

Assessment Of Drought Tolerance In Maize (*Zea Mays L.*) Genotypes At Early Growth Stages By Using Principle Component And Biplot Analysis

ABSTRACT

Drought has drastic effects on performance of plants. With deficient moisture availability normal physiology and chemistry of the plants is disturbed. To evaluate the performance of maize genotypes against drought stress current study was planned. Total 34 maize accessions were evaluated at different moisture levels i.e. 70%(T1), 50%(T2) and 30%(T3) of field capacity (FC). Seeds were sown in polythene bags by following triplicated CRD (two factor factorial). Evaluation of maize accessions was made against 10 different parameters related to early growth stages (fresh root weight, fresh shoot weight, dry root weight, dry shoot weight, root length, shoot length, stomata size, leaf temperature, root density and relative water contents). Results were analyzed by using CRD two factor factorial analysis of variance, principal component analysis and biplot analysis for selection of tolerant genotypes. Highly significant differences among accessions for all the characters were found under water deficit stress conditions. A considerable reduction in almost all parameters was revealed under drought stress. First three, out of nine, principle factors showed more than 1 eigen value under all of three water treatments. First two factors (F1 & F2) contributed 78.01%, 72.54% and 69.24% cumulative variability in T1, T2 and T3 respectively. Dry root weight, dry shoot weight, fresh root weight and length and fresh shoot weight and length were proved the most effective selection indicators against drought stress at early growth stage. EV-329, EV-342, Islamabad, F-151, F-134 at 70%FC, EV-329, Islamabad, F-151, B-316, B-316 at 50%FC, Islamabad, F-151, EV-342, EV-329, B-304 at 30%FC were better performer and relatively were tolerant to water deficit.

Key words: Maize, drought, morphology, physiology, principle component analysis

INTRODUCTION

Globally maize (*Zea mays L.*) is ranked third in cereals after wheat and rice and is proved as cash grain crop. Textile, foundry, corn starch, corn syrup, corn oil dextrose, corn flakes, gluten, grain cake, lactic acid and acetone are among main products of maize. As the livestock and poultry industries are expanding and getting advanced, consumption of maize as feed and its demand increased. Maize is consumed in both fresh and processed forms. The expected demand of maize will increase up to 784 million tons in 2020 and developing countries are the expected consumers of this increased demand (Rosegrant et al, 1999). Value addition is proved an economic driver in corn markets.

Average yield of maize in Pakistan is much lesser than that of world. In 2009-2010 maize yield was 817 million tons from an area of 159 million hectares with an average yield of 5 tons per hectare. Maize yield of Pakistan in 2010-2011 was 3341 thousand tons from an area of 939 thousand hectares (Economic survey of Pakistan, 2011). Pakistan statistical data showed that maize yield was declined relative to previous years. There were many biotic and abiotic disfavorers which contributed in this loss of yield. Drought stress contributed significantly in the reduction of maize yield in Pakistan. The crop plants which are efficient water users and can give more production under stress conditions are the only hope. Survival of plants under drought conditions varies from plant to plant as every plant has different genetic makeup. Water requirement of maize is 500-800 mm during its life cycle of 80 to 110 days (Critchley and Klaus, 1991). Problem of water scarcity for crop production in Pakistan is very serious. Rainfed agricultural area of Pakistan is 25-30% of the total agriculture area. In 1982 irrigated area of Pakistan was 15.48 million hectares which increased up to 18.22 million hectares after 20 years in 2002. In Pakistan wastage of water by means of seepage and leakage is almost 40% which is a huge loss of

irrigation water (Pakistan Agriculture Research Council, 2007). Increase in irrigation water dependent area, reduced rainfall and loss of irrigation water through seepage and leakage are the potential threats on sufficient moisture availability for agriculture. In Pakistan availability of irrigation water will be almost half in next few years as the water resources are becoming limited day by day.

Along with better management to reduce water losses and increase availability, development of moisture stress, tolerant maize germplasm is most suitable solution to this problem. Better varieties with drought tolerance can be developed through plant breeding. Sound knowledge of genetics can help a breeder to understand the mechanism of inheritance of different traits which can ultimately help in the selection of a good genotype. Agricultural practices have their own importance but genetic improvement of plants can fill the gap up to 30% between theoretical yield and the actual yield under drought conditions (Edmeades *et al*, 2004). This study was conducted to identify some best genotypes with very good performance at low moisture availability. These genotypes will be an asset in future breeding program for the development of low moisture tolerant maize synthetics and hybrids.

