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SELECTION OF SCREENING CRITERIA AGAINST DROUGHT STRESS AT EARLY GROWTH STAGES IN MAIZE (*Zea mays* L.)

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Globally, water stress is the major abiotic stress, which contributes huge in yield losses of major crops including maize. To breed for drought tolerance, the first and foremost step is to search resistance at genotypic level among genetically diverse maize germplasm on the basis of most reliable traits. To find out the best responsible and reliable traits, the responses of 60 maize accessions under three different moisture levels at seedling against different standards were examined following triplicated completely randomized design. Principal component analysis and level of association among studied parameters were computed to mark most relevant standards to tolerance and susceptibility. The accessions were grouped in to different categories on the basis of their performances. On the basis of different evaluation standards, the accessions 19191, 15233, 15155 and 14927 were identified as drought tolerant while the accessions 15035, 14880, 15188 and 24685 were observed as drought sensitive. Selected accessions may be used as contrasting parents for the development of drought tolerant hybrids in the next breeding program. Principal component analysis and graphical representation of the traits indicated that root length, shoot length, root fresh weight, shoot fresh weight, root dry weight, cell membrane thermo-stability and leaf temperature were verified as very important variables linked to drought stress. This study is helpful for the identification of selection criterion linked to drought tolerance in maize to be used in further selection and breeding programs.
Keywords: Drought stress, maize, genetic variability, principal component analysis, seedling traits, correlation analysis.

INTRODUCTION

Food security is a great global challenge for at least another 40 years due to the exponential increase in the population size and demand for food (Ma *et al.*, 2016). Inadequate water availability is very crucial and limiting factor which affects the growth and development of crops ultimately decreasing the food production. Water stress is major abiotic stress causing yield reduction throughout the world especially in arid and semi-arid areas (Viscardi *et al*., 2016; Tian *et al*., 2016). Moreover, severe droughts are expected in future due to climate change causing reduced production of food crops including staple cereals. Among the cereals, maize is the 2nd most important cultivated crop in the world after wheat with the average production value of over 1 billion tons per annum. It is widely used in industry for food, feed and bioenergy production (Nuss and Tanumihadrjo, 2010). Under the favorable environmental and crop management conditions, maize is highly productive crop, however, it is very sensitive to drought stress. About 15-20% loss from the production potential of maize is witnessed due to drought stress all over the world (FAOSTAT, 2008; Lobell *et al*., 2011).

Drought stress can occur at various developmental stages of the crop (Aslam *et al*., 2015; Maqbool *et al*., 2015a, 2015b; Maqbool *et al*., 2016; Maqbool *et al*., 2017). In maize, stress during and after anthesis reduces the grain yield up to 20% (Kebede *et al*., 2001; Cakir, 2004). Drought stress can also reduce the biomass production (Ashraf, 1989). Reduction in yield can also be observed in cell membrane thermostability (Thakur and Rai, 1984; Chohan, 2012). Genetic improvement of plants is very important strategy along with proper management practices to fill the gap between theoretical and actual yield, which is up to 30% (Edmeades *et al.*, 2003). Therefore, it is need of the time to develop drought tolerant maize cultivars which can express their full yield potential even at sub-optimal water availability for sustainable production.

To develop drought tolerant varieties or hybrids, the basic step is the selection of suitable parental lines for breeding from the available germplasm. Field screening is relatively difficult due to uncontrolled environmental conditions, soil heterogeneity, large amount of plant material, time and labor investment. Hence greenhouse experiments are preferred over field trials because of the ease, precision and reliability. The main objectives of this study were to develop selection criteria for the identification of drought tolerant and drought sensitive accessions and to find the correlation among different seedling traits of maize.

MATERIALS AND METHODS

***Experimental Details*:** Experiment was conducted in the greenhouse at 31.4336° latitude and 73.0683° longitude during spring. The average range for minimum and maximum temperature in the greenhouse throughout the experiment was 30°C and 35°C, respectively. Total 60 maize (*Zea mays* L.) accessions were collected from Plant Genetic Resource Institute (PGRI), National Agricultural Research Center (NARC), Islamabad (Table 1). These accessions were evaluated in greenhouse at seedling stage based upon different evaluating standards against various levels of moisture. Seeds were planted in polythene bags (18×9 cm) following triplicated completely randomized design under factorial structured treatments having three sets of treatments as follows:

1. $T_{80\%}$ = 80% of field capacity (Control)
2. $T_{60\%}$ = 60% of field capacity
3. $T_{40\%}$ = 40% of field capacity

Field capacity was measured according to the following formula:

$$\text{Field Capacity (\%)} = \frac{T2-T1}{T1} \times 100$$

Where, T1: Weight of the oven-dried soil, T2: Weight of the saturated soil.

Five seedlings of each accession were kept in each replication and equally measured 150 ml water was applied to facilitate germination in all the bags. Each bag was irrigated after seven days of sowing for each moisture level. Measuring cylinder was used for the measurement of each moisture level.

Parameters**:** After 21 days of sowing at three leaf stage data were recorded of five plants for various important morphological, physiological and biochemical standards which include root fresh weight (RFW; g), root dry weight (RDW; g), root length (RL; cm), shoot length (SL; cm), shoot fresh weight (SFW; g), shoot dry weight (SDW; g), root-shoot ratio (RSR), leaf temperature (LT; ˚C), stomatal conductance (SC; mmol m^{-2} s^{-1}), cell membrane thermostability (CMT; %), chlorophyll *a* contents (*Chl a*; mg/ml), chlorophyll *b* contents (*Chl b*; mg/ml), carotenoid contents (Caro; mg/ml), proline contents (Pro; µmol/g) and ascorbic acid contents (AA; µg/g). Root-shoot ratio was estimated according to the formula given by Nour *et al*. (1978).

$$\text{Root} - \text{shoot ratio} = \frac{\text{Root dry weight}}{\text{Shoot dry weight}}$$

For leaf temperature, infrared thermometer (RAYPRM 30 CFRJ, RAYTEK, USA) was used. Cell membrane thermostability (CMT) was estimated according to Ibrahim and James (2001). Steady state porometer (Model L-1 1600 SSP1674 Li cor. Ink, USA) was used to measure the stomatal conductance. Chlorophyll *a, b* and carotenoid contents were estimated by following a protocol used by Nagata and Yamashita (1992). In plant shoot samples, ascorbic acid contents were determined by following Kampfenkel *et al*. (1995). Proline contents of leaf were determined by following the method devised by Bates *et al*. (1973).

