

Genetic Divergence in Sorghum [*Sorghum bicolor* (L.) Moench] Genotypes under Contrasting Moisture Environments

Abstract

Sorghum is one of the most important cereal crops in Ethiopia which is grown most dominantly in the low land area where drought predominates. In this area farmer's preference to improved sorghum variety is dependent on earliness and drought tolerance traits. The objective of the study was to evaluate the genetic diversity of early maturing sorghum genotypes for drought tolerance by using principal component and cluster analysis. Twenty three early maturing sorghum genotypes were phenotyped under post-flowering moisture stressed and non-stressed environment using RCBD design in adjacent experiment. The analysis of variance revealed significant variation among genotypes for most of the traits for both moisture environments. Post-flowering drought reduce the value for all of the traits except flag leaf area and average grain yield was reduced by 21%. Five genetically divergent clusters which showed significant inter cluster distance were observed in both environments. Genotypes in cluster one showed best performance for grain yield and yield components under non-stress environment. Under stressed environment, genotypes under C1, and C2 revealed best performance for drought tolerance and yield traits, respectively. Therefore, the performance of genotypes under these clusters and different clustering pattern observed depicts the divergence of genotypes for drought response which creates opportunity for further improvement through selection and hybridization. Principal component analysis revealed five and seven PC captured 80% and 87% of total variation observed under stressed and non-stressed environment, respectively.

Key Words: Clustering, Early maturing, Genetic divergence, Post-flowering drought, Stress

1. INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is one of the most important cereal crops ranking fifth in the world after wheat, rice, maize, and barley. In Ethiopia Sorghum is one of major staple food and third most important cereal crop after tef(*Eragrostis tef*) and maize (*Zea mays*. L) in terms of sown area (1,854,710.93 ha) and it is the third in total production next to maize and tef which is 4,752,095.6 tons and mean yield of 2.525 t ha⁻¹. It covers 14.97% and 16.36% of the cropped area and total production from the total grain produced in Ethiopia (CSA, 2017). East Africa is considered to be the center of origin and diversity for sorghum and Ethiopia is the third largest producer from Africa next to Nigeria and Sudan (Rakshit et al., 2014; FAOSTAT, 2017).

In Ethiopia, the dry land areas cover 66% of the total area, in these areas crop production is mainly rain-fed. Because of the low amount, uneven distribution and erratic nature of the rainfall, crop production is seriously affected in these areas. Cultivation of sorghum takes the third larger area under wide agro-ecology of Ethiopia and highly preferred in dry lowlands where drought predominates (Demeke and Marcantonio, 2013; FAOSTAT, 2017). Even if sorghum has

the ability to copewith many stresses including heat and moisture, itsproduction highly affected by drought occurred duringreproductive stage in arid and semi-arid regions of theworld (Ejeta and Knoll, 2007)

Terminal drought stress is a major occurrence in Ethiopia (Brhaneet al. 2006). Moisture stressed areas in Ethiopia are more extensive in southern, southeastern, eastern and northeastern part of the country. From these where sorghum is the major cereal crop grown in Konso and Derashe in the south, Miesso, Asebot, Babile and Jijiga plains in the east, North Shoa (ShoaRobit), Wello, Raya valley and Sheraro and Humera areas in the north (ICRA, 1997). In these areas, the livelihood of the population is mainly dependent on sorghum (Geremew et al., 2004). A research conducted in North Eastern Ethiopia indicated moisture stress within and between seasons was a common phenomenon in all the areas surveyed (Amelework. 2012). In comparison between farmers variety Vs improved variety higher ranking was given by farmers for improved varieties for early maturity and drought resistance traits (Firew, 2008). Thus, the contribution of these traits for the adoption of newly released varieties is crucial and needs to be considered in the breeding program.

There is a significant genotypic difference in water use pattern in plants, early maturing genotypes use less water in comparison with medium to late maturing genotypes during the growing season (FAO, 2002). Also, the daily water requirement varies greatly depending on the growth stage. Since, higher water demand by crop exists in flowering and the stress occurring in this time result a great yield penalty(Stichler and Fipps, 2003)Therefore, breeders should look for a solution to reduce the yield loss under such conditions.

The success in obtaining a highly heterotic group for hybrid program depends on the creation of variability and selection of genetically divergent parents. To select genotypes that are superior and/or divergent for the trait of interest we should group genotypes having similar value for the trait that will lead to ease of selection. Thus, cluster analysis and principal component analysis (PCA) are powerful tools used for crop modeling and parental selection in breeding programs (Ali et al., 2011; Brown, 1991). Principal component analysis is appropriate when you have obtained measures on several observed variables and try to reduce to a smaller number of artificial variables or principal components. Interpretation of the PCA is based on finding which variables are most strongly correlated with each component, i.e., which of these numbers are large in magnitude, the farthest from zero in either direction influence the clustering more than those with a lower value closer to zero (Chahal and Gosal. 2002).