MATERIALS AND METHODS:

This study was carried out in the greenhouse of the department of Plant Breeding and Genetics, University of Agriculture Faisalabad during Feb-2013. Total 34 maize genotypes; F-151, Islamabad gold, EV-342, EV-329, EV-78, RAKAPOSHI, F-134, B-326, VB-06, B-316, EV-323, F-127, B-304, POP-209, BF-236, B-308, F-121, EV-335, EV-1097, EV-336, F-114, EV-79, B-327, POP-2007, F-136, EV-330, F-113, EV-77, VB-51, EV-345, E-322, EV-349, F-150 and E-337 were collected from different research institutes (Department of Plant Breeding and Genetics, University of Agriculture Faisalabad & Ayub Agricultural Research Institute, Faisalabad, Pakistan) were planted in polythene bags (5×9”) by following completely randomized design. Sowing was done in three different sets of experiments each containing different treatment. Following are the treatments applied:

T1= Moisture level 70% of field capacity (control).

T2= Moisture level 50% of field capacity.

T3= Moisture level 30% of field capacity.

Following morphological and physiological parameters were estimated; Fresh root weight (g), fresh shoot weight (g), root length (cm), shoot length (cm), dry root weight (g), dry shoot weight (g), leaf temperature (°C), root density (mm cm⁻²), stomata size (µm²) and relative water contents (RWC). RWC were estimated according to following formula.

$$\text{RWC} = \frac{\text{Fresh weight} - \text{dry weight}}{\text{turgid weight} - \text{dry weight}}$$

Stomata size (µm²) was measured by sampling five strips from the middle part of the leaf and dipped into Carnoy's solution to remove chlorophyll and arrest stomatal movement. After 24 hours leaf strips were washed with acetone and stored in formalin solution for further examination. Stomata size was measured by using medium power (40X) objective and 12.5X eye piece of the microscope. Stomatal area was measured in microns by measuring five stomata from each strip using 100X objective lens and average was calculated.

Two factor factorial analysis of variance was computed to estimate the significance of genotypic differences for each studied trait (Steel and Torrie, 1984; Steel *et al*, 1997). Data recorded for different morphological and physiological traits were statistically analyzed by using principal component analysis (Sneath and Sokal, 1973). Principal component analysis is defined as “a method of data reduction to clarify the relationship between two or more characters and to divide the total variance of the original characters into a limited number of uncontrolled new variables”. Biplot graph on the basis of principal component analysis was used for estimation of association among different drought tolerance indices and grain yield at different conditions. Correlation between variables is described as angle between vectors; acute angle

(<90°) showed positive correlation, acute angle (<45°) showed strong positive correlation, right angle (=90°) showed independence or no correlation, obtuse angle (>90°) showed negative correlation and obtuse angle of >135°&<180° showed strong negative correlation.

RESULTS:

Two factor factorial analysis of variance was conducted for data of studied traits. Genotypic and treatment effects were found highly significant for root length, shoot length, fresh root weight, fresh shoot weight, dry root weight, dry shoot weight, leaf temperature, stomata size, root density and relative water contents. Genotype into treatment interaction (G×T) was highly significant for all studied morphological and physiological traits except for leaf temperature (Table-1). Principle component analysis was conducted for all of three water treatments separately. PCA converted the 10 traits into 9 different factors or components and only three (F1, F2 and F3) factors had eigen value greater than 1, in all of three water treatments. First two factors (F1 & F2) contributed 78.01%, 72.54% and 69.24% cumulative variability in T1, T2 and T3 respectively (Table-2). Cumulative variability and eigen values for three treatments were also presented in scree plot (Figure-1, 2 and 3).

