maize yield per unit area in Pakistan, it is imperative for the plant breeders to develop maize varieties that are high yielding, early maturing, disease resistant, responsive to improved production practices and adjustable in the exiting cropping pattern (Aziz *et al*., 1992). In view of the economic importance of maize crop, the present study was undertaken to determine the effect of maydis leaf blight on maturity, morphological traits and yield related attributes in maize variety Sarhad white. Moreover selection and recombination of desirable lines will increase the frequency of favorable alleles for resistance to maydis leaf blight, early maturity, improve morphological traits and to enhance yield related characteristics using S_1 line recurrent selection. Broad sense heritability (h^2_{BS}), selection differential (S) and expected response to selection (R_e) were computed for all the parameters to find out the most promising lines among the experimental material for further necessary action.

MATERIALS AND METHODS

The experiment was conducted at Agricultural Research Farm (Malakandher), NWFP Agricultural University, Peshawar during 2006-07. Standard cultural practices including irrigation, fertilizer application, hoeing and thinning were carried out throughout the growing seasons. One cycle of recurrent selection 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 2005. These infected leaves were sun dried and kept in oven for 24 hours at 60°C. After 24 hours, the leaves were grinded with the help of a grinder to make inoculum in powder form (Shah *et al*., 2006, Durrishahwar *et al*., 2008). This powder was preserved for artificial inoculation in the proceeding seasons.

Preparation of genetic material (S_1 line production phase)

Improvement in Sarhad White, an open pollinated commercial maize variety, has been initiated in Plant Breeding and Genetics Department of Agriculture University, Peshawar using S_1 recurrent selection breeding procedure since 2003-04. Sarhad White is a long maturity, medium tall stature, semi dense tassel with profuse branching, open pollinated variety having semi-dent white kernel grain. Three selection cycles have been completed in this variety before launching the present investigation. One hundred and fifty five half-sib families from improved version of Sarhad White (SW-C_2) were selected and planted during 2006 (February-June). About 400 S_1 were generated using manual self pollination procedure (Russell and Hallauer, 1980). Out of these 400 lines, 76 lines were selected on the basis of their performance for disease resistance and seed setting at harvest for 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, P-3025) were planted for screening during July 2006 in a square lattice design with two replications.

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 (Smith and Hooker, 1973; Miles *et al*., 1980; Khalil *et al*., 2010).

Days to 50 % tasseling

Through daily visual observation during the flowering period, days from planting to 50% tasseling were counted when 50% of the plant had extruded tassels in each plot.

Days to 50 % pollen shedding

Data on days to 50% pollen shedding was worked out by visual observation when 50% of the plants in a plot shed pollen. The days were counted from date of sowing.

Days to 50 % silking

When more than 50% plants in a plot were showing silks coming out, the date was recorded and the duration from sowing time was recorded as days to 50% silking.

Plant height (cm)

Plant height in each plot was recorded after completion of male flowering, as the distance between the ground surface and node bearing the flag leaf, using five randomly selected plants in each row. Average data were used for analysis of variance.

Ear height (cm)

Ear height was measured in cm from the ground level to the base of apical ear as an average of five randomly selected plants per plot and then means were calculated.

All the data were subjected to ANOVA appropriate for Lattice Square Design using computer program "MSTATC" package (Freed, 1990). The following formulae were used to estimate broad sense heritability, Selection differential and Expected response.

Broad Sense Heritability (h^2_{BS}) = $\sigma^2_G / (\sigma^2_E + \sigma^2_G)$

Selection differential (S) = $\mu_{S1} - \mu$

μ_{S1} = Mean of the selected S_1 lines

μ = Population means (comprising all S_1 lines)

Expected response (R_e) = $S \times h^2$ was calculated as the product of selection differential and heritability.

Selection and recombination (S_1 line recombination phase)

Based upon field evaluation for maturity and morphological traits, a total of 13 promising S_1 lines were selected. These selected S_1 lines were recombined during the off season (November, 2006- February, 2007) in the glass house of Department of Plant Breeding and Genetics, NWFP Agricultural University, Peshawar to constitute the improved version (C_4) for further improvement program.

