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Identification of Drought Tolerant Maize Genotypes and Seedling based Morpho-Physiological Selection Indices for Crop Improvement

(Pengenalpastian Genotip Jagung yang Tahan Kemarau dan Indeks Pemilihan Morfo-Fisiologi berasaskan Anak Benih untuk Pembaikan Tanaman)

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ABSTRACT

Maize is an imperative grain crop used as a staple food in several countries around the world. Water deficiency is a serious problem limiting its growing area and production. Identification of drought tolerant maize germplasm is comparatively easy and sustainable approach to combat this issue. Present research was conducted to evaluate 50 maize genotypes for drought tolerance at early growth stage. Drought tolerance was assessed on the basis of several morphological and physiological parameters. Analysis of variance showed significant variation among the tested maize genotypes for recorded parameters. Principal component analysis revealed important morpho-physiological traits that were playing key role in drought tolerance. Correlation studies depicted significant positive correlation among the attributes such as fresh shoot length (FSL), fresh root length (FRL), dry shoot weight (DSW), dry root weight (DRW), relative water contents (RWC) and total dry matter (TDM) while a strongly negative correlation was observed among RWC and excised leaf water loss. Results concluded that the parameters fresh shoot weight, fresh root weight, FRL, DRW, TDM, cell membrane thermo stability (CMT) and RWC can be useful for rapid screening of maize germplasm for drought tolerance at early growth stages. Furthermore, the genotypes 6, 16, 18, 40, 45 and 50 can be used as a drought tolerant check in breeding programs. Moreover, biplot analysis along with other indices was proved to be a useful approach for rapid and cost efficient screening of large number of genotypes against drought stress condition.

Keywords: Cell membrane thermo stability; correlation; drought tolerance; principal component analysis; relative water contents

ABSTRAK

Jagung adalah tanaman bijirin penting yang digunakan sebagai makanan ruji di beberapa negara di seluruh dunia. Kekurangan air adalah masalah serius yang membatasi kawasan dan pengeluarannya yang semakin meningkat. Pengenalpastian germplasma jagung yang tahan kemarau adalah pendekatan yang agak mudah dan mampan untuk memerangi isu ini. Penyelidikan kini dijalankan untuk menilai 50 genotip jagung untuk toleransi kemarau pada peringkat pertumbuhan awal. Ketahanan kemarau dinilai berdasarkan beberapa parameter morfologi dan fisiologi. Analisis varians menunjukkan variasi ketara antara genotip jagung yang diuji untuk parameter yang direkodkan. Analisis komponen utama mendedahkan ciri morfo-fisiologi yang memainkan peranan penting dalam ketahanan kemarau. Kajian korelasi menunjukkan korelasi positif yang signifikan antara ciri seperti panjang pucuk segar (FSL), panjang akar segar (FRL), berat pucuk kering (DSW), berat akar kering (DRW), kandungan air relatif (RWC) dan jumlah bahan kering (TDM) manakala korelasi yang sangat negatif diperhatikan dalam kalangan RWC dan mengurangkan kehilangan air daun. Keputusannya menyimpulkan bahawa parameter pucuk berat baru, berat akar segar, FRL, DRW, TDM, kestabilan thermo membran sel (CMT) dan RWC adalah berguna untuk penapisan pantas germplasma jagung untuk ketahanan kemarau pada peringkat pertumbuhan awal. Selain itu, genotip 6, 16, 18, 40, 45 dan 50 boleh digunakan sebagai pemeriksaan ketahanan kemarau dalam program pembiakan. Selain itu, analisis biplot bersama-sama dengan indeks lain telah terbukti merupakan pendekatan yang berguna untuk penapisan pantas dan cekap kos untuk sejumlah besar genotip terhadap keadaan tekanan kemarau.