To develop sorghum cultivars that best-fit farmers preference in lowland area of Ethiopia a breeder needs to conduct targeted breeding byincluding traits related to earliness and drought tolerance in to the dataset. Characterizing and grouping the genotypes based on their phenotypic traits under different moisture environment could help the breeding program to develop superior varieties. **Therefore, breeding program need to have effective screening criteria for selection for stress environment and also to make crosses between genetically divergent genotypes to increase**

genetic gain per generation. Hence the objective of the study was to evaluate the genetic diversity of early maturing sorghum genotypes for drought tolerance under different moisture environment by using principal component and cluster analysis.

2. MATERIAL AND METHODS

The experiment was conducted at Werer Agricultural Research Center, Eastern Ethiopia experimental site in the 2017/18 post rainy season. The center is located at 9°16'8" N, 40° 9'41" E with an altitude of 750 m.a.s.l. The area is characterized by a drought-prone and semi-arid climate. The mean maximum and minimum temperature is 40.8°C and 19°C, respectively. The soil in the testing field of Werer is predominantly Fluvisols with silty clay textural class and a pH of 8.5. The experiment consists of 23 early maturing sorghum genotypes and here is the name along with their designation number in parenthesis: 76T1#23 (1), Birhan (2), B-35 (3), ETSL100674 (4), Macia (5), A2267-2 (6), Dekeba (7), E36-1 (8), ESH-1 (9), ESH-3 (10), Girana-1 (11), ICSR14 (12), Emahoye (13), Misikir (14), Meko-I (15), SC103-14E (16), Teshale (17), Abshir (18), ICSV 93046 (19), ICSV745 (20), Melkam (21), Khwangphang (22), and ICSV700 (23). The experiment was carried out using randomized complete block design under two moisture environments (non-stress and moisture stress) each replicated twice. The non-stressed moisture environment received full irrigation as per the area recommendation while the stressed moisture environment was created through withholding irrigation at the booting stage to induce post-flowering drought stress. The soil moisture content of the experimental site was determined by using a gravimetric method as described by Klute (1986). All recommended agronomic practices were carried out.

Data were recorded from the plot for: Days to 50% flowering (DF), Days to 75% physiological maturity, (DM), Seedling vigor (SVG), Stay-green (SG), Overall plant aspect (PAS), Drought Score (DRS): under stressed environment, Leaf senescence (LSC), Disease Score (Dis), Grain filling period (GFP), Grain filling rate (GFR): kg ha⁻¹ day⁻¹, above ground biomass (AGBM): kg ha⁻¹, Harvest index (HI) in percentage, Thousand grain weight (TGW): in grams at moisture content were adjusted to 12% and Grain yield (YLD) kg ha⁻¹.

Five representative plants were used for: plant height (PH) in cm, Panicle length (PL) in cm, Panicle weight (PW) in g, Panicle exertion (PEX) in cm, Flag leaf area (FLA) (Stickler et al., 1961), chlorophyll content (SPAD) using Chlorophyll Meter SPAD-502, number of tillers (NT) and Root angle (Singhet al., 2011).

The value of all the recorded data were subjected to analysis of variance (ANOVA) using General Linear Model (GLM) procedure of SAS statistical version 9.2, software (SAS, 2009). Mean comparisons were done by using Duncan's Multiple Range Test (DMRT). Phenotypic characters which showed significant variation among genotypes were used to estimate genotypic divergent and clustered into different groups. Accordingly, eighty and twenty one traits were used under non-stressed and stressed environment, respectively for clustering of genotypes in to

different groups by using Ward's method as described by Singh (2001). Estimation of distance between clusters was done according to D^2 statistics (Mahalanobis, 1936) by using Minitab version 17.1.0.0 (2013) package. Significance of the squared distances for each cluster was tested against the tabulated χ^2 values at p degree of freedom at 1% and 5% probability level where p=number of characters used for clustering genotypes. PCA transforms large sets of related variables into a smaller set of variables, termed principal components that reveal the degree of variation or correlation. Eigenvalues and eigenvectors for PCA were analyzed using Past version 3.14 statistical software.

3. RESULTS AND DISCUSSION

The analysis of variance revealed significant ($P=0.05$) to highly significant ($P=0.01$) variation between genotypes for most of the traits studied except grain filling period for both moisture environments and SPAD chlorophyll reading and disease score for non-stressed environment (Table 1). Post-flowering drought reduces the value for most of the traits except for flag leaf area. The mean value indicated an increase in flag leaf area as drought was imposed, these, in turn, imply the importance of flag leaf area as selection criteria for drought tolerance breeding. Ali et al. (2009) in sorghum reported that increased flag leaf area could serve as selection criteria for drought tolerance. Highly significant ($P=0.01$) variations among the tested genotypes were observed in both environments for grain yield. All the tested genotypes showed a reduction in grain yield due to moisture stress with a mean percentage yield reduction of 21.2%. Similar findings were reported by Sory et al. (2017); Khaton et al. (2016); Menezes et al. (2014) and they found that drought significantly affected the yield of sorghum lines, causing a significant yield reduction compared to the full-irrigation condition. The variation observed in most of the traits allows further improvement by selection and hybridization.

