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Morpho-physiological diversity and its implications for improving drought tolerance in grain sorghum at different growth stages

Muhammad Amjad Ali[1a*], K. Jabran[2], S. I. Awan[3], A. Abbas[1], Ehsanullah[4], M. Zulkiffal[5], Tuba Acet[6], J. Farooq[7] and A. Rehman[1]

[1]Wheat Research Institute, Ayub Agricultural Research Institute (AARI), Jhang Raod, Faisalabad, Pakistan, [a]Present address: Institute of Plant Protection, DAPP, University of Natural Resources and Life Sciences, Vienna, Austria
[3]Department of Plant Breeding and Molecular Genetic, University of Azad Jammu and Kashmir, Rawalakot, Pakistan
[2] ARI, AARI, Faisalabad, [5]VRI, AARI, Faisalabad, Pakistan, [7] CRI, AARI, Jhang Raod, Faisalabad
[4]Department of Agronomy, University of Agriculture, Faisalabad, Pakistan,
[6]Plant Physiology and Biochemistry Laboratory, Department of Biology, Karadeniz Technical University, Trabzon, Turkey

*** Corresponding author: Amjad.Ali2001@gmail.com**

Abstract

Sorghum grown under rain-fed conditions is usually affected by drought stress at different stages resulting in negative effect on yield. The assessment and quantification of morpho-physiological diversity for the traits contributing towards drought tolerance at these stages is of vital importance. For this purpose, drought stress was imposed on 44 sorghum accessions at seedling stage and natural incidence of water stress at post anthesis stage. The data of 21 different morpho-physiological traits were subjected to different multivariate techniques, including correlation, principal component (PC) and cluster analysis to assess the diversity for drought tolerance in sorghum. The correlation analysis revealed that selection for long roots; higher root/shoot ratio, leaf area and leaf dry matter could be performed simultaneously. There was positive association between relative water contents and cell membrane stability but both of these traits were negatively correlated with residual transpiration and excised leaf weight loss. Principal component (PC) analysis showed first 7 PCs having Eigen value >1 explaining 77.653% of the total variation with head width, head weight, grain yield per plant, fresh and dry shoot weight being the most important characters in PC1. Cluster analysis classified 44 accessions into four divergent groups. The members of first two clusters exhibited adequate degree of drought tolerance on the basis of majority of morpho-physiological traits, whereas, cluster 3 and 4 included genotypes with lower level of drought tolerance. The D^2 statistics revealed the highest distances between 2nd and 3rd clusters, while 3rd and 4th clusters displayed maximum similarity. Scatter plot and tree diagrams demonstrated sufficient diversity among the sorghum accession for various traits and some extent of association between different clusters. The results concluded that morpho-physiological diversity in the studied material is structured by genotypes and this diversity could be utilized for cultivar breeding and germplasm conservation programs aimed at improving drought tolerance in sorghum.

Keywords: Sorghum. Drought tolerance; Diversity; Morpho-physiological traits; Post anthesis

Introduction

The grain sorghum (*Sorghum bicolor* L. Moench) is used as staple food in human diet as well as used in animal feed. It is the fifth leading cereal crop in the world after wheat, maize, rice and barley. Sorghum grain is the staple food of poor and the most food-insecure people, living mainly in the semiarid tropics (Ali et al., 2009a; Bibi et al., 2010). It performs better under adverse soil and weather conditions as compared to other crops (Ejeta and Knoll, 2007). However, sorghum grown in arid and semi-arid regions is influenced by water stress at terminal growth stages like anthesis and post-anthesis which renders the most adverse effect on yield in sorghum (Tuinstra et al., 1997; Prasad et al., 2008). Water stress is a problem in 45 % of the world's geographical area and is a major limitation to the productivity of agricultural systems and food production worldwide (Boyer, 1982). In cereal crops that are major carbohydrate staples for humans, water stress at critical stages like seedling establishment, tillering and reproductive stages may result in significant yield reduction and even lethal to the crops (Westgate et al., 1989; Ludlow and Muchow, 1990). Moreover, moisture deficit at the reproductive stage causes the principal decline in yield as compared to stress happening at any other growth stages. Fluctuations in water balance and soil available water are critical to crop yields because they directly disturb plant physiological processes and responses (Kramer and Boyer, 1995; Miyashita et al., 2005). Water deficits tend to shift the