Kata kunci: Analisis komponen utama; kandungan air relatif; kestabilan thermo membran sel; ketahanan kemarau; korelasi

Introduction

Maize is an imperative cereal crop of the world serving as a source of food and feed for humans and animals, respectively. Several biotic and abiotic stresses affect its growth and yield (Abbasi et al. 2016; Waqas et al. 2017; Zafar et al. 2015). However, drought stress has been the major limiting factor affecting its yield potential (Khodarahmpour & Hamidi 2011; Seghatoleslami et al. 2008). It affects various morpho-physiological processes including plant biomass, root length, shoot length, photosynthesis, water use efficiency (WUE) and leaf water content (Egilla et al. 2005; Jaleel et al. 2008).

Screening for drought tolerant germplasm is a pre-requisite for any breeding program. However, assessment of germplasm for abiotic stresses like heat and drought under natural field conditions is difficult due to uncertain environmental conditions like rainfall and humidity. (Zafar et al. 2017). The task becomes more challenging when dealing with large germplasm due to requirement of large area, labor, resources and long time period. Alternatively, its rapid evaluation can be done at early growth stages under controlled environmental conditions based on several growth and physiological parameters (Zafar et al. 2017). Various growth related to morpho-physiological traits have been potentially utilized for evaluating genotypes of different crops against water stress (Ali et al. 2009; Chohan et al. 2012; Javed 2012; Taiz & Zeiger 2006). Root to shoot ratio of plants has been reported to increase under water deficit conditions (Wu & Cosgrove 2000). This ratio deviates because roots are less sensitive to growth inhibition by low water potential as compared to shoots. Significant variation exists in maize seedlings for root to shoot ratio and shoot dry weight under water deficit condition (Gonzales & Ayerbe 2011). Dry root weight was found to be a valuable selection criterion for selection against water stress (Mehdi et al. 2001). Assessment of drought tolerance in cereals indicated a significant positive correlation for root length with grain weight, grain yield (Shrimali 2001) and harvest index (Kashiwagi et al. 2005). Among various water stress resistance traits targeted in crop breeding, cell membrane thermo-stability (CMT) (Chohan et al. 2012) and coleoptile length (Qayyum et al. 2011) have been considered as the key physio-morphological traits affected under drought (Bayoumi et al. 2008).

Selection for best performing genotypes under stress condition is the primary objective for any crop improvement program (Thiry et al. 2016). However, low heritability of drought tolerant traits and unavailability of effective selection approach limits the development of drought tolerant cultivars (Kirigwi et al. 2004). Various morphological, physiological and biological features of higher plants show adaptability in response to water stress. Existence of variability for drought tolerance in different plant species has been reported (Frova et al. 1999; Guttieri et al. 2004). According to 'stress gradient hypothesis' the results of seedling analysis determine the structure and dynamics of plant population (De La Cruz et al. 2008; Kitajima & Fenner 2000). Therefore, phenotypic evaluation at the seedling stage is regarded an attractive approach having high throughput and cost efficient, saves space and time (Meeks et al. 2013). This approach has been successfully utilized to develop drought tolerant varieties in different crop species (Meeks et al. 2013).

When dealing with a large number of genotypes, multivariate biometrical techniques are utilized to assess biodiversity irrespective of data set. Among these biometrical techniques, biplot analysis or principal component analysis (PCA), principal coordinate analysis (PCoA), cluster analysis and multidimensional scaling (MDS) are utilized by plant breeders (Brown-Guedira et al. 2000). Biplot analysis interpreted easily and requires only a glance to understand the tolerance potential of genotypes due to graphical presentation of data. Moreover, biplot also illustrates average or overall performance of genotypes across the environments which makes it a reliable technique (Ali et al. 2012).

Present study was conducted with the objectives: to identify the genetic variation in maize germplasm and genotypes which can perform well under limited water conditions, and to evaluate the various morpho-physiological parameters related to drought tolerance which can be used efficiently as selection criteria in breeding programs.

