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COMPARISON OF 10 SORGHUM (*Sorghum bicolor* L) GENOTYPES UNDER VARIOUS WATER STRESS REGIMES

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ABSTRACT

Ten different accessions of sorghum (*Sorghum bicolor* L.) were evaluated for their ability to drought tolerance at seedling stage. Three water stress levels (50%, 75%, and 100%) were applied under controlled conditions. After twenty eight days of seedling emergence, data were recorded for seedling and physiological traits as shoot length (SL), root length (RL), shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), root dry weight (RDW), leaf area (LA), relative water contents (RWC), residual transpiration (RT), chlorophyll ‘a’ and Chlorophyll ‘b’ under control as well as two water stressed conditions. Significant differences were observed among the genotypes, treatments and their interactions for evaluated plant traits suggesting a great amount of variability for water stress tolerance in sorghum. However, shoot related traits were the most sensitive against the water stress. Stress tolerance indices of SL, RL, SDW, RWC, SFW and LA, RT, RFW, RDW, Chl ‘a’ Chl ‘b’ was 84%, 73 %, 61%, 59%, 56%, 43%, 43% 33%, 32%, 16% and 15%, respectively. Soot length (84%) was the highest towards drought susceptible, indicating shoot length was most affected by water stress among all the seedling traits. The most promising drought tolerant accession (NO.1749) and drought susceptible F-2007 and F-2008) were screened.

Key word: *Sorghum bicolor* L., Seedling traits, Physiological traits, Water stress.

INTRODUCTION

Sorghum has an extensive range of adaptability and can be cultivated in different kinds of environments. Mostly cultivated for feed, food and industrial uses. It can also be used in the biofuels industry. Sorghum is an ideal crop for a more concerned crop improvement program in agriculture to utilize marginal lands, to meet food and energy demands which might be increased in the near future (Bibi *et al.* 2012). Sorghum has potential to compete with many types of stresses, including high temperature stress, water stress, salts stress and over irrigation (Ejeta and Knoll, 2007). Abiotic stresses 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. Under water deficit conditions, plants urgently need available water in root zone, and tolerant genotypes will extract water from deep layers of soil (Xiong *et al.* 2006). Generally, it has been observed that drought tolerant crop species has longer roots with more root density (Achakzi (2009); Kaydan and Yagmur, 2008). Dhanda *et al*. (2004) reported that the decrease in water availability affects the crop production at different growth stages but generally resulted in decreased coleoptile length, higher roo:shoot ratio and longer roots. 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 (Balota *et al.* (2008) Wajid *et al.* 2011), sorghum (Bibi *et al,* 2010, Ali *et al*. (2011), Achakzai (2011) maize (Hajibabaee *et al.* (2012), Qayyum *et al*. 2012).

The objective of presented research work was to make a comparison of different elite lines of sorghum under various water stress treatments and to identify the physiological markers attributable to drought stress.

MATERIALS AND METHODS

Ten genotypes were sown in the greenhouse of Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan during the growing season of 2011-2012 to find out the effect of water stress on various morphological and physiological characters of sorghum. Mean Minimum and maximum temperature in green house was maintained by steam and electric heaters during day and night time at 25-32ºC respectively. At the day time, plants were subjected to natural sunlight supplemented and at night with artificial lightening to have photoperiod of 16 hours. All the experimental plants were sown in complete randomized design (CRD) with three replications. Field saturation percentage/field capacity was determined before sowing. Agromoterological data of Year 2013 has been given in Figure 12. The three water regimes were 100% (normal), 75% and 50 % according to saturation percentage. The 10 seeds of each genotype were sown in plastic pots filled with sand and soil in equal proportion.

The genetic material used in research experiment was collected from Fodder Research Substation, Ayub Agricultural Research Institute, Faisalabad, Pakistan; F.C-26-I, NO.1749, F.S.9902, F-2007, AK-113, NOOR, F-2-2007, F-2008, M.R-SORG-2011,F-114. After four weeks of germination, the shoot length, root length, fresh shoot weight, dry shoot weight, fresh root weight, dry root weight, chlorophyll a andchlorophyll b contents, leaf area, relative water content and residual transpiration were recorded.

Shoot length (cm): The shoot length was measured from emergence of plant up to the plant tip with the help of measuring tape. The average data was calculated and recorded.

Root length (cm): After harvesting plants roots were washed and the total length of primary roots was measured with the help of measuring tape and recorded.

Shoot Fresh Weight (g): After harvesting the plants, shoots was separated from the roots and fresh weight was recorded.

Shoot Dry Weight (g): Shoots were placed in paper bags, these were air-dried first and then oven dried at 65 + 5^0C till constant weight. After that the dry weight was recorded with electrical weighing balance. (Kaydan and Yagmur, 2008).

Fresh Root weight (g): Root samples were washed and weighed with the electrical weighing balance and recorded the fresh weight.

Dry Root weight (g): Roots were placed in paper bags, these were air-dried first and then oven dried at 65 +5till constant weight. After that the dry weight was recorded with electrical weighing balance.

