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SCREENING OF SORGHUM (*Sorghum bicolor* Var Moench) FOR DROUGHT TOLERANCE AT SEEDLING STAGE IN POLYETHYLENE GLYCOL.

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ABSTRACT

Twenty different accessions of sorghum (*Sorghum bicolor* var Moench) were evaluated for their genetic potential to drought tolerance at seedling stage. Water stress was simulated by non-ionic water soluble polymer polyethylene glycol of molecular weight 6000. After fourteen days data were recorded for easily measurable seedling traits as shoot length, root length, fresh shoot weight, dry shoot weight, fresh root weight and dry root weight under control as well as water stress conditions. Significant differences were observed among the accessions, treatments and their interactions for evaluated plant traits suggesting a great amount of variability for drought tolerance in sorghum. Differential sensitivity of seedling traits was noted due to water stress created by PEG. However, shoot related traits were the most sensitive against the water stress. Proportional contribution of RL, SL, FSW, DSW, FRW and DRW was 56.6%, 20.2 %, 10.5%, 8.5%, 3.5% and 0.7% to drought stress as measured by Principle component analysis. Root length (56.6%) was the highest towards drought tolerance, indicating root length was least effected by water stress among all the seedling traits. The most promising drought tolerant accessions (80353, 80365, 80199, 80204 and 80319) were screened through multivariate scoring index.

Key words: *Sorghum bicolor* L., Polyethylene glycol (PEG), water stress, seedling traits.

INTRODUCTION

Sorghum (*Sorghum bicolor*) is a multipurpose crop grown for food, animal feed and industrial purposes. It is considered more tolerant to many stresses, including heat, drought, salinity and flooding as compared to other cereal crops (Ejeta and Knoll, 2007; Ali *et al.* 2011a), however, the crop grown in rain fed areas is highly effected by drought stress (Kebede *et al*. 2001).

Plant abiotic stresses such as drought stress along with the growing world population stress and per capita food consumption threaten stable global food availability. Drought or any other abiotic stresses results in reduction of yield and plant growth. They limit the photosynthesis and consequently, limited availability of photosynthetic assimilates and energy to the plant. It is imperative for plants to use this limited supply of nutrients to their maximal advantage to survive under stress. Apparently, under drought stress conditions, an urgent need for plants would be to increase the uptake of water, which is usually more available deep down in the soil (Xiong *et al.* 2006). Identification and understanding the mechanisms of drought tolerance in sorghum have been major goals of plant physiologists and breeders include prolific root system, ability to maintain stomatal opening at low levels of leaf water potential and high osmotic adjustment and various seedling parameters (Rajendran *et al.* 2011) .Water stress affects almost every developmental stage of the plant. However, damaging effects of this stress was more noted when it coincided with various growth stages such as germination; seedling shoot length, root length and flowering (Rauf, 2008; Khayatnezhad, *et al*. 2010).

Significant progress has been made in understanding plant growth under drought stress. Water deficit is sensed by the roots which begin to synthesize ABA within 1 hour of the onset of the water stress. ABA is transported via xylem from roots to leaves within minutes to hours. Root length is an important trait against drought stress in plant varieties; in general, variety with longer root growth has resistant ability for drought (Leishman and Westoby, 1994; Kaydan and Yagmur, 2008). Dhanda *et al*. (2004) reported that the osmotic membrane stability of the leaf segment was the most important trait, followed by root-to-shoot ratio and root length on the basis of their relationships with other traits for drought tolerance. These water sensitive stages may be exploited to discriminate genotypes on the basis of their resistance to water stress. Among these critical stages, water stress induced during seedling stage has been exploited in various crop species to screen germplasm or breeders populations i.e. Wheat (Dhanda *et al*. 2004), sorghum (Gill *et al*. 2002; Bibi *et al,* 2010), maize (Mohammadkhani and Heidari, 2008; Farsiani and Ghobadi, 2009; Khayatnezhad, 2010) and sunflower (Rauf *et al*. 2008). Rauf (2008) narrated several benefits of screening genotypes at seedling stages such as low cost, ease of handling, less laborious and getting rid of susceptible genotypes at earliest. Furthermore seedling traits have also shown moderate to high heritability with additive type of genetic variance within and over environments (Rauf *et al.* 2008).