Table 1 Trait means and analysis of variance for 23 sorghum genotypes under Non-stressed (NS) and stressed (DS) moisture environments

Variable	NS				DS			
	Mean	σ^2	σ_x	R^2	Mean	σ^2	σ_x	R^2
DF	71.0**	11.80	3.44	0.97	70.9**	5.54	2.35	0.84
DM	107.8**	8.61	2.93	0.86	106.5**	4.34	2.08	0.80
GFP	36.8	2.41	1.55	0.57	35.7	2.37	1.54	0.51
SVG	2.1*	0.32	0.57	0.67	2.1**	0.32	0.56	0.81
SPAD	58.7	29.28	5.41	0.57	50.8**	39.52	6.29	0.76
PAS	2.8**	0.45	0.67	0.79	3.2**	0.44	0.66	0.78
PH	188.2**	3569.0	59.74	0.99	175.5**	3175.0	56.34	0.99
FLA	222.7**	7290.0	85.38	0.74	253.0**	7934.0	89.07	0.84
DS	-	-	-	-	3.0*	0.49	0.70	0.70
LSC	3.2**	0.90	0.95	0.73	4.3**	1.64	1.28	0.74
YLD	4926**	1739912	1319	0.91	3112**	798245.0	893.4	0.91
GFR	134.3**	1386.00	37.23	0.88	109.2**	995.66	31.55	0.90
TGW	36.6**	29.79	5.46	0.95	31.3**	41.34	6.43	0.88

AGBM	19116**	73969057	8601	0.90	12479**	24789759	4979	0.92
HI	29.1**	82.61	9.09	0.81	27.6**	82.51	9.08	0.89
SG	2.6*	0.58	0.76	0.72	3.1**	0.53	0.73	0.81
PEX	7.0**	56.40	7.51	0.99	4.6**	36.64	6.05	0.99
NT	0.4**	0.26	0.51	0.98	0.1**	0.14	0.38	1.00
HDL	28.2**	25.06	5.01	0.80	26.5**	19.81	4.45	0.92
HDW	115.2**	928.29	30.47	0.86	102.7**	833.26	28.87	0.75
Dis	2.2	0.06	0.25	0.51	2.6*	0.39	0.63	0.67
RA	19.1**	14.47	3.80	0.99	-	-	-	-

*, ** significant at 5% and 1% level of probabilities, respectively, σ^2 :variance, σ_x : standard deviation, R^2 : R-Square and trait abbreviation as indicated in material and method

3.1 Clustering and genetic divergence of genotypes under non-stressed environment

Significance difference among varieties for majority of the traits tested would justify further calculation of D^2 statistics (Sharma, 1998). Accordingly, eighty phenotypic traits were employed to cluster genotypes based on Ward's linkage and squared Euclidean distance matrix. Cluster analysis grouped the 23 genotypes into five distinct groups of which the first cluster consisted of 11 genotypes (47.8%), the second cluster seven genotypes (30.4%), the fourth cluster consists of three genotypes (13%) and the rest of two clusters each consist of one or solitary genotypes (4.35%) (Figure 1).

Cluster analysis revealed genotypes under cluster one were characterized by higher mean values for grain yield, grain filling rate, panicle weight, thousand grain weight and moderate root angle (Table 2). As per the result from cluster analysis genotypes under this cluster showed the best performance for grain yield and yield components therefore, promising genotypes for the non-stress environment for hybridization purpose could be found from this cluster. Cluster two is characterized by maximum value from all clusters for SPAD chlorophyll reading and harvest index and shorter plant height. Under non-stressed moisture environment, there are two solitary clusters. Cluster three with solitary of genotype, ETSL100674 was characterized by longer flowering date, better seedling vigor, larger flag leaf area, long plant height and better agronomic score, leaf senescence and stay green, with narrow root angle and least score for thousand grain weight and harvest index. Genotype under this cluster shown better agronomic performance but does lower grain yield. Thus, genotypes could be utilized in the breeding program for specific traits of interest other than grain yield.

Table 2 Mean value of eighty phenotypic traits for the five clusters of 23 sorghum genotypes under non-stressed environment

Traits	Clusters				
	C1	C2	C3	C4	C5
Days to flowering	71.32	68.07	77.00	75.67	67.50
Days to maturity	108.05	105.50	112.00	111.67	105.50
Seedling vigor	2.05	1.89	1.50	2.25	3.25

SPAD	58.64	61.40	59.89	54.76	50.62
Plant agronomic aspect	2.86	2.50	2.00	3.42	4.25
Plant height (cm)	184.23	135.00	280.00	272.00	260.00
Flag leaf area (cm ²)	234.04	239.49	315.63	161.75	70.14
Leaf senescence	3.45	2.57	2.00	4.00	3.00
Yield (kg ha ⁻¹)	5778.84	4336.01	3159.16	4878.03	1585.79
Grain filling rate (kg ha ⁻¹ day ⁻¹)	157.59	116.38	90.33	136.38	41.69
Stay green	2.86	2.07	2.00	3.17	3.00
Panicle exertion (cm)	4.38	8.79	2.58	4.35	35.75
Number of tiller	0.22	0.14	0.70	0.80	2.00
Panicle length (cm)	26.95	30.14	32.60	22.18	32.75
Panicle weight (g)	130.71	92.02	124.87	132.64	44.05
Thousand grain weight (g)	39.83	34.42	23.39	37.66	25.96
Above ground biomass (kg ha ⁻¹)	19438.38	12741.76	35936.11	31625.93	5837.94
Harvest index	30.93	35.00	8.80	16.15	27.51
Root angle (°)	20.37	18.10	13.75	19.55	15.00