Relative water content (%): After 28 days of sowing, leaf samples were collected from the selected plants. Samples were covered with polythene bag after excision. Fresh weight was recorded by using electronic balance in the laboratory. Turgid leaf weight was recorded after keeping the leaf samples in water for overnight. The leaf dry weight was calculated after oven drying at 70°C. Relative water content was calculated using the formula proposed by Ritchie et al. (1990). RWC (%)= FW DW/TW-DW ×100.
While,
RWC= Relative water contents FW= Fresh weight (leaf)
DW= Dry weight TD= Turgid weight

Residual transpiration: The “residual transpiration” (RT, the rate of water transpired at minimum stomatal aperture in total water limitation) was measured according to Clarke *et al*. (1991). The leaves were excised and immediately brought to the laboratory. Then the leaves remained in the darkness for stomatal closure for half an hour under ambient room conditions. Leaves were weighed for two times; (W_1 after half an hour of stomatal closure) and after 180 minutes (W_2 in g); the leaf area (LA in cm^2) was also determined using leaf area meter (LI-3000/Lambda Instr. Corp. Lincolin, Nebraska, USA). Residual transpiration on leaf area basis (g $H_2O/min/cm^2/105$) was determined as given below:

$$RT = (W_1 – W_2)/ (LA \times 180)$$

Chlorophyll a and Chlorophyll b contents (%): Leaves chlorophyll a contents were measured in percentage in the laboratory. The amount of chlorophyll ‘a’ was calculated according to Arnon's equation (1949).

Chl a (mg/g) = (12.7 Abs663) - (2.6 Abs645) ml Aseton)/mg leaf tissue

Similarly, Chlorophyll a contents plants were treated with chemical and data of chlorophyll b contents was measured in laboratory. Chlorophyll b was calculated according to Arnon's equation (1949).

Chl b (mg/g) = ((22.9 Abs645) - (4.68 Abs663) ml Aseton) / mg leaf tissue

Statistical Analysis

Analysis of variance and covariance: The methodology given by Steel *et al.* (1997) was used for statistical analysis to compute variance and covariance from the data collected for the traits to ascertain the difference among various genotypes for variability and co-variability. Total variance was partitioned into genotypic and phenotypic components

RESULTS

Analysis of variance described highly significant differences among 10 genotypes of sorghum for all the traits under normal and two water stressed levels. Analysis of variance also described the significant interaction of both factors; genotypes and water stress. Variances of all genotypes showed the different responses against particular treatment of each water stress. Least Significant DifferenceTest (LSD) was used to calculate the means which showed significant differences among genotypes for all traits. For genotypes comparison, means of studied parameters were calculated and compared. Mean squares from analysis of variance (Table 1) for all the seedling traits demonstrated considerable range and variability among 10 sorghum genotypes under control and water stress conditions. The statistical analysis also showed highly significant differences among all the treatments and interactions in sorghum (Table 1). Expression of 10 genotypes of sorghum for various traits under water stress was variable due to genetic variability. The expression of mean performance for all seedling traits SL (Fig. 1), RL (Fig. 2), SFW (Fig. 3), SDW(Fig. 4), RFW (Fig. 5) and RDW (Fig. 6), LA (Fig.7), RWC (Fig.8), RT(Fig. 9), Chl. 'a' (Fig. 10), Chl.' b' (Fig. 11) was decreased under water stress. At 50% water stress the highest percentage reduction was noted for traits SL (84%) followed by RL (73%), SDW (61%), RWC (59%), SFW (56%), LA (43%), RT(43%), RFW (33%), RDW (32%), Chl. 'a' (16%) and Chl. 'b' (15%)at 50% water stress compare to saturation percentage/field capacity.While, at 75% water stress compare to 100% saturation percentage/field capacity the highest percentage reduction was noted for RL (98%) followed by SL (91%), RFW (80%), SFW (76%), LA (71%), RT (59),RWC (59%), SDW (55%), RDW (48%), Chl. 'a' (36%) and Chl. 'b' (32%). The results suggested that chlorophyll b was least effected by drought stress compared with other seedling traits.

F-2008 was found to be most drought susceptible as among all genotypes maximum reduction in SL and RL had been observed in it, at both 50% and 75% water stress applied; for RWC, FSW & RT highest decrease in phenotypic expression was in F-2007 respectively again at both water stress regimes. Chl. a and Chl. b was found to be least effected by drought stress in NO.1749 under various water stress regimes. 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).

DISCUSSION

Analysis of variances had key role in screening of genotypes and also for future breeding programs (Dhanda *et al*. (2004) and Khan *et al.* (2004). They also found significant variation among seedling traits in wheat, maize, sorghum and pearl millet.

Drought stress reduced the phenotypic expression of all the seedling traits starting from germination percentage to other growth parameters as shoot length, number of leaves, leaf area, and total dry matter including both fresh weight and dry weight of the plants till tips of the root systems. All the rapidly growing divisions in different organs are strictly effected by drought stress. The same results have been observed in the present research work where all seedling paramerts got negatively affected by water deficit. And the severity of water deficit stress was more in 50% water applied as compare to 75% water stress. The results verified the previous findings as (Meo, 2000, Bibi *et al.* 2010; Ali *et al.* 2011). Bibi *et al*. 2010 observed that most of the morphological and physiological characters at seedling stage were 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; 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 first 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.Water uptake by the root is a complex parameter that depends on root structure, root anatomy, and the pattern.Root length at seedling stage provides a fair estimate about the root growth in field (Ali *et al*., 2011; Rajendran *et al*. 2011).