Statistical Analysis**:** Maize genotypes and treatments applied were treated as two different factors, hence, Two-Factor Factorial Analysis of Variance under CRD (Steel and Torrie, 1997) was conducted to estimate the effects of treatments, genotypes and their interaction. Correlation analysis was used to estimate the correlation coefficients between different traits following the method described by Kwon and Torrie (1964). Drought stress effect of various parameters with variable field capacity were combined in a graph to visualize most of the variable parameters using Microsoft Excel. Moreover, Principal Component Analysis (PCA) based Biplots (Gabriel, 1981) were made for each drought stress treatment separately using principle factors which have most of variability. Biplot was two-dimensional scatter diagram that depicted the scattering pattern of genotypes and traits.

RESULTS

Analysis of variance**:** Among the accessions, significant differences were observed for all the traits under study (Table 2). Water treatments ($T_{80\%}$, $T_{60\%}$ and $T_{40\%}$) were also significantly different in their effects. Genotype × treatment interaction was also observed to be significant for all the subjected traits. Under $T_{40\%}$, all the traits showed decrease in their expression as compared to that of $T_{60\%}$ except leaf temperature and proline contents.

Table 1. List of maize germplasm used in experiments.

Sr. No.		Sr. No.		Sr. No.		Sr. No.		Sr. No.		Sr. No.	
1	15129	11	15091	21	15157	31	15047	41	24669	51	15352
2	15979	12	14997	22	14970	32	19198	42	15317	52	19176
3	15327	13	15220	23	19180	33	19201	43	15038	53	15280
4	19206	14	14965	24	14969	34	19195	44	15035	54	15169
5	15023	15	24684	25	15233	35	15127	45	14971	55	15175
6	15085	16	15123	26	15104	36	15064	46	24672	56	15202
7	14967	17	14927	27	15158	37	14968	47	19191	57	15188
8	15341	18	19179	28	14985	38	15334	48	15155	58	15257
9	15066	19	19203	29	24685	39	14880	49	15139	59	15268
10	15345	20	19186	30	15124	40	15162	50	15153	60	15044

Table 2. Mean square values from analysis of variance for seedling traits of maize accessions under treatments.

SOV	Accessions (A)	Treatments (T)	A×T	Error
DF	59	2	118	358
RL	96.10^{**}	1978.41^{**}	3.77^{**}	0.60
SL	90.39^{**}	2399.08^{**}	4.17^{**}	0.07
RFW	28.25^{**}	1065.16^{**}	2.13^{**}	0.01
SFW	28.44^{**}	1436.82^{**}	2.21^{**}	0.00188
RDW	1.57^{**}	3.75^{**}	0.011^{**}	0.00056
SDW	1.04^{**}	1.68^{**}	0.006^{**}	0.00039
RSR	0.17^{**}	0.28^{**}	0.001^{**}	0.00013
SC	0.04^{**}	0.047^{**}	0.038^{**}	3.43
LT	8.44^{**}	387.64^{**}	1.25^{**}	0.02
CMT	1218.42^{**}	53260.3^{**}	69.38^{**}	4.41
Chl *a*	0.08^{**}	1.62^{**}	0.003^{**}	0.00016
Chl *b*	0.08^{**}	1.09^{**}	0.001^{**}	0.00010
Caro	0.06^{**}	1.03^{**}	0.0009^{**}	0.00013
Pro	4.84^{**}	181.68^{**}	0.38^{**}	1.21
AA	0.12^{**}	5.07^{**}	0.013^{**}	0.0005

****** Highly significant differences (P<0.01)

SOV= Source of variation, DF= Degree of freedom, A×T= Accession × Treatment, RL= Root length, SL= Shoot length, RFW= Root fresh weight, SFW= Shoot fresh weight, RDW= Root dry weight, SDW= Shoot dry weight, RSR= Root/shoot ratio, SC= Stomatal conductance, LT= Leaf temperature, CMT= Cell membrane thermostability, Chl *a*= Chlorophyll a contents, Chl *b*= Chlorophyll b contents, Caro=carotenoid contents, Pro=Proline contents, AA= Ascorbic acid contents

Selection of traits: Changes in the response of plants were visualized with respect to change in field capacity through graphical representation. This graph showed that how much difference is there in the performance of traits under changing field capacity. It can be seen from the graph (Fig. 1) that with the decrease of field capacity (from $T_{80\%}$ to $T_{60\%}$ to $T_{40\%}$), a general trend of decrease in root length (RL), shoot length (SL), root fresh weight (RFW), shoot fresh weight (SFW) and cell membrane thermostability (CMT) along with increase in leaf temperature (LT) and proline contents (Pro) was observed as response of all the accessions under studied.

Biplot analysis (Fig. 2, 3 and 4) also indicated the response of traits under all the treatments ($T_{80\%}$, $T_{60\%}$ and $T_{40\%}$). PCA biplot for $T_{80\%}$ depicted that Chl *a*, Chl *b*, RL, SL, RFW, SFW, RDW, SDW and RSR were the most discriminating parameters (Fig. 2). Among all the studied traits Chl *a*, Chl *b*, SDW, SFW, RFW, RL, RDW, SL and RSR were most discriminating traits under $T_{60\%}$ of the drought stress treatment (Fig. 3). PCA biplot for $T_{40\%}$ representing that Chl *a*, Chl *b*, SFW, RFW, RDW, SDW and RL were the most discriminating variables among all the studied traits (Fig. 4).

