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# Genetic diversity of sorghum [*Sorghum bicolor* (L.)] germplasm for drought tolerance

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**Abstract**
Drought is a major cause which affects production and productivity of sorghum. Sorghum drought is associated with pre and post flowering stages. Stay-green is the important drought tolerant trait which is associated with post flowering stage. One hundred accessions of sorghum were sown in two different situations in RBD with two replications in Coimbatore, *i.e.* one with complete irrigation till physical maturity and another with imposed moisture stress from flowering to maturity. Genotype B35 was used as drought resistant check and variety CO26 as drought susceptible check. Significant variation was found on stress indicators like relative water content (RWC), SPAD chlorophyll and stay-green traits. The drought stress indices also showed significant variation which indicated selection of drought tolerance may involve either high yield potential or stable yield under water deficit condition. RWC and SPAD chlorophyll had a positive association with grain yield. Negative association with stay-green indicated RWC, SPAD chlorophyll and stay-green as important for selection under drought stress condition. Cluster analysis was performed based on physiological expression of stay-green trait. In cluster analysis tolerant genotypes and susceptible genotypes clustered separately, different clusters given an opportunity to select drought tolerant genotypes which can be used as donors for hybridization programme for drought tolerance.

**Keywords:** Cluster analysis, Drought tolerance, Genetic diversity, Physiological traits, Stay-green, *Sorghum bicolor*

## Introduction

Drought acts as a major limiting factor in agricultural production. Improving drought tolerance is an important objective in many crop-breeding programs. Sorghum [*Sorghum bicolor* (L.)] is an important forage and food crop in arid and semiarid regions of India. More than half of the world's sorghum is grown in semi-arid tropics of India and Africa, where it is a staple food for millions of poor people (Iqbal *et al.,* 2010). Genetic improvement of sorghum is considered as an essential effort, since it is a simple long-term measure. Sorghum grown under rainfed conditions is usually affected by drought stress at different stages of its growth period resulting in negative effect on yield (Ali *et al.,* 2011). Drought response in sorghum has been characterized at both pre and post flowering stages. Drought stress during the post flowering stage needs serious consideration because the negative impact of post-flowering drought on yield can be very drastic. The stay-green is the important drought tolerant indicator in sorghum crop. The stay-green trait can be defined as the ability to retain green leaves, fill grain normally, and resist lodging under conditions of post-flowering drought stress (Rosenow *et al*., 1983). Knowledge on genetic diversity among the plant populations and its quantitative assessment usually helps the breeder in choosing the parents for breeding program on the basis of divergence analysis. Hence, understanding the genetic diversity in sorghum will facilitate for further improvement in this crop to overcome the yield decline under water stress condition experienced in recent years.

## Materials and Methods

***Plant materials***: The field experiment was conducted at Millet breeding station, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The experimental materials for this study comprised of 100 accessions of sorghum, which include local landraces adapted to different agro climatic regions of India and accessions from other countries (Table 1). The experiment was laid out in two different situations, one with complete irrigation till physiological maturity and another under post flowering moisture stress imposed by withholding irrigation from flowering phase to maturity. It was raised in a randomized block design (RBD) with two replications. In this study genotype B 35, was used as the drought resistant check and variety CO 26 as the drought susceptible check. It was ensured that no rain was recorded from anthesis to crop maturity. The experimental plots with normal irrigations from planting to maturity served as control. Normal recommended cultural practices were adopted during the crop period. Data were recorded for various traits on five randomly selected plants in two replications.