Fresh and dry root weight was also decreased due to water stress in sorghum and many other crop plants. Similar results were reported by Shiralipour and West (1984). Dry and fresh root weights were decreased during the drought period as their leaf size remained small to minimize transpiration, ultimately plant dry and fresh weight also reduced. Dry root weight (DRW) has been utilized as a selection criterion for drought tolerance by many plant breeders. A reason for decrease in chlorophyll contents as drought or any other abiotic stress by producing reactive oxygen species (ROS) such as O_2- and H_2O_2, can lead to lipid peroxidation and consequently, chlorophyll destruction (Foyer *et al*., 1994). Also, with decreasing chlorophyll contents due to the changing green color of the leaf into yellow, the reflectance of the incident radiation increased (Schlemmer *et al.,* 2005). Decrease in RWC in plants under drought stress may depend on plant vigor reduction and have been observed in many plants (Liu *et al.,* 2002). Lonbani and Arzani (2009) and Bibi*et al.* (2010) found that leaf area was decreased during the drought period as cell division process slow down and their leaf size remained small to minimize the evapotranspiration loss. Ultimately plant leaf area remains constant and not grow with the time during drought stress. There were ten genotypes of sorghum and they were arranged on the basis of descending order of percentage decrease under highly water stressed condition. A review of the figure (1-11) showed shoot length was most sensitive character to drought stress (as 84% reduction) While chlorophyll a and b was least effected at both water stress treatments. So the genotypes having more chlorophyll contents under water stress can be screened for utilization in drought breeding programs.

Table 1. Combine mean square values of analysis of variance (ANOVA) of sorghum seedling traits under water stress.

S.O.V	D.F	SL	RL	SFW	SDW	RFW	RDW	LA	RWC	RT	Chl.a	Chl.b
Drought	2	137.2**	110.23**	0.0513**	0.0089**	0.152**	0.013**	443**	4748**	0.003**	70.126**	18.444**
Genotype	9	56.4**	13.75**	0.005**	0.0003**	4.493**	1.21**	4.7**	23.7**	4.419**	0.0261**	0.013**
DxG	18	0.24*	2.27**	0.00005**	0.00002**	7.422**	9.181**	1.1**	1.49N.S	3.122*	0.0011**	0.0035**
Error	60	0.12	0.035	0.00002	0.00001	3.956	2.133	0.02	1.12	1.411	0.0004	0.0007
Total	89											

* = Significant at 5% probability level
SL = Shoot Length
RL = Root Length
SFW = Shoot Fresh Weight
SDW = Shoot Dry Weight
RFW = Root Fresh Weight

** = Significant at 1% probability level
RDW = Root Dry Weight
LA = Leaf Area
Chl. a = Chlorophyll a
Chl. b = Chlorophyll b
RT = Residual trans

RWC = Relative water content

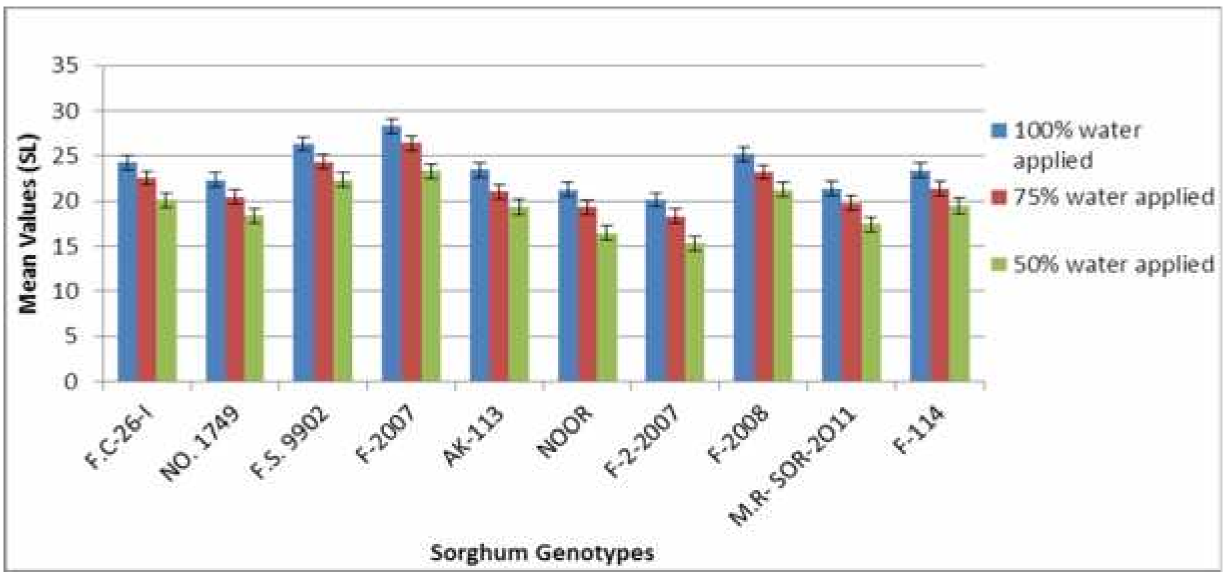

Figure 1: Comparison of varietal means in sorghum for shoot length under various water stress levels.