Field experiments related to water stress has been difficult to handle due to significant environmental or drought interactions with other abiotic stresses (Rauf, 2008). An alternative approach is to induce water stress through polyethylene glycol (PEG) solutions for screening of the germplasm (Nepo-muceno *et al.* 1998; Kulkarni and Deshpande, 2007; Khodarahmpour, 2011; Rajendran *et al.* 2011). Polyethylene glycol glycols with molecular mass of 6000 and above are non-ionic, water soluble polymers which are not expected to penetrate intact plant tissues rapidly. This solution interferes with the roots to absorb water due to reduction of osmotic potential (Dodd and Donovan, 1999; Sidari *et al*. 2008).

An artificially created water-stress environment is used to provide the opportunity in selecting superior genotypes out of a large population. On the basis of these grounds, experiment was carried out to categorize sorghum germplasm against drought stress; to select suitable accessions for drought tolerance and also to determine the suitability of various seedling traits for selection of tolerant or susceptible genotypes to drought stress.

MATERIALS AND METHODS

Twenty accessions of sorghum (*Sorghum bicolor* L.) were chosen on the basis of their morphological and agronomic diversity to formulate the representative core sample of the species. Ten seeds of each accession were sown at the depth of 1cm in earthen pots filled with sand and supplemented with Hoagland solution (20 ml pot^{-1}) as nutritive media. The experiment was laid out in a factorial completely randomized design (FCRD) with three replications and two factors. Two factors were genotypes and irrigation levels i.e. control (T_1) and stress (T_2). In control treatment, plants were irrigated daily with 50 ml of tap water per pot and created an osmotic potential of 0.0MPa and -1.03MPa created using a polyethylene glycol solution (PEG-6000, 80g/kg) as suggested by Michel and Kaufman (1973). Temperature was maintained by steam and electric heaters during day and night time at 25^oC. Plants were subjected to natural sunlight supplemented with artificial lightening to have photoperiod of 16 hours. Experiments were terminated after fourteen days of water stress. Five plants of each accession from each replication and treatment were evaluated for shoot length (**SL**), root length (**RL**), fresh shoot weight (**FSW**), dry shoot weight (**DSW**), fresh root weight (**FRW**), and dry root weight (**DRW**). Length based traits were measured with measuring tape after carefully uprooting the seedling and dissecting into roots and shoot. Fresh shoot or root weight was measured on digital analytical balance while dry shoot and root weight was measured by putting shoots and roots in kraft paper bags separately and dried in the oven at 70ºC for constant dry weight (Kaydan and Yagmur, 2008). The average dry shoot and root weight was then calculated.

Statistical analysis / biometrical approach: The recorded data were subjected to analysis of variance Steel *et al*. (1997). Then multivariate scoring index (Minitab software) was applied to find out the best accessions performing well under water stress for all the six seedling traits.

RESULTS

Mean squares from analysis of variance (Table 1) for all the seedling traits demonstrated considerable range and variability among 20 sorghum accessions under control and water stress conditions. The statistical analysis also showed highly significant differences among the treatments and interactions in sorghum (Table 1). Expression of 20 accessions of sorghum for various traits under water stress was erratic due to genetic variability. The expression of mean performance for all seedling traits **SL** (Fig. 1), **RL** (Fig. 2), **FSW** (Fig. 3), **DSW** (Fig. 4), **FRW** (Fig. 5) and **DRW** (Fig. 6) was decreased under osmotic stress simulated by PEG. The highest percentage reduction was noted for **DSW** (48.95 %;) followed by **SL** (41.95%), **FRW** (44.08%), **FSW** (38.65%), **DRW** (33.37%) and **RL** (17.05%) due to water stress (-1.03MPa). The results suggested that root length was least effected by drought stress compared with other seedling traits. However **FSW** was most affected by the drought stress as maximum reduction was observed in **FSW.**