Materials and Methods

A set of 50 diverse maize inbred lines were collected from various national and international research organizations (Table 1). The genotypes were evaluated for water stress tolerance on the basis of various seedling based morpho-physiological parameters. The seeds were sown in plastic pots of one liter (L) volume filled with sand during spring 2014 in completely randomized design (CRD) in wire house with three replications under both normal and water deficit conditions. One seedling in each pot was maintained through thinning after uniform emergence. Five seedlings of each genotype were kept in each replication and equal amount of water was applied to facilitate germination to each pot in both the treatments. The first irrigation was applied after seven days of sowing to both normal and water deficit treatments. After two weeks of sowing the second irrigation was supplied to entries only under normal condition. Seedlings under both treatments were allowed to grow further for seven days. Data pertaining to physiological and morphological parameters were recorded before and after uprooting seedlings by applying adequate amount of water to entries in both treatments. The average range for minimum and maximum temperature throughout experimental duration in the wire house was 30 ± 2 °C and 35 ± 2 °C, respectively.

Data were recorded for various traits before uprooting of seedlings. Coleoptile length was recorded after seven days of sowing and was measured from the base to the tip of coleoptile in evening about 18:00 h in centimeters (cm). Leaf temperature (LT) was measured for each treatment at 13:00 - 15:00 h from fully expanded leaves. Measurements were recorded from three leaves of each seedling with infrared thermometer (RAYPRM 30 CFRJ, RAYTEK, USA).

CMT an indicator of relative cell injury percentage was determined according to protocol described elsewhere

(Ibrahim & Quick 2001). Relative water contents (RWC) were determined from five leaves of each sample per treatment using the standard method (García-Mata & Lamattina 2001). Fresh, dry and turgid weight from leaves were measured by using standard methods. Relative water contents were determined by using the formula.

$$\text{RWC } \% = \frac{[\text{Fresh Leaf Weight} - \text{Dry Leaf Weight}]}{[\text{Turgid Leaf Weight} - \text{Dry Leaf Weight}]} \times 100.$$

Excised leaf weight loss (ELWL) were determined by using following formula (Clarke & Townley-Smith 1986).

$$\text{ELWL} = \frac{(\text{Fresh Leaf Weight} - \text{weight after 6 h})}{(\text{Fresh Leaf Weight - Dry Leaf weight})} \times 100.$$

Fresh root weight, fresh shoot weight, fresh root length and fresh shoot length were recorded immediately after uprooting the seedling. Dry root weight, dry shoot weight and total dry matter were recorded after complete drying of samples in incubator. Root to shoot ratio (RSR) was obtained according to the formula described elsewhere (Nour & Weibel 1978).

Root to shoot ratio = Dry root weight/Dry shoot weight

Genotypic and phenotypic variance and heritability were calculated by using following formulas.

$$\text{Genotypic variance } (\sigma^2 g) = \frac{[\text{Genotype mean square (GMS)} - \text{Error mean square (EMS)}]}{\text{number of replications (r)}}$$

$$\text{Phenotypic variance } (\sigma^2 p) = \sigma^2 g + \sigma^2 e/r$$

$$\text{Heritability } (H^2) = \sigma^2 g/\sigma^2 p.$$

STATISTICAL ANALYSIS

Data related to seedling based morphological and physiological traits were analyzed using GenStat version 10 for analysis of variance (ANOVA) and GGE biplot analysis. Xl-stat version 2012 software was used to compute the correlation analysis.

RESULTS AND DISCUSSION

Analysis of variance (ANOVA) showed significant variation among the tested maize genotypes for recorded parameters

TABLE 1. List of Maize germplasm under study evaluated for drought tolerance

Sr. No.	Inbred lines	Sr. No.	Inbred lines
1	NCIL-10-4	26	D-130
2	NCIL-10-5	27	D-135
3	NCIL-10-23	28	D-132
4	NCIL-20-4	29	D-133
5	NCIL-20-11	30	D-134
6	NCIL-20-20	31	D-136
7	NCIL-30-4	32	D-137
8	NCIL-30-5	33	D-143
9	NCIL-30-15	34	D-148
10	NCIL-40-8	35	D-149
11	NCIL-40-12	36	D-150
12	NCIL-40-18	37	D-152
13	D-103	38	D-153
14	D-105	39	D-155
15	D-106	40	D-157
16	D-109	41	D-158
17	D-111	42	W 64 TMS
18	D-114	43	W 64 SP
19	D-118	44	OH 54-3A
20	D-120	45	OH-8
21	D-122	46	Antigua-1
22	D-123	47	AES-204
23	D-126	48	A-638
24	D-131	49	A-556
25	D-124	50	M-14