Different traits have different pattern of contribution For $T_{80\%}$ traits SDW, SFW, RFW, SL, RL, RDW and RSR were in positive direction while SC, CMT and Pro were negatively contributing (Fig. 2). SFW, SDW, RFW, RSR and RL were positively contributing and LT, SC and Pro were negatively contributing under $T_{60\%}$ (Fig. 3). Variables RL, RFW, SDW, SFW, RDW and RSR were in positive direction under $T_{40\%}$ and SC and Pro contents were negatively contributed (Fig. 4).

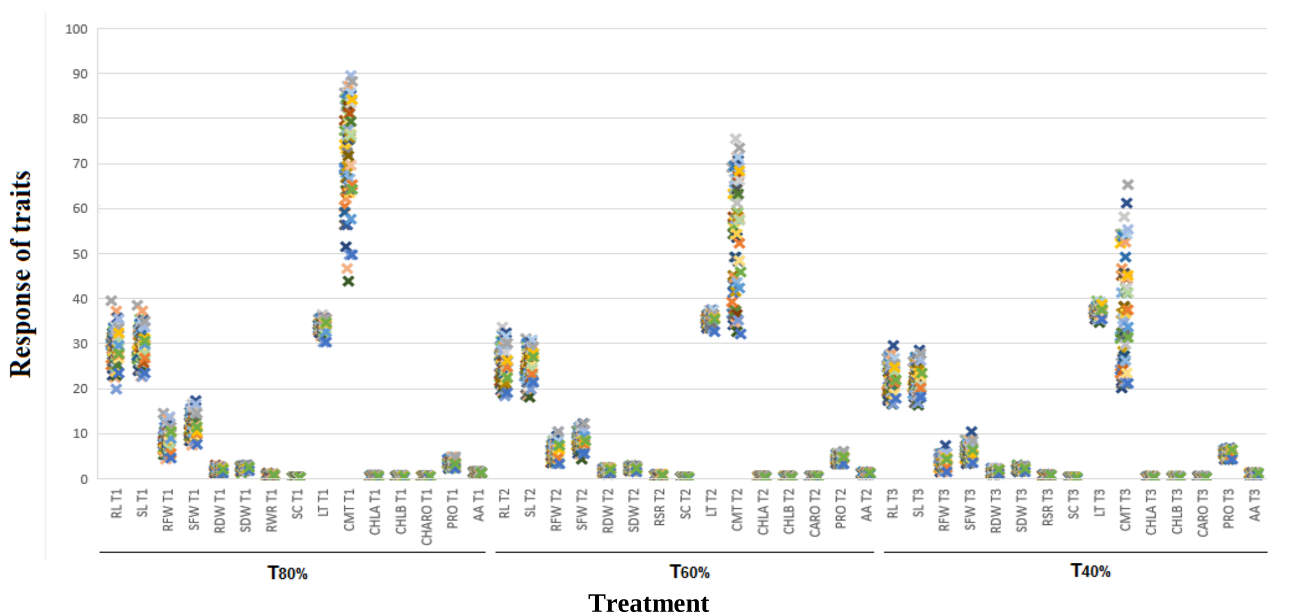

Figure 1. Response graph for various studied traits under changing field capacity.

RL= Root length, SL= Shoot length, RFW= Root fresh weight, SFW= Shoot fresh weight, RDW= Root dry weight, SDW= Shoot dry weight, RSR= Root/shoot ratio, SC= Stomatal conductance, LT= Leaf temperature, CMT= Cell membrane thermostability, Chl *a*= Chlorophyll a contents, Chl *b*= Chlorophyll b contents, Caro=carotenoid contents, Pro=Proline contents, AA= Ascorbic acid contents