The highest reduction in **SL** was observed in accession 80204 i.e., 57.65%; in **RL** was in 80121 i.e., 54.01%; in **FSW** in SS-97-7 i.e., 52.53%; in **DSW** in accession 80091 i.e., 55.88%; in **FRW** in accessions 80361 i.e., 59.16% and in **DRW** in accession 80361 i.e., 58.06%; The lowest reduction in **SL** was observed in accession 80205 i.e., 20.79%; in **RL** was in 80236 i.e., 1.09%; in **FSW** was in SS-98-5 i.e., 13.73%; in **DSW** was in accession 1728. i.e., 32.17%; in **FRW** was in accessions 80265 i.e., 28.96% and in **DRW** was in accession 1728 i.e., 12.44%. That differential performance of accessions indicated a great deal of variability in sorghum for drought tolerance. Significant interaction of accessions with treatments supported the differential behavior of various accessions under water stress (Table 1).

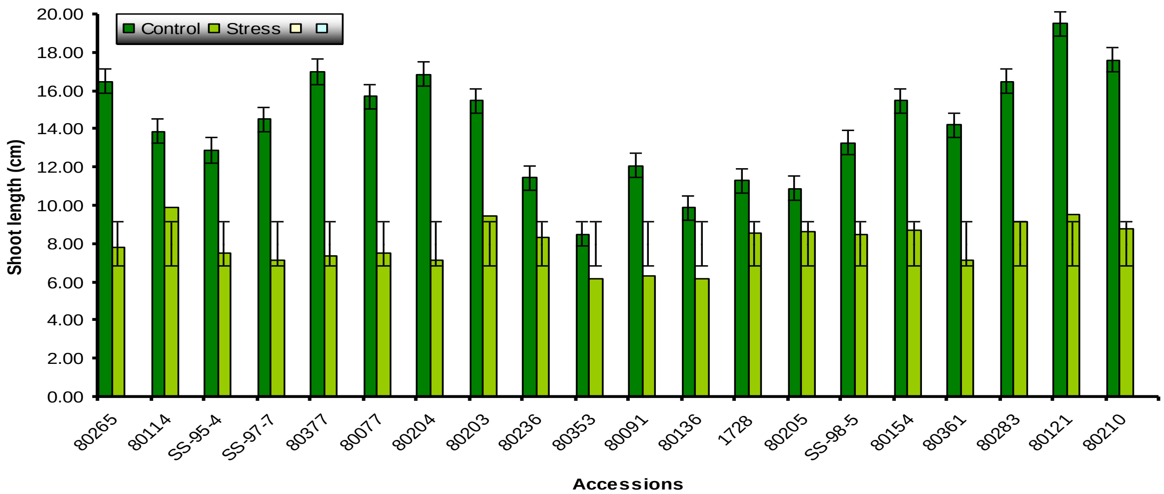
Multivariate scoring was carried out using principle components analysis (Fig. 7 and 8) using replicated data of shoot length and root length, fresh and dry shoot weight and fresh and dry root weight. Based upon principle component analysis proportional contribution of root length and shoot length, fresh and dry shoot weight and fresh and dry root weight was 56.6%, 20.2 %, 10.5%, 8.5%, 3.5% and 0.7% for drought tolerance (Fig. 8). Root length was the highest contributor towards drought stress, so it can be utilized as a selection parameter at seedling stage in sorghum.

Table 1. Mean squares of 20 Sorghum accessions for various seedling traits under control and water stress conditions.