Different traits had different percent contribution in total variability under different treatments. Root length contributed 15.97%, 15.03% and 13.14% variability in 1st factor whereas, this trait had contributed 0.64%, 3.37% and 6.96% variability in 2nd factor for T1, T2 and T3 respectively (Table-3). Shoot length had very low percent contribution for F1 and F2 of T1, T2 and T3 but for F3 of T1 and T2 this trait had contributed 94.96% and 45.03% variability respectively. Fresh root weight, fresh shoot weight, dry root weight, dry shoot weight and stomata size contributed 15.20%, 15.01%, 14.06%, 14.06% and 10.79% variability of F1 under 70%FC (Table-3). Leaf temperature, root density and relative water contents had contributed 31.93%, 28.04% and 25.82% variability of F2 under 70%FC (Table-3). Root length, fresh root weight, fresh shoot weight, dry root weight, dry shoot weight and relative water contents had more than ten percent contribution in variability of F1 under 50%FC. Leaf temperature, stomata size, root density and relative water contents had contributed individually more than 18% in variability of F2 under 50%FC. Root length, fresh root weight, fresh shoot weight, root density and relative water contents had more than 10% contribution in variability of F1 under 30%FC. Leaf temperature, stomata size, root density and relative water contents had more than 10% contribution in variability of F2 under 30%FC whereas, only dry root weight and dry shoot weight has above 10% contribution in variability of F3 under 30%FC (Table-3).

All studied traits contributed positively for F1 of 70%, 50% and 30% FC except stomata size which had negative contribution under all of three treatments (Table-4). Root length, fresh root weight, dry root weight and dry shoot weight had negative contribution but leaf temperature, stomata size, root density and relative water contents had positive contribution in F2 of 70%, 50% and 30%FC (Table-4). Shoot length had positive contribution in F3 under 70%, 50% and 30%FC while other traits had either positive contribution under one treatment or negative for other and vice versa (Table-4).

Biplot graphs were made by using F1 and F2 of principle component analysis for all of three water treatments separately. Length of vector and cosine of angle were used for grouping of traits into different groups. These groups showed the similar performance for discrimination of genotypes. Biplot categorized the traits into three groups under 70%FC. Group-1 had shoot length, fresh shoot weight, dry root weight, dry shoot weight, root length. Group-2 had root density, leaf temperature and relative water contents. Group-3 had stomata size. Within the group traits had strong positive correlation with each other but among groups traits had very weak positive and negative correlation (Figure-4). At 50%FC, shoot length was present in group-2 instead of group-1 (Figure-5). At 30%FC, groups of parameters were not distinct as these were in 70% and 50%FC (Figure-6).

Length of vector showed the discrimination power of trait for differentiation of genotypes. Under 70%, 50% and 30% FC, vector for shoot length was of shorter length which showed the poor discrimination power of this trait which showed that this trait should not be used for evaluation of maize genotypes in this study (Figure-4, 5 and

6). Traits with longer vector length and shorter cosine of angle with other traits showed the strength of correlation between traits. Root length, fresh shoot weight, fresh root weight, dry shoot weight and dry root weight had strong positive correlation at 70% and 50% FC whereas there correlation was somewhat weaker at 30%FC. Shoot length had shorter vector length so, its correlation was not significant. Stomata size had larger cosine of angle with morphological traits (Root length, fresh shoot weight, fresh root weight, dry shoot weight and dry root weight) so, it had negative correlation with these traits (Figure-4, 5 and 6).

Genotypes which were present away from origin in the positive direction of discriminating traits showed their better performance whereas, genotypes which were farther away from the origin in the negative direction of traits showed their poor performance. EV-329, EV-342, Islamabad, F-151, F-134, EV-323, POP-2007, EV-77 and VB-06 were better performer whereas, E-337, F-114, EV-79, EV-336, EV-349, EV-330 and EV-345 were poor performer under 70% FC (Figure-4). EV-329, Islamabad, F-151, B-316, B-316, EV-342, EV-323 and F-134 were better performer but EV-337, F-114, EV-79, EV-349, EV-336, EV-345, EV-330 and F-136 were poor performer under 50% FC (Figure-5). Islamabad, F-151, EV-342, EV-329, B-304, EV-323, F-134 and POP-209 were better performer but E-337, EV-79, EV-349, F-114, EV-336, F-136, EV-330 and F-121 poor performer under 30%FC (Figure-6).

DISCUSSION:

Biotic and abiotic stresses are the main threats across the world for agricultural crops (Aslam et al., 2013a; Aslam et al., 2013b; Aslam et al., 2013c; Naveed et al., 2013; Aslam et al., 2014). Different techniques are used for evaluation of germplasm and multivariate analysis are most appropriate among them. Data mining is very helpful technique for selection, exploration and modeling of large datasets to find novel patterns and trends to make the interpretation more attractive and decisive. Multivariate analysis are used for data mining. Principle component analysis is unsupervised (no response variable or no dependent variable) multivariate analysis which has wide applicability. Main objective of principal component analysis is to reduce dimensionality and to achieve parsimony by retrieving the smallest number of components which accounts for most of original variation of original multivariate data. So, in this way data is summarized with little loss of information (Fernandez, 2002). Objective of principal component analysis (PCA) are achieved if few first factors explains most of variation. In current study, factor-1 and factor-2 explained 78%, 72% and 69% cumulative variation which showed that PCA was appropriate for this data and biplot on the basis of this data explained most of variation.