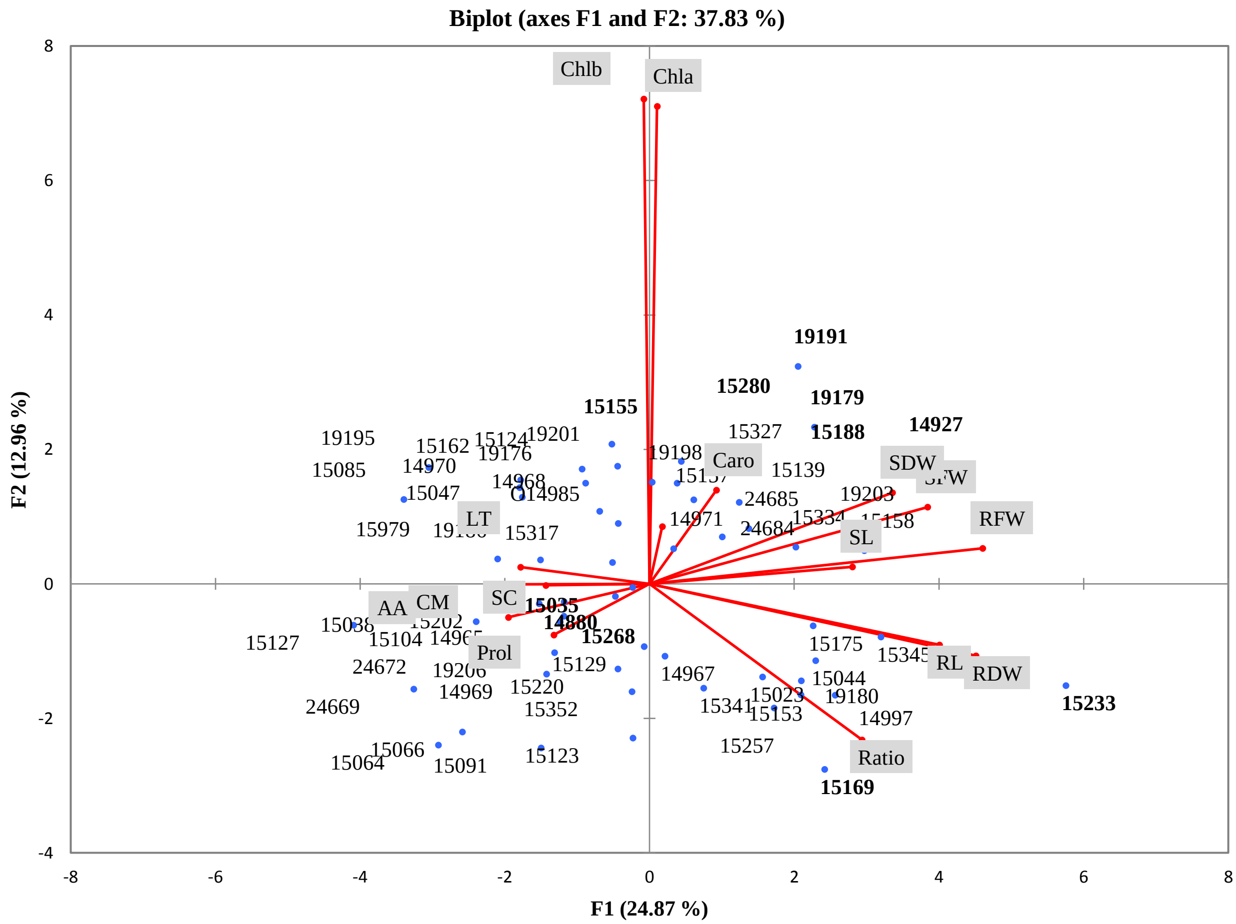

Figure 2. Biplot analysis based on principal components analysis (PCA) for seedling traits of maize accessions under $T_{80\%}$.

RL= Root length, SL= Shoot length, RFW= Root fresh weight, SFW= Shoot fresh weight, RDW= Root dry weight, SDW= Shoot dry weight, RSR= Root/shoot ratio, SC= Stomatal conductance, LT= Leaf temperature, CMT= Cell membrane thermo-stability, Chl *a*= Chlorophyll a contents, Chl *b*= Chlorophyll b contents, Caro=carotenoid contents, Pro=Proline contents, AA= Ascorbic acid contents

***Selection of genotypes*:** Selection of genotypes is facilitated by the principal component analysis (PCA) when there is substantial number of accessions to be selected and many traits to be involved. For each drought stress treatment i.e., $T_{80\%}$, $T_{60\%}$ and $T_{40\%}$, biplot analysis was accomplished with the help of two main factors (F_1 and F_2). Genotypes and different variables were merged in a single biplot to further facilitate the visualization. Biplot graphs based on principal component analysis for $T_{80\%}$, $T_{60\%}$ and $T_{40\%}$ were presented in Figures 2, 3 and 4, respectively.

Results indicated presence of significant genetic variability among all the studied accessions under both normal and stress conditions. For the analysis of genotypes, biplot displayed through PCA technique was divided into four quartiles. PCA biplot for $T_{80\%}$ showed 37.83% of total variability. Biplot for $T_{80\%}$ (Fig. 2) revealed that the accessions 19191, 14927, 19179, 15280, 15233, 15155, 15188 and 15169 were genetically most distinct and scattered towards the positive value region reflecting much better performance than the other accessions. Accessions 19191, 14927, 15233 and 15123 had high mean values for all the studied traits except leaf temperature and proline contents under $T_{80\%}$ of moisture stress level. Accessions 15035, 14880 and 15268 were present closer to biplot origin and distributed in negative value region with low variability and poor adaptability for studied traits.

$T_{60\%}$ explained 37.82% of the total variability. PCA for $T_{60\%}$ (Fig. 3) showed that there was a huge dispersion of accessions which depicted variable response of accessions with respect to drought stress. Accessions 19191, 15327, 15233, 15123, 15155 and 14927 were present in positive quartile farthest away from the biplot origin showing better performance as compared to the rest of accessions under $T_{60\%}$. Under $T_{60\%}$