Table 1. The 44 genotypes used in this study alongwith their sources and status

Sr. No.	Name	Source	Status
1	Chakwal Sorghum	BARI, Chakwal	Approved variety
2	BMR-14	FRI, Sargodha	Germplasm accession
3	FJSS-1	BARS, Fateh Jang	Local land race
4	FJSS-2	BARS, Fateh Jang	Local land race
5	FJSS-3	BARS, Fateh Jang	Local land race
6	FJSS-4	BARS, Fateh Jang	Local land race
7	FJSS-5	BARS, Fateh Jang	Local land race
8	FJSS-6	BARS, Fateh Jang	Local land race
9	FJSS-7	BARS, Fateh Jang	Local land race
10	FJSS-8	BARS, Fateh Jang	Local land race
11	FJSS-9	BARS, Fateh Jang	Local land race
12	FJSS-10	BARS, Fateh Jang	Local land race
13	FJSS-11	BARS, Fateh Jang	Local land race
14	FJSS-15	BARS, Fateh Jang	Local land race
15	FJSS-16	BARS, Fateh Jang	Local land race
16	FJSS-17	BARS, Fateh Jang	Local land race
17	FJSS-20	BARS, Fateh Jang	Local land race
18	FJSS-21	BARS, Fateh Jang	Local land race
19	G-160	FRI, Sargodha	Germplasm accession
20	JS-2002	FRI, Sargodha	Approved variety
21	JS-61	FRI, Sargodha	Germplasm accession
22	H-118	FRI, Sargodha	Germplasm accession
23	H-18	FRI, Sargodha	Germplasm accession
24	PARC-SS-1	PARC, Islamabad	Advanced line
25	PGRI-141	PGRI, Islamabad	Germplasm accession
26	PGRI-191	PGRI, Islamabad	Germplasm accession
27	PGRI-29	PGRI, Islamabad	Germplasm accession
28	PGRI-35	PGRI, Islamabad	Germplasm accession
29	S-149	FRI, Sargodha	Advanced line
30	S-1563	FRI, Sargodha	Advanced line
31	S-171	FRI, Sargodha	Advanced line
32	S-58	FRI, Sargodha	Advanced line
33	YSS-1	MMRI, Yousafwala	Advanced line
34	YSS-10	MMRI, Yousafwala	Advanced line
35	YSS-18	MMRI, Yousafwala	Advanced line
36	YSS-2	MMRI, Yousafwala	Advanced line
37	YSS-3	MMRI, Yousafwala	Advanced line
38	YSS-4	MMRI, Yousafwala	Advanced line
39	YSS-5	MMRI, Yousafwala	Advanced line
40	YSS-6	MMRI, Yousafwala	Advanced line
41	YSS-7	MMRI, Yousafwala	Advanced line
42	YSS-8	MMRI, Yousafwala	Advanced line
43	YSS-9	MMRI, Yousafwala	Advanced line
44	YSS-98	MMRI, Yousafwala	Approved variety