High degree of correlation among multiple variables is very useful for making the PCA very effective analysis. Correlation matrix is most commonly used for PCA which is very effective when variables have different units or have different variances. Correlation matrix is equivalent to standardizing the variables with zero mean and unit standard deviation. Root length, fresh shoot weight, fresh root weight, dry shoot weight and dry root weight had strong positive correlation with each other under all of three water treatments. Stomata size had negative correlation with other morphological traits. Eigenvalue is very important criteria for selection of principle factors and 1 is used as cutoff value for selection of principle factors. Eigenvalue >1 showed that factor accounts more variance than one of the original variables (Gabriel, 1981). In current study, three factors (F1, F2 and F3) have eigenvalue greater than one and only these factors are duly important under three water treatments.

Root length (Terbea and Ciocazanu, 1999; Wu and Cosgrove, 2000), shoot length (Hussain, 2009), fresh root and shoot weight (Efeoglu et al, 2009), dry root and shoot weight (Mehdi et al, 2001), leaf temperature (Hirayama et al, 2006; Hussain, 2009) and relative leaf water contents (Matsuura et al, 1996) were extensively used by scientists for evaluation of different crops. These traits were having significantly variation among maize genotypes under three different water treatments.

Contrasting genotypes were selected for three different water treatments i.e. 70%, 50% and 30%FC which showed their suitability for different locations with different levels of water availability. As different areas in Pakistan, had different levels of water availability across the seasons but pattern is somewhat uniform in any specified region.

Genotypes which performed better under 30% of field capacity, were most appropriate for regions with very scarce water availability. Genotypes which were better performer under 50% of field capacity, these were most suitable for the regions with average water availability. Genotypes which were superior under 70% of field capacity, these have suitability for the regions of about normal or optimum water availability. Contrasting genotypes were selected for each of three water treatments which showed that these selected parents could be used for recombination breeding for further development of drought tolerant accessions. EV-329, Islamabad, F-151, EV-342, EV-323 and F-134 genotypes performed better under all of three water treatments i.e. 70%, 50% and 30% of field capacity. Better performance of genotypes over wide range of water availability showed that these genotypes have resistant genetic background. E-337, F-114, EV-79 and EV-330 genotypes were consistently poor for studied three water treatments i.e. 70%, 50% and 30% of field capacity which showed poor genetic background of these genotypes for drought tolerance. Performance of the genotypes was not similar for all traits i.e. performing well for one but not for others or genotype ranking for differences on the basis of different traits. Genotypes were declared as tolerant and susceptible on the basis of discrimination traits like, root length, dry root weight, fresh root weight, fresh shoot weight, relative water contents and root density.

Table -1: Mean Squares with level of significance for different maize parameters subjected to drought stress

SOV	df	RL	SL	FRW	FSW	DRW	DSW	LT	SS	RD
Replication	2	205.36	225.92	103.149	92.14	20.74	30.68	5.0	377094	0.094
Genotypes	33	259.75**	81.06**	66.13**	35.13**	4.14**	4.14**	9.6**	103588**	1.888**
Treatment	2	355.24**	5015.6**	1313.10**	1378.42**	304.63**	304.63**	36658.1**	3359548**	578.97**
G*T	66	0.99**	3.44**	2.61**	1.91**	0.74**	0.73**	5.3ns	4152.2**	0.485**
Error	202	1.378E-30	0.28	0.00496	2.417E-31	9.187E-32	9.731E-32	4.0	3.734E-27	0.029
Total	305									

Note: RL: root length, SL: shoot length, FRW: fresh root weight, FSW: fresh shoot weight, DRW: dry root weight, DSW: dry shoot weight, LT: temperature, SS: stomata size, RD: root density, RWC: relative water contents