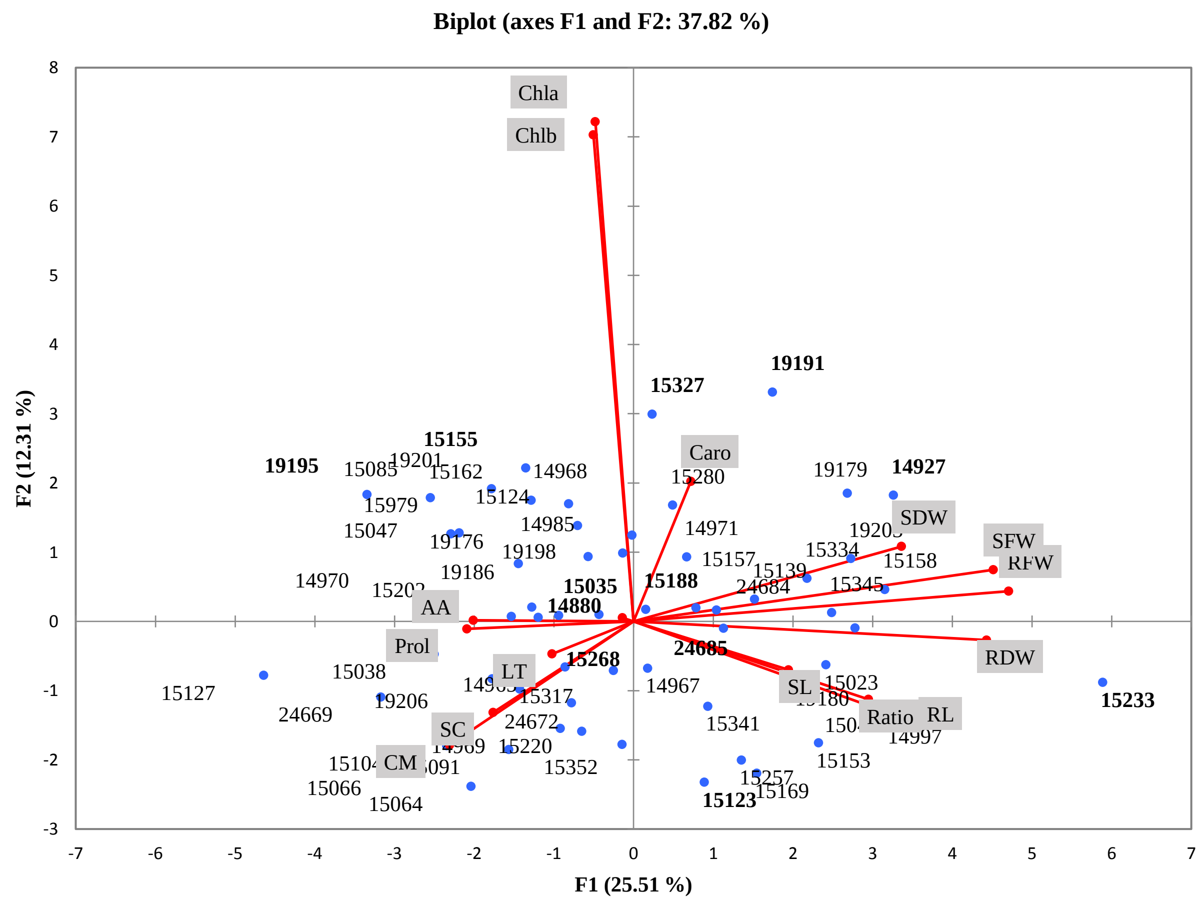

Figure 3. Biplot analysis based on principal components analysis (PCA) for seedling traits of maize accessions under $T_{60\%}$.

RL= Root length, SL= Shoot length, RFW= Root fresh weight, SFW= Shoot fresh weight, RDW= Root dry weight, SDW= Shoot dry weight, RSR= Root/shoot ratio, SC= Stomatal conductance, LT= Leaf temperature, CMT= Cell membrane thermos-stability, Chl *a*= Chlorophyll a contents, Chl *b*= Chlorophyll b contents, Caro=carotenoid contents, Pro=Proline contents, AA= Ascorbic acid contents

15127, 19191, 15233 and 14927 showed highest means for all the studied traits. PCA biplot for $T_{40\%}$ showed 33.82% of the total variability. Accessions 15188, 24685, 15035 and 14880 were located near the biplot origin and showed comparatively poor adaptability and had lowest means for most of the studied traits. From the results of PCA for $T_{40\%}$, the accessions 15280, 19191, 15233, 14927, 15155, 15169 and 15127 were well adapted and best performing as secured position farther from origin in positive quadrant on biplot graph. Accession 15035 was in the biplot origin and reflecting the least variability under $T_{40\%}$ (Fig. 4).

All the genotypes performed differently under all the stress levels. Accessions 19191, 15233, 15155 and 14927 showed least change in mean performance under different treatments and performed fairly well under all the treatments for seedling traits. These genotypes were hence declared as comparatively very good performer under normal and low moisture stress. Accessions 15035, 14880 and 15268 which did not perform well under $T_{80\%}$ so these are genetically poor performing lines. Accessions 19191 and 14927 did not changed their behavior at any kind of stress which indicating that these lines are resistant at all the moisture stress levels. Accessions 15188 and 24685 showed better performance under $T_{80\%}$ but had poor adaptability under $T_{60\%}$ and $T_{40\%}$ so these lines were selected as drought sensitive.

Correlation studies**:** Genotypic correlation coefficients were estimated for all the seedling morpho-physiological and biochemical traits under all the applied treatments (Table 3). RL had positive and significant correlation with SL, RFW, SFW, RDW, SDW, Chl *a*, Chl *b*, RSR and Caro under stressed conditions. Pro and LT showed negative and significant correlation with RL under all the treatments.

SL and SFW had positive and significant correlation with Chl *a*, Chl *b* and Caro, while negative correlation was observed