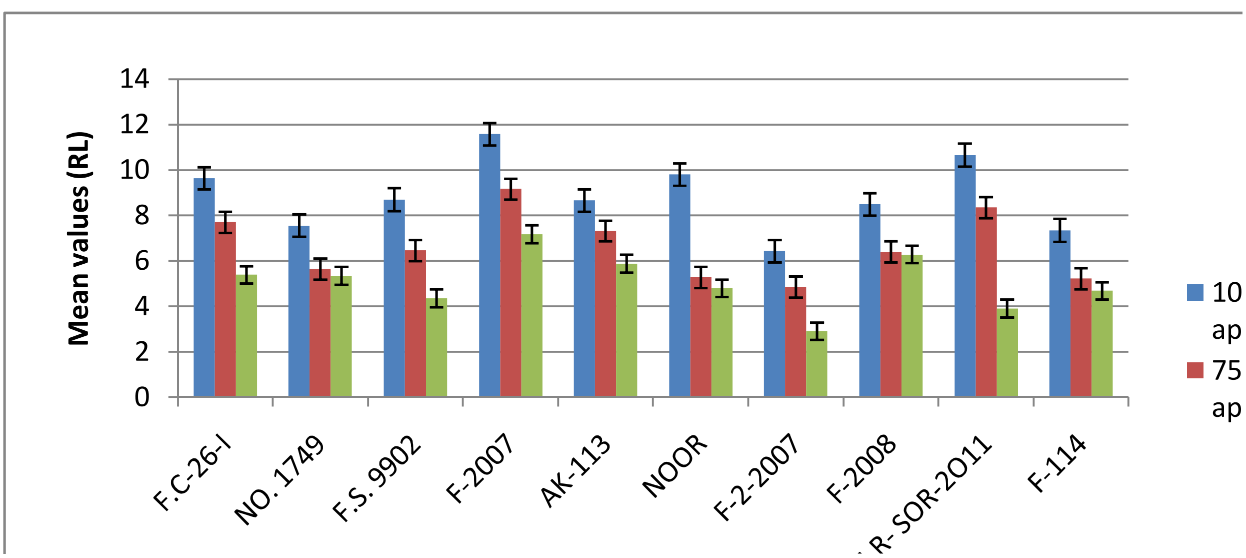

Figure 2: Comparison of varietal means in sorghum for root length under various water stress levels.

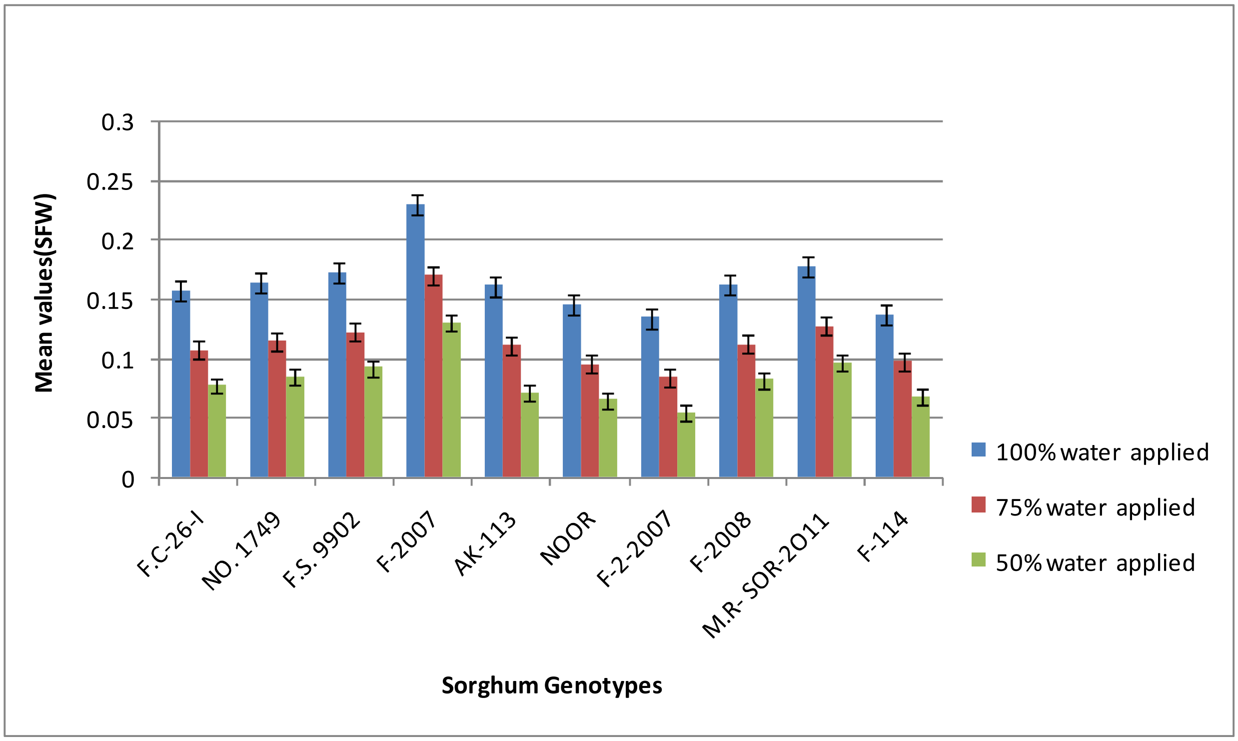

Figure 3: Comparison of varietal means in sorghum for Shoot Fresh Weight under various water stress levels.