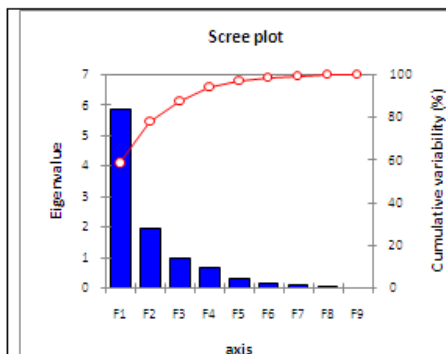


Figure-1: Scree Plot for 70% FC

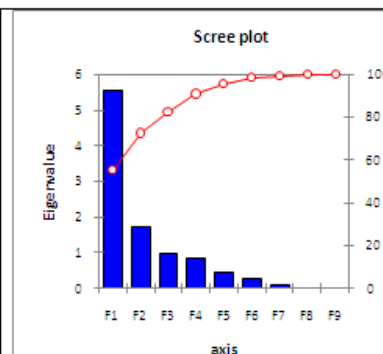


Figure-2: Scree Plot for 50% FC

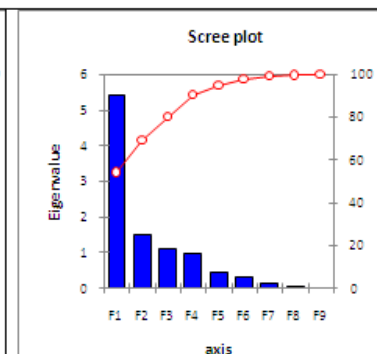


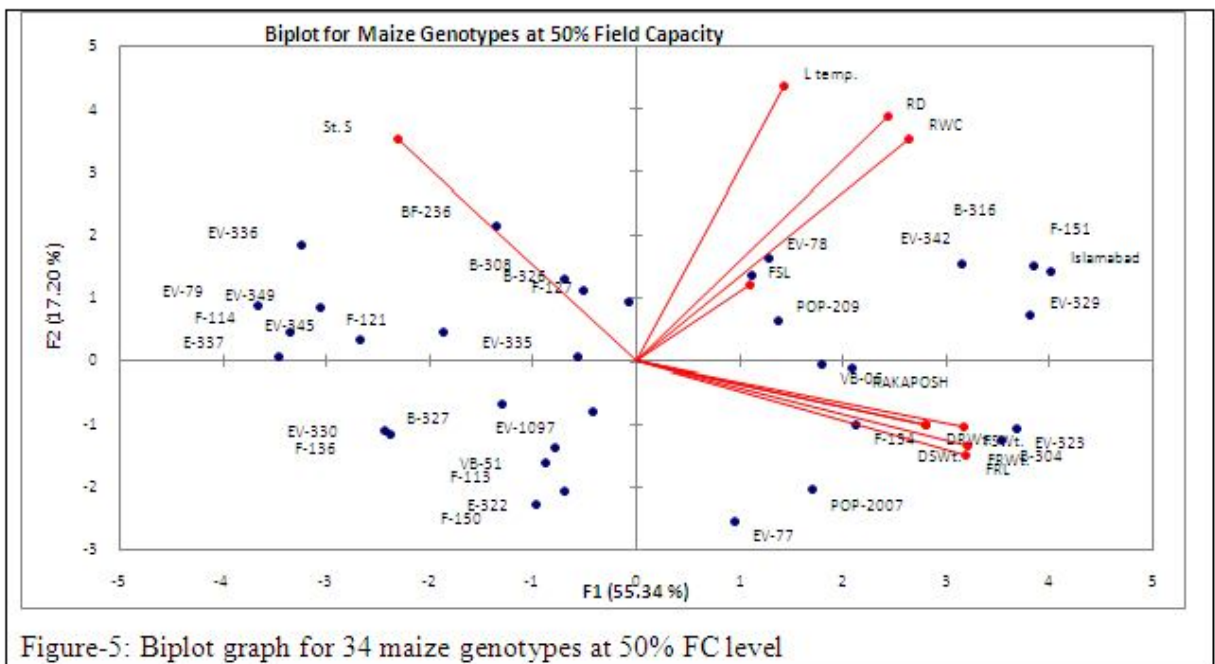
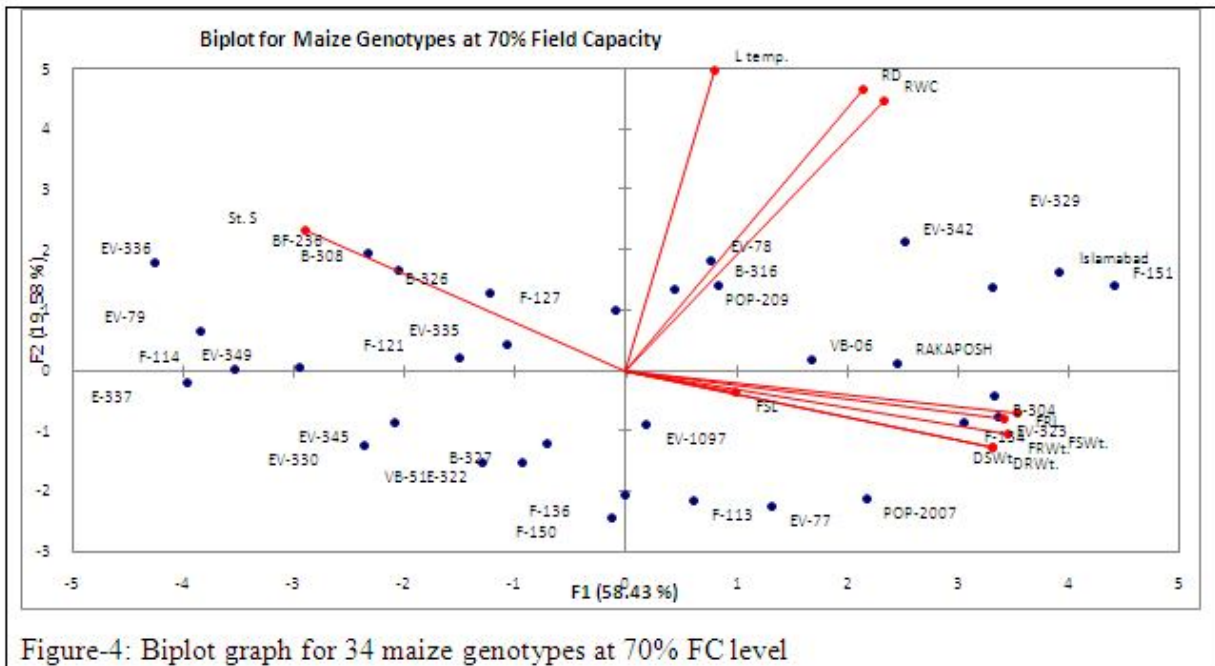
Figure-3: Scree Plot for 30% FC