The generalized squared distances under non-stressed environment revealed highly significant ($P=0.01$) inter-cluster distance for most of the clusters except between cluster one and two (Table 3). The result implies that there was diversity in the performance of early maturing sorghum genotypes. Non-significant inter cluster difference observed between cluster one and two implies low degree of genetic divergence among genotypes of these clusters. The minimum significant squared distance was between cluster one and cluster four. On the contrary, maximum squared distance was between cluster three and cluster five thus, the observed genetic divergence creates a potential that could be resulted in a hybrid vigor if breeders utilized genotypes under this clusters for crossing block. However, in exploiting the genetic distance to get hybrid vigor the actual performance and desirable characteristics of the genotypes or cluster mean should be taken into account.

Table 3 Inter-cluster divergence D^2 value for 23 sorghum genotypes under non-stressed (**bold**) and stressed environments

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
Cluster 1		22.35	367.75**	63.11**	219.57**
		73.55**	147.10**	34.87*	3296.74**
Cluster 2			357.57**	102.60**	173.00**
			106.05**	58.78**	3980.06**
Cluster 3				178.96**	444.56**
				69.72**	3938.95**
Cluster 4					251.72**
					3440.19**
Cluster 5					

For Non-stressed, *significant ($\chi^2=27.587$) and **highly significant ($\chi^2=33.409$)

For Stressed, *significant ($\chi^2=31.410$) and **highly significant ($\chi^2=37.566$)

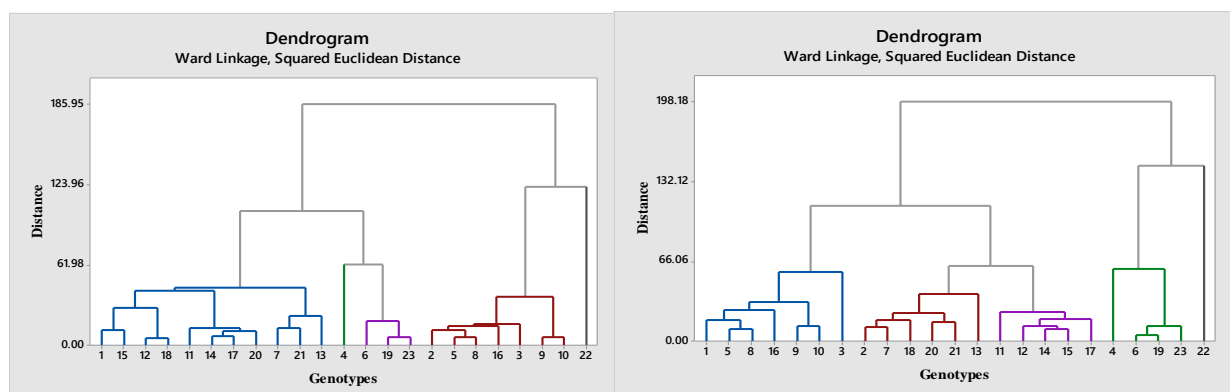


Figure 1(left) and 2(right) Dendrogram of 23 sorghum genotypes constructed using Ward's method based on phenotypic traits under non-stressed (left) and stressed environment (Genotypes codes as given in material and method)

3.2 Clustering and genetic divergence of genotypes under stressed environment

The cluster analysis had shown a quite different grouping from non-stressed moisture environment. The clustering analysis under a stressed environment also classified the 23 genotypes into five different groups (Figure 2). More number of genotypes were observed in cluster one which consists of seven genotypes. Cluster 1 is characterized by better value for seedling vigor, drought score, leaf senescence, stay green and higher SPAD chlorophyll reading with shorter plant height (Table 4). Genotypes under this cluster viz. 76T1#23, B-35, Macia, E36-1, ESH-1, ESH-3 and SC103-14E showed best performance for traits related to drought tolerance. However, having these drought tolerance characteristics doesn't guarantee yield under stress environments hence, introgression of these important traits to high yielding and adapted genotypes should be the focus in resistance breeding program.

Six genotypes were found under cluster two and characterized by higher flag leaf area, grain yield, grain filling rate and Harvest index with moderately wider root angle. Cluster three consists of four lines characterized by higher value for plant height, panicle weight and aboveground biomass also showed lower harvest index and grain yield with a higher level of leaf senescence. Cluster four consists of five genotypes and has characteristics of the maximum value for flag leaf area and thousand grain weight. Genotype Kwangphang found to be solitary in both moisture regimes which showed susceptible and least agronomic performance for most of the traits tested in both environments.