**Table 1.** List of sorghum genotypes used in the study

| S. No. | Genotypes | Source |
|---|---|---|
| 1 | B 35 | NRCS |
| 2 | CO 26 | Coimbatore |
| 3 | M 35-1 | NRCS |
| 4 | CO(S) 28 | Coimbatore |
| 5 | IS 21756 | Sudan |
| 6 | IS 21757 | Sudan |
| 7 | IS 22005 | Maharashtra |
| 8 | IS 22212 | USA |
| 9 | IS 22215 | USSR |
| 10 | IS 22233 | Botswana |
| 11 | IS 22243 | Botswana |
| 12 | IS 22244 | Botswana |
| 13 | IS 22248 | Botswana |
| 14 | IS 22251 | Botswana |
| 15 | IS 22334 | Botswana |
| 16 | IS 22335 | Botswana |
| 17 | IS 22339 | Botswana |
| 18 | IS 22349 | Botswana |
| 19 | IS 22360 | Botswana |
| 20 | IS 22697 | Sudan |
| 21 | IS 22764 | Somalia |
| 22 | IS 22765 | Somalia |
| 23 | IS 22794 | Somalia |
| 24 | IS 22959 | Somalia |
| 25 | IS 23158 | Sudan |
| 26 | IS 23390 | Tanzania |
| 27 | IS 23392 | Sudan |
| 28 | IS 23397 | India |
| 29 | IS 23402 | India |
| 30 | IS 23408 | India |
| 31 | IS 23418 | India |
| 32 | IS 23419 | India |
| 33 | IS 23422 | India |
| 34 | IS 23429 | India |
| 35 | IS 23430 | India |
| 36 | IS 23440 | India |
| 37 | IS 23442 | India |
| 38 | IS 23446 | India |
| 39 | IS 23453 | India |
| 40 | IS 23455 | India |
| 41 | IS 23460 | India |
| 42 | IS 23477 | India |
| 43 | IS 23590 | India |
| 44 | IS 24484 | Ethiopia |
| 45 | IS 24693 | South Africa |
| 46 | IS 24978 | India |
| 47 | IS 25004 | Sudan |
| 48 | IS 25071 | Sudan |
| 49 | IS 25098 | Ghana |
| 50 | IS 25400 | Ghana |
| 51 | IS 25601 | ICRISAT |
| 52 | IS 25602 | ICRISAT |
| 53 | IS 25760 | ICRISAT |
| 54 | IS 25779 | ICRISAT |
| 55 | IS 26103 | ICRISAT |
| 56 | IS 26700 | ICRISAT |
| 57 | IS 26742 | ICRISAT |
| 58 | IS 39690 | ICRISAT |
| 59 | IS 26760 | ICRISAT |
| 60 | IS 27874 | ICRISAT |
| 61 | IS 27875 | ICRISAT |
| 62 | IS 29115 | ICRISAT |
| 63 | IS 29218 | ICRISAT |
| 64 | IS 29231 | ICRISAT |
| 65 | IS 29239 | ICRISAT |
| 66 | IS 29251 | ICRISAT |
| 67 | IS 29277 | ICRISAT |
| 68 | IS 29278 | ICRISAT |
| 69 | IS 29306 | ICRISAT |
| 70 | IS 29307 | ICRISAT |
| 71 | IS 29322 | ICRISAT |
| 72 | IS 29323 | ICRISAT |
| 73 | IS 29341 | ICRISAT |
| 74 | IS 29344 | ICRISAT |
| 75 | IS 29358 | ICRISAT |
| 76 | IS 29359 | ICRISAT |
| 77 | IS 29379 | ICRISAT |
| 78 | IS 29386 | ICRISAT |
| 79 | IS 29389 | ICRISAT |
| 80 | IS 29393 | ICRISAT |
| 81 | IS 29440 | ICRISAT |
| 82 | IS 29450 | ICRISAT |
| 83 | IS 29451 | ICRISAT |
| 84 | IS 29458 | ICRISAT |
| 85 | IS 29459 | ICRISAT |
| 86 | IS 29484 | ICRISAT |
| 87 | IS 29487 | ICRISAT |
| 88 | IS 29496 | ICRISAT |
| 89 | IS 29498 | ICRISAT |
| 90 | IS 29509 | ICRISAT |
| 91 | IS 29515 | ICRISAT |
| 92 | IS 29523 | ICRISAT |
| 93 | IS 29545 | ICRISAT |

| S. No. | Genotypes | Source |
|---|---|---|
| 94 | IS 29554 | ICRISAT |
| 95 | IS 29573 | ICRISAT |
| 96 | IS 29589 | ICRISAT |
| 97 | IS 29611 | ICRISAT |
| 98 | IS 29625 | ICRISAT |
| 99 | IS 29629 | ICRISAT |
| 100 | IS 29640 | ICRISAT |

