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Contribution of Various Leaf Morpho-physiological Parameters Towards Grain Yield in Maize

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ABSTRACT

Knowledge of interrelationship between grain yield and its contributing components improves the efficiency of breeding programs through the use of appropriate selection indices. Significant differences were found among ten maize genotypes for grain yield and some leaf morpho-physiological parameters (excise leaf water content, stomata size, stomatal frequency, leaf venation, cell membrane thermostability & leaf area). Most leaf parameters, such as excise leaf water content, stomata size, leaf venation, cell membrane thermostability and leaf area had negative direct effect on grain yield. However, positive correlation due to indirect effects for stomata size, leaf venation and leaf area indicated that indirect selection for these parameters may be possible for grain yield improvement in maize. Stomatal frequency had positive direct effect on grain yield but negative correlation with it, which suggested that direct selection of this parameter might be effective to reduce the undesirable indirect effects on grain yield. Investigations of yield differences between maize genotypes using phenotypic and genotypic correlation showed that leaf area had significant and positive genotypic and phenotypic correlation with yield. Both genotypic and phenotypic correlations also confirmed leaf area as an indirect indicator for yield improvement in maize.

Key Words: Maize; Grain yield; Leaf parameters; Correlation; Path analysis

INTRODUCTION

Maize (*Zea mays* L.) is the world's most widely grown cereal and is the primary staple food in many developing countries (Morris *et al*., 1999). Its grain is used for making different products like glucose and starch. Stalk is used for making paper, insulator and cardboard. It is also a source of useful polyunsaturated fatty acids, which is beneficial for human health. It possesses a wide range of adaptation and is grown extensively in the temperate, sub tropical and tropical zones. Its range of cultivation stretches from 50°N to 40°S latitude and from sea level to mountains of 3300 m altitude. About 26% of the world's total cultivable land falls in arid and semi arid areas, (Paylore & Greenwell, 1979) and about 40 million hectares are planted annually in Asia, producing 130 million metric tones of grains, which is approximately 30% of the total world maize production (Logrono & Lothrop, 1997).

The average yield of maize in Pakistan is very low as compared to the developed countries and even to the world average. Maize hybrids are cultivated on only a limited area in the developing countries despite of their higher yield potential (Vasal *et al*., 1994). Paterniani (1990) discussed several characteristics of temperate and tropical maize production and suggested that the problems facing maize cultivation in the tropics are numerous and are of greater magnitude and more challenging than in temperate areas.

The usefulness of maize is enhanced by its extreme diversity of form, quality and growth habit. For example, in grain size alone there are more than fifty variations between the normal kernel. Maize being C_4 plant, can capture energy efficiently is capable of producing maximum food grains per unit area as compared to other cereal, and thus play a dominant role in the agricultural economy. Many crop scientists are concerned with, how environmental factors and plant genotypes can be altered to increase yield of agronomic crops. Yield being a complex character involves a number of complex morpho-physiological characters. It can be predicted on the basis of performance of yield components that these components are genetically correlated with yield. The information about phenotypic and genotypic interaction of various morpho-physiological traits is of immense importance to a plant breeder for selection and breeding of different varieties of maize with increased yield potential. Correlation between various characters is of great value as it indicates the degree to which various characters of a plant are associated with the economic productivity.

Knowledge of genetic variation and relationships between accessions or genotypes is important: (i) to understand the genetic variability available and its potential use in breeding programs, (ii) to estimate any possible loss of genetic diversity, (iii) to offer evidence of the evolutionary forces shaping the genotypic diversities and

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(iv) to choose genotypes to be given priority for conservation (Thormann *et al.*, 1994). The present study is an attempt to estimate direct and indirect contribution of indicated parameters to grain yield and to estimate associations both at phenotypic and genotypic levels between grain yield and its various morpho-physiological traits. The information so derived might be helpful to make useful selection criteria and selecting most promising genotypes for further future breeding program.

MATERIALS AND METHODS

General experimental details. The present study was carried out in the Department of Plant Breeding and Genetics, University of Agriculture Faisalabad during the summer season 2005-06. The experimental material comprised the ten maize genotypes viz., Agaiti 85, FHY425, Golden, V1089, Sonari, FHY 421, EV5089, DTC, FHY 396 and IZ Population. Sowing was done in a single row of 15.3 m length by dibble. Two seeds per hill were sown, which later thinned to one seedling after germination. Thinning was done at 4 to 5 leaves stage. Plant-to-plant and row-to-row distances were kept at 15 and 75 cm, respectively. Normal cultural and agronomic practices were applied throughout the crop growing season. Ten guarded plants were selected from each genotype at random and data were recorded for the following plant parameters.