RESULTS AND DISCUSSIONS

Days to 50 % tasseling

The analysis of variance for mean of 50% tasseling showed highly significant differences among the treatments at 0.01 level of probability (Table-1). Maximum heritability (0.65) was calculated among all the parameters for days to 50 % tasseling (Table-1) while Shah *et al*. (2006) observed a little higher value of heritability (0.79) in two cycles of S_1 line recurrent selection in Sarhad White population. Mean of the selected S_1 lines was 52 (days) while the populations mean comprising the selected, unselected S_1 lines and the 5 checks used in the experiment were 51 (days). The selection differential worked out from the selected lines mean and population mean was 0.33 (Table-1). Response to selection exerted in improving the population mean was 0.21 (Table-1). Data regarding days to 50 % tasseling is an index selection parameter for maturity. The lower the numbers of days to 50 % tasseling the earlier the genotype will mature. The selected E-1 exhibited the least number of days to 50 % tasseling (50 days) while maximum number of days needed for 50% tasseling (54 days) was observed for the selected E-31 and E- 44. On the other hand, the two checks Jalal and P-3025 needed 51 and 52 days, respectively, to reach 50 % tasseling (Table-2). Days to 50 % tasseling are an important character related to early maturity as well as improving resistance to diseases. Ceballos *et al*. (1991) expressed that late maturing genotypes with the same genetic background were more resistant to diseases than early maturing genotypes. Choi *et al*. (1994) suggested that inbreds with stay-green character had high photosynthetic activities and high protein and lipid content. They also noticed that the inbred lines were very resistant to *H. maydis*. Days regarding mean of the population for 50% tasseling (51 days) equal that observed by Rahman *et al*., 2005. Minimum co-efficient of variation 1.73 % was observed for days to 50 % tasseling showing the accuracy of the experiment.

Days to 50 % pollen shedding

Analysis of variance showed highly significant differences ($P \leq 0.01$) among the S_1 lines for days to 50 % pollen shedding (Table-1). Shah *et al*., (2006) divulged highly significant variation for mid pollen shedding using recurrent selection for MLB resistance in two corn populations. Mean of the selected S_1 lines (55 days) was greater from the population mean (54.77 days) conferring a selection differential of 0.54 (Table-1). High value of heritability (0.64) was observed for 50 % pollen shedding (Table-1). Response to selection (0.35 days) was the product of heritability and selection differential indicating the improvement of the population. These results were against the finding of Shah *et al*. (2006). S_1 lines at E-31 and 22 exhibited maximum days to reach 50 % pollen shedding (58 days) followed by E-44 (57). Minimum (52) days for 50 % pollen shedding were recorded for the selected E-1 (Rehman *et al*., 2005). The ability of the crop to accumulate photosynthates as easily as possible will help in reducing the severity of this disease. Among the checks Pahari and Azam needed 50 days to reach this point (Table-2). The character is directly related to the reproductive stage of the crop. The co-efficient of variation was 2.01 %, second small value among the analyzed parameters (Table-1).

Days to 50 % silking

The statistical analysis for days to 50 % silking revealed highly significant ($P \leq 0.01$) differences among the S_1 lines including the respective checks used in the experiment (Table-1) Abendon and Tracy (1998) noted significant variation for mid silking using recurrent selection for rust resistance in three sweet corn populations. Leonard *et al.,* (1990) observed that based on disease ratings three to four weeks after mid-silk, grain yield is reduced by an estimated 4.2% per 10% diseased leaf tissue but infection before silking is considerably more damaging. On the other hand non-significant differences have been reported for days to 50 % silking in short season variety tests and generally late silking have been recorded in Swabi white and earlier silking in the rest of the populations (Aziz *et al.,* 1992) Value of heritability regarding days to 50 % silking was 0.62. Variance component, the environmental and genetic variance were 2.10 and 3.64, respectively. Selected S_1 lines mean (55 days) was less than the population mean (55.19 days) showing negative value for the selection differential (-0.15 days) (Table-1). In our experiment population mean regarding 50% silking (55 days) resembles the results of Rahman *et al.* (2005). They observed the lowest number of days (53) for Sarhad White population. Shah *et al*., (2006) observed average of 60 days in cycle 1 and 57 in cycle 2 for Sarhad White population using two cycles of S_1 line recurrent selection for MLB. Response to selection pertaining days to 50 % silking was -0.10 (Table-1). Selected E-22 exhibited 59 days, maximum among the selected lines while E-1 exhibited minimum 51 days among the selected and UN selected lines for days to 50% silking. The checks Azam and Pahari needed 50 and 49 days, respectively (Table-2). The estimated co-efficient of variation for days to 50 % silking was 6.67 % (Table-1). Damage is most critical if infection occurs prior to silking and if weather conditions are favourable for disease development during the reproductive growth stages. If cultivating varieties with the Texas source of male sterility, infection by Race T pathotypes can lead to extensive stalk and ear rots. Seedlings grown from kernels infected with the pathogen may die four weeks following planting. Increased application of nitrogen fertilizer and increased crop density are associated with increased disease severity.