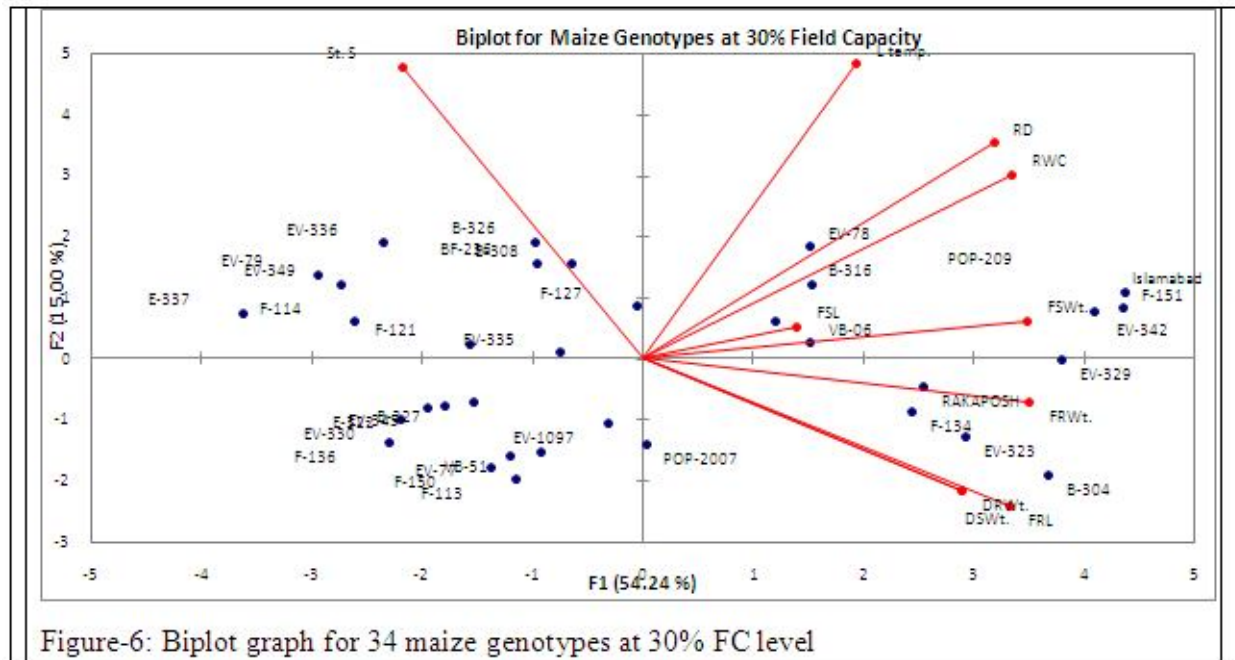


Table-2: Eigenvalue, variability and cumulative variability of different factors based on Principal Component Analysis under different drought treatments										
	Treatment	F1	F2	F3	F4	F5	F6	F7	F8	F9
Eigenvalue	70%FC	5.84	1.96	1.01	0.65	0.29	0.17	0.08	0.03	0.03
	(50%FC)	5.53	1.72	1.09	0.85	0.47	0.29	0.09	0.03	0.03
	(30%FC)	5.43	1.50	1.11	0.99	0.47	0.30	0.14	0.04	0.03
Variability (%)	70%FC	58.43	19.59	9.52	6.49	2.90	1.73	0.81	0.29	0.24
	50%FC	55.34	17.20	9.95	8.45	4.69	2.86	0.92	0.33	0.26
	30%FC	54.24	14.99	11.04	9.94	4.67	3.01	1.38	0.41	0.32
Cumulative %	70%FC	58.43	78.01	87.54	94.04	96.98	98.66	99.47	99.76	100.0
	50%FC	55.34	72.54	82.49	90.94	95.63	98.49	99.42	99.74	100.0
	30%FC	54.24	69.24	80.28	90.22	94.89	97.90	99.28	99.68	100.0

Table-3: Contribution of the variables (%) in variability of different factors having eigenvalue greater than 1 under different drought treatments.									
	70%FC			50%FC			30%FC		
	F1	F2	F3	F1	F2	F3	F1	F2	F3
RL	15.97	0.64	0.09	15.03	3.37	3.29	13.14	6.96	9.14
SL	1.25	0.16	94.96	1.79	2.13	45.03	2.33	0.33	0.13
FRW	15.20	1.43	0.19	15.16	2.71	1.65	14.62	0.59	9.67
FSW	15.01	0.82	0.23	14.77	1.64	1.07	14.46	0.45	6.07
DRW	14.06	2.12	0.72	11.62	1.54	20.14	9.97	5.64	32.98
DSW	14.06	2.12	0.72	11.62	1.54	20.14	9.97	5.64	32.98
LT	0.82	31.93	1.82	3.03	28.11	3.95	4.46	27.64	3.63
SS	10.79	6.90	0.38	7.91	18.26	4.11	5.63	27.20	4.93
RD	5.84	28.04	0.06	8.74	22.18	0.47	12.09	14.81	0.11
RWC	7.00	25.82	0.82	10.33	18.51	0.18	13.33	10.75	0.36

Total	100%	100%	100%	100%	100%	100%	100%	100%	100%
Table-4: Contribution of Factors for different maize traits under different drought levels									
	70%FC			50%FC			30%FC		
	F1	F2	F3	F1	F2	F3	F1	F2	F3
RL	0.40	-0.08	-0.03	0.39	-0.18	0.18	0.36	-0.26	-0.30
SL	0.11	-0.04	0.97	0.13	0.15	0.67	0.15	0.06	0.04
FRW	0.39	-0.12	-0.04	0.39	-0.16	0.13	0.38	-0.08	-0.31
FSW	0.39	-0.09	-0.05	0.38	-0.13	0.10	0.38	0.07	-0.25
DRW	0.37	-0.15	-0.08	0.34	-0.12	-0.45	0.32	-0.24	0.57
DSW	0.37	-0.15	-0.08	0.34	-0.12	-0.45	0.32	-0.24	0.57
LT	0.09	0.56	0.13	0.17	0.53	-0.20	0.21	0.53	0.19
SS	-0.33	0.26	-0.06	-0.28	0.43	-0.20	-0.24	0.52	0.22
RD	0.24	0.53	-0.02	0.29	0.47	0.07	0.35	0.38	-0.03
RWC	0.26	0.51	-0.09	0.32	0.43	0.04	0.36	0.33	-0.06





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