The χ^2 - test for the five clusters under stressed environment revealed significant ($P=.05$) to highly significant ($P=.01$) inter cluster distance for all of the clusters (Table 3). The minimum squared distance was observed between cluster one and cluster four which is significant at 5% probability level. On the contrary, the maximum squared distance was between cluster two and cluster five which revealed that these clusters were genetically more divergent from each other

which could be an opportunity to exploit by hybridization for moisture stress breeding. As per Amsalu and Endeshaw (1999) crossing genotypes belonging to distant clusters could maximize transgressive segregation. Accordingly, genotypes under clusters one and two could be more effective for drought tolerance breeding hence they revealed best performance for drought tolerance and yield traits, respectively.

The patterns of clustering under stressed and non-stressed environment were somewhat different. But clusters two, four and five under non-stressed environment are more or less similar with clusters one, three and five of stressed environment, respectively. Genotypes found in cluster one under non-stressed environment become two distinct groups (clusters) under stressed environment, implies the divergence of the genotypes in stress response which creates the opportunity for selection.

Table 4 Mean value of 21 phenotypic traits for the five clusters of 23 sorghum genotypes under stressed environment

Traits	Clusters				
	C1	C2	C3	C4	C5
Days to flowering	68.64	72.00	73.25	71.10	69.00
Days to maturity	104.86	107.58	108.38	106.50	104.50
Seedling vigor	2.00	2.17	2.19	2.00	3.25
SPAD	53.47	53.30	46.65	49.09	42.31
Plant agronomic aspect	3.00	2.83	3.50	3.55	4.50
Plant height (cm)	128.71	148.67	252.75	195.60	253.50
Flag leaf area (cm ²)	235.57	295.59	203.73	298.43	90.30
Drought score	2.54	2.83	3.31	3.60	3.00
Leaf senescence	3.57	3.92	5.13	5.10	4.50
Grain yield (kg ha ⁻¹)	3641.14	4738.74	3069.85	4470.00	911.00
Grain filling rate (kg ha ⁻¹ day ⁻¹)	100.63	133.37	87.17	126.61	25.66
Stay green	2.64	2.92	3.44	3.65	3.00
Panicle exertion (cm)	6.28	1.46	1.84	3.82	27.00
Number of tiller	0.04	0.00	0.07	0.00	1.81
Disease score	2.75	2.33	2.75	2.45	3.00
Panicle length (cm)	28.54	28.76	23.23	24.32	22.90
Panicle weight (g)	82.25	116.96	119.30	113.84	38.82
Thousand grain weight (g)	30.58	35.43	25.75	34.63	16.85
Above ground biomass (kg ha ⁻¹)	11250.01	14184.72	26113.38	17127.58	4827.73
Harvest index (%)	32.79	33.97	11.91	26.81	18.87
Root angle (°)	18.24	22.63	18.10	17.47	15.00

3.3 Principal component analysis for non-stressed environment

During principal component analysis determination of the level of the correlation matrix is of paramount importance based on the data set. Which numbers we consider to be large or small is, of course, is a subjective decision. As per Kline, (2002) loadings of 0.30 or higher can be considered significant, or at least salient. Accordingly, here a correlation above 0.3 is considered

important in both moisture regimes, even if a lower score signifies the absence of a strong correlation between the variables and the component.

Under non-stressed moisture environment five principal components captured 79.98% of the total variations in the whole dataset of the 21 variables have been identified (Table 5). The eigenvalues for the principal component PC1 – PC5 were 6.559, 5.236, 2.636, 1.741 and 1.423, respectively. The first two contributes more to the total variation which accounts for 53.61% implies the two PC plays greater role for observed total variation. Therefore, characters having relatively high factor loading in the first PC1 were flowering date and above ground biomass contributed 29.81% of the total variation observed in the data set. This suggests that these two traits vary together. If one increases, then the remaining ones tend to increase as well if the sign is similar and had more contribution to the total diversity observed. Characters having higher loading in PC2 include grain yield and grain filling rate whereas panicle exertion and number of tiller showed high negative loading. Since this principal component comprises yield and yield components so that we could name it as ‘Yield factor’ and contribute 23.8% of the total variation. Genotypes found under cluster one showed high value for positive loading traits under PC2 so that could be exploited to enhance grain yield under non-stressed environment (Figure 3). Several Authors indicated the important contribution of the first PCs in total variability while conducting a research on different traits (Mustafa et al., 2015; Ali et al., 2011; Mujaju and Chakuya, 2008)

According to the current study PC3 accounts, 11.98% of the variation and higher loading were observed from plant agronomic aspect and thousand grain weight. Maturity date and panicle length had positive loading while flag leaf area had higher negative loading in PC4 which accounts 7.91% of total variability. Principal component 5 accounts for 6.47% of the variation with higher negative loading on seedling vigor and root angle.