Characters	Accessions (A)	Treatment (T) (water levels)	Interaction(A x T)	Error
D.F.	19	1	19	80
SL	18.67**	1148.74**	9.85**	0.02
RL	43.46**	4.47**	4.47**	0.05
FSW	0.03**	2.46**	0.31**	0.03
DSW	0.03**	0.11**	0.32**	0.14
FRW	0.03**	0.78**	0.01**	0.02
DRW	0.02**	0.32**	0.01**	0.33

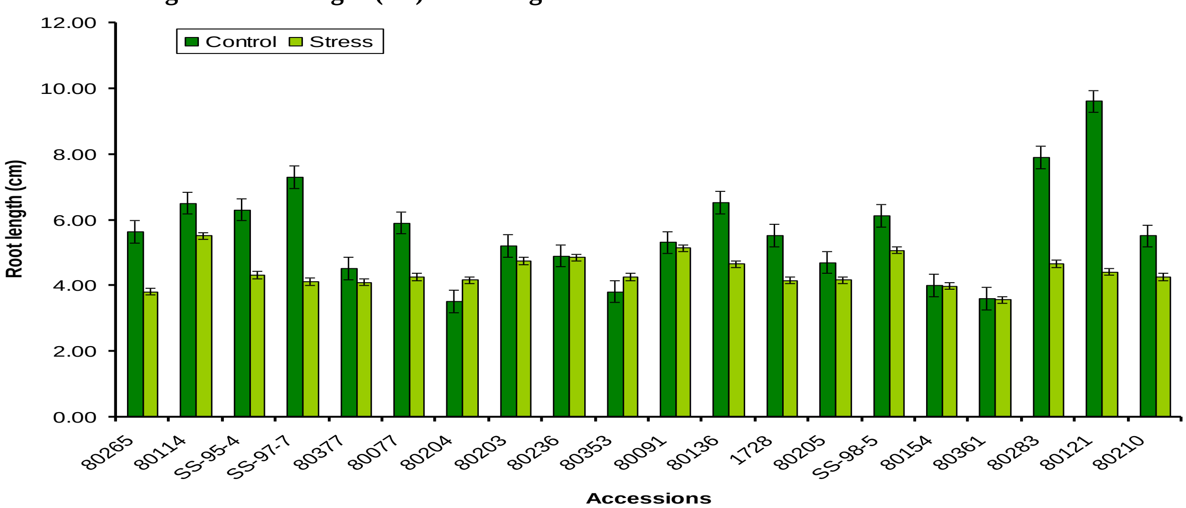
- *= Significant at 0.05% probability level Significant at 0.01% probability level*
- *D.F = Degree of freedom*

Abbreviations: Shoot length (SL), Root length (RL), Fresh Shoot Weight (FSW), Dry Shoot Weight (DSW), Fresh Root Weight (FRW), Dry Root Weight (DRW), PCA (Principle Component Analysis)



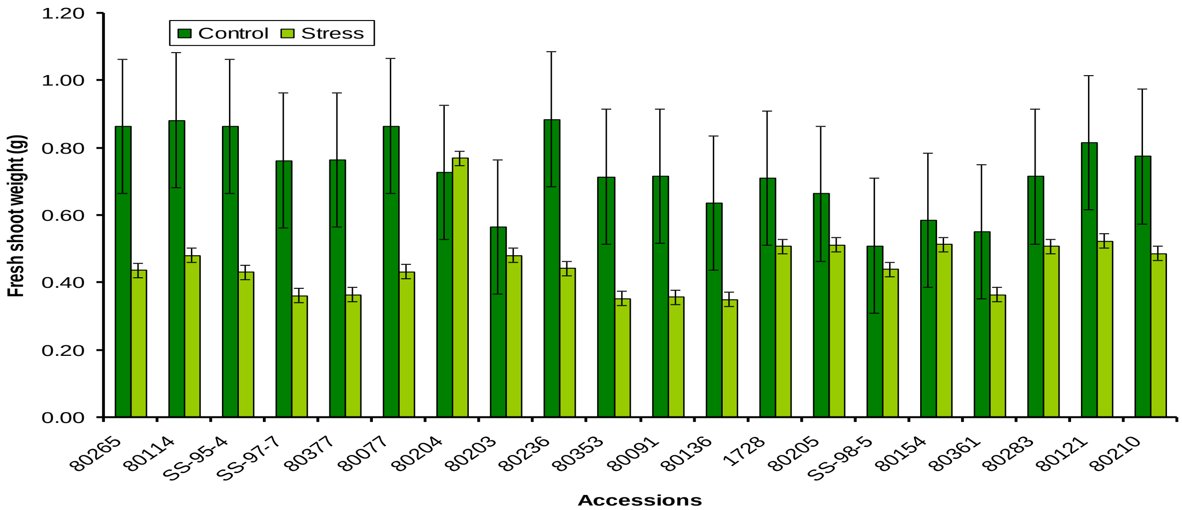
CD_1=0.031, CD2= 0.101

Fig. 1. Shoot length (SL) of 20 sorghum accessions under control and water stress.