Water stress indicator traits like relative water content (RWC) was calculated using the formula suggested by Barrs and Weatherly (1962) and total leaf chlorophyll contents were measured in both stressed and fully irrigated trials with a Minolta chlorophyll meter SPAD-502. The stay-green was estimated visually on a plot basis on a scale of 1 to 5 based on the degree of leaf and plant death at physiological maturity in the field both under post-flowering drought stress and control, by following the visual ratings of stay-green trait suggested by Wanous *et al.* (1991) in sorghum. Under the drought tolerance measurement indices, the drought susceptibility index (DSI) was calculated for all the genotypes under stress conditions using the formula suggested by Fischer and Maurer (1978). Stress tolerance index was calculated. Yield stability ratio (YS) was also calculated by taking the ratio of grain yield under stress and irrigated conditions as suggested by Lewis (1954) and superiority measures or relative yield was calculated based on Lin and Binns (1988).

***Construction of phenotypic dendrogram*:** The data on 10 quantitative traits for all the 100 accessions under stress condition were subjected to multivariate hierarchial cluster analysis. The mean data over two replications were computed for hierarchial cluster analysis. The computer software NTSYS version 2.1 software was used (Rohlf 1992). A phenetic tree was constructed using the TREEPLOT program of NTSYS after standardization of the data.

**Results and Discussion**

The mean performance of one hundred sorghum genotypes for the biometrical and physiological traits (Table 2) under both stress and control showed high variation among the genotypes as it was evident from the range of values. Under the variability parameters, all the characters showed significant variation among the genotypes studied (Table 3). Genotypic variance was lesser than the phenotypic variance for all the ten characters studied under stress condition. The phenotypic (2541.37) and genotypic (2539.93) variances were the highest for the plant height and also higher values of phenotypic (31.00) and genotypic (30.99) coefficients of variation were recorded for plant height. Patil and Thombre (1985) reported that it is due to the influence of environment on expression of traits.

Higher values of genotypic and phenotypic coefficients of variation for plant height stay green, grain yield, leaf area index and SPAD chlorophyll reading under stress justified the relative contributions of these traits to the variability of genotypes. The present finding substantiates the findings of Geleta and Daba (2005) in that higher value of genotypic and phenotypic coefficients of variation for plant height and grain yield per plant were observed. Significant improvement of these traits can be made through effective selection in the genotypes. Heritability in broad sense was the highest for relative water content (0.99), SPAD chlorophyll reading (0.99) and plant height (0.99), followed by stay-green (0.98), grain yield (0.91), days to 50% flowering (0.91), biological yield (0.88), leaf area index (0.81), days to normal maturity (0.67) and days to physiological maturity (0.67). In the present study all the traits investigated under stress showed high heritability estimates, which indicated that most likely the heritability was due to additive gene effects and selection may be effective. Subashri (2005) reported high heritability for the traits like grain yield, biological yield and senescence under stress. Kebede *et al.* (2001) reported high heritability for stay-green ranging from 72% to 84% suggesting it as useful selection criteria under drought. Relative comparison of heritability estimates and expected genetic advance as percentage of mean will give an idea about the nature of gene action governing a particular character. High heritability along with high genetic advance as percentage of mean was observed for plant height, stay green, grain yield, relative water content, SPAD chlorophyll reading and leaf area index. Patil and Thombre (1985) observed high heritability coupled with high genetic advance as per cent of mean for plant height and grain yield per plant. The genetic advance as percentage of mean was the highest for plant height (63.83) followed by stay green, (60.83), relative water content (46.57), grain yield (46.51) and SPAD chlorophyll (44.39).