Plant height (cm)

Plant height is an important morphological trait affecting the final yield. Extremely dwarf varieties have the problem of crowded canopy, aeration and transmission of sun light to the lower parts resulting in drastic reduction in yield. Moreover the high stature plants are highly susceptible to lodging. In this study the statistical analysis of the data revealed highly significant differences ($P \leq 0.01$) among the experimental material (Table-1). Stromberg and Campton (1989) also reported significant differences regarding plant height after 10 cycles of full-sib recurrent selection in Nebraska Krung open pollinated maize. Non significant variation was achieved for plant height and rust resistance to *Exserohilium turcicum* in 8 sub tropical maize population while practicing recurrent selection (Ceballos *et al*., 1991). Their results were against our finding associated to plant height. Environmental variance and genetic variance was 127.76 and 142.90, respectively. The variance components revealed heritability of 0.53 for plant height (Table-1). Ahsan and Mehdi (2000) also divulged low heritability value (0.56) for plant height in maize for higher green fodder yield using S_1 family selection. Mean value for the population (127.61 cm) lower than the selected lines mean (131cm). Selection differential calculated from the difference of selected S_1 lines mean and population means (3.96 cm Table-1). Response to selection 2.04 cm was obtained for plant height (Table-1). Plant height is directly related to lodging and results in significant yield losses. Plant breeders are mostly interested in short stature genotypes because of their lodging resistance and higher response to fertilizer application (Hassan., 2000; Rahman *et al*., 2005). Maximum plant height 163 cm was observed for E-13 followed by hybrid P-3025. Among the selected lines the maximum plant height 152 cm was observed for E-44 followed by E-22 (Table-2). Among the non selected lines minimum plant height 87 cm was observed for E-54 (Table-1). Co-efficient of variation regarding plant height was 8.85 % (Table-1).

Ear height (cm)

Statistical analysis of the data showed highly significant differences ($P \leq 0.01$) among the experimental material pertaining ear height in cm (Table-1). Low value of heritability (0.48) was observed for ear height (Table-1). These results were against the finding of Jonson *et al*., (1986) they reported very high h^2 value in their experimental material. Mean of the population including selected lines (50.36 cm) was less than the mean of the selected S_1 lines (53 cm) resulting a selection differential of 2.23 (Table-1). Response to selection was (1.13 cm) was observed for ear height (Table-1). The effect of ear height has a great impact on production of successful maize crop. If the ears are placed above the middle, plants are liable to be damaged by stalk lodging, but if present too low, the wild animals will cause havoc, so a breeder must give proper attention to these parameters in order to provide lodging resistance in maize population. Among the selected lines the ear was placed 67 cm above the ground level showing maximum value of ear height for E-71 while the minimum value for ear height (42 cm) was observed for E-31. Alam (1999) reported that central or near central placement of top ear were desirable for resistance to lodging. Results of Lamkey and Duddley (1984) inferred that selection methods were not very effective to plant morphology because of low heritability, high instability and increase environmental influences in maize crop. The value of co-efficient of variation was 14% (Table-1).

Table-1. Mean square values (MS), heritabilities (h^2_{BS}), selection differential (S), expected response (R_e) and coefficient of variation (CV) for different parameters observed during Cycle-3 in maize population Sarhad White.

Parameters	MS	h^2_{BS}	S	R_e	CV
Days to mid tasseling	3.74**	0.65	0.33	0.21	1.73
Days to mid P. shedding	5.53**	0.64	0.54	0.35	2.01
Days to mid silking	9.46**	0.62	-0.15	-0.10	6.67
Plant height (cm)	413.56**	0.53	3.86	2.04	8.85
Ear height (cm)	160.65**	0.48	2.33	1.13	14

Table-2. Mean values of the 13 selected S_1 lines and checks for 50 tasseling, pollen shedding, silking, plant height (cm) and ear height (cm).

S_1 Lines and checks	50 Tasseling	Pollen shedding	Silking	Plant height (cm)	Ear height (cm)
E-1	50	52	52	115	43
E-10	51	55	54	123	54
E-14	52	56	54	136	57
E-22	53	58	59	147	63
E-31	54	58	57	124	42
E-44	54	58	59	152	60
E-51	52	54	53	122	43
E-55	52	55	55	126	43
E-64	51	54	54	137	56
E- 65	52	56	56	124	43
E-67	53	56	56	121	57
E-71	51	55	54	144	67
E-72	53	55	56	143	60
S. White	49	52	52	148	54
Pahari	48	50	50	130	47
Azam	49	51	50	141	67
Jalal	51	54	53	142	66
P-3025	52	55	54	157	60

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