TABLE 2. Mean Square of various morpho physiological traits in 50 Maize genotypes evaluated for drought tolerance

	MS	σ^2e	σ^2g	σ^2p	ECV%	GCV%	PCV%	H2%	GA
RSRW	1.88**	0.083	0.9	0.98	17.601	57.72	60.34	91.49	1.86
TDM	7.14**	0.074	3.53	3.61	6.72	46.27	46.76	97.93	3.83
CL	6.227**	0.55	2.83	3.39	19.82	44.86	49.05	83.67	3.17
CMT	243.47**	21.45	111.09	132.464	11.7	26.63	29.1	83.8	19.86
RWC	246.73**	5.49	120.61	126.11	3.56	16.68	17.06	95.64	28.57
ELWL	324.44*	24.77	149.83	174.61	12.29	30.23	32.64	85.81	23.35
LT	9.04*	2.8	3.11	5.92	4.57	4.82	6.65	52.65	2.63
FSL	25.62*	0.769	12.42	13.19	3.12	12.54	12.92	94.17	9.09
FRL	43.708**	0.76	21.47	22.23	3.19	16.99	17.29	96.58	9.38
FSW	29.56**	0.243	14.62	14.9	5.11	39.6	39.93	98.36	7.822
FRW	23.65**	0.58	11.53	12.11	8.31	36.96	37.89	95.18	6.82
DSW	0.180*	0.058	0.061	0.119	15.732	16.099	22.509	51.15	0.47
DRW	6.154**	0.027	3.063	3.091	6.61	69.27	69.58	99.1	3.58

* = significance at 0.05 while ** = significant at 0.01 percent probability level, respectively. MS = Mean square σ^2e = environmental variance, σ^2g = genotypic variance σ^2p = phenotypic variance, H2= broad sense heritability, GA= genetic advance, RSR = root shoot ratio, TDM= total dry matter, CL= coleoptile length, CMT= cell membrane thermo-stability, RWC= relative water content, ELWL= excised leaf weight loss, LT= leaf temperature, FSL= fresh shoot length, FRL= fresh root length, FSW= fresh shoot weight, FRW= fresh root weight, DSW= dry shoot weight, DRW= dry root weight

(Table 2), pointing out considerable genetic diversity in germplasm.

COEFFICIENT OF VARIATION AND VARIANCE COMPONENTS

The estimates of genetic traits such as genotypic mean square (MS), broad sense heritability (H^2), genetic advance (GA), genotypic, phenotypic and environmental variances (σ^2g, σ^2p and σ^2e, respectively) along with genotypic, phenotypic and environmental coefficient of variation (GCV, PCV and ECV, respectively) are given in Table 2. For all the traits under investigation, values of phenotypic variance were greater as compared to genotypic variance. ELWL showed highest phenotypic and genotypic variance whereas, dry shoot weight (DSW) showed minimum genotypic and phenotypic variance. Similarly, values of phenotypic and genotypic variances were high as compared to environmental variance for all the recorded traits.