Drought tolerance measurement indices were estimated for all the genotypes studied and these showed significant variation among the genotypes. Selection for drought tolerance typically involves evaluating genotypes for either high yield potential or stable performance under varying degrees of water stress. Drought susceptibility index (DSI) and relative yield (RY) values were used to describe the yield stability and yield potential. Ahmed *et al*. (1999) and Pinter *et al*. (1990) proposed the combination of drought susceptibility index vs. relative yield as useful selection criteria in identifying the genotypes with yield potential and relatively stable yield performance under different moisture environments. Based on findings of Ahmed *et al.* (2003), the genotypes having low drought susceptibility index and high relative yield under drought condition were selected as drought tolerant genotypes because of high relative yield performance under drought.

**Table 2.** Mean, range, standard error difference (S.Ed), and critical difference (CD) for agronomic and physiological traits in sorghum

| Characters | | Mean | Range | SEd | CD |
|---|---|---|---|---|---|
| Days to 50% flowering (DF) | S | 67.8 | 62.0-78.0 | 0.86 | 1.70 |
| | I | 64.6 | 58.0-75.0 | 0.85 | 1.72 |
| Days to physiological maturity (DPM) | S | 89.0 | 81.0-93.0 | 1.45 | 2.89 |
| | I | 92.0 | 84.0-97.0 | 1.40 | 2.77 |
| Days to normal maturity (DNM) | S | 99.6 | 94.0-105.0 | 1.26 | 2.51 |
| | I | 94.8 | 89.0-100.0 | 1.25 | 2.49 |
| Relative water content (RWC) (%) | S | 52.9 | 22.4-66.9 | 1.15 | 2.28 |
| Plant height (PHT) (cm) | S | 162.5 | 97.7-286.4 | 1.19 | 2.38 |
| | I | 166.8 | 101.7-292.7 | 1.25 | 2.48 |
| SPAD chlorophyll reading (CHL) | S | 39.1 | 26.3-62.6 | 0.08 | 0.16 |
| | I | 52.8 | 28.2-67.8 | 1.31 | 2.61 |
| Leaf area index (LAI) | S | 2.7 | 1.6-3.8 | 0.27 | 0.54 |
| | I | 4.4 | 2.2-6.3 | 0.30 | 0.60 |
| Biological yield (BY) (g) | S | 150.9 | 116.7-192.2 | 6.23 | 12.36 |
| | I | 158.0 | 123.8-201.8 | 6.22 | 12.35 |
| Grain yield (GY) (g) | S | 26.4 | 11.4-43.6 | 1.08 | 2.16 |
| | I | 36.8 | 13.5-53.1 | 0.09 | 0.18 |
| Stay green (SG) (score) | S | 3.6 | 2.0-5.0 | 0.25 | 0.50 |
| | I | 2.5 | 1.0-4.0 | 0.09 | 0.19 |

**Table 3.** Genetic parameters for the biometrical and physiological traits in sorghum

| Characters | | $V_P$ | PCV% | $V_g$ | GCV% | $h^2$ | GA | GA% |
|---|---|---|---|---|---|---|---|---|
| DF | S | 8.39 | 4.27 | 7.65 | 4.08 | 0.91 | 5.56 | 8.00 |
| | I | 8.40 | 4.49 | 7.60 | 4.28 | 0.91 | 5.57 | 8.42 |
| DPM | S | 6.55 | 2.87 | 4.42 | 2.36 | 0.67 | 3.89 | 3.99 |
| | I | 7.22 | 2.91 | 5.26 | 2.49 | 0.72 | 4.33 | 4.38 |
| DNM | S | 5.00 | 2.44 | 3.39 | 1.84 | 0.67 | 3.41 | 3.13 |
| | I | 4.93 | 2.30 | 3.35 | 1.93 | 0.68 | 3.37 | 3.27 |
| RWC | S | 145.93 | 22.81 | 144.60 | 22.71 | 0.99 | 24.71 | 46.57 |
| | I | - | - | - | - | - | | - |
| PHT | S | 2541.37 | 31.00 | 2539.93 | 30.99 | 0.99 | 103.80 | 63.83 |
| | I | 2411.78 | 29.43 | 2410.22 | 29.42 | 0.99 | 101.11 | 60.59 |
| CHL | S | 71.36 | 21.55 | 71.36 | 21.55 | 0.99 | 17.40 | 44.39 |
| | I | 95.67 | 18.49 | 93.94 | 18.32 | 0.98 | 19.87 | 37.41 |
| LAI | S | 0.41 | 23.58 | 0.33 | 21.31 | 0.81 | 1.13 | 39.70 |
| | I | 1.72 | 24.23 | 1.08 | 23.26 | 0.92 | 2.09 | 46.00 |
| BY | S | 350.38 | 12.39 | 311.56 | 11.69 | 0.88 | 35.27 | 22.71 |
| | I | 339.83 | 11.66 | 301.08 | 10.97 | 0.88 | 34.65 | 21.28 |
| GY | S | 63.44 | 24.56 | 62.26 | 23.55 | 0.91 | 16.17 | 46.51 |
| | I | 118.64 | 29.54 | 118.63 | 29.54 | 0.99 | 22.43 | 60.85 |
| SG | S | 0.81 | 30.08 | 0.74 | 29.81 | 0.98 | 1.74 | 60.83 |
| | I | 0.59 | 30.62 | 0.58 | 30.36 | 0.98 | 1.56 | 62.03 |

