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Genetic diversity and assessment of drought tolerant sorghum landraces based on morph-physiological traits at different growth stages

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Abstract

Root morphology and leaf water relations are important morph-physiological traits for screening various crop plants under water stress. In this study these morph-physiological traits were measured to sort out drought tolerant local landraces (LLRs) of sorghum. The results revealed that the landrace FJSS-1 performed the best for most of the characters followed by FJSS-11 and FJSS-17 which also performed well for various traits contributing towards water stress tolerance at seedling and post-flowering stages. The landrace, FJSS-10 revealed the contrasting parents showing drought susceptibility. Cluster analysis clearly divided the LLRs in two groups out of which Cluster I displayed more scope for selection against water stress. Dry root weight exhibited the highest genotypic coefficient of variation among seedling traits while excise leaf weight loss among the flag leaf related characters. Considerable degree of variation among the LLRs for most of the traits proposed these genotypes as significant source for the selection of water stress tolerance. In the same way, higher amount of heritability and genetic advance for the most of the morph-physiological parameters advocated that elevated amount of genetic gain for these parameters might be possible followed by hybridization. Our results suggest that these morpho-physiological traits could be efficiently used as selection criteria for drought tolerance in sorghum at different growth stages.

Key words: Morph-physiological traits, drought tolerance, seedling, flag leaf, sorghum landraces

Abbreviations: LLRs-Local landraces; FJSS-Fateh Jang Sorghum Selection; FRW-fresh root weight; FSW-Fresh; shoot weight; DRW-Dry root weight; DSW-Dry shoot weight; RL-Root length; SL-Shoot length; CL-Coleoptile length; R/S ratio-Root/shoot ratio; FLA-Flag leaf area; SFLW-Specific flag leaf weigh; SFLA-Specific flag leaf area; LDM-Leaf dry matter; ELWL-Excise leaf weight loss; RDW-Relative dry weight; RWC-Relative water content; RT-Residual transpiration; CMS-Cell membrane stability; GY-Grain yield per plant

Introduction

Sorghum (*Sorghum bicolor* L.), an important crop in many parts of the world is grown for food, feed and industrial purposes. It is also a major crop in many parts of Africa and some Asian countries. Compared to other cereals, sorghum is more tolerant to many stresses, including heat, drought, salinity and flooding (Ejeta and Knoll, 2007). However, this crop usually grown in arid and semi-arid regions is affected by drought at the reproductive stage especially during and post-flowering stage (Tuinstra *et al*., 1997; Kebede *et al*., 2001).

Water deficit is one of the most severe stresses faced by the sustainable crop productivity all over the world (Bot et al., 2000). Worldwide, yield losses each year due to drought are estimated to be around USD 500 million (Sharma and Lavanya 2002). Drought is a complex phenomenon, and is considered one of the most important factors limiting crop yields around the world (Beltrano and Ronco, 2008) and continues to be a challenge to agricultural scientists in general and plant breeders in particular, despite many decades of research. Breeding based on the plant characters associated with drought tolerance has been very popular. Drought tolerance is a function of various morphological (early leaf emergence, flowering and maturity, reduced leaf area, leaf rolling, wax content, coleoptile length, awns, stability in yield, stomatal density, reduced tillering, root characteristics and cell membrane stability), physiological (low transpiration rate, high water-use efficiency, stomatal conductance, osmotic adjustment, relative water content (RWC) and leaf turgor) and biochemical (accumulation of proline, polyamine, trehalose, etc., increased nitrate reductase activity and increased storage of carbohydrate) characters (Mitra, 2001). Drought response in sorghum has been classified into two distinct stages, pre-flowering and post-flowering (Tuinstra *et al*., 1997). A severe drought stress during post-flowering stages like anthesis or post anthesis causes loss of chlorophyll, cell electrolyte leakage, flag leaf yellowing and grain pre-maturation (Beltrano *et al*., 1999; Beltrano and Ronco, 2008).

In past, different morpho-physiological traits have been potentially utilized for screening genotypes of different crops under water stress conditions. These include seedling traits like shoot weight, root weight, root and shoot lengths, root:shoot ratio and coleoptile length at seedling stage (Sharp and Davies, 1979, Passioura, 1983; Turner, 1986; Ludlow and Muchow, 1990; Zekri, 1991; Takele, 2000; Matsui and Singh 2003; Dhanda *et al*., 2004; Kashiwagi *et al*., 2004; Pathan *et al*., 2004; Taiz and Zeiger 2006). Physiological traits such as the prevention of fatal relative water content and high cell membrane stability are well-defined component of adaptation to water deficit in sorghum (Sullivan and Ross, 1979; Premachandra *et al*., 1989). Water deficit also results in severe changes in cell membrane properties including selective permeability, stability, fluidity and microviscosity (Beltrano *et al*., 1994; 1999). Some other leaf hydraulics related parameters such as flag leaf area (Ahmad *et al*., 2004), relative dry weight (Wilson *et al*., 1980; Jones *et al*., 1980), excise leaf weight loss (Clarke 1987; McCaig and Romogosa 1991) and residual transpiration (Clarke at al., 1991; Balota, 1995) have gained utmost importance for breeding programs aimed at increasing drought tolerance in crop plants.

However, success for breeding under stress condition is limited (Hollington and Steele, 2007) but an understanding of genetic basis of drought tolerance in crop plants based on various morpho-physiological traits is also a pre-requisite for a geneticist to evolve superior genotype through either conventional breeding methodology or genetic engineering (Mitra, 2001, Chen et al., 2004). Therefore identification and analysis of plant traits with sound and positive association with drought tolerance and high productivity under drought is necessary (Richards, 2004; Rauf and Sadaqat, 2008). Similarly, the local landraces (LLRs), which are still the backbone of agricultural production in many developing countries, are well adapted in stress environments and farmers prefer landraces due to their ability to produce some yield even in difficult conditions where modern cultivars are less reliable (Brush, 1999).