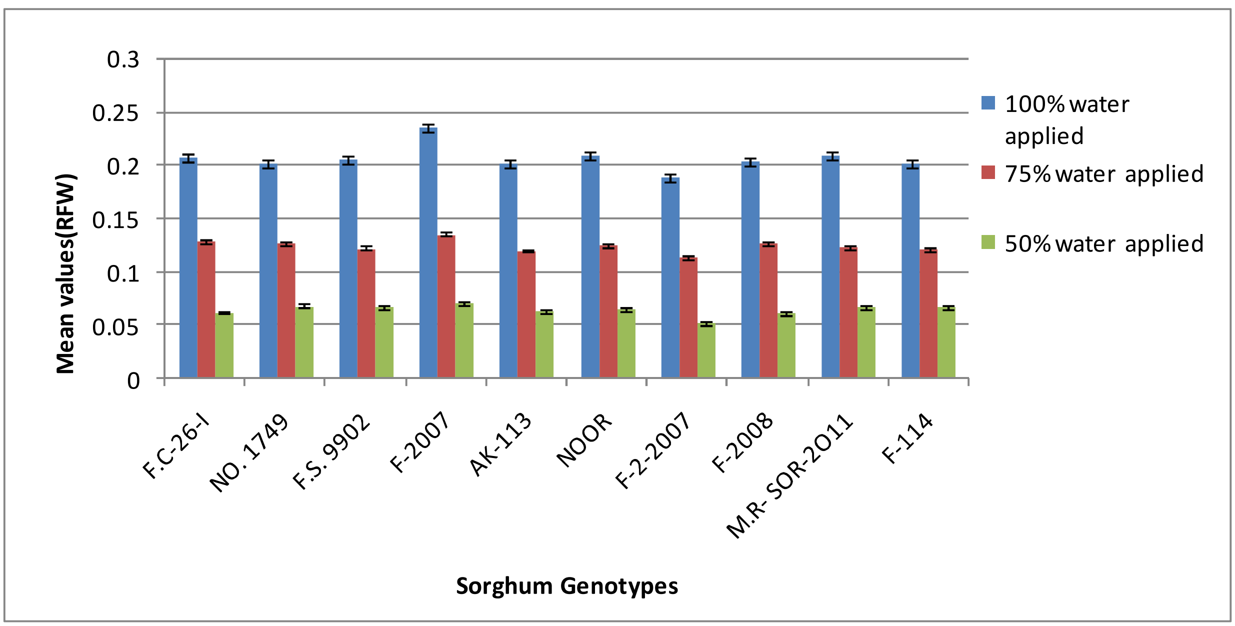

Figure 4: Comparison of varietal means in sorghum for Root Fresh Weight under various water stress levels.

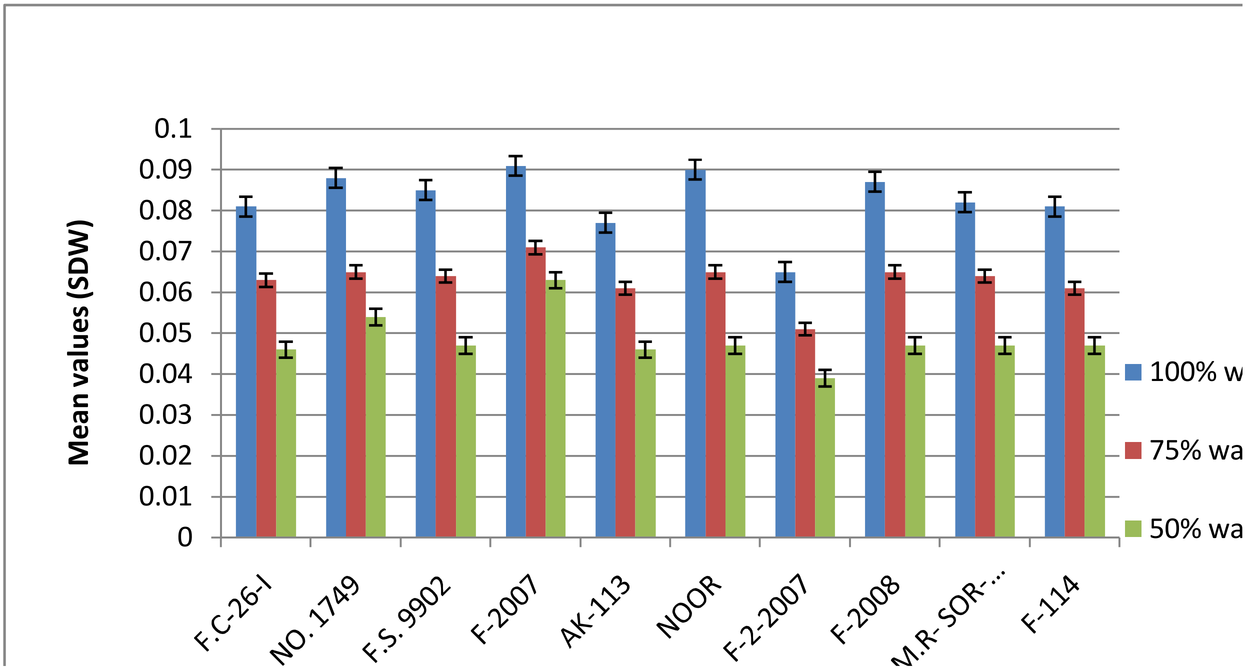

Figure 5: Comparison of varietal means in sorghum for Shoot dry weight under various water stress levels.