Table 5 Eigenvalues and eigenvectors of the correlation matrix for the five main components extracted from 21 traits of the 23 tested genotypes under non-stressed environment

Parameters	PC1	PC2	PC3	PC4	PC5
Eigenvalue	6.559	5.236	2.636	1.741	1.423
% Variance	29.81	23.8	11.98	7.91	6.47
Cumulative	29.81	53.61	65.59	73.51	79.98
Traits	Eigenvectors				
Flowering date	0.313	-0.003	-0.270	0.146	-0.225
Maturity date	0.288	-0.034	-0.245	0.306	-0.183
Grain filling period	-0.235	-0.070	0.212	0.292	0.227
Seedling Vigor	0.030	-0.264	0.227	0.087	-0.436
SPAD	-0.230	0.232	-0.031	-0.113	0.146
Plant agronomic aspect	0.128	-0.251	0.338	-0.286	-0.087
Plant height	0.291	-0.196	-0.162	0.059	0.282

Flag leaf area	-0.076	0.274	-0.114	-0.357	-0.168
Leaf senescence	0.291	-0.016	0.264	0.152	-0.023
Grain yield	0.157	0.337	0.183	0.032	0.230
Grain filling rate	0.181	0.332	0.163	-0.005	0.197
Thousand grain weight	0.131	0.245	0.333	0.007	0.016
Harvest index	-0.288	0.138	0.294	0.101	-0.020
Stay green	0.271	-0.054	0.278	-0.180	0.019
Panicle exertion	-0.121	-0.346	0.073	0.109	0.096
Number of tiller	0.064	-0.333	0.020	-0.211	0.183
Panicle length	-0.229	-0.030	-0.232	0.301	0.077
Panicle weight	0.269	0.257	0.006	0.138	0.091
Disease score	0.143	-0.247	0.100	-0.261	0.195
Root angle	0.072	0.128	0.183	0.136	-0.533
Above ground biomass	0.327	0.060	-0.248	-0.117	0.124

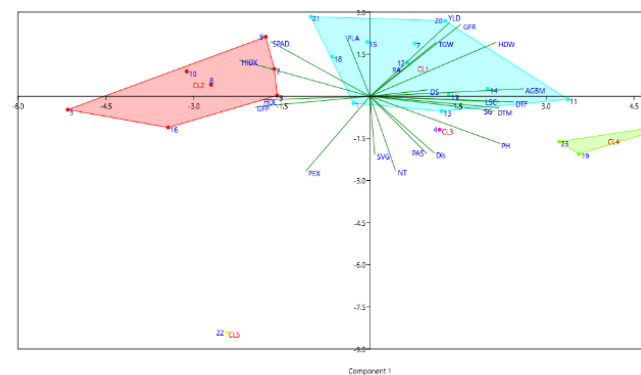


Figure 3 Scatter plot of PC1 and PC2 of 23 sorghum genotypes based on 21 phenotypic traits under non-stressed environment (code as description given in material and method)

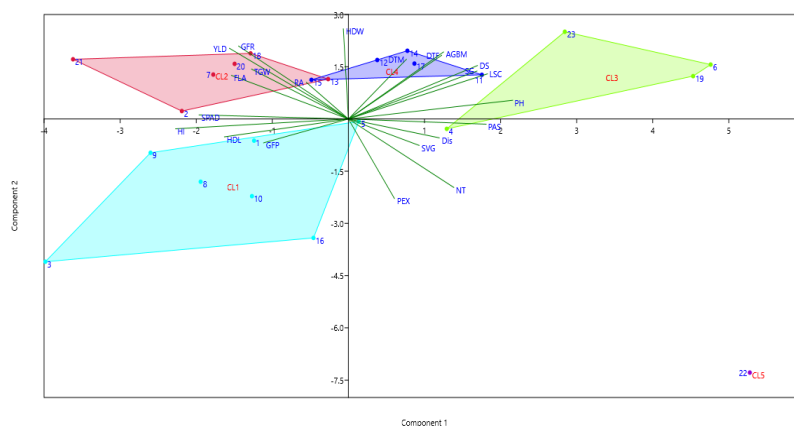


Figure 4 Scatter plot of PC1 and PC2 of 23 sorghum genotypes based on 22 phenotypic traits under stressed environment (code as description given in material and method)

3.4 Principal component analysis for stressed environment

The principal component analysis based on 22 phenotypic traits revealed seven principal components captured 87% of the total variation observed among the genotypes. Eigenvectors and eigenvalues along with their contribution to total variation were summed under Table 6. The first principal component captured 28.29% which contributed larger portion to the total variation. The traits having relatively higher loading in these components are plant height and harvest index which showed higher positive and negative loading, respectively. Principal component 2 captured 24.87% of the total variation most of the characters having higher loading in this component related to yield and yield component so that could differentiate as ‘yield factor’. In this component higher positive loadings were observed from grain yield, grain filling rate and panicle weight whereas panicle exertion had negative loading. All of these characters had positive correlation except panicle exertion which showed a decrease in value when the rest increases. Genotypes found under cluster two showed higher value for the traits which have higher positive loading in PC2 (Figure 4). Therefore, could be important to look genotypes under this cluster to breed for drought tolerant high yielding varieties under stressed environment.