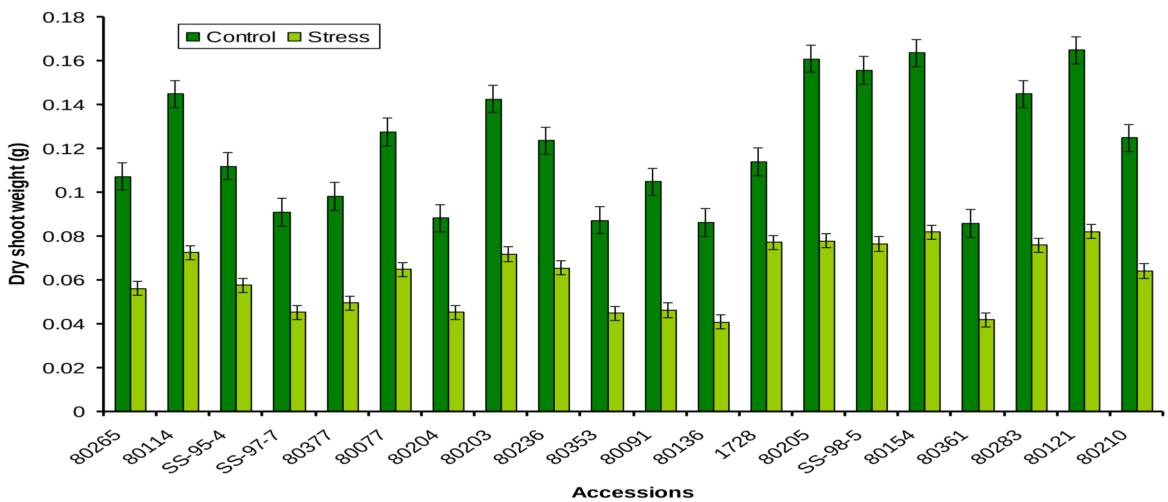
CD_1=0.02, CD2= 0.04

Fig. 2. Root length (RL) of 20 sorghum accessions under control and water stress.