In this study local landraces of sorghum were collected from farmer's field grown year after year by the farmers. The objectives of this research were: (i) to evaluate their performance for seedling traits at seedling stage and plant water relations under water stress at anthesis stage and ultimately grain yield, (ii) to estimate genetic heritable variation for these parameters and (iii) to assess correlated response of seedling and physiological traits among them and with grain yield. The ultimate goal of the study was to establish possible selection criteria for sorghum helpful for combating water stress.

Material and Methods

Plant material

The experiment used plots at the Barani Agricultural Research Station, Fatehjang (33º34´ N, 72º38´ E), Pakistan during the year 2007 and 2008. Seventeen sorghum genotypes/ local land races FJSS-1, FJSS-2, FJSS-3, FJSS-4, FJSS-5, FJSS-6, FJSS-7, FJSS-8, FJSS-9, FJSS-10, FJSS-11, FJSS-15, FJSS-16, FJSS-17, FJSS-20 and FJSS-21 including one approved variety Chakwal sorghum (developed at Barani Agricultural Research Institute, Chakwal, Pakistan) were evaluated for drought tolerance at seedling and post-flowering stage under rainfed environment and the average of the two years data was taken. The local landraces were collected during 2001 from farmer fields and pure lines were developed by inbreeding of these landraces after consecutive five years.

Seedling traits

Water deficit condition at seedling stage was achieved by watering the plants with quantity of water 50% of normal condition (Khan *et al*. 2004). Ten seeds per genotype were grown in iron trays (20 cm ×20 cm with 10 cm depth) filled with river sand

Table 1. Performance and statistical differences among sorghum LLRs for seedling traits under drought stress

	FRW (g)	FSW (g)	DRW (g)	DSW (g)	RL (cm)	SL (cm)	CL (cm)	R/S ratio	GY (g)
Chakwal sorghum	0.421 bc	3.42defg	0.040 cde	0.617 bcd	3.56 bc	21.09 a	1.64 cdefg	0.17 ef	25.89 a
FJSS-1	0.620 a	5.76 a	0.040 cde	0.910 a	3.02 de	18.41 abcd	2.01 bc	0.16 efg	23.85 ab
FJSS-10	0.280 defgh	5.14ab	0.013 e	0.643 bc	2.89 de	15.60 ed	1.32 fg	0.19 de	12.47 ef
FJSS-11	0.303 cdef	2.57 fgh	0.100 a	0.470 fgh	4.82 a	18.40 abcd	1.76 cdef	0.26 a	8.93 fg
FJSS-15	0.460b	3.34defg	0.115 a	0.573 cde	2.56 e	17.40 bcde	1.93 bcd	0.15 efg	23.57ab
FJSS-16	0.263 efgh	3.67 cdef	0.060 cd	0.530 def	3.06 cde	18.43 abcd	1.66 cdefg	0.17 efg	25.51 a
FJSS-17	0.380 bcde	4.24 bcde	0.010 e	0.703 b	2.70 de	19.52 abc	1.60 cdefg	0.14 fgh	23.51 ab
FJSS-2	0.240 fgh	4.18 bcde	0.107 a	0.697 b	3.94 b	16.50 cde	2.77 a	0.24 ab	8.87 fg
FJSS-20	0.360 bcdef	3.36 defg	0.013 e	0.497 efg	4.01 b	19.23 abc	1.81 bcde	0.21 cd	17.52 cd
FJSS-21	0.220 h	3.96 cde	0.020 e	0.390 hi	2.72 de	17.20 bcde	1.96 bcd	0.16 efg	13.86de
FJSS-3	0.240 fgh	3.24 efg	0.030 de	0.637 bc	2.88 de	20.32 ab	1.83 bcde	0.14 fg	10.80 efg
FJSS-4	0.343 bcdefg	2.82 fgh	0.118 a	0.640 bc	3.91 b	17.76 abcde	1.39 efg	0.22 bc	20.79 bc
FJSS-5	0.357 bcdef	4.41 bcd	0.092 ab	0.657 bc	3.05 cde	17.20 bcde	2.21 b	0.18 de	7.27 g
FJSS-6	0.243 fgh	2.50 gh	0.063 bc	0.313 i	1.66 f	14.66 e	1.53 defg	0.11 h	9.19 fg
FJSS-7	0.223 gh	3.41 defg	0.040 cde	0.563 cdef	2.65 e	20.00 ab	1.26 g	0.13 gh	11.70 ef
FJSS-8	0.300 defgh	1.90 h	0.013 e	0.417 gh	4.00 b	19.20 abc	1.80 bcde	0.21 bcd	19.58 bc
FJSS-9	0.397 bcd	4.74 bc	0.020 e	0.637 bc	3.21 cd	17.33 bcde	1.90 bcd	0.18 de	13.88 de

Where the values carrying same alphabets are statistically similar, FRW=Fresh root weight (g), FSW=Fresh shoot weight (g), DRW=Dry root weight (g), DSW=Dry shoot weight (g), RL=Root length (cm), SL=Shoot length, (cm), CL=Coleoptile length (cm), R/S ratio =Root:shoot ratio and GY=Grain yield (g)