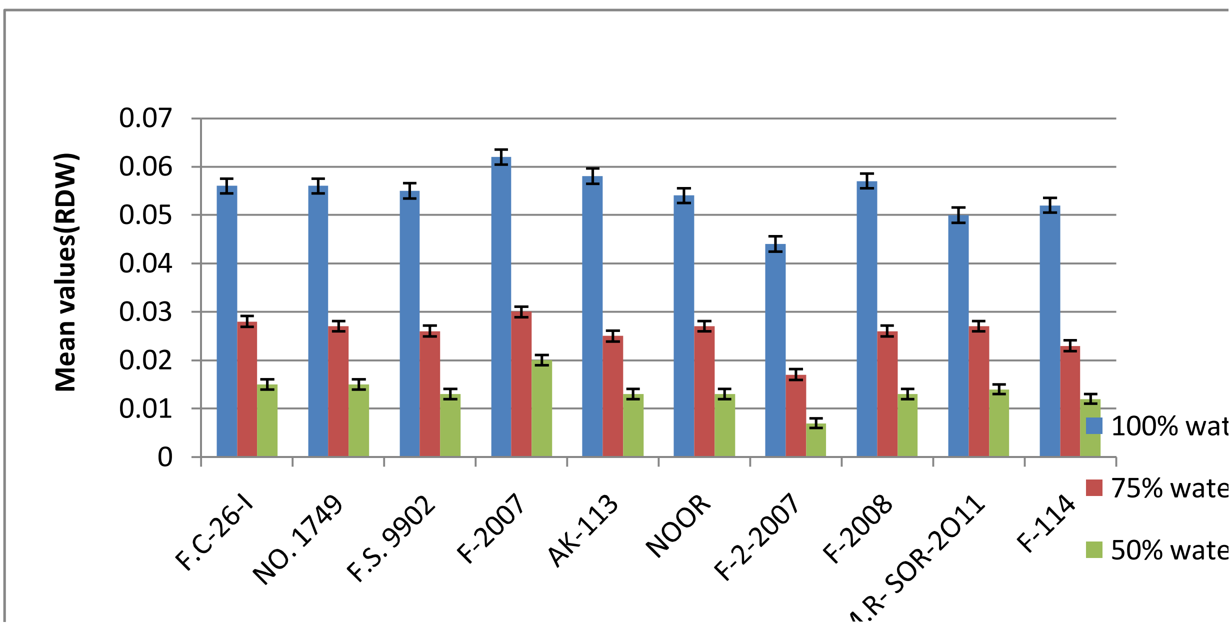

Figure 6: Comparison of varietal means in sorghum for Root dry weight under various water stress levels.

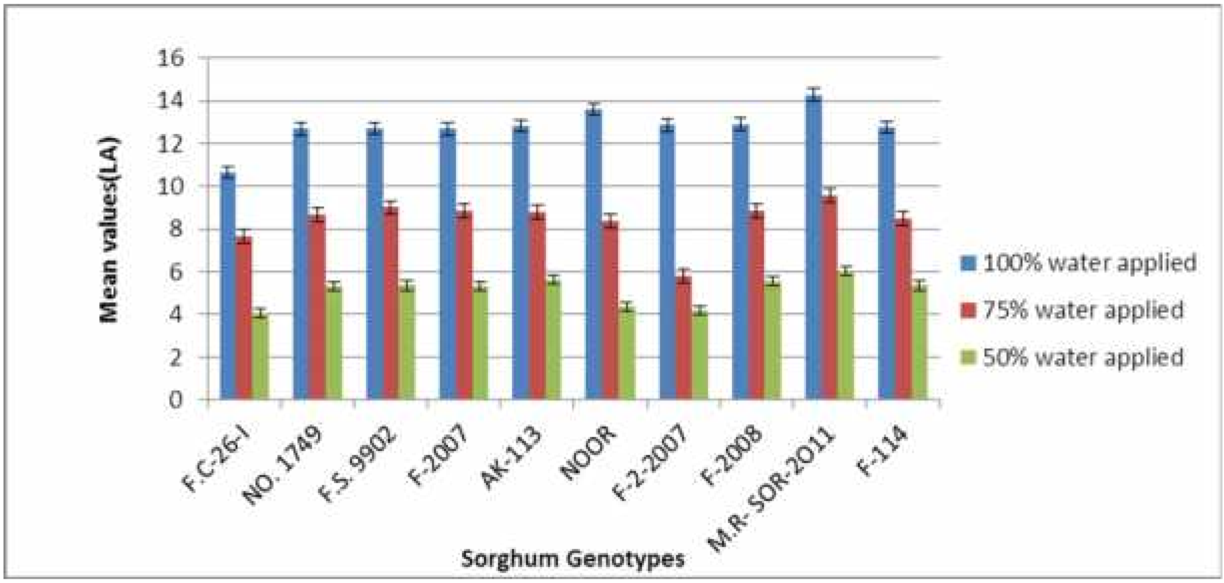

Figure 7: Comparison of varietal means in sorghum for leaf area under various water stress