Excise leaf water content. Third leaf was used to measure excised leaf water content. Leaf samples were collected and surface dried gently with tissue paper, then wrapped into properly tagged polythene bags. Fresh weight of the excised leaves was measured soon after arriving in the laboratory. The leaf samples were then spread on a laboratory bench to wilt for six h at room temperature (20˚C). Leaves were weighed again after six h to obtain wilted weight dried in an oven for 72 h at 70˚C and dry weight was measured. Excise leaf water content was calculated by the following equation:

$$ELWC = (Fresh\ weight - Wilted\ weight.)/Dry\ weight.$$

Stomatal size and frequency. The leaf strips taken for counting the leaf venation were also used to measure the stomata size. Length and breath of the stomata were measured using a compound microscope. Ten observations were recorded for each leaf and the average was calculated. The stomatal frequency counts per unit area was made on the upper surface of the leaf of each randomly selected plant. The strips, which were taken from the middle part of the leaf were dipped in Carnoy's solution to arrest stomatal movement and remove chlorophyll. After 24 h, the strips removed from the solution, washed in Acetone and stored in Farmaline solution for the further examination for stomatal frequency.

Leaf venation. For recording leaf venation the above strips were examined under microscope for counting the numbers of parallel veins of leaf of selected plant. Five observations were taken from each strip and the average was calculated.

Cell membrane thermostability. CMT was measured following the methods proposed by Sullivan (1972). CMT was measured on the youngest fully expanded leaves (20-22 days after germination). Samples were collected in paired sets from both sides of the leaves midrib. A special steal puncture was designed with 10 mm inner diameter for quick punching of leaf discs. One sample set was used for heat treatment and second as control. A 10 mm diameter leaf discs were excised for 1300 to 1500 h. Excised samples were immediately placed in glass vials containing 2 mL de-ionized water. Vials were quickly brought to the laboratory. Leaf discs were thoroughly rinsed thrice in de-ionized water to wash out any adherent electrolytes or those already released into the water. After final washing, 2 mL water was added to each tube and capped to avoid desiccation and evaporation during heat treatment. One set of vials was treated in a controlled temperature water bath maintained at 50°C for one h. The control tubes were kept at 25°C for the same period. After heat treatment, 10 mL deionized water was added to each vial and held at 10°C for 24 h to allow diffusion of electrolytes. Vials were brought to 25°C and shaken to mix the contents. Electric conductivity (EC) was measured with a EC- meter. Vials were autoclaved for 10 min at 0.10 MPa pressure to completely kill tissues and release all the electrolytes. Vials were then brought to 25°C and final EC was measured with the same instrument. Percentage relative cell injury (RCI), an indicator of CMT, was calculated by following formula:

$$RCI\ \% = 1 - \frac{1-(T1/T2)}{1-(C1/C2).} \times 100$$

Leaf area. Leaf area was measured as the product of the length from base to the tip and the maximum breadth. Physiologically matured leaves of ten randomly selected competitive plants from each treatment were collected and area of each was measured by using the formula suggested as Mckee (1964).

Grain yield per plant. Grain yield per plant in grams was recorded separately by using an electronic balance and then average yield of ten plants was computed.

Statistical analysis. Analysis of variance and covariance for all the characters were computed using the method given by Steel *et al.* (1996). The individual comparisons of genotypic means were accomplished by using Duncan's new multiple range (DMR) test. Correlation coefficients were determined by using the method as outlined by Kwon and Torrie (1964). The procedure for path coefficient analysis was used as given by Dewey and Lu (1959).

RESULTS AND DISCUSSION

Highly significant differences were found among the genotypes for all indicated leaf physiological and genetic parameters and yield (Table I). Konak *et al.* (1997a) and Mahmood *et al.* (2004ab) reported significant differences among the maize genotypes for grain yield. However,

Table I. Mean squares for some leaf morpho-physiological parameters in maize

SOV	df	Excise leaf water content	Stomata size	Stomatal frequency	Leaf venation	Cell membrane thermostability	Leaf area	Grain yield
Replication	2	5.31^{NS}	25.60^{NS}	38.07^{NS}	1.03^{NS}	39.85^{NS}	738.23^{NS}	8.03^{NS}
Genotype	9	7296.32**	434.92**	1536.83**	2.07**	7162.27**	6920.50**	2549.84**
Error	18	18.86	23.27	20.18	0.55	12.0	1055.49	4.85

NS= non-significant, ** = significant at 1% of probability level.