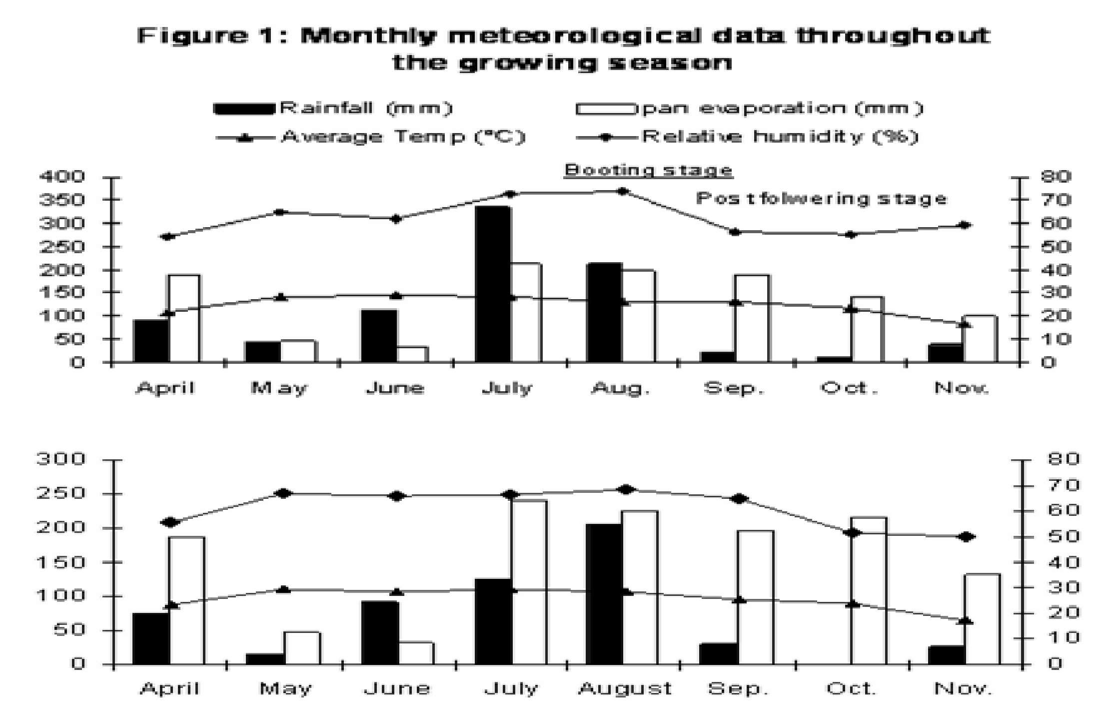


Fig 1. The meteorological data during 2007 (upper figure) and 2008 (lower figure)

by keeping row to row and plant to plant distance of 5 and 3 cm, respectively. After two weeks data were recorded for root, coleoptile and shoot length (cm), fresh root and shoot weight (g), dry root and shoot weight (g) and root: shoot ratio.

Field experiment

The sorghum genotypes were planted on second week on July, 2007 and first week of July, 2008 in triplicate Randomized Complete Block Design (RCBD) with experimental plots that comprising of two rows, 4m long and 30 cm apart. Four soil samples from each replication were taken for soil analysis which resulted in maximum average water holding capacity of the soil was 35% of soil dry weight, and the permanent wilting point was 12%. Plots were treated alike for all the cultural practices and nutrient application from sowing till harvest. Meteorological data regarding minimum and maximum temperature, relative humidity, pan evaporation and monthly rainfall were taken throughout the growing season. Patterns of rainfall, temperature, relative humidity and pan evaporation (Figure 1) showed sufficient period for the crop to be exposed to water stress at booting, anthesis and post-anthesis stages. At post-anthesis stage in both the year flag leaf of the plants were utilized for recording of the data for various physiological characters.

Morph-physiological traits related to flag leaf

Flag leaf area (FLA) of 10 randomly selected plants from each replication was obtained during early morning hours when leaves were fully turgid. Flag leaf area was measured in centimeters (cm^2) by using leaf area meter (LI-3000/Lambda Instr. Corp. Lincolin, Nebraska, USA). The leaves were oven dried at 80 ºC during 48 hours and specific flag leaf area (SFLA) was calculated as a ratio of flag leaf area to the oven dry weight (g) of the leaves. Specific flag leaf weight (SFLW) was determined by the ratio of oven dry weight (g) of the leaves to flag leaf area (cm^2). The specific flag leaf weight (SFLW) was calculated as SFLW = DW/LA. For excised leaf weight loss (ELWL) the leaves were weighed at three stages, viz., immediately after sampling (fresh weight), then dried in an incubator at 28 ºC at 50% R.H. for 6 h, and then dried again in an oven for 24 h at 70 ºC as proposed by Clarke and Townley-Smith (1986). ELWL was calculated from the following formula:

ELWL= [(Fresh weight -Weight after 6 h)/ (Fresh weight - Dry weight)] ×100

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) leaves were excised and immediately brought to the laboratory. Then, they remained in the darkness for stomatal closure for half an hour under ambient room conditions. They were weighted (W1 in g) after this period and again 180 min later (W2 in g); the leaf area (LA in cm^2) was determined using leaf area meter (LI-3000/Lambda Instr. Corp. Lincolin, Nebraska, USA). Residual transpiration on leaf area basis (g $H_2O/min/cm^2/10^5$) was determined as given below according to Clarke *et al*. (1991);

RT = (W1 – W2)/ (LA.180)

Relative water content (RWC) was determined for detached leaves using the method of Mata and Lamattina (2001).The relative water content (RWC) was calculated on flag leaf blades from the following equation:

RWC (%) = (FW – DW)/ (TW – DW) × 100

The fresh weight (FW) was measured immediately after excision, the full turgid weight (TW) was determined after the rehydration of the leaves placing them in a test tube containing distilled water for 24 hours at 4ºC in darkness, and the dry weight (DW) after oven drying at 80ºC during 48 hours. Leaf dry matter was determined by taking the average of dry weight in RWC and dry weight in ELWL. The relative dry weights of the leaves (RDW) were calculated following using the following formula:

RDW= DW/ (TW-DW)

For the measurement of cell membrane stability (CMS), plant material (0.4 g) was washed with double distilled deionized water, placed in tubes with 20 ml of 3d water and incubated for 2 h at 25°C. Subsequently, the electrical conductivity of the solution (L1) was determined using conductivity meter (Model, 145 A+, Thermo Electron USA). Samples were then autoclaved at 120°C for 20 min and the final conductivity (L2) was measured after equilibration at 25°C. The CMS was the mean percentage of five leaf sample and was calculated as follows:

CMS (%) = [(1-(L1/L2)] × 100

At maturity, the heads of ten randomly selected plants were detached from the plants and threshed to determine grain yield per plant (g).