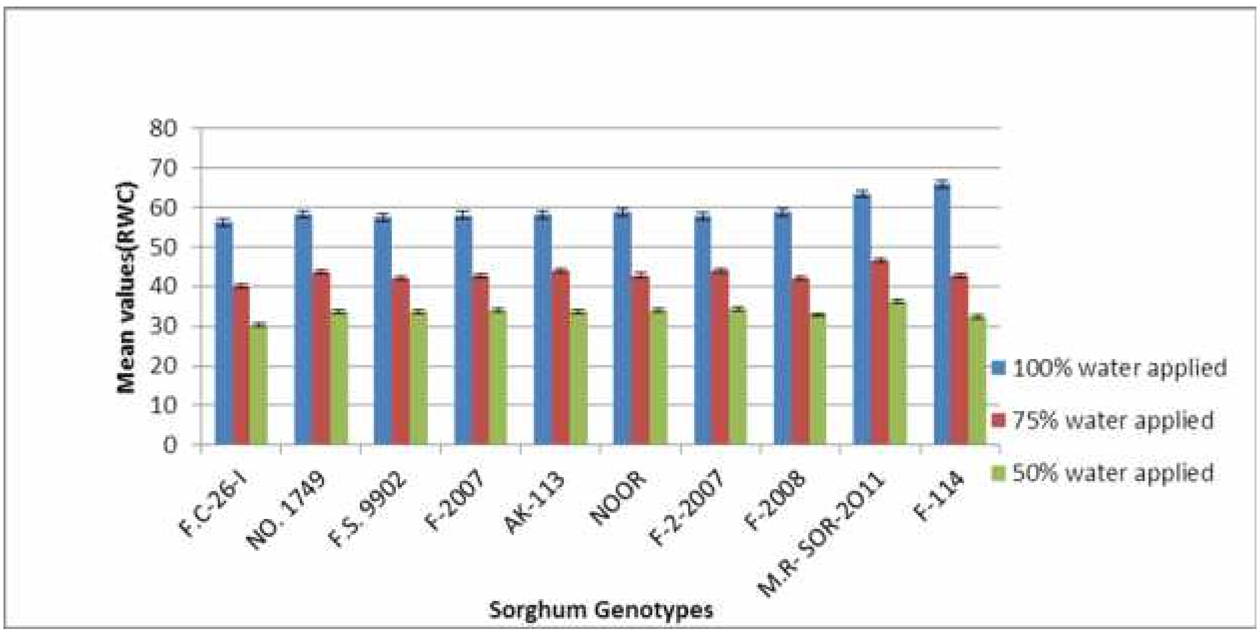

Figure 8: Comparison of varietal means in sorghum for Relative Water Contents under various water stress levels.

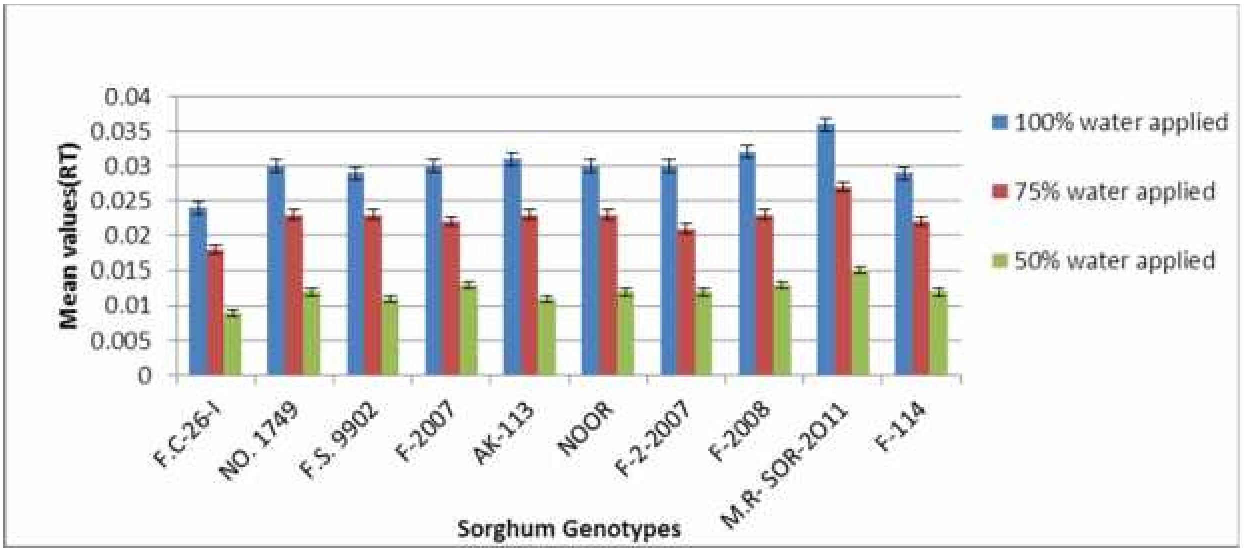

Figure 9: Comparison of varietal means in sorghum for residual transpiration under various water stress levels.