(S= Stress, I= Irrigated)

**Table 4.** Correlation coefficients for component traits of 100 sorghum genotypes under drought conditions

| | | DF | DPM | DNM | RWC | PHT | CHL | LAI | BY | SG | GY |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DF | G | DF | 0.053 | 0.117 | -0.135 | 0.031 | -0.145 | -0.064 | 0.278** | 0.076 | -0.216* |
| | P | | 0.055 | 0.130 | -0.127 | 0.030 | -0.138 | -0.061 | 0.276** | 0.066 | -0.206* |
| DPM | G | | DPM | 0.533** | -0.501** | -0.339** | -0.467** | -0.351** | -0.404** | 0.261** | -0.543** |
| | P | | | 0.521** | -0.472** | -0.276** | -0.383** | -0.274** | -0.292** | 0.207* | -0.445** |
| DNM | G | | | DNM | -0.390** | -0.019 | -0.078 | -0.111 | -0.119 | 0.145 | -0.198* |
| | P | | | | -0.319** | -0.014 | -0.064 | -0.099 | -0.058 | 0.111 | -0.158* |
| RWC | G | | | | RWC | 0.236** | 0.570** | 0.406** | 0.312** | -0.380** | 0.555** |
| | P | | | | | 0.234** | 0.567** | 0.368** | 0.294** | -0.365** | 0.554** |
| PHT | G | | | | | PHT | 0.551** | 0.368** | 0.596** | -0.181* | 0.398** |
| | P | | | | | | 0.550** | 0.347** | 0.562** | -0.175* | 0.395** |
| CHL | G | | | | | | CHL | 0.555** | 0.553** | -0.197* | 0.629** |
| | P | | | | | | | 0.553** | 0.552** | -0.189* | 0.623** |
| LAI | G | | | | | | | LAI | 0.388** | -0.212* | 0.555** |
| | P | | | | | | | | 0.298** | -0.184* | 0.553** |
| BY | G | | | | | | | | BY | -0.262** | 0.435** |
| | P | | | | | | | | | -0.238** | 0.411** |
| SG | G | | | | | | | | | SG | -0.104 |
| | P | | | | | | | | | | -0.099 |
| GY | G | | | | | | | | | | GY |
| | P | | | | | | | | | | |

*,** significant at 0.05 and 0.01 probability level, (P-Phenotypic correlation coefficient, G-Genotypic correlation coefficient)