Statistical analyses

The average data recorded for seedling traits and physiological characters during 2007 and 2008 were subjected to the analysis of variance according to Steel *et al*. (1996) using MSTATC software to evaluate significant differences among the varieties for seedling parameters. Genetic parameters were obtained as outlined by Johnson *et al*. (1959) and Mahmud and Kramer (1951). Heritability estimates were calculated following the method of Allard (1960). Cluster diagrams were sketched using Ward's method based on linkage distances by Statistica software version 5.0 (www.statsoft.com).

Results

Performance of local landraces (LLRs) for various morph- physiological traits

Considerable statistical differences were observed between studied local landraces for various seedling traits (Table 1). Most of the local landraces (LLRs) demonstrated their worth for drought tolerance as they exhibited greater dry root weight, lengthy roots and higher root:shoot ratios over the control variety. The LLR, FJSS-1 exhibited the highest fresh root weight (FRW) followed by FJSS-15, however FJSS-21 displayed minimum value for FRW. Similarly, the highest and lowest fresh shoot weight (FSW) was observed for FJSS-1 and FJSS-10 respectively. Dry root weight (DRW) was maximum for FJSS-4 and minimum for FJSS-17. Five LLRs exceeded form the control variety Chakwal sorghum for root length (RL) with the highest value for FJSS-11 and the lowest for FJSS-15. However, coleoptile length (CL) was the maximum for FJSS-2 followed by FJSS-5 while minimum for FJSS-7. Most of the landraces performed better for CL over the check variety. Root: shoot ratio, a well known selection criterion for drought tolerance was the highest for FJSS-11 followed by FJSS-2 while lowest value was presented by FJSS-6, however most of the other landraces showed more root: shoot ratio over the control variety.

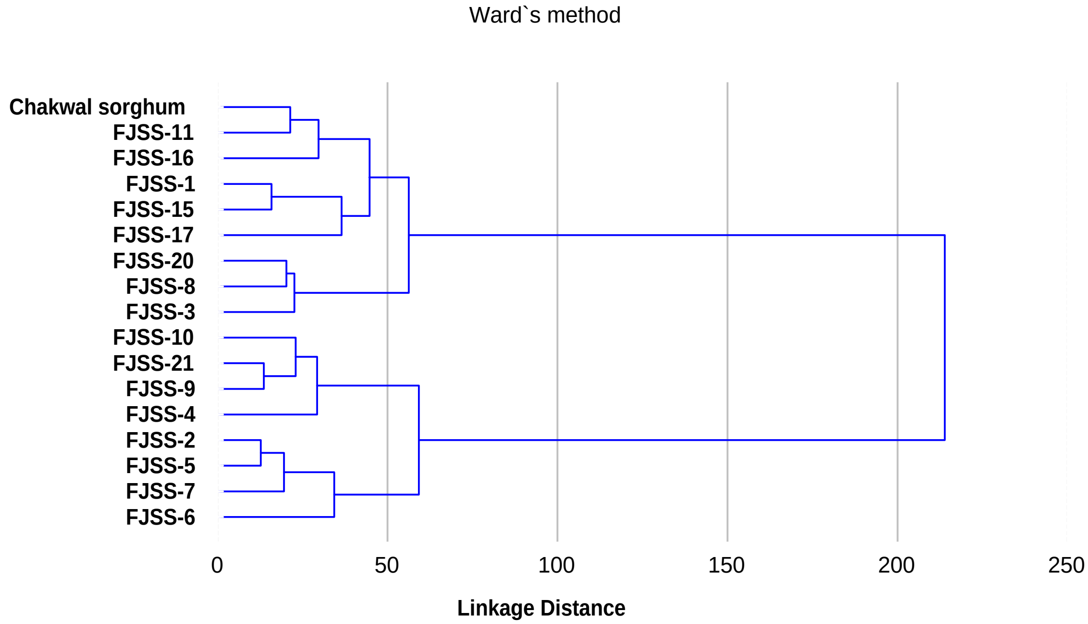
Various physiological parameters contributing towards drought tolerance revealed statistically significant variation among different LLRs (Table 2). Flag leaf area (FLA) which has positive correlation with grain yield for many crops was the greatest for FJSS-16 and FJSS-17, whereas, out of other landraces FJSS-1, FJSS-11 and FJSS-15 demonstrated more FLA over the control. FLA was the lowest for FJSS-2. Likewise FJSS-17, FJSS-3 and FJSS-20 raised the topper values for specific flag leaf area (SFLA) while FJSS-9 revealed the lowest. Among the remaining landraces FJSS-11, FJSS-15, FJSS-16, FJSS-2, FJSS-6 and FJSS-8 superseded the control variety Chakwal sorghum for specific flag leaf area. All the landraces performed well for leaf dry matter (LDM) over the check except FJSS-2, FJSS-21, FJSS-5 and FJSS-7; however FJSS-8 and FJSS-2 gave the highest and lowest dry matter accumulation in leaves respectively. The LLR, FJSS-2 presented more relative dry weight than control variety which was the maximum whereas, FJSS-10 revealed lowest value. All other landraces displayed less relative dry weight as compared to the check except FJSS-2. Residual transpiration a usual part of selection criteria for drought resistance was the lowest one for FJSS-6