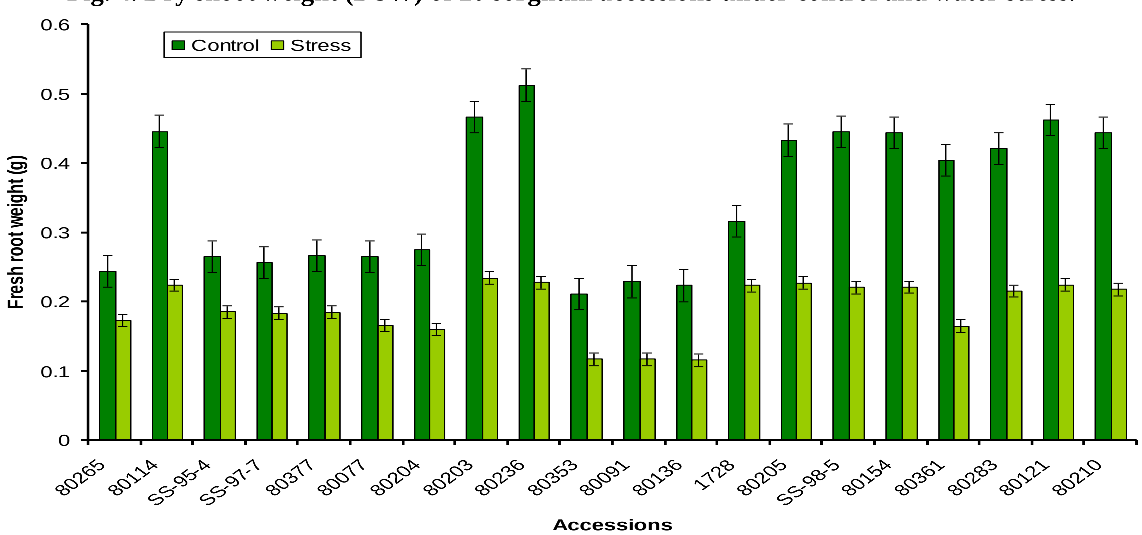
CD_1=0.0018, CD_2= 0.0023

Fig. 3. Fresh shoot weight (FSW) of 20 sorghum accessions under control and water stress



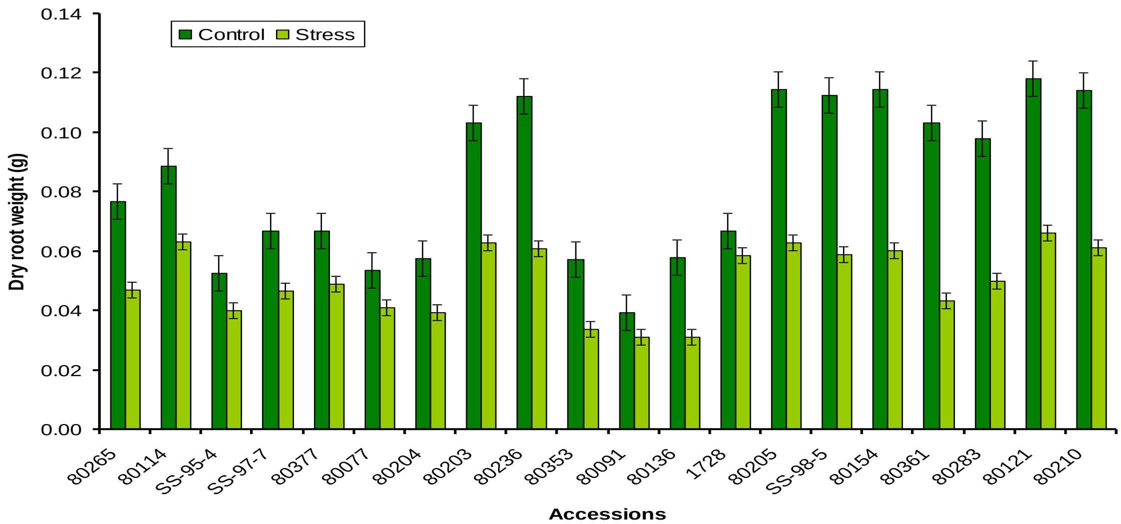
CD_1=0.0018, CD2= 0.0020

Fig. 4. Dry shoot weight (DSW) of 20 sorghum accessions under control and water stress.



CD1= 0.003, CD2=0.002

Fig. 5. Fresh root weight (FRW) of 20 sorghum accessions under control and water stress.



CD1= 0.0025, CD2=0.0032

Fig. 6. Dry root weight (DRW) of 20 sorghum accessions under control and water stress.