Table II. Mean and statistical significance for some leaf morpho-physiological parameters in maize

Genotype	Excise leaf water content	Stomata Size (μ^2)	Stomatal frequency	Leaf venation	Cell membrane thermostability	Leaf area (cm^2)	Grain yield (g)
EV5089	203.2^{a}	95.0^{b}	122.0^{cd}	14.0^{abc}	131.5^{b}	311.7^{bc}	108.0^{g}
FHY425	168.5^{b}	95.7^{b}	116.7^{cd}	12.7^{cd}	99.3^{de}	291.3^{cd}	115.3^{f}
Sonari	153.6^{c}	95.7^{b}	125.0^{bc}	13.3^{abcd}	95.7^{f}	363.3^{ab}	152.0^{b}
IZ Population	128.6^{d}	93.7^{b}	115.7^{cd}	12.7^{cd}	105.3^{d}	385.0^{a}	207.7^{a}
FHY396	128.4^{d}	91.0b^{c}	138.3^{a}	13.0^{bcd}	98.5^{e}	315.7^{bc}	110.3^{g}
Golden	112.7^{e}	110.0^{a}	124.7^{bc}	13.0^{bcd}	119.4^{c}	321.0^{bc}	130.0^{a}
DTC	95.4^{f}	95.7^{b}	135.0^{a}	12.0^{cd}	98.7^{e}	344.7^{abc}	132.7^{de}
Agaiti 85	94.6^{f}	72.7^{d}	123.0b^{cd}	14.7^{a}	249.3^{a}	291.0^{cd}	134.3^{d}
FHY421	85.2^{g}	69.0^{d}	120.0^{cd}	14.3^{ab}	79.6^{g}	239.0^{d}	141.0^{c}
EV1089	26.0^{h}	84.7^{c}	131.3^{ab}	13.7^{abc}	100.4^{de}	238.0^{d}	118.0^{f}

"Values sharing the same letters are non-significant at 5 % probability level".

Table III. Genotypic (r_G) and phenotypic (r_P) correlation coefficients for some leaf morpho-physiological parameters in maize

		Grain yield per plant	Excise leaf water content	Stomata Size	Stomatal frequency	Leaf venation	Cell membrane thermostability
Excise leaf water content	(r_G)	-0.055^{NS}					
	(r_P)	-0.054^{NS}					
Stomata Size	(r_G)	0.020^{NS}	0.095^{NS}				
	(r_P)	0.017^{NS}	0.078^{NS}				
Stomatal frequency	(r_G)	-0.493^{NS}	-0.401^{NS}	0.248^{NS}			
	(r_P)	-0.419**	-0.317^{NS}	0.148^{NS}			
Leaf venation	(r_G)	-0.222^{NS}	-0.148^{NS}	-0.857*	-0.231^{NS}		
	(r_P)	-0.146^{NS}	-0.124^{NS}	-0.609**	-0.247^{NS}		
Cell membrane thermostability	(r_G)	-0.076^{NS}	-0.046^{NS}	-0.402^{NS}	-0.133^{NS}	0.612^{NS}	
	(r_P)	-0.074^{NS}	-0.048^{NS}	-0.366*	-0.100^{NS}	0.428**	
Leaf area	(r_G)	0.627*	0.534^{NS}	0.544^{NS}	-0.103^{NS}	-0.826*	-0.083^{NS}
	(r_P)	0.492**	0.432**	0.312*	0.001^{NS}	-0.308^{NS}	-0.061^{NS}

NS= non-significant, * = significant at 5 % probability level, ** = significant at 1% probability level.

Table IV. Direct (bold) and indirect effects of some leaf morpho-physiological parameters on grain yield in maize

	Excise leaf water content	Stomata Size	Stomatal frequency	Leaf venation	Cell membrane thermostability	Leaf area
Direct effects	-0.382	-1.217	1.255	-0.464	-0.048	-0.030
Correlation	-0.054	0.017	-0.493	0.222	-0.048	0.627
		Indirect effects				
Excise leaf water content		-0.036	0.153	0.057	0.017	-0.204
Stomata Size	-0.115		-0.302	1.043	0.489	-0.663
Stomatal frequency	-0.504	0.312		-0.291	-0.167	-0.129
Leaf venation	0.068	0.397	0.107		-0.284	0.384
Cell membrane thermostability	0.002	0.019	0.006	-0.029		0.004
Leaf area	-0.016	-0.016	0.003	0.025	0.002	

morphological variation does not always reflect real genetic variation because of genotype × environment interaction and the largely unknown genetic control of polygenic morphological and agronomic traits (Smith & Smith, 1992).