Table 2. Performance and statistical differences among sorghum LLRs for flag leaf related traits under drought stress at reproductive stage

	LA	SFLA	SFLW	LDM	ELWL	RDW	RWC	RT	CMS
Chakwal sorghum	96.46 e	39.32 cde	0.031 f	2.806 abc	15.80 cd	0.490 ab	54.373 f	0.0081 bcd	56.83 d
FJSS-1	96.80 e	36.64 def	0.022 gh	2.99 abc	10.63 d	0.468 ab	78.65 bc	0.0063 def	75.82 a
FJSS-10	65.85 k	30.67 fg	0.043 e	3.04 ab	17.25 cd	0.252 d	52.86 f	0.0100 b	59.65 cd
FJSS-11	101.34 d	47.51 b	0.020 h	2.98 abc	10.63 d	0.492 ab	60.98 ef	0.0062 def	57.58 d
FJSS-15	102.08 c	47.51 b	0.026 g	3.17 a	17.58 cd	0.400 c	72.35 cd	0.0092 bc	74.44 a
FJSS-16	121.31 a	45.87 bc	0.017 hi	3.14 a	12.25 d	0.475 ab	53.37 f	0.0103 b	64.41 bc
FJSS-17	108.87 b	70.71 a	0.013 i	2.88 abc	11.17 d	0.388 c	70.66 cd	0.0071 cde	72.16 a
FJSS-2	47.59 p	39.45 cde	0.054 c	2.17 d	44.50 a	0.519 a	63.55 de	0.0150 a	72.04 a
FJSS-20	87.64 f	65.21 a	0.015 i	2.86 abc	11.17 d	0.485 ab	61.33 ef	0.0098 b	59.56 cd
FJSS-21	61.15 m	27.10 g	0.051 cd	2.66 abcd	17.32 cd	0.437 bc	64.52 de	0.0082 bcd	68.67 ab
FJSS-3	77.27 i	65.28 a	0.026 g	2.94 abc	14.57 d	0.479 ab	74.37 c	0.0073 cde	75.37 a
FJSS-4	84.41 g	19.58 h	0.047 de	3.19 a	24.55 c	0.439 bc	65.15 de	0.0072 cde	73.06 a
FJSS-5	52.03 o	36.11 ef	0.073 a	2.48 cd	33.82 b	0.491 ab	87.11 a	0.0070 cde	73.18 a
FJSS-6	56.14 n	43.93 bcd	0.048 de	2.76 abc	14.63 d	0.485 ab	44.04 g	0.0042 f	74.41 a
FJSS-7	64.10 l	30.95 fg	0.054 c	2.55 bcd	38.98 ab	0.462 ab	65.48 de	0.0052 ef	70.82 ab
FJSS-8	83.45 h	51.17 b	0.035 f	3.13 a	10.70 d	0.465 ab	64.97 de	0.0071 cde	72.61 a
FJSS-9	68.28 j	17.55 h	0.060 b	3.07 ab	19.67 cd	0.475 ab	83.80 ab	0.0069 cde	73.59 a

Where the values carrying same alphabets are statistically similar, FLA=Flag leaf area (cm), SFLW=Specific flag leaf weigh, SFLA=Specific flag leaf area, LDM=Leaf dry matter (g), ELWL=Excise leaf weight loss (%), RDW=Relative dry weigh, RWC=Relative water content (%), RT=Residual transpiration (g H2O/min/cm2/105), CMS=Cell membrane stability (%)

Figure 2: Dendrgram for 17 sorghum genotypes based on all morph-physiological traits under drought stress at seedling and post-flowering stages



closely followed by FJSS-7, FJSS-11 and FJSS-1 respectively while highest value was demonstrated by FJSS-2. Minimum excised leaf weight loss (ELWL) was observed for FJSS-1 and FJSS-11 however, FJSS-2 showed the greatest ELWL. Six landraces demonstrated lower ELWL than the control variety. Considerable variation was observed for relative water content (RWC) which was uppermost for FJSS-5 followed by FJSS-9 and FJSS-1. Most of the landraces showed more RWC as compared to the control Chakwal sorghum. The data regarding cell membrane stability (CMS), an efficient and widely utilized parameter for screening against many types of stresses, revealed that all the LLRs presented higher CMS as compared to the check. FJSS-1 and FJSS-3 had the most stable membranes narrowly followed by FJSS-15 and FJSS-6. Grain yield was the highest for the control variety, while no significant difference was observed between this variety and FJSS-1, FJSS-15, FJSS-16 and FJSS-17 (Table 1).

Cluster diagram based on the means of all morph-physiological traits under water stress at seedling and reproductive stages clearly divided the sorghum local landraces (LLRs) into two main groups (Figure 2 and 3). The first group formed by the control variety Chakwal sorghum and eight landraces FJSS-1, FJSS-3, FJSS-8, FJSS-11, FJSS-15, FJSS-16 FJSS-17 and FJSS-20, and second group by the remaining nine LLRs.

Variability parameters for different morph-physiological traits

Diversity for various morph-physiological traits was assessed to determine patterns of genetic variation for these characters among the LLRs (Table 3). Amongst seedling characters shoot length revealed highest phenotypic and genotypic variance followed by FSW and RL. Furthermore, RDW showed the highest values of phenotypic and genotypic coefficients of variations followed by FRW and FSW. All the seedling parameters displayed high broad-sense heritability values, except shoot length, with highest levels observed for RDW and root:shoot ratio traits. Genetic advance was the uppermost for shoot length followed by FSW and root length. Similarly, FLA displayed the maximum values for phenotypic and genotypic variances followed by SFLA and RWC. Phenotypic coefficients of variability were the highest for LDM while ELWL showed maximum genotypic coefficients of variability followed by SFLW. All the physiological parameters exhibited high heritability values exception for LDM. FLA revealed the highest heritability and genetic advance followed by SFLA. Estimates of genetic advance were lowest for CMS coupled with high heritability in broad sense.