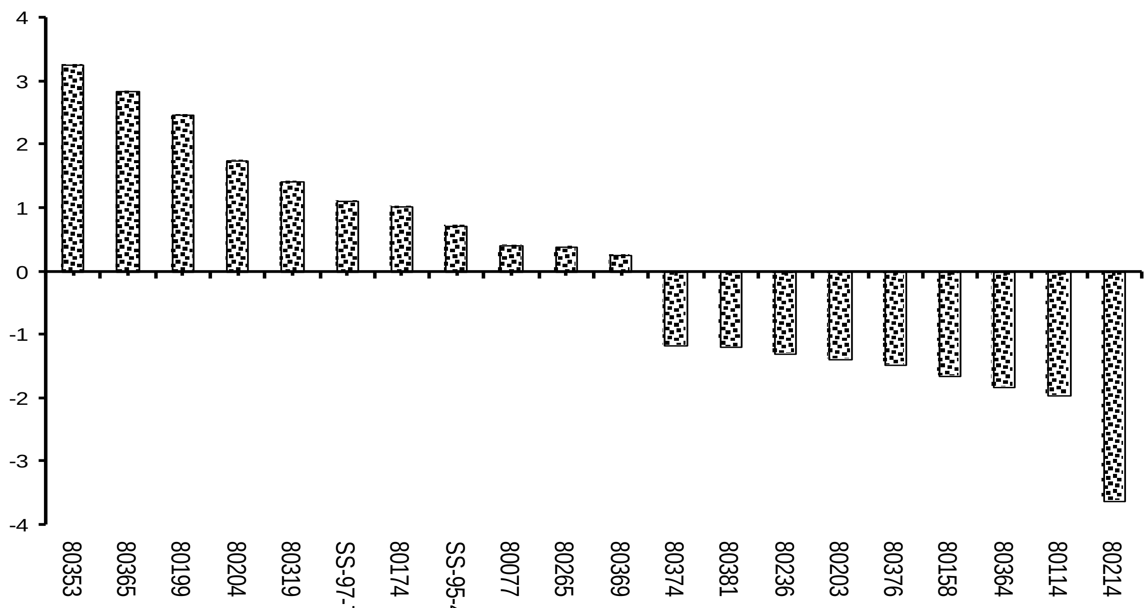


Fig. 7. Multiple scores over SL, RL, FSW, DSW, FRW and DRW in 20 sorghum accessions under control and water stress.

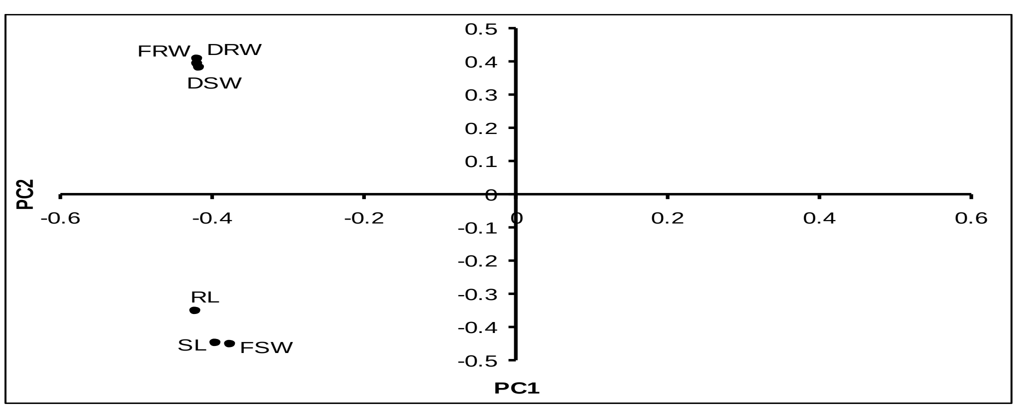


Fig. 8. PCA performed on SL, RL, FSW, DSW, FRW and DRW in 20 sorghum accessions under control and water stress.

DISCUSSION

Drought stress reduced the phenotypic expression of all the seedling traits SL, RL, FSW, DSW, FRW, and DRW as clear from figures (1-6) The results are in accordance of Meo, 2000, Bibi *et al.* 2010; Ali *et al.* 2011a). Bibi *et al*. 2010 observed that most of the morphological and physiological characters at seedling stage are affected by water stress in sorghum. Drought stress suppressed shoot growth more than root growth and in certain cases root growth increased (Salih *et al*. 1999; Younis *et al*. 2000; Okçu *et al*. 2005; Bibi *et al*. 2010). Reduction in seedling growth is the result of restricted cell division and enlargement, as drought stress directly reduces growth by deceasing cell division and elongation (Kramer, 1983). Roots are the place where plants first encounter water stress, it is likely that roots may be able to sense and respond to the stress condition (Xiong *et al*. 2006; Khodarahmpour, 2011). It plays an important role in water stress tolerance by reduction in leaf expansion and promotion of root growth. (Singh, 2009). Root length at seedling stage provides a fair estimate about the root growth in field (Ali *et al*., 2011a, b; Rajendran *et al*. 2011).