**Table 5.** Cluster mean performance of sorghum genotypes under drought condition

| Clusters | DF | DPM | DNM | RWC | PHT | CHL | LAI | BY | GY | SG |
|---|---|---|---|---|---|---|---|---|---|---|
| Grp.1 | 68.73 | 91.54 | 100.62 | 32.77 | 162.12 | 27.76 | 2.20 | 145.57 | 17.96 | 3.77 |
| Grp.2 | 67.25 | 86.13 | 99.25 | 66.40 | 284.55 | 61.58 | 3.71 | 191.12 | 42.35 | 2.75 |
| Grp.3 | 67.82 | 88.79 | 98.90 | 54.48 | 139.88 | 36.94 | 2.66 | 147.08 | 26.15 | 3.68 |
| Grp.4 | 67.54 | 89.12 | 100.18 | 55.79 | 144.91 | 43.37 | 2.65 | 148.70 | 26.03 | 3.92 |
| Grp.5 | 68.30 | 88.70 | 100.20 | 56.51 | 245.13 | 33.92 | 2.81 | 152.44 | 24.97 | 3.20 |
| Grp.6 | 65.50 | 90.00 | 100.00 | 62.48 | 103.55 | 30.85 | 3.07 | 150.25 | 22.10 | 3.00 |
| Grp.7 | 64.50 | 87.50 | 102.50 | 37.09 | 117.45 | 31.53 | 3.45 | 138.95 | 42.90 | 4.00 |
| Grp.8 | 71.50 | 90.00 | 101.00 | 47.72 | 241.95 | 40.55 | 3.54 | 155.55 | 28.10 | 4.00 |
| Grp.9 | 65.50 | 92.00 | 102.00 | 60.88 | 268.85 | 47.30 | 2.95 | 131.45 | 28.90 | 5.00 |

Information about correlation among the traits is important for initiation of any breeding program because it provides a chance for selection of desirable genotypes with desirable traits simultaneously (Ali *et al*., 2009). In association analysis, other than stay-green, the remaining all other stress indicators had significant positive association with grain yield under stress condition. SPAD chlorophyll reading had significant negative correlation (-0.197) with stay green under stress condition (Table 4). Relative water content had highly significant positive correlation with SPAD chlorophyll reading (0.570) and significant negative correlation with stay-green (-0.380).

Selection based on physiological traits may provide new criteria of selection for improvement of productivity. For this, understanding on association of the physiological traits with yield and other component traits is essential. Relative water content had highly significant positive correlation with SPAD chlorophyll reading and grain yield under stress. Omanya *et al.* (1997) also obtained the similar results who reported that genotypes with high leaf relative water content exhibited less reduction in biomass and yield. Mokote *et al.* (1998) also reported the significant positive association of grain yield with relative water content. Relative water content and SPAD chlorophyll reading had highly significant negative association with stay-green trait, the stay-green cultivars with more internal plant water status coupled with high chlorophyll content influenced the grain yield positively. Xu *et al.* (2000) investigated the relationship between the visual stay-green rating and the leaf chlorophyll concentration in sorghum and reported that the chlorophyll content was significantly correlated to stay-green rating (r = -0.90). Relative water content and SPAD chlorophyll reading were found to be more important while executing selection of traits under water stressed environments. Plants with more relative water content, lower stay-green score and good amount of chlorophyll on normal leaf will be better yielding genotype under moisture stress condition. This finding can be used for developing ideotype for drought prone environments.

In this study, diversity among one hundred sorghum accessions was estimated using hierarchical cluster analysis for ten quantitative traits under drought stress conditions. The genotypes were classified based on the morpho-physiologial traits and the type and level of expression of stay-green. The clustering pattern of the analysis indicated the categorization of the drought resistant and drought susceptible genotypes into separate clusters. Genotypes which had delayed onset of senescence or the tolerant genotypes were found clustered in separately as well as the genotypes which had early senescence or susceptible were found grouped in different clusters. Genotypes with the moderate level of senescence had clustered in another separate clusters which indicate that they are less useful as the source of trait for drought tolerance genotypes with delayed onset of senescence. However, the susceptible genotypes grouped under separate cluster would similar in their reaction to drought hence these genotypes will perform in irrigated conditions. This is similar to the findings of Mahalakshmi and Bidinger (2002). This is also consistent with the hypothesis of Thomas and Howarth (2000) where they observed that the genetic and physiological routes to the stay-green trait are diverse and classified the stay-green phenotypes into five types A, B, C, D, and E. This stay-green trait is likely to be controlled by different genes that in turn, are triggered by the specific pattern of stress development (Dunwell 2000). The grouping of accessions into different clusters (Table 5) gives us an opportunity to identify and select the drought tolerant genotypes, which can be used as the donors in hybridization programme for drought tolerance.

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