Discussion

Selection of drought tolerant plant species has been considered to be an economic and efficient means of alleviating agricultural problems especially in dry areas (Ashraf *et al*., 1992). To achieve this goal, a set of reliable traits that can be rapidly and relatively inexpensively screened is needed. For successful selection, presence of considerable magnitude of variability in the available germplasm is prerequisite (Ali et al. 2008). Significant statistical differences were observed among the local landraces for seedling as well as physiological parameters (Table 1 and 2) and it was in accordance with results of Dhanda *et al*. (2004). Drought-adapted plants are often characterized by deep and vigorous root systems (Blum, 1979). In this study FJSS-1 revealed the highest FRW and FSW which indicated its significance for drought tolerance. Nour *et al*. (1978) also reported root weight is the best and easiest attribute to determine drought tolerance in grain sorghum. The LLR FJSS-15 and FJSS-11 showed maxmum DRW suggesting considerable scope of this genotype for crop improvement under water stress (Takele 2000). Furthermore, highest DSW value was observed for FJSS-1. Nour *et al*. (1978) correlated high RDW/SDW ratios of young plants with superior drought resistance in sorghum genotypes which again displayed the worth of FJSS-15 and FJSS-11 as potential genotypes for selection against drought. Matsuura *et al*. (1996) also reported a positive correlation between drought tolerance and root length in sorghum and millet (*Pennisetum glaucum*). On the other hand, Zekri (1991) stated that the decrease in water supply is contributed to increase in R/S ratio of seedlings. In our study, FJSS-11 was at the top for both the root length as well as root-shoot ratio (R/S) which in consistence with the results of Matsuura *et al*. (1996) which advocated the importance of this landrace fot improvement in drought tolerance. Moreover, water stress at seedling stage significantly affected the R/S ratio (Zekri, 1991). Plaut *et al*. (1996) and Pace *et al*. (1999) reported that seedlings under water stress caused an increase in root length with reduced diameter. In addition, numerous seedling traits have been suggested as important relative to drought tolerance including root weight, lateral root number and root-to-shoot ratios (Cook 1985; Pace *et al*., 1999). The genotype FJSS-2 gave rise to the maximum coleoptile length followed by FJSS-5 and FJSS-2 respectively. Deep rooting, root length density, root distribution (Passioura 1983; Turner 1986; Ludlow and Muchow 1990; Matsui and Singh 2003; Taiz and Zeiger 2006) and coleoptile length (Dhanda *et al*., 2004) have also been regarded as drought tolerance contributing traits but a great limitation of this work exists in assessing the root system using seedlings and, by contrast, assessing production in field-grown adult plants (DaMatta, 2004). These studies suggested that the LLRs, FJSS-1 and FJSS-11 are the most appropriate candidates to be selected against water stress at seedling stage based on these important measured seedling traits. The difficulties in evaluating root systems such as large environmental influences and the complex inheritance of root characteristics hold back the use of these characters in selection programs in spite of their apparent positive relationship with yield under drought conditions (Medrano *et al*., 1998).

Drought tolerance in sorghum is a complex trait influenced by many other characters (Blum, 1979). Plants have different life strategies to cope drought stress, like drought avoidance and drought tolerance (Blum, 1996). A wide variety of drought tolerance mechanisms; both morphological and physiological have been developed in plants. Physiological traits related to the flag leaf like FLA, SFLA, SFLW, LDM, ELWL, RDW, RWC, RT and CMS (Table 2) have been extensively utilized by plant physiologists and breeders to evaluate their significant role for drought tolerance in crop plants. These traits revealed significant differences among the LLRs as shown in Table 1. Tsuji *et al*. (2003) reported that drought tolerance in sorghum is associated with its smaller leaf area. However, in drought conditions optimum flag leaf area (FLA) is also important for optimum photosynthetic activity (Khaliq *et al*., 2008). The landrace FJSS-16 demonstrated the maximum while FJSS-2 revealed the lowest one suggesting that drought considerably reduced FLA in FJSS-2 to save loss of water through evapotranspiration but it may cause in lower photosynthetic activity (Khaliq *et al*., 2008) which is also unwanted. Moreover, traits like reduced leaf area and prolonged stomata closure, decrease water losses, but result in reduced dry matter production and, therefore, reduced final yield (Karamanos and Papatheohari, 1999; Fischer and Wood, 1979). Hence, optimum leaf area is important for producing high dry matter as well as grain yield under water stressed situations (Fischer and Wood, 1979) as in this study flag leaf area showed positive association with LDM which was also the highest for FJSS-16. Therefore, the LLRs having average FLA were found to be more promising for selection against water stress under rainfed conditions.