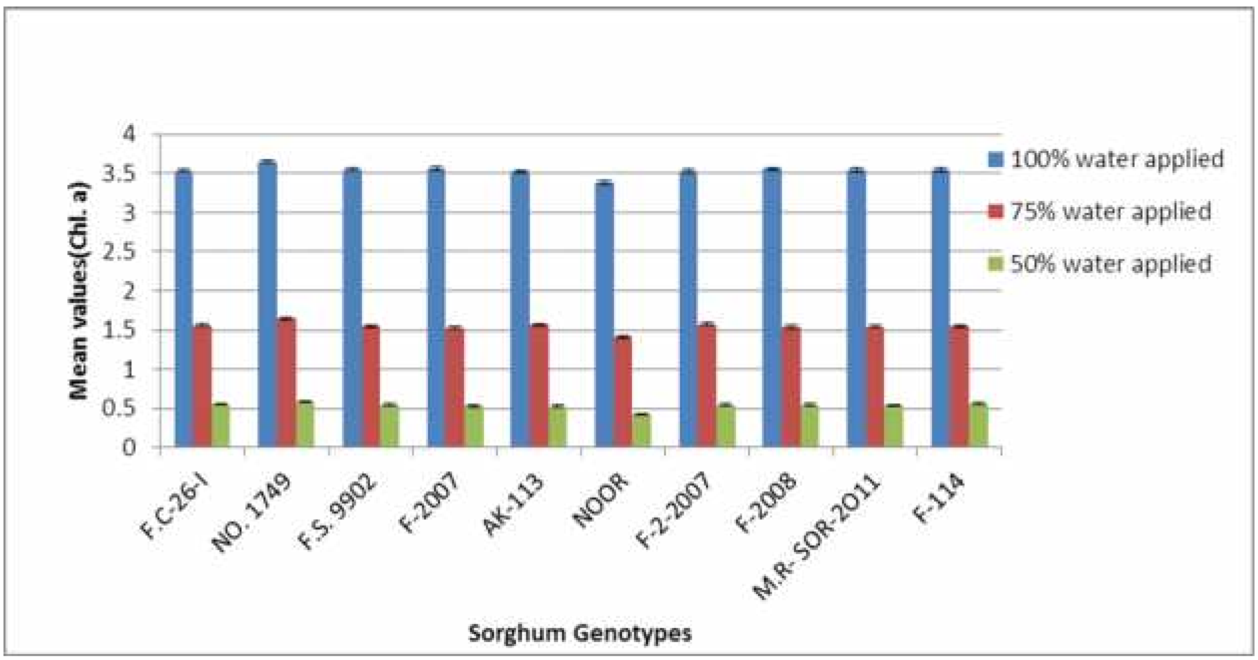

Figure 10: Comparison of varietal means in sorghum for chlorophyll a under various water stress levels.

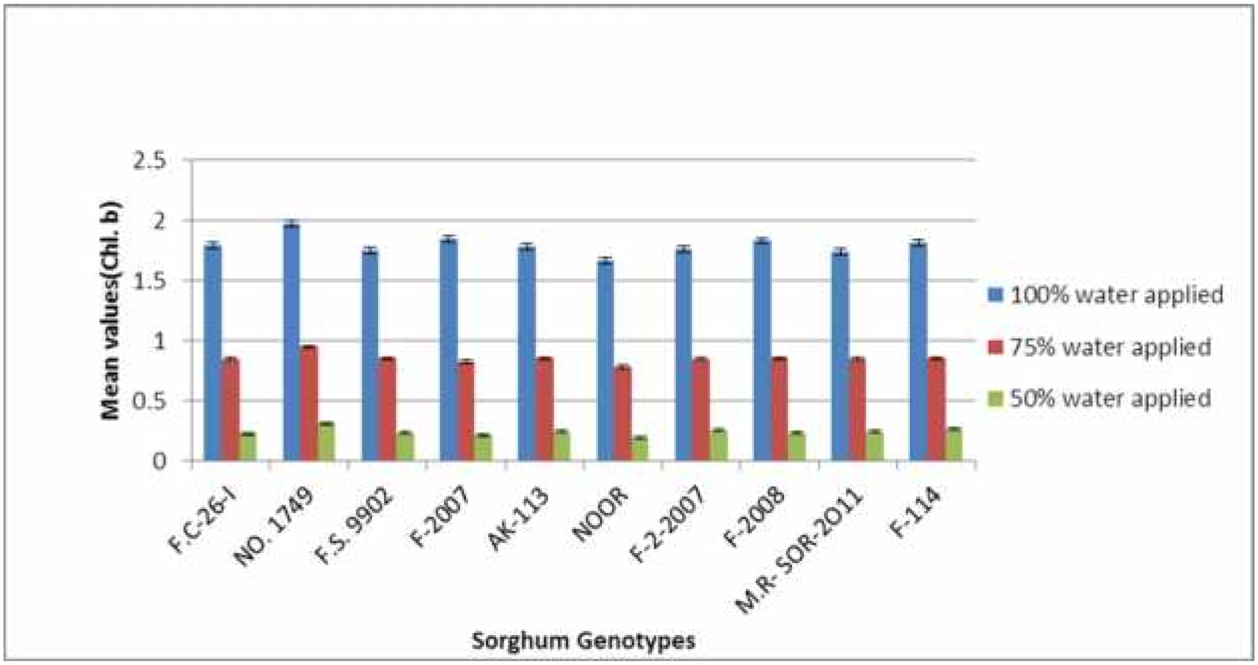

Figure 11: Comparison of varietal means in sorghum for chlorophyll b under various water stress levels.

Conclusion: The genotypes F-2007, F-2008 was found to be most drought susceptible while NO.1749, found superior against water stress and might be productive in further breeding programs for drought tolerance. Selection can be made on the basis of these characters at early growth stages 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.

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