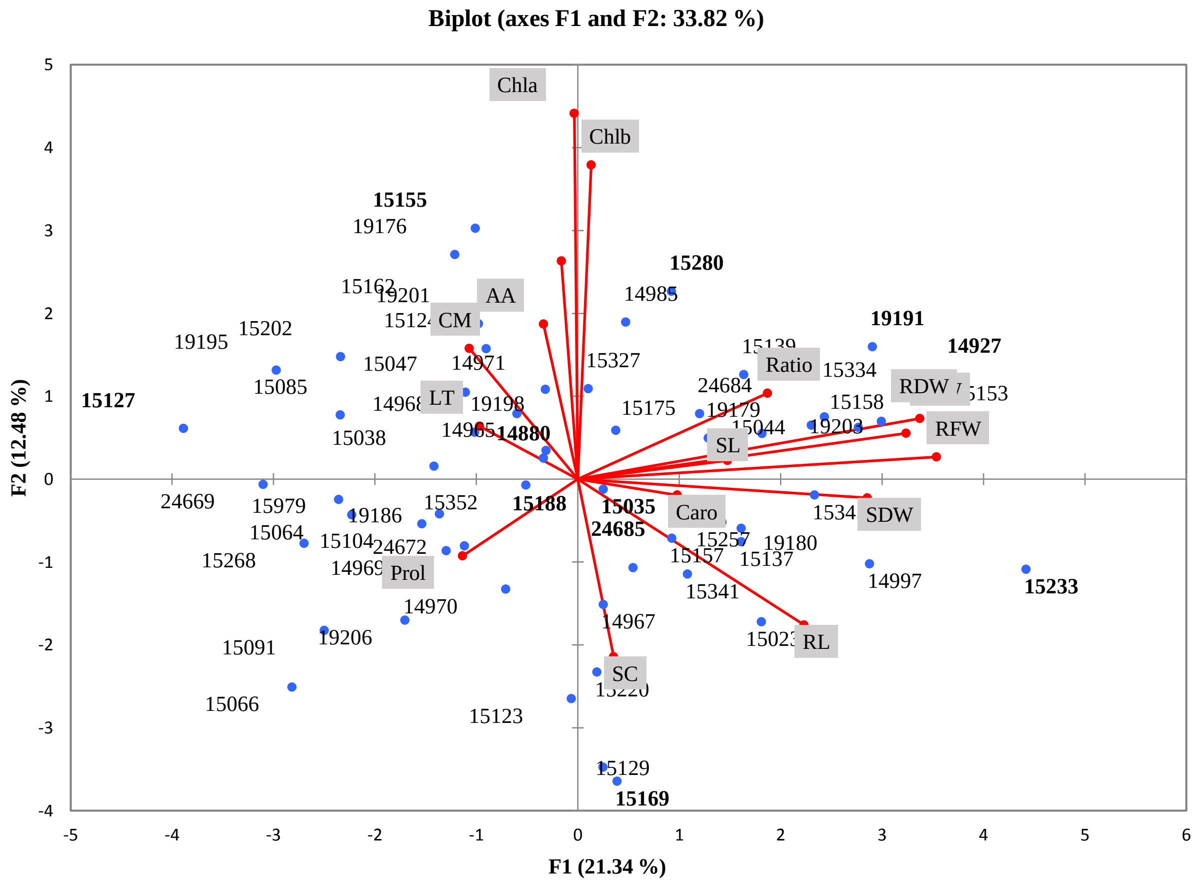

Figure 4. Biplot analysis based on principal components analysis (PCA) for seedling traits of maize accessions under $T_{40\%}$.

RL= Root length, SL= Shoot length, RFW= Root fresh weight, SFW= Shoot fresh weight, RDW= Root dry weight, SDW= Shoot dry weight, RSR= Root/shoot ratio, SC= Stomatal conductance, LT= Leaf temperature, CMT= Cell membrane thermos-stability, Chl *a*= Chlorophyll a contents, Chl *b*= Chlorophyll b contents, Caro=carotenoid contents, Pro=Proline contents, AA= Ascorbic acid contents

with SC, CMT, LT and Pro. Similarly, significant positive correlation of SDW was found with Chl *a* and Chl *b*, while negative correlation with LT, Pro and AA was observed. SC, LT and AA showed significant and negative correlation with RFW under all the treatments. RDW showed positive and highly significant correlation with RSR and *Chl b* while negative correlation was found of RDW with Pro and LT.

CMT and AA showed positive and significant correlation with SC under all the treatments. SC had significant and negative correlation with Chl *a*, Chl *b* and Pro. Negative and significant correlation of Chl *a*, Chl *b*, Caro, Pro with CMT was observed at all the levels of moisture treatments. CMT showed significant and negative correlation with Caro and Pro. LT had positive and highly significant correlation with AA at all the levels. LT showed negative and significant correlation with Chl *b* and with Caro. Under all the treatments levels Chl *a* had positive and significant correlation with Chl *b* and Caro. Pro and AA contents had negative and significant correlation with Chl *b*, which showed significant and positive correlation with Car. Pro had positive and significant correlation with AA.

DISCUSSION

Globally, the production of maize is badly impacted by different abiotic stresses including low moisture stress (Aslam *et al.*, 2014). Maize plant is very sensitive to drought stress throughout its life cycle with different morpho-physiological and biochemical adverse effects at various stages of growth and development (Aslam *et al.*, 2006, 2014; Anjum *et al.*, 2017). Thus, for the evaluation of maize accessions against low moisture stress, the choice of a proper screening standard is the need of the hour. Different low moisture stress treatments ($T_{80\%}$, $T_{60\%}$ and $T_{40\%}$) were used for to categorize

Table 3. Genotypic correlation coefficients among seedling traits of maize under $T_{80\%}$, $T_{60\%}$ and $T_{40\%}$.