The outcome of present investigation showed that environmental variation was greater enough to modify expression of gene systematizing the trait under examination. Selection pressure might be applied on traits to spot superlative genotypes from given germplasm. Similar outcomes were obtained for GCV, PCV and ECV (Table 2). The differences between GCV and PCV were very less for almost all the traits under study which showed a small effect of environment on these traits suggesting that selection of these traits could be effective. Our results were compatible with previous findings (Ali et al. 2013; Mustafa et al. 2013). In current study, dry root weight (DRW) showed maximum PCV and GCV followed by RSR with PCV and GCV. These attributes can be used as a reliable selection criteria for drought tolerance in maize at early growth stage. On the other hand, LT showed minimum PCV and GCV followed by FSL with PCV and GCV. Thus, these parameters which showed minimum PCV and GCV were regarded as poor index for selection of genotypes against drought tolerance. Our results were in line with a previous report (Ali et al. 2011).

HERITABILITY AND GENETIC ADVANCE OF TESTED MORPHO-PHYSIOLOGICAL PARAMETERS

Heritability is a good indicator for transmission of attributes from parents to offspring and scope of trait improvement through selection breeding relies on the value of heritability (Neelima & Reddy 2008). GCV coupled with heritability estimate presents a more reliable approximate of quantity of GA to be anticipated through phenotypic selection. Heritability values <59% are considered as low while values ranged between 60-79% are referred to as moderately higher. Whereas, values greater than 80% are considered as higher values (Singh & Singh 1999).

Higher heritability was observed for DRW, FSW, TDM, FRL, RWC and CMT, respectively (Table 2). Similar results were obtained in some previous investigations for these traits (Chohan et al. 2012; Rezaeieh & Eivazi 2011). RWC, ELWL, CMT and FRL showed higher GA. However, GA was less for DSW and RSR. Our results were in agreement with past findings (Mustafa et al. 2013; Nzuve et al. 2014). The attributes under observation showed higher values for broad sense heritability and GA indicating additive gene action with less environmental influence. Thus, these traits can be employed as useful selection criteria for maize improvement programs.

PRINCIPAL COMPONENT ANALYSIS (PCA) REVEALED USEFUL PARAMETERS FOR DROUGHT TOLERANCE

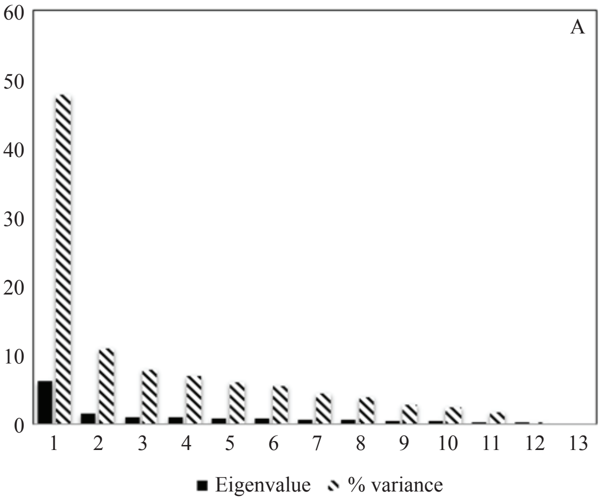
Out of 13 principal components (PCs), first three PCs exhibited Eigen values more than 1 with a combined variance of 66.31% (Figure 1(A)) whereas, remaining 10 PCs showed only 33.68% cumulative variability. First two PCs showed a combined variance of 58.50% suggesting that these were playing key role in overall variation. The PC 1 had a high match with DRW followed by TDM. While PC 2 was more related to FRL followed by FSW (Figure 1(B)). Factor loadings for genotypes showed that genotypes 50, 18, 16, 40, 6 and 45 exhibited higher values for PC 1 (Figure 2). Whereas, second PC was more related to genotype 42 followed by genotype 3 (Figure 2). Genotypes with higher PC 1 and lower PC 2 values are considered stable genotypes while genotypes with lower PC 1 and higher PC 2 are perceived as unstable genotypes (Seghatoleslami et al. 2008). Use of this biometrical technique for pin pointing water deficit stress tolerance maize genotypes has previously been reported in maize crop (Ahmadzadeh 1997). Since FRW, DRW, TDM, RWC, CMT, CL and RSR showed higher values in PC 1 and lower values in PC 2, so it is presumable that these traits can be used as a selection marker for drought tolerance in maize. Similarly, genotypes 50, 18, 16, 40, 6 and 45 showed higher values for first PC accompanied with lower second PC values; hence these genotypes can be considered as stable for selection breeding.