Maize genotype EV5089 had significantly higher excise leaf water content (203.2) as compared to all other genotypes in this study (Table II). A further perusal of this table reflected that greatest stomata size (110 μ^2) was depicted by Golden amongst the genotypes. Jones (1979) also recorded significant differences in stomata size in maize. FHY396 had significantly higher stomatal frequency (138.3) as compared to other all genotypes except DTC and EV1089. Agaiti 85 had maximum leaf venation (14.7 $leaf^{-1}$) followed by FHY421, EV5089 and EV1089. It also had significantly higher value of cell membrane thermostability (249.3) than all other genotypes. IZ population had

significantly greater leaf area (385.0 cm^2) and higher grain yield (207.7 g) as compared to other maize genotypes in this study.

Genotypic and phenotypic associations. Excise leaf water content, leaf venation and cell membrane thermostability were not correlated with grain yield per plant both at genotypic and phenotypic levels (Table III). There was positive but non-significant correlation between stomata size and grain yield per plant at both levels. But leaf area had positive and significant correlation with grain yield at both genotypic and phenotypic levels suggesting that increased leaf area was an effective contributor towards grain yield in maize. These data corroborate the findings of Ahsan (1999) for six elite/exotic maize inbred lines. The stomatal frequency had no correlation with grain yield per plant at genotypic but significant at phenotypic level. Sen and Misra (1981) reported positive correlation between stomatal frequency and grain yield in wheat.

Stomata size had no correlation with excise leaf water content at both genotypic and phenotypic levels. Stomatal frequency, leaf venation and cell membrane thermostability were negatively and non-significantly correlated with excise leaf water content at both geno-and phenotypic levels. The leaf area had positive and non-significant correlation with excise leaf water content at genotypic but highly significant at phenotypic level. There was positive and non-significant correlation between stomatal frequency and stomata size at both levels. Leaf venation was negatively and significantly correlated with stomata size at both genotypic and phenotypic levels. There was a negative correlation at phenotypic but none at genotypic level between cell membrane thermostability and stomata size. Leaf area had correlation with stomata size at genotypic but positive one at phenotypic level.

Leaf venation, cell membrane thermostability had negative and non-significant correlation with stomatal frequency at both levels. Leaf area also indicated no correlation with stomatal frequency at genotypic and phenotypic levels. Cell membrane thermostability had no correlation with leaf venation at genotypic but positive one at phenotypic level. There was a negative and significant correlation between leaf area and leaf venation at genotypic but none at phenotypic level. Leaf area had no correlation with cell membrane thermostability both at genotypic or phenotypic levels.

Path analysis. Path coefficient analysis is simply a measurement of the influence of each variable upon the resultant variable directly as well as indirectly by partitioning the genetic correlation. It helps in choosing the plant traits amenable to manipulate the breeding programs of crop plants. Excise leaf water content (-0.382), stomata size (-1.217), leaf venation (-0.464), cell membrane thermostability (-0.048) and leaf area (-0.030) had negative direct effects on grain yield per plant (Table IV). Konak *et al*. (1997b) reported that leaf area had direct negative effects on yield in maize. The direct effects for cell membrane thermostability and leaf area were negligible because they found lower than 0.1 as given in scales for path Coefficients by Lenka and Mishra (1973). This indicated that direct selection of these parameters for grain yield improvement cannot be made. Although stomatal frequency had high and positive direct effects (1.255) on grain yield but correlation for this was negative (-0.493) it suggested that direct selection for this parameter should nullify the undesirable indirect effects. However excise leaf water content had indirect positive but low effects on grain yield via leaf venation (0.068) and cell membrane thermostability (0.002). Stomata size had positive and high indirect effects via stomatal frequency (0.312) and leaf venation (0.397) on grain yield. Stomatal frequency also had positive and high indirect effects on grain yield via excise leaf water content (0.153) and leaf venation (0.107), but low via cell membrane thermostability (0.006) and leaf area (0.003). Contrary to this Khaliq *et al.* (2000) reported direct positive effects of stomatal frequency on grain yield in wheat. Leaf venation and cell membrane thermostability were also affected positively and indirectly by grain yield via stomata size, leaf area and excise leaf water content. Leaf area had high and positive indirect effects on grain yield via leaf venation (0.384) but low via cell membrane thermostability (0.004).

CONCLUSION

Differences in yield, phenotypic and genotypic associations showed that leaf area had significant and positive genotypic and phenotypic correlation with yield. Positive correlation coefficients of leaf area and indirect selection via leaf venation and cell membrane thermostability may improve grain yield in maize. Positive direct effects of Stomatal frequency on grain yield but negative correlation with it might be effective to reduce the undesirable indirect effects on grain yield while developing new cultivars.

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