		RL	SL	RFW	SFW	RDW	SDW	RSR	SC	CMT	LT	Chl *a*	Chl *b*	Caro	Pro	AA
SL	$T_{80\%}$	0.245**	**1.000**													
	$T_{60\%}$	0.394**	**1.000**													
	$T_{40\%}$	0.272**	**1.000**													
RFW	$T_{80\%}$	0.322**	0.088	**1.000**												
	$T_{60\%}$	0.212**	0.122	**1.000**												
	$T_{40\%}$	0.169*	0.200**	**1.000**												
SFW	$T_{80\%}$	0.452**	0.417**	0.284**	**1.000**											
	$T_{60\%}$	0.470**	0.411**	0.362**	**1.000**											
	$T_{40\%}$	0.354**	0.318**	0.443**	**1.000**											
RDW	$T_{80\%}$	0.231**	0.086	0.046	0.185**	**1.000**										
	$T_{60\%}$	0.301**	0.002	0.067	0.181**	**1.000**										
	$T_{40\%}$	0.226**	0.031	-0.025	0.185**	**1.000**										
SDW	$T_{80\%}$	0.216**	0.055	0.092	0.091	0.576**	**1.000**									
	$T_{60\%}$	0.306**	-0.002	0.084	0.010	0.608**	**1.000**									
	$T_{40\%}$	0.248**	0.044	0.012	0.077	0.607**	**1.000**									
RSR	$T_{80\%}$	0.082	0.071	-0.005	0.152*	0.784**	-0.048	**1.000**								
	$T_{60\%}$	0.134*	0.024	0.012	0.172**	0.767**	-0.034	**1.000**								
	$T_{40\%}$	0.091	0.013	-0.023	0.182**	0.772**	-0.027	**1.000**								
SC	$T_{80\%}$	-0.201**	-0.156**	-0.229**	-0.362**	0.047	0.022	0.028	**1.000**							
	$T_{60\%}$	-0.084	-0.178**	-0.225**	-0.377**	0.047	-0.009	0.023	**1.000**							
	$T_{40\%}$	-0.027	-0.201**	-0.250**	-0.404**	0.009	0.000	-0.015	**1.000**							
CMT	$T_{80\%}$	0.018	-0.278**	0.049	-0.308**	0.075	0.053	0.058	0.447**	**1.000**						
	$T_{60\%}$	-0.060	-0.251**	-0.103	-0.240**	-0.003	0.036	-0.040	0.391**	**1.000**						
	$T_{40\%}$	0.006	-0.288**	-0.255**	-0.413**	-0.031	0.020	-0.041	0.483**	**1.000**						
LT	$T_{80\%}$	-0.101	-0.148*	-0.125*	-0.420**	-0.137*	-0.190**	-0.029	0.065	0.062	**1.000**					
	$T_{60\%}$	-0.086	-0.178**	-0.161*	-0.368**	-0.130*	-0.110**	-0.002	0.005	0.083	**1.000**					
	$T_{40\%}$	-0.150*	-0.270**	-0.330**	-0.313**	-0.084	-0.174**	0.032	-0.013	-0.046	**1.000**					
Chl *a*	$T_{80\%}$	0.301**	0.392**	0.481**	0.293**	0.050	0.220**	-0.114	-0.187**	-0.256**	0.022	**1.000**				
	$T_{60\%}$	0.274**	0.328**	0.388**	0.324**	0.062	0.185**	-0.053	-0.212**	-0.353**	-0.043	**1.000**				
	$T_{40\%}$	0.279**	0.267**	0.325**	0.329**	0.051	0.125*	-0.041	-0.094	-0.276**	-0.055	**1.000**				
Chl*b*	$T_{80\%}$	0.220**	0.242**	0.387**	0.228**	0.186*	0.334**	-0.015	-0.142*	-0.260**	0.110	0.501**	**1.000**			
	$T_{60\%}$	0.240**	0.111	0.297**	0.138*	0.164*	0.252**	0.012	-0.195**	-0.202**	-0.096	0.548**	**1.000**			
	$T_{40\%}$	0.252**	0.114	0.190**	0.319**	0.197**	0.282**	-0.028	-0.146*	-0.120**	-0.157*	0.563**	**1.000**			
Caro	$T_{80\%}$	0.095	0.197**	0.275**	0.444**	0.046	-0.200	0.059	0.056	-0.255**	0.114	0.339**	0.338**	**1.000**		
	$T_{60\%}$	0.183**	0.157*	0.140*	0.435**	0.026	0.024	-0.003	0.010	-0.164*	-0.170*	0.439**	0.396**	**1.000**		
	$T_{40\%}$	0.336**	0.073	0.074	0.439**	0.159*	-0.005	0.180**	-0.035	-0.110	-0.094	0.436**	0.380**	**1.000**		
Pro	$T_{80\%}$	-0.280**	-0.311**	-0.027	-0.128*	-0.449**	-0.266**	-0.348**	-0.127*	-0.292**	0.029	-0.108	0.019	0.039	**1.000**	
	$T_{60\%}$	-0.310**	-0.110**	0.019	-0.078	-0.410**	-0.260**	-0.332**	0.110	-0.087	0.133*	-0.069	-0.034	0.052	**1.000**	
	$T_{40\%}$	-0.253**	-0.080	0.036	-0.151*	-0.401**	-0.280**	-0.292**	-0.160*	-0.132*	0.097	-0.205**	-0.065	-0.124	**1.000**	
AA	$T_{80\%}$	-0.209**	0.047	-0.272	-0.271**	-0.128*	-0.140*	-0.050	0.210**	0.015	0.537**	-0.111	-0.148*	0.070	0.137*	**1.000**
	$T_{60\%}$	0.115	0.070	-0.211**	-0.146*	-0.119	-0.110**	0.008	0.257**	0.104	0.496**	-0.133	-0.187**	0.024	0.004	**1.000**
	$T_{40\%}$	0.209**	0.031	-0.225**	-0.188**	-0.086	-0.161*	0.026	0.207**	0.039	0.503**	-0.138*	-0.226**	-0.014	0.065	**1.000**

RL= Root length, SL= Shoot length, RFW= Root fresh weight, SFW= Shoot fresh weight, RDW= Root dry weight, SDW= Shoot dry weight, RSR= Root/shoot ratio, SC= Stomatal conductance, LT= Leaf temperature, CMT= Cell membrane thermbility, Chl *a*= Chlorophyll a contents, Chl *b*= Chlorophyll b contents, Caro=carotenoid contents, Pro=Proline contents, AA= Ascorbic acid contents

the low moisture stress tolerant and sensitive accessions in the current study; these accessions were evaluated based on different morphological, physiological and biochemical standards to finalize most efficient and effective selection standard. Results showed that among the accessions regarding low moisture stress treatments and their interaction, significant differences were found for all the morphological, physiological and biochemical standards of evaluation. The performance level of all the standards decreased with the increasing level of water stress except leaf temperature and proline contents. The reduced moisture availability reduces overall physiological and biochemical life of the plants; this reduction in physiological reactions ultimately results in less availability of photosynthetic synthesis and finally stunted growth. Same type of results was published by different researchers in different era like Hussain (2009); Olaoye *et al*. (2009); Ali *et al*. (2011a); Chohan *et al*. (2012); Iqbal *et al.* (2012); Chen *et al*., (2012); Aslam *et al*. (2014) and Anjum *et al*. (2017). These researchers reported that with high level of moisture stress there was reduction in overall physiological life of the plants in all the phases of life.

Response of roots to low moisture stress condition is very important as indicator of resistance and susceptibility. Change in root length under moisture stress provides a good immediate sign of response. Being sensitive to reduction in moisture availability, root length rapidly reduced with increase in low moisture severity. In current study accessions 19191 and 14927 were reported with maximum root and shoot length under $T_{60\%}$ and $T_{40\%}$ whereas accessions 15035 and 14880 had minimum root and shoot length at $T_{80\%.}$ Partheeban *et al*. (2017) published that with the increase in low moisture stress level always there was reduction in root and shoot lengths. Fresh root weight is most commonly used as selection criterion at seedling stage against drought stress tolerance Hamayun *et al.* (2010). Fresh root and dry root weights of the plant under water deficit conditions significantly reduced by inhibiting the penetration of root into the dry soil (Borrell and Hammer, 2000; Thomas and