BIPLOT ANALYSIS SHOWED SIGNIFICANT DIVERSITY AMONG TESTED MAIZE GERMPLASM

In present investigation, biplot analysis revealed high genetic diversity among the tested maize genotypes for all the recorded parameters (Figure 3). Closest the distance of genotype clog position on graph from the point of origin least will be the diversity and vice versa. Maize genotypes which are located at the vertex of the polygon were regarded as most diversified compared to others. Genotypes 50, 18, 45, 6, 11, 44, 14 and 42 clogged at the vertex of polygon hence considered more diversified and of more breeding value. Interestingly the genotypes 50, 18, 6, 16, 40 and 45 were also proved stable based on high PC1 and lower PC2 (Figure 2), thus can be utilized in maize improvement programs for drought tolerance Moreover, the traits FRL, FSW, TDM, DSW, DRW and RSR were clogged away from the point of origin which depicted them as stable and reliable for selection against drought tolerance in maize.

CORRELATION ANALYSIS AMONG MORPHO-PHYSIOLOGICAL TRAITS FOR DROUGHT TOLERANCE

Correlation analysis revealed significant positive as well as negative association among several traits under examination (Table 3). A significant (p<0.01) positive correlation was observed between LT and ELWL. Previously, strong positive correlation was also observed between LT and ELWL (Khan et al. 2014). However, LT was negatively correlated with CMT, FSW, FRW, DSW and DRW.



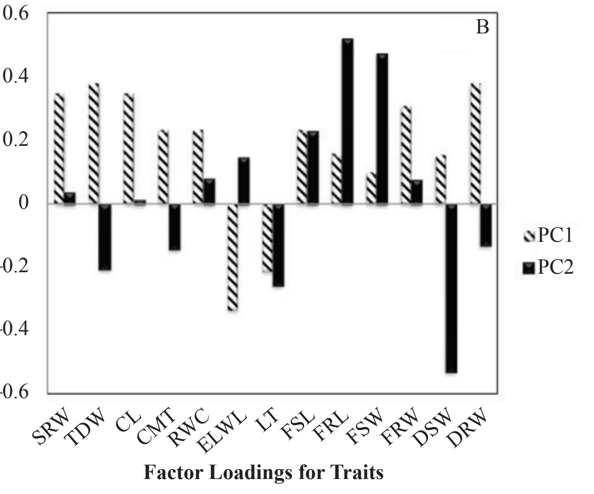


FIGURE 1. Data showing eigen value more than 1 and cumulative percent variance of 66.31% in first three PCs (A), and factor loadings for traits (B) based on 50 maize genotypes evaluated under normal and water deficit conditions