Reduction in shoot length perhaps due to less water absorption and decrease in external osmotic potential created by PEG (Kaydan and Yagmur, 2008) but higher root: shoot ratios than the susceptible lines, which may have been responsible for their higher leaf water-potentials in the stressed environments. It appears that vigorous shoot growth corresponds to vigorous root growth under water stress. Drought has drastically affected fresh shoot and root weight in some cultivars of sorghum, wheat, maize and sunflower.

Where only in one accession (80204) showed increase in fresh shoot weight under stress that may be attributed to the accumulation of organic and inorganic solutes and due to the higher growth because of osmotic adjustment. Under water stress conditions the increase in fresh root weight (80204 and 80353) could be attributed to the fact that roots become increased in search of water and may also be attributed to increased weight due to accumulation of different solutes. Moreover, a longer root is the characteristic of a drought tolerant variety as root growth is relatively less affected by water stress.

Fresh and dry root weight was also decreased due to water stress in sorghum. Similar results were reported by Shiralipour and West (1984). Dry and fresh weights of roots were decreased during the drought period as their leaf size remained small to minimize transpiration, ultimately plant dry weight also reduced. Dry root weight (**DRW**) has been utilized as a selection criterion for drought tolerance by many plant breeders. Water uptake by the root is a complex parameter that depends on root structure, root anatomy, and the pattern by which different parts of the root contribute to overall water transport (Cruz *et al*. 1992).

Multivariate scoring was carried out using principle components analysis (Fig. 7 and 8) using replicated data of **SL**, **RL**, **FSW**, **DSW**, **FRW** and **DRW**. Based upon principle component analysis proportional contribution of **RL, SL, FSW, DSW, FRW and DRW** was 56.6%, 20.2 %, 10.5%, 8.5%, 3.5% and 0.7% for drought tolerance (Fig. 8).

There were twenty accessions of sorghum and they were arranged on the basis of descending order of multivariate scoring. A review of the figure showed that in sorghum accessions 80353, 80365, 80199, 80204 and 80319 showed the highest multivariate scores. While the accessions 80214, 80114, 80364, 80158 and 80376 showed the lowest scores. Therefore, five accessions from upper side and five accessions from lower side were selected as drought tolerant genotypes (Fig. 7).

Multivariate analysis handles simultaneously a number of variables of common effects whereby similar data patterns being summarized, noise removed and the internal or some times hidden structures of the data being elucidated. Principal component analysis (PCA) is the most frequently used multivariate method. The 10 accessions (five each from upper & lower side of the graph) represent two distinct patterns or groups with differing responses to water stress could be advanced for further testing to drought tolerance. The results exhibited that accessions 80265, 80114, SS-95-4, SS-97-7 and 80377 are water stress tolerant among the accessions studied based on the seedling traits and can be further exploited in hybridization programme. Principle component analysis revealed that higher root length, shoot length with lower leaf water potential, osmotic potential and turgor pressure under water stress could be utilized as selection criteria for drought tolerance in sorghum at seedling stage. The most drought tolerant and susceptible genotypes might be used further in hybridization programme to create maximum genetic variability.

Conclusion: The present research work was conducted to evaluate the genetic potential of twenty sorghum genotypes through artificially created water stress by PEG of molecular weight 6000 in laboratory conditions followed by selection of genotypes based on easily measurable and inherited seedling traits contributing to drought tolerance. The genotypes 80353, 80365, 80199, 80204 and 80319 found superior and might be productive in further breeding programmes for drought tolerance. Selection can be made on the basis of these characters at early growth stage to screen a large population for drought stress. It would be cost effective, less time consuming and less laborious to screen the germplasm at early stage. So is suggested that the findings may be helpful and fruitful for selection of drought stress in sorghum under the discussed traits.

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