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

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Author’s contribution
The sole author designed, analysed, interpreted and prepared the manuscript.

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 depicits 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.
1. INTRODUCTION

Sorghum \([Sorghum \text{ bicolor} \ (L.) \text{ Moench}]\) 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 \((\text{Eragrostis tef})\) and maize \((\text{Zea mays. \ L})\) in terms of sown area \((1,854,710.93 \ \text{ha})\) and it is the third in total production next to maize and tef which is \(4,752,095.6 \ \text{tons}\) and mean yield of \(2.525 \ \text{t \ ha}^{-1}\). It covers \(14.97\%\) and \(16.36\%\) of the cropped area and total production from the total grain produced in Ethiopia \([1]\). 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 Nigériya and Sudan \([2,3]\).

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 \([4,3]\). Even if sorghum has the ability to cope with many stresses including heat and moisture, its production highly affected by drought occurred during reproductive stage in arid and semi-arid regions of the world \([5]\).

Terminal drought stress is a major occurrence in Ethiopia \([6]\). 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 (Shoa Robit), Wello, Raya valley and Sheraro and Humera areas in the north \([7]\). In these areas, the livelihood of the population is mainly dependent on sorghum \([8]\). A research conducted in North Eastern Ethiopia indicated moisture stress within and between seasons was a common phenomenon in all the areas surveyed \([9]\). In comparison between farmers variety Vs improved variety higher ranking was given by farmers for improved varieties for early maturity and drought resistance traits \([10]\). 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 \([11]\). 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 \([12]\). 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 \([13,14]\). 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 \([15]\).

To develop sorghum cultivars that best-fit farmers preference in lowland area of Ethiopia a breeder needs to conduct targeted breeding by including 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. MATERIALS 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 Fluvisol's 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), Miskir (14), Mekol (15), SC103-14E (16), Teshale (17), Abshir (18), ICSV 39046 (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 [16]. 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) [12], chlorophyll content (SPAD) using Chlorophyll Meter SPAD-502, number of tillers (NT) and Root angle [17].

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 [18]. 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 Ward and Hook [19]. Estimation of distance between clusters was done according to D² statistics [20] by using Minitab version 17.1.0.0 (2013) package. Significance of the squared distances for each cluster was tested against the tabulated χ² 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. [21] 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. [22], Khaton et al. [23], Menezes et al. [24] 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.
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 [25]. 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%) (Fig. 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 1. Trait means and analysis of variance for 23 sorghum genotypes under non-stressed (NS) and stressed (DS) moisture environments

| Variable | NS | | | | DS | | |
|----------|----|----|----|----|----|----|----|
|          | Mean | $\sigma^2$ | $\sigma_x$ | $R^2$ | Mean | $\sigma^2$ | $\sigma_x$ | $R^2$ |
| 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, $\sigma$: variance, $\sigma_x$: standard deviation, $R^2$: $R$-Square and trait abbreviation as indicated in material and method.
The generalized squared distances under non-stressed environment revealed highly significant \((P=.01)\) 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.

### 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

| 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 (cm²)          | 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⁻¹day⁻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) | 19434.38 | 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    |
genotypes into five different groups (Fig. 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 χ² 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 [26] 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.

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, [27] 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.

Table 3. Inter-cluster divergence D² 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**  | 58.78**   |
|           | 178.96**  | 444.56**  | 3980.06** |
| Cluster 3 | 251.72**  | 58.78**   | 3938.95** |
|           | 69.72**   | 444.56**  | 3938.95** |
| Cluster 4 | 251.72**  | 173.00**  | 444.56**  |
| Cluster 5 | 251.72**  | 3980.06** | 3938.95** |

*For Non-stressed, *significant (χ²=27.587) and **highly significant (χ²=33.409); For Stressed, *significant (χ²=31.410) and **highly significant (χ²=37.566)
Table 4. Mean value of 21 phenotypic traits for the five clusters of 23 sorghum genotypes under stressed environment

| Traits                          | 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 (cm²)           | 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   | 22.90  |
| 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  |

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 (Fig. 3). Several Authors indicated the important contribution of the first PCs in total variability while conducting a research on different traits [28],[13], [29].

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.

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 (Fig. 4). Therefore, could be important to look genotypes under this cluster to breed for drought tolerant high yielding varieties under stressed environment.

![Scatter plot of PC1 and PC2 of 23 sorghum genotypes based on 21 phenotypic traits](image)

**Fig. 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)

**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     |
|---------------------------------|---------|---------|---------|---------|---------|
| **Traits**                      |         |         |         |         |         |
| 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   |
Fig. 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)

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  |
|----------------------------|------|------|------|------|------|------|------|
| Eigenvector                | 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                     |      |      |      |      |      |      |      |
| 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|      | 0.032| 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|
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.