The third principal component contributed 9.28% of the total variation with positive loading in thousand grain weight and harvest index while above ground biomass having negative loadings. Characters contribute to the PC4 were maturity date and seedling vigor which contribute 7.68% of the total variation. Panicle length, disease score and root angle have higher positive loading while flag leaf area showed negative loading in the PC5 which accounts for 6.68% of the total variation. Under PC6 higher loading of the variables were observed in grain filling period, panicle length positively whereas negative loading for root angle and accounts for 5.67% of the total variation. Grain filling period showed higher loading in the last component PC7 with a contribution of 5.1% to the total variation.

Table 6 Eigenvalues and eigenvectors of the correlation matrix for the seven main components extracted from 22 traits of the 23 tested genotypes under stressed environment

Parameter	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Eigenvalue	6.507	5.721	2.134	1.766	1.536	1.303	1.072
% Variance	28.29	24.87	9.28	7.68	6.68	5.67	4.66
Cumulative	28.29	53.17	62.45	70.12	76.8	82.47	87.13
Traits	Eigenvectors						
Flowering date	0.194	0.254	-0.186	0.294	0.120	-0.101	-0.270
Maturity date	0.130	0.244	-0.116	0.462	0.206	0.066	0.076
Grain filling period	-0.160	-0.086	0.170	0.206	0.115	0.310	0.660
Seedling Vigor	0.138	-0.115	0.283	0.486	-0.222	-0.228	-0.020
SPAD	-0.281	0.037	0.032	0.169	-0.276	-0.146	0.104
Plant agronomic aspect	0.257	-0.041	0.265	-0.203	-0.197	-0.268	0.268
Plant height	0.317	0.057	-0.142	0.027	0.019	0.130	0.124
Flag leaf area	-0.210	0.198	-0.004	-0.047	-0.301	0.231	0.243
Drought score	0.260	0.207	0.207	-0.244	-0.004	0.204	-0.014

Leaf senescence	0.278	0.171	0.160	-0.192	-0.059	0.210	-0.087
Grain yield	-0.203	0.312	0.151	-0.048	-0.014	0.040	-0.033
Grain filling rate	-0.186	0.320	0.133	-0.075	-0.028	-0.001	-0.123
Thousand grain weight	-0.167	0.223	0.374	0.074	0.056	-0.153	0.103
Above ground biomass	0.191	0.264	-0.321	0.021	-0.036	-0.070	0.293
Harvest index	-0.323	-0.015	0.312	-0.067	0.060	0.073	-0.253
Stay green	0.260	0.185	0.280	-0.156	0.068	0.141	-0.013
Panicle exertion	0.073	-0.335	0.153	0.172	-0.018	0.076	0.061
Number of tiller	0.189	-0.297	0.147	0.116	-0.092	0.065	-0.130
Panicle length	-0.238	-0.058	-0.146	0.044	0.394	0.398	-0.062
Panicle weight	0.016	0.378	-0.025	0.145	-0.148	-0.043	0.104
Disease score	0.166	-0.091	0.185	-0.123	0.527	-0.120	0.240
Root angle	-0.071	0.158	0.203	0.106	0.441	-0.396	-0.028

4. CONCLUSION

Twenty three early maturing sorghum genotypes were phenotyped under post-flowering moisture stress and unstressed environments and the result revealed that post-flowering drought reduce the value for all of the traits except flag leaf area. The increase in flag leaf area could serve as selection criteria for drought tolerance in sorghum. Cluster analysis grouped the 23 genotypes in to five genetically divergent clusters which showed significant inter cluster distance in both environments. Different clustering pattern observed depicts the divergence of genotypes for drought response which creates opportunity for further improvement through selection and hybridization. Genotypes under cluster I Viz. 76T1#23, B-35, Macia, E36-1, ESH-1, ESH-3 and SC103-14E showed best performance for traits related to drought tolerance. However, having these drought tolerance characteristics doesn't guarantee yield under stress environments hence, introgression of these important traits to high yielding and adapted genotypes should be the focus in resistance breeding program. Principal component analysis revealed flowering date and above ground biomass under non-stressed environment whereas plant height and harvest index for stressed environment have larger contribution for the observed total variation in the dataset.

Conflict of Interest

The author declares that there is no conflict of interest.

Acknowledgement

I would like to thank Ethiopian Institute of Agricultural Research (EIAR) for financial and technical support provided.