Howarth, 2000). Maximum fresh and dry root weight was observed in accessions 19191 and 15233 under moisture stress levels while minimum was found in 14880 and 15268 under normal conditions. Under drought stress, a significant reduction in fresh and dry shoot weight of seedlings was also observed by Sharp *et al.* (1988) due to the reduction in growth of shoot, increase in senescence and changing the trend of the plant growth from shoot towards root. In this study highest fresh and dry shoot weight and root shoot ratio was observed in accessions 19191, 14927 and 15169 under all the stress levels whereas lowest fresh and dry shoot weight and root shoot ratio was gained in sensitive genotypes 15188 and 24685. Consistent with other studies, root growth and shoot growth were inhibited by low moisture availability and root-shoot ratio was typically reduced as reported by Li *et al.* (2014). Moreover, root-shoot ratio has also been used as selection criteria for low moisture stress tolerance in different studies (Wu and Cosgrove, 2000; Grzesiak, 2001; Khan *et al*., 2010; Ali *et al*., 2011a; Chohan *et al*., 2012). Due to extent of relationship of root length with other parameters as compared to shoot length, it can be said that root length is more promising selection criterion under low moisture stress conditions, because root acts as more important sink at low moisture availability opposite to shoot which acts as important sink during normal conditions with excessive water availability. Moreover, the shifting of plant growth or tolerant genotypes from shoot growth to root growth under stress conditions is another evidence.

Leaf temperature and proline contents increased with the increase in water stress level. Similar findings have been reported by Chandrasekar *et al*. (2000); Siddique *et al*. (2000) and Hola *et al*. (2017). In maize and other plants, highest leaf and canopy temperatures were observed in different studies under severe drought due to reduced transpiration (Siddique *et al*., 2000; Hirayama *et al*., 2006). In the present study genotypes 19191, 14927 and 15280 had highest leaf temperature at $T_{60\%}$ and $T_{40\%}$ of stress level. Sensitive genotypes 15188 and 14880 had lowest leaf temperature with the increase of stress. Proline and soluble sugars are the key osmolytes (important for osmotic adjustment in plant). The increase in proline contents as a response to increasing level of stress was observed by Yoshiba *et al*. (1997) and Mundree *et al*. (2002). Increased proline contents were observed in the accessions 15280 and 19191 under $T_{60\%}$ and $T_{40\%}$. Cell membrane thermostability can also be used as selection criterion against low moisture stress tolerance; as increasing stress levels decreased the cell membrane thermostability in studied maize accessions. Under water deficit conditions ultimately there is reduction in transpiration rate which increases temperature at cellular level, this increase in temperature causes structural damage to membranes, which results in leakage of solutes from cell, hence, membrane thermostability of the cell reduces. These results are in accordance with the findings of Rehman *et al.* (2004), Munjal *et al.* (2004) and Aslam *et al*. (2006). Maximum stability of membrane was found in the genotypes 15127, 19191 and 15155. Genotypes 14880 and 24685 had minimum membrane stability under $T_{80\%}$. A reduction was observed in chlorophyll *a*, *b* and carotenoid contents under low moisture stress because due to leaf senescence acceleration, chlorophyll *a* and *b* contents reduces significantly under stressed conditions (Mohammadkhani and Heidari, 2007; Efeoglu *et al.*, 2009). Genotypes 15155, 15280 and 19191 showed maximum chlorophyll *a, b* and carotenoid contents under $T_{60\%}$ and $T_{40\%}$. Maximum variation in data explained by first principal component is indication of successful achievement of objectives of PCA. In our findings PCA biplots explained 37.83%, 37.82% and 33.82% of the total variability for $T_{80\%,}$ $T_{60\%}$ and $T_{40\%}$ respectively. These findings were in accordance with Aslam *et al*. (2014) who reported 78.01%, and Maqbool *et al.* (2016) who reported 88.23% cumulative contribution. From this study it was found that RL, SL, RFW, SFW and RDW were identified as very good variables for the selection of maize genotypes under low moisture stress environments. Principal component analysis has been extensively used in the research to partition the observed variability of data. This analysis is very effective for the selection of genotypes under drought stress. For three different drought treatments, biplots and genotypic selections were made separately. Different researchers evaluated different crops through biplots under diverse environmental conditions (Yan and Kang 2011; Maqbool *et al*., 2015a, 2015b, 2016). PCA biplots for $T_{80\%}$, $T_{60\%}$ and $T_{40\%}$ showed that accessions 19191, 15233, 15155 and 14927 were comparatively showed good performance under different stress levels based on their distribution in the positive quartile and distance from the origin point. On the other hand, accessions 15035 and 14880 showed susceptibility based on the closer to the origin under all the stress levels but 15188 and 24685 under $T_{60\%}$ and $T_{40\%}$.

Based on correlation studies, all the traits were grouped into two categories. First category contained root length, root-shoot ratio, chlorophyll *a*, chlorophyll *b*, carotenoid contents and cell membrane thermo-stability, while the other category contained leaf temperature, proline contents and ascorbic acid contents. With the increase in stress, decrease in the parameters under first category was observed with increase in the ones from the other. It means that as the water deficit increases, parameters like root length, root-shoot ratio, chlorophyll *a*, chlorophyll *b*, carotenoid contents and cell membrane thermo-stability decrease while other parameters like leaf temperature, proline contents and ascorbic acid contents tend to increase. Comparable results were witnessed by Khan *et al.* (2004); Ali *et al.* (2011b) and Ali *et al.* (2016). Study on the physiological traits by adjusting osmotic regulation of the plant is an effort to lower down the adverse effects of low moisture stress.

***Conclusion*:** Presence of genetic variability for all the traits suggests that different traits interact differently under low moisture stress condition. Through different analyses, it was concluded that accessions 19191, 15233, 15155 and 14927 were tolerant to low moisture stress, while 15035, 14880, 15188 and 24685 were poor performing. The selected accessions may be used in the future breeding programs for the study of low moisture stress tolerance and for the development of low moisture tolerant hybrids in maize. Changes in parameters like RL, SL, RFW, SFW, RDW, LT and CMT can be observed with induction of drought stress, so these traits can be verified as standard indicators of low moisture stress and can be declared as selection criteria for screening of tolerant genotypes against drought stress. More prominently cell membrane thermostability could be used as very efficient selection criteria for screening of tolerant and susceptible accessions.

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