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.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. CSA (Central Statistical Agency). 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; 2017.
2. Rakshit S, Hariprasanna K, Gomashe S, Ganapathy KN, Das IK, Ramana OV, Dhandapani A, Patil JV. Changes in area, yield gains, and yield stability of sorghum in major sorghum-producing countries, 1970 to 2009. Crop Science. 2014;54(4): 1571-1584.
3. FAO STAT. Food and Agricultural Organization Statistical Data base: Production and Trade; 2017. Accessed on August 2, 2020. Available: http://www.fao.org/faostat/en/#data/QC
4. Demelk F, Di-Marcantonio F. Analysis of incentives and disincentives for sorghum in Ethiopia. Technical notes series, MAFAP, FAO, Rome; 2013.
5. Ejeta G, Knoll JE. Marker-assisted selection in sorghum. Genomics-assisted Crop Improvement. Springer, Dordrecht. 2007;187-205.
6. Brhane G, Wortmann CS, Mamo M, Gebrekidan H, Belay A. Micro-basin tillage for grain sorghum production in semiarid areas of Northern Ethiopia. Agronomy Journal. 2006;98(1):124-128.
7. ICRA. International Center for Development Oriented research in Agriculture. 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; 1997.
8. Geremew G, Asfaw A, Taye T, Tesfaye T, Ketema B, Michael HS. Development of sorghum varieties and hybrids for dryland areas of Ethiopia. Uganda Journal of Agricultural Sciences. 2004;9(1):594-605.
9. Amelework Beyen. 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; 2012.

10. Firew Mekbib. Genetic erosion of sorghum (Sorghum bicolor (L.) Moench) in the centre of diversity, Ethiopia. Genetic Resource and Crop Evolution. 2008; 55:351–364.

11. FAO. Crop water management. Online. AGLW Water Management Group, United Nations FAO, Rome, Italy; 2002.

12. Stickler FC, Wearden S, Pauli AW. Leaf Area Determination in Grain Sorghum 1. Agronomy Journal. 1961 May;53(3):187-8.

13. Ali MA, Jabran K, Awan SI, Abbas A, Ehsanullah Zulkiffl M, Acet T, Farooq J, Rehman A. Morpho-physiological diversity and its implications for improving drought tolerance in grain sorghum at different growth stages. Australian Journal of Crop Science. 2011;5(3):311-320.

14. Brown JS. Principal component and cluster analyses of cotton cultivar variability across the US cotton belt. Crop Science. 1991;31(4):915-922.

15. Chahal GS, Gosal SS. Principles and procedures of plant breeding: Biotechnological and conventional approaches. CRC Press; 2002.

16. Klute A. (Ed.). Methods of soil analysis Part 1: Physical and mineralogical methods. American Society of Agronomy, Madison, Wisconsin, USA; 1986:188.

17. Singh V, van Oosterom EJ, Jordan DR, Hunt CH, Hammer GL. Genetic variability and control of nodal root angle in sorghum. Crop Science. 2011 Sep;51(5):2011-20.

18. SAS S. STAT user’s guide, version 9.2. Cary, NC, USA: SAS Inst.

19. Ward Jr JH, Hook ME. Application of an hierarchical grouping procedure to a problem of grouping profiles. Educational and Psychological Measurement. 1963 Apr;23(1):69-81.

20. Mahalanobis PC. On the generalized distance in statistics. National Institute of Science of India; 1936.

21. Ali MA, Abbas A, Niaz S, Zulkiffl M, Ali S. Morpho-physiological criteria for drought tolerance in sorghum (Sorghum bicolor) at seedling and post-anthesis stages. International Journal of Agricultural Biology. 2009;11: 674–680.

22. Sory S, Gaoussou DA, Mory CM, Niaba T, Gracen V, and Eric D. Genetic analysis of various traits of hybrids sorghum (Sorghum bicolor (L) Moench), correlated with drought tolerance. Journal of Plant Biology and Soil Health. 2017;4(1): 9.

23. Khaton MA, Sagar A, Tajkia JE, Islam MS, Mahmud MS, Hossain AKMZ. Effect of moisture stress on morphological and yield attributes of four sorghum varieties. Progressive Agriculture. 2016;27(3):265-271.

24. Menezes CB, Ticona-Benavente CA, Tardin FD, Cardoso MJ, Bastos EA, Nogueira DW, Portugal AF, Santos CV, Schaffert RE. Selection indices to identify drought-tolerant grain sorghum cultivars. Genetics and Molecular Research. 2014; 13(4):9817-9827.

25. Sharma JR. Statistical and biometrical techniques in plant breeding. New Age International (P) Limited Publishers, New Delhi. 1998:432.

26. Amsalu A, Endashew B. Multivariate analysis of morphological variation in sorghum (Sorghum bicolor (L) Moench) germplasm from Eritrea and Ethiopia. Genetic Resources and Crop Evolution. 1999;46(3): 273-284.

27. Kline P. An easy guide to factor analysis. London: Routledge; 2014.

28. Mustafa HSB, Farooq J, Bibi T, Mahmood T. Cluster and principle component analyses of maize accessions under normal and water stress conditions. Journal of Agricultural Sciences, Belgrade. 2015;60(1):33-48.

29. Mujaju C, Chakuya E. Morphological variation of sorghum landrace accessions on-farm in Semi-arid areas of Zimbabwe. International Journal of Botany. 2008;4: 376-382.

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