REFERENCES

- Ali, M.A., Abbas A., Niaz S., Zulkiffal M. and Ali S. 2009. Morpho-physiological criteria for drought tolerance in sorghum (*Sorghum Bicolor*) at seedling and post-anthesis stages. *International Journal of Agricultural Biology*, 11: 674–680.
- Ali., M.A., Jabran, K., Awan, S.I., Abbas, A., Ehsanullah, Zulkiffal, M., Acet, T., Farooq, J., Rehman, A. (2011): Morpho-physiological diversity and its implications for improving drought tolerance in grain sorghum at different growth stages. *Australian Journal of Crop Science* 5(3):311-320.
- Amsalu, A. and Endashew, B. 1999. Multivariate analysis of morphological variation in sorghum (*Sorghum bicolor* (L) Moench) germplasm from Eritrea and Ethiopia. *Genetic Resources and Crop Evolution* 46(3):273-284.
- Amelework Beyen. 2012. Genetic Diversity Analysis of Lowland Sorghum [*Sorghum bicolor* (L.) Moench] Landraces under Moisture Stress Conditions and Breeding for Drought Tolerance in North Eastern Ethiopia. Doctoral Dissertation. University of KwaZulu-Natal, Republic of South Africa.
- Brhane, G., Wortmann, C.S., Mamo, M., Gebrekidan, H. and Belay, A., 2006. Micro-basin tillage for grain sorghum production in semiarid areas of Northern Ethiopia. *Agronomy Journal*, 98(1), 124-128.
- Brown, J.S., 1991. Principal component and cluster analyses of cotton cultivar variability across the US cotton belt. *Crop Science*, 31(4), pp.915-922.
- Chahal, G.S. and Gosal, S.S., 2002. Principles and Procedures of Plant Breeding: Biotechnological and Conventional Approaches. CRC Press.
- CSA (Central Statistical Agency). 2017. Agricultural sample survey 2016/2017. Volume I. Report on area and production of major crops for private peasant holdings, *meher* season. Statistical bulletin 578, Central Statistical Agency, Addis Ababa, Ethiopia.
- Demeke M, Di-Marcantonio F. 2013. Analysis of incentives and disincentives for sorghum in Ethiopia. Technical notes series, MAFAP, FAO, Rome.
- Demeke, M. and Di Marcantonio, F., 2013. Analysis of incentives and disincentives for sorghum in Ethiopia. Technical notes series, MAFAP, FAO, Rome.
- Ejeta G, Knoll JE. 2007. Marker-assisted selection in sorghum. *Genomics-assisted Crop Improvement* pp. 187-205. Springer, Dordrecht.
- FAO STAT. 2017. Food and Agricultural Organization Statistical Data base: Production and Trade. <http://www.fao.org/faostat/en/#data/QC>. Accessed on August 2, 2020.

- FAO. 2002. Crop water management. Online. AGLW Water Management Group, United Nations FAO, Rome, Italy.
- FirewMekbib. 2008. Genetic erosion of sorghum (*Sorghum bicolor* (L.) Moench) in the centre of diversity, Ethiopia. *Genetic Resource and Crop Evolution*. 55:351–364.
- Geremew, G., Asfaw, A., Taye, T., Tesfaye, T., Ketema, B. and Michael, H.S., 2004. Development of sorghum varieties and hybrids for dryland areas of Ethiopia. *Uganda Journal of Agricultural Sciences*, 9(1), 594-605.
- ICRA. International Center for Development Oriented research in Agriculture. 1997. Strengthening farmer participatory research and development in Jijiga zone: The case of moisture stress reduction in the plains and soil fertility management in the hills. Working document series, 60 Jijiga Ethiopia.
- Khaton MA, Sagar A, Tajkia JE, Islam MS, Mahmud MS and Hossain AKMZ. 2016. Effect of moisture stress on morphological and yield attributes of four sorghum varieties. *Progressive Agriculture* 27 (3): 265-271.
- Kline, P., 2014. An easy guide to factor analysis. London: Routledge.
- Klute, A. (Ed.), 1986. Methods of soil analysis Part 1: Physical and mineralogical methods. American Society of Agronomy, Madison, Wisconsin, USA, pp188.
- Mahalanobis, P.C. 1936. On the generalized distance in statistics. National Institute of Science of India.
- Menezes, C.B., Ticona-Benavente, C.A., Tardin, F.D., Cardoso, M.J., Bastos, E.A., Nogueira, D.W., Portugal, A.F., Santos, C.V. and Schaffert, R.E., 2014. Selection indices to identify drought-tolerant grain sorghum cultivars. *Genetics and Molecular Research*, 13(4), 9817-9827.
- Mujaju, C., and Chakuya, E. 2008: Morphological variation of sorghum landrace accessions on-farm in Semi-arid areas of Zimbabwe. *International Journal of Botany* 4:376-382.
- Mustafa, H.S.B., Farooq, J., Bibi, T. and Mahmood, T., 2015. Cluster and principle component analyses of maize accessions under normal and water stress conditions. *Journal of Agricultural Sciences, Belgrade*, 60(1), pp.33-48.
- Rakshit, S., Hariprasanna, K., Gomashe, S., Ganapathy, K.N., Das, I.K., Ramana, O.V., Dhandapani, A. and Patil, J.V., 2014. Changes in Area, Yield Gains, and Yield Stability of Sorghum in Major Sorghum-Producing Countries, 1970 to 2009. *Crop Science*, 54(4), pp.1571-1584.
- Sharma, J.R., 1998. Statistical and Biometrical Techniques in Plant Breeding. New Age International (P) Limited Publishers, New Delhi.432p.

Sory S, Gaoussou DA, Mory CM, Niaba T, Gracen V, and Eric D. 2017. Genetic Analysis of Various Traits of Hybrids Sorghum (*Sorghum bicolor* (L) Moench), Correlated with Drought Tolerance. *Journal of Plant Biology and Soil Health*.;4(1): 9.

Stichler, C. and Fipps, G., 2003. Irrigating sorghum in south and south central Texas. Texas FARMER Collection.

UNDER PEER REVIEW