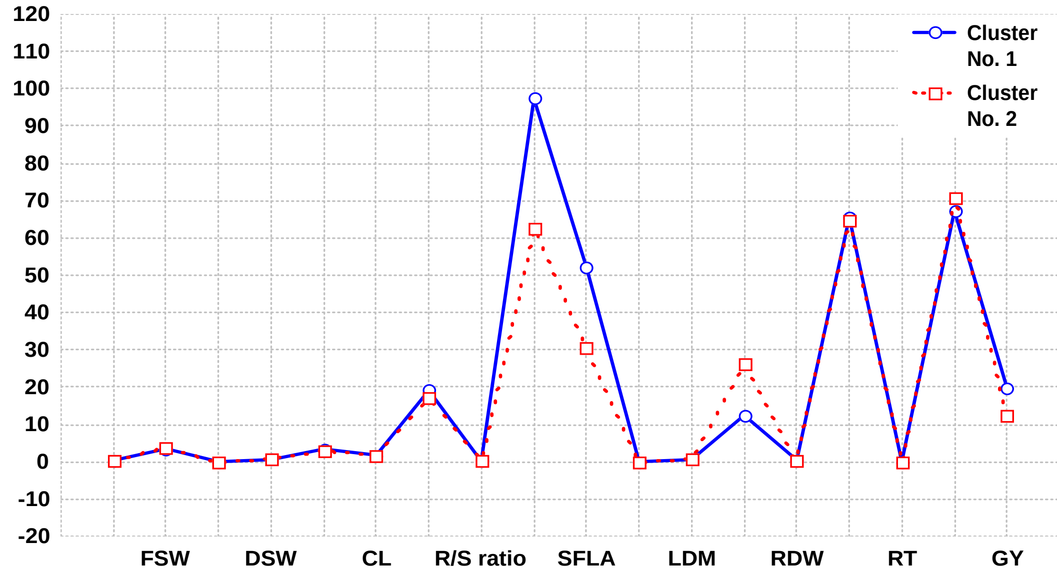
Assessment of excise leaf weight loss (ELWL) is an important selection criterion for water stress tolerance in plants (Clarke 1987; McCaig and Romogosa, 1991). This trait is moderately heritable (Clarke and Townley-Smith, 1986) and can be easily estimated in a large population (Dhanda and Sethi, 1998). In our study, FJSS-1 and FJSS-11 displayed

Table 3. Estimates of variability parameters among sorghum LLRs for morph-physiological characters under drought stress

Characters	$\sigma^2 p$	$\sigma^2 g$	*CVp (%)*	*CVg (%)*	h^2_{BS} *(%)*	*GA*
Seedling tratis						
Fresh root weight (g)	0.014	0.010	35.19	29.60	70.73	17.06
Fresh shoot weight (g)	1.211	0.880	29.81	25.41	72.66	164.93
Dry root weight (g)	0.002	0.002	79.08	76.77	94.23	8.09
Dry shoot weight (g)	0.022	0.019	25.29	23.48	86.15	26.16
Root length (cm)	0.614	0.532	24.34	22.66	86.64	140.01
Shoot length (cm)	4.911	1.828	12.22	7.460	37.23	170.20
Root: Shoot ratio	0.002	0.002	23.34	22.65	94.23	8.09
Coleoptile length (cm)	0.163	0.111	22.55	18.60	68.10	56.72
Flag leaf related physiological traits						
Flag leaf area (cm^2)	466.78	466.69	26.656	26.654	99.98	5498.14
Specific flag leaf weight	0.0003	0.0003	0.043	0.042	97.61	4456.34
Specific flag leaf area	244.08	226.60	41.930	40.458	92.84	3.61
Leaf dry matter (g)	0.128	0.054	62.276	40.374	42.03	2992.25
Excise leaf weight loss (%)	123.03	101.03	58.257	52.793	82.12	31.03
Relative dry weight	0.004	0.004	14.456	13.125	82.44	1879.21
Relative water content (%)	143.17	121.543	18.348	16.905	84.89	11.13
Residual transpiration (g $H_2O/min/cm^2/10^5$)	0.00001	0.00001	33.486	29.803	79.21	2095.57
Cell membrane stability (%)	51.852	37.880	10.451	8.933	73.05	0.43
Grain yield (g)	46.815	41.328	41.776	39.252	88.28	1085.24

Where, σ^2p=Phenotypic variance, σ^2g=Genotypic variance, CVp (%) =Phenotypic coefficient of variation, CVg (%) =Genotypic coefficient of variation, h^2BS (%) =Heritability in broad-sense and GA=Genetic advance

Figure 3: Plot of means of all morph-physiological traits for each cluster



the lowest values for ELWL suggesting the worth of these LLRs for drought tolerance and exploitation in future programs. The RDW values were equal to the amount of dry matter per unit volume of water at the saturated condition (Wilson *et al*., 1980). Higher RDW values are believed to be escorted by vigorous accumulation of osmotica (osmotic adjustment) resulting in ability of plants to withstand against severe drought conditions (Jones *et al*., 1980). The landraces FJSS-2, FJSS-1, and the control cultivar Chakwal sorghum exhibited maximum values for RDW.

One of the initial indicators of water deficit in plant tissues is the reduction of relative water content (RWC). The decrease of RWC in stressed plants might be associated with the decrease in plant vigour as was observed in many plant species (Halder and Burrage, 2003; Lopez *et al*., 2002). Relative water content had been identified as potential physiological marker for drought tolerance in many crop plants such as barley (*Hordium Vulgare* L.) (Martin *et al*., 1989), sunflower (*Halianthus annus* L.) (Rauf and Sadaqat, 2008), sugarcane (*Saccharum officinarum* L.) (Silva *et al*., 2007), durum wheat (*Triticum durum*) (Merah, 2001) wheat and its wild relatives (Farooq and Azam, 2002). Our results revealed that FJSS-5 gave the highest RWC followed by FJSS-9 and FJSS-1, however most of the landraces exhibited high values RWC and surpassed over control variety also except FJSS-10. This suggested that most of the landraces were drought tolerance having enough water content to withstand the acute shortage of water (Fischer and Wood, 1979). Likewise, cuticular or residual transpiration (RT), which signifies the key system of water loss during night under optimal conditions and during noon under drought conditions, was suggested as selection criterion in wheat breeding for drought tolerance (Clarke at al., 1991; Balota, 1995). In this way FJSS-6 showed minimum RT closely chased by FJSS-11 and FJSS-1 showing considerable tolerance to water stress at night and noon times (Clarke at al., 1991).