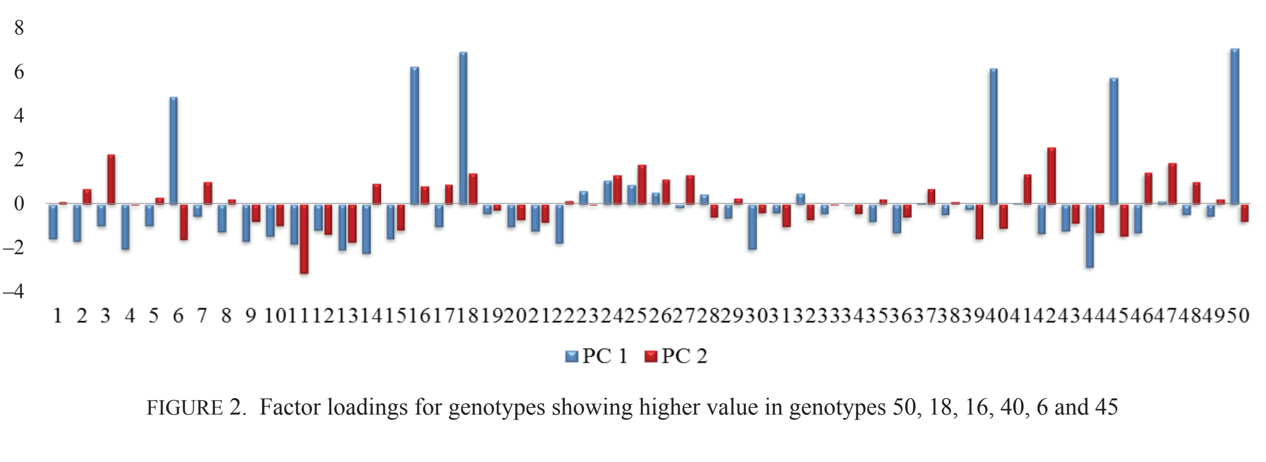


FIGURE 2. Factor loadings for genotypes showing higher value in genotypes 50, 18, 16, 40, 6 and 45

TABLE 3. Correlation among various seedlings based morpho-physiological parameters under normal and water deficit conditions in maize

	RSRW	TDM	CL	CMT	RWC	ELWL	LT	FSL	FRL	FSW	FRW	DSW	DRW
RSRW	1												
TDM	0.88542	1											
CL	0.70801	0.78341	1										
CMT	0.36841	0.49074	0.50394	1									
RWC	0.44615	0.4844	0.56538	0.28333	1								
ELWL	-0.6576	-0.7812	-0.6297	-0.5113	-0.3815*	1							
LT	-0.4123	-0.3901	-0.3881	-0.1695	-0.2036	0.42216**	1						
FSL	0.46617*	0.45903	0.48992	0.23006	0.29303	-0.4307	-0.1993	1					
FRL	0.27359	0.22894	0.35427	0.08357	0.17863	-0.2624	-0.3709	0.37591*	1				
FSW	0.15399	0.12216	0.18813	0.23968	0.23191	-0.1039	-0.1696	0.13475	0.24696	1			
FRW	0.6191	0.65063	0.58797	0.41771	0.36433	-0.5988	-0.494	0.45575	0.2848	0.166	1		
DSW	0.08188^{NS}	0.51552	0.30144	0.26883	0.14176	-0.4114	-0.1185	0.07636	0.00456	-0.0904	0.21502	1	
DRW	0.94003	0.98915	0.79247	0.48271	0.49765	-0.7713	-0.4*	0.48153**	0.24591	0.14712	0.66421	0.38406*	1

* = significance at 0.05 while ** = significant at 0.01 percent probability level, respectively. RSR = root shoot ratio, TDM= total dry matter, CL= coleoptile length, CMT= cell membrane thermo-stability, RWC= relative water content, ELWL= excised leaf weight loss, LT= leaf temperature, FSL= fresh shoot length, FRL= fresh root length, FSW= fresh shoot weight, FRW= fresh root weight, DSW= dry shoot weight, DRW= dry root weight