Measurement of cell membrane stability (CMS) is a technique that has frequently been used for screening against drought tolerance in various crops like sorghum (Sullivan and Ross, 1979), wheat (Blum and Ebrecon, 1981) and wheat relatives (Farooq and Azam, 2002), maize (*Zea mays*) (Premachandra *et al*., 1989) and rice (*Oryza sativa*) (Tripathy *et al*., 2000). Additionally, it has also been used for assessing tolerance to frost (Dexter, 1956), heat (Martineau *et al*., 1979) and desiccation (Bewley, 1979). A view of Table 2 demonstrated that most of the LLRs were drought tolerant based on CMS with FJSS-1 at the top followed by FJSS-3, FJSS-15, FJSS-6 and FJSS-9 and can further be used in sorghum breeding program for the evolution of drought tolerant genotypes at post anthesis stage. In case of grain yield per plant, Chakwal sorghum and FJSS-16 were at the top while FJSS-1, FJSS-17 and FJSS-15 also performed well for grain yield among the LLRs and this supported the well established fact that yield of crop plants in drying soil reduces even in tolerant lines of that crop species (Ashraf and Mehmood, 1996; Tahir and Mehdi, 2001).

Cluster analysis obviously separated the 17 sorghum genotypes into two groups; one having characters contributing more towards drought tolerance and the other with less input for drought tolerance. Cluster I formed by LLRs (FJSS-1, FJSS-3, FJSS-8, FJSS-11, FJSS-15, FJSS-16 FJSS-17 and FJSS-20) along with control variety Chakwal sorghum revealed more drought tolerance due to higher value of FRW, DSW, SL, RL, R/S ratio, FLA, SFLA, LDM, RDW, RWC, GY while lower values for ELWL and RT. Hence this group was more stable against drought stress at seedling as well as post-flowering stage. No doubt the cluster II demonstrated higher values for FSW, DRW, CL, SFLW and CMS, but most of the traits contributing towards drought tolerance were more frequent in cluster I.

Genetic improvement of crops for drought tolerance necessitates the investigation of the possible attributes of drought tolerance as well as exploration of the genetic variation of the crops for the traits contributing towards drought tolerance (Dhanda *et al.* 2004). The results revealed that all the characters had considerable values of phenotypic and genotypic coefficients of variation (CVp and CVg respectively) among the LLRs except SFLW. However, DRW gave rise to the highest coefficients of variation (both CVp and CVg) followed by FRW in case of seedling parameters which suggested that these traits had sufficient scope of selection among the LLRs. Dhanda *et al.* (2004) observed considerable genetic variability for root length, shoot length, root-to-shoot length ratio and coleoptile length in bread wheat. Similarly, in case of flag leaf related traits ELWL exhibited maximum CVg closely followed by LDM and SFLA which advocated a vide range of genetic variability for ELWL among the LLRs and in turn considerable selection pressure for drought tolerant lines could be possible. On the other hand, CMS displayed the minimum value for CVg demonstrating less genotypic variation among the genotypes. A very little difference between CVp and CVg for most of the characters showed that their variation was genotypic in nature, whereas, a large difference between the CVp and CVg for LDM revealed the substantial involvement of blocking effects for this parameter. Tripathy *et al.* (2000) also indicated that the variation in CMS was genotypic in nature in case of rice. Baldini *et al.* (1997) on the other hand found considerable variability for RWC in sunflower.

Heritability in broad-sense was high for most of the seedling and physiological traits among the LLRs exception for SL and LDM. Dhanda *et al.* (2004) reported moderate to high broad sense heritability for root and shoot length, root-to-shoot ratio and coleoptile length in wheat genotypes evaluated under both water stress and non stress conditions. Flag leaf area and its related traits contributing towards drought tolerance revealed high heritability in wheat (Ahmad *et al*., 2004). Similarly, Songsri *et al*. (2008) found high heritability for specific leaf area in peanut (*Arachis hypogea* L.). Clarke and Townley-Smith (1986) concluded that ELWL was moderately heritable in durum wheat. CMS also revealed high heritability which was in agreement with the results of Dhanda *et al*. (2004) in wheat and in contrast with those of Tripathy *et al*. (2000) in rice. Reliable heritability estimates displayed by these morph-physiological traits will not point to only the scope of assembling genetic characters imparting stress tolerance but also allow us to formulate predictions about the possible progress in this effort. Fresh shoot weight, root length, flag leaf area, specific flag leaf weight, leaf dry matter, relative dry weight, residual transpiration and grain yield exhibited considerable amount of genetic advance that indicated high magnitude of selection gain followed by hybridization for these parameters.

Our results suggest that this set of sorghum landraces might play a significant role for incorporation of drought tolerance in this important crop on the basis of various seedling and other morpho-physiological traits. The landrace FJSS-1 performed the best for most of the characters especially in case of seedling traits followed by FJSS-11 and FJSS-17 which also showed their better performance for various traits contributing towards water stress tolerance at seedling and post-flowering stage in sorghum. The landrace, FJSS-10 revealed the contrasting parents showing drought susceptibility for most of the parameters which suggested that this LLR could be used for hybridization with the tolerant ones (FJSS-1, FJSS-11 and FJSS-17). The resultant segregating generations then would be utilized for QTL genetic analysis based on these drought tolerance attributes in sorghum especially at post-anthesis stage. Considerable magnitude of variation among the LLRs for most of the traits proposed these genotypes as significant source for the selection of water stress tolerance. Similarly, higher amount of heritability and genetic advance for the majority of the characters advocated high amount of genetic gain followed hybridization for these parameters.

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