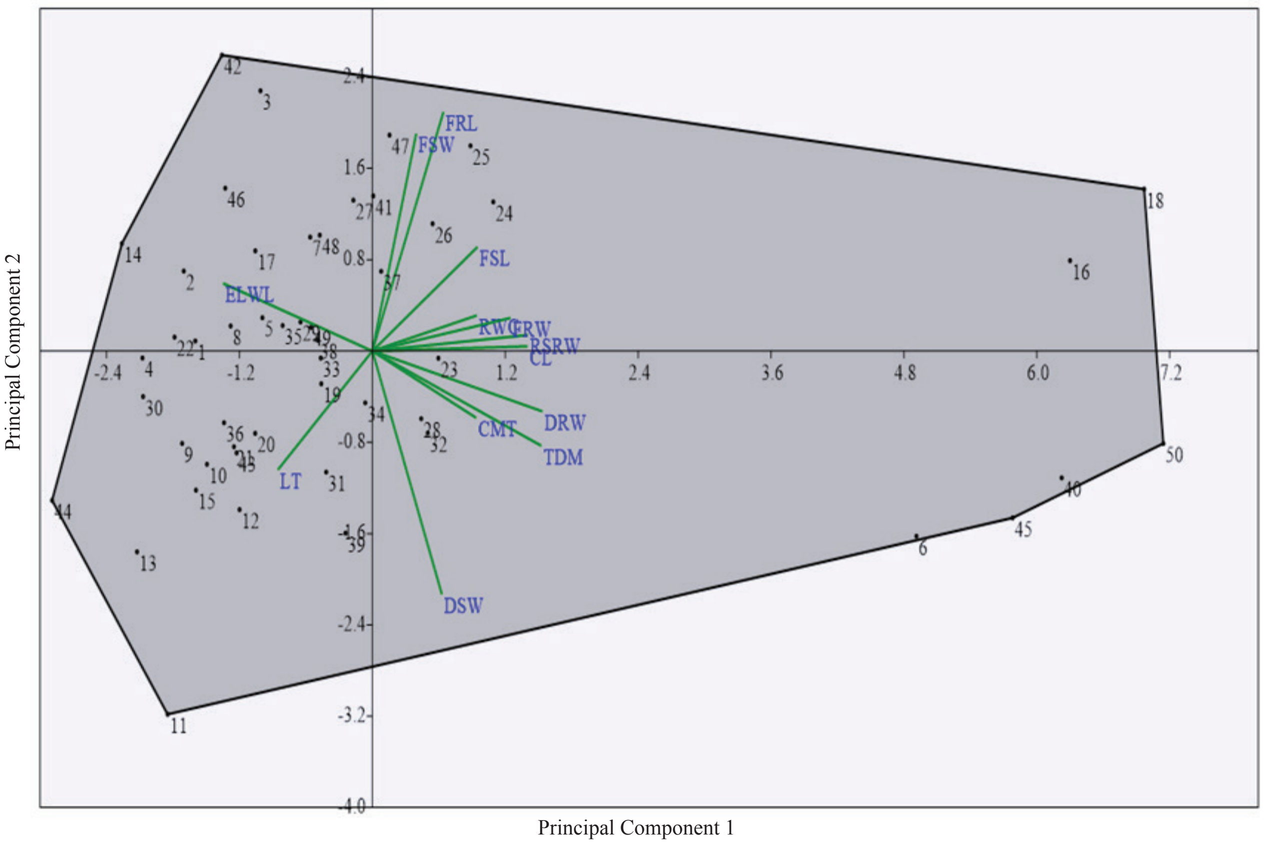


FIGURE 3. Biplot Analysis showing different attitude of 50 Maize genotypes for various morphological attributes under drought stress condition. * = significance at 0.05 while ** = significant at 0.01 percent probability level, respectively

The reason for the increased ELWL under high LT seems to be the increased transpiration and reduced CMT level due to heat shock. FSL also showed significant positive association with FRL and DRW. Mustafa et al. (2013) also reported a significant positive association among FSL, FRL and DRW. Positive correlation was also noticed between DSW and DRW. Some previous studies were found to support our results (Ali et al. 2011). There exists a negative correlation among RWC and ELWL (Amini et al. 2013). Present study also showed a significant negative correlation between RWC and ELWL. The reason for the decreased RWC under increased ELWL seems to be due to excessive water loss by high transpiration rate and reduced CMT.

Conclusion

Significant variation was observed among the tested maize genotypes for drought tolerance using biplot analysis. Genotypes 6, 16, 18, 40, 45 and 50 were proved to be relatively drought tolerant based on tested morpho-physiological indices. In addition, FRL, FSW, FRW, DRW, TDM, CMT and RWC were identified as useful morpho-physiological indices based on principal component analysis along with higher heritability and GA. Moreover, Biplot analysis along with other indices was proved to be a useful approach for rapid and cost efficient screening of large number of genotypes against drought stress condition.

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