source-sink relation out of balance one way or the other. The key effect of water stress is generally considered a decline in photosynthesis and growth as a result of stomatal closure (Mwanamwenge et al., 1999). Water stress at different growth stages causes various morpho-physiological changes in the plant to acclimatize under such conditions. For example, water stress at seedling stage might lead to higher dry root weights, longer roots, coleoptiles and higher root/shoot ratios which could be exploited as selection criteria for stress tolerance in crop plants at very early stage of growth (Takele, 2000; Dhanda et al., 2004; Kashiwagi et al., 2004). However, at later growth phase like reproductive stage, flag leaf area (Karamanos and Papatheohari, 1999; Ali et al., 2010), specific leaf weight, leaf dry matter (Aggarwal and Sinha, 1984), excised leaf weight loss (McCaig and Romogosa, 1991; Bhutta 2007), relative dry weight (Jones et al., 1980), relative water content (Fischer and Wood, 1979; Colom and Vazzana, 2003), residual transpiration (Clarke at al., 1991; Sabour et al., 1997) and cell membrane stability (Premachandra et al., 1992, Ali et al., 2009b) are the characters of interest and had been widely exploited as reliable morph-physiological markers contributing towards drought tolerance for various crop plants in addition to sorghum. In completely independent experiments we demonstrated that most of these traits could be exploited for selecting drought tolerant sorghum genotypes at different growth stages (Ali et al., 2009a & b). The presence of significant genetic variability for these traits among the germplasm genotypes suggests an opportunity for improvement of grain yield and drought tolerance through hybridization of genotypes related to divergent groups and subsequent selection from the segregating generations. This could help the plant breeder to select promising drought tolerant genotypes because the selection of plant species with drought tolerance has been considered to be an economic and efficient means of alleviating agricultural problems especially in rain-fed areas (Ashraf et al., 1992). In plant breeding program, several characters are simultaneously considered that make it feasible to approximate the genetic divergence by using multivariate techniques. These multivariate techniques include principal component and cluster analysis which have analogous efficacy to establish the most suitable cross combinations (Machado et al., 2000). Similarly, the D^2 statistics proposed by Mahalnobis (1936) based on cluster analysis is the most appropriate method for selecting agronomically or morpho-metrically divergent parents as it furnishes a measure of actual variation between any pair of populations (Rao, 1952, Assefa et al., 2001). In past, multivariate analysis had mostly been exploited to assess and differentiate the genotypes for various morphological traits in sorghum (Teshome et al., 1997; Ayana and Bekele, 1999; Hasanuzzaman et al., 2002; Tesso et al., 2005; Chozin, 2007; Aruna and Audilakshm, 2008; Mujaju and Chakuya, 2008). However, Ahlawat et al. (2002) utilized multivariate analysis to ascertain diversity for stay green character in 36 wheat genotypes. Similarly, Golabadi et al. (2006) and Tesso et al. (2005) worked out multivariate analysis for drought tolerance in durum wheat and sorghum respectively. Very recently, Bibi et al. (2010), working on 80 sorghum genotypes, found osmotic potential as the most important physiological marker for drought tolerance in addition to root length. But up to the date, no study has been carried out with the objective to assess the morpho-physiological diversity in sorghum for drought tolerance at different growth phases based on different morpho-physiological traits mentioned above by using multivariate analysis. This is why the present study was planned with the objectives 1) to investigate important associations between various morpho-physiological traits, 2) to assess genetic diversity in sorghum accessions, and 3) to classify the genotypes into drought tolerant and drought susceptible groups on the basis of these traits at different growth stages.

Materials and methods

Plant materials

The experiment was carried out at Barani Agricultural Research Station (BARS), Fatehjang (33º34´ N, 72º38´ E, elevation from sea level 512 m), Punjab, Pakistan during the kharif seasons of 2007 and 2008. The germplasm comprised of 44 sorghum genotypes including 3 approved varieties, 9 germplasm accessions, 16 local land races and 16 advance lines (Table I). The germplasm accessions were collected from different research institutes including Barani Agricultural Research Institute (BARI), Chakwal, Plant Germplasm Resource Institute (PGRI), Islamabad, Pakistan Agriculture Research Council (PARC), Islamabad, Fodder Research Institute (FRI), Sargodha and Maize and Millet Research Institute (MMRI), Yousafwala, Sahiwal located in various agro-ecological zones of Punjab province i.e. Central Punjab, Southern Punjab and Northern Punjab. The local landraces were collected during the year 2001 from farmer fields. Pure lines were developed by inbreeding for five consecutive years from these landraces. These 44 accessions were evaluated for drought tolerance at seedling and post-anthesis stages under rain-fed environment.

Pot experiment and seedling traits

Water deficit conditions at seedling stage were achieved by watering the plants with quantity of water 50% of normal conditions (Khan et al., 2004). Fifteen seeds per genotype were grown in trays (20 cm × 20 cm with 10 cm depth) filled with river sand by keeping row to row and plant to plant distance of 5 cm and 3 cm respectively. Triplicate Randomized Complete Block Design with 5 seeds per replication was used. 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 from ten plants.

Weather and soil

The meteorological data during both the growing seasons of 2007 and 2008 showed nearly similar patterns of rainfall (mm), temperature (ºC) and relative humidity (%); however elevation in pan evaporation (mm) revealed that in the second year there was more stress as compared to first one (Fig 1). The outline of Fig 1 clearly demonstrated that at post-anthesis stage, significant drought stress due to lower rainfall as well as high rate of evaporation affected the growth of sorghum plants. Before recording the data at post-anthesis stage, the maximum average water holding capacity of the sandy loam soil was 37% of soil dry weight and the permanent wilting point was 12%. Data from soil analysis also hinted towards the limited amount of water available in the soil.

Field experiment and flag leaf related morpho-physiological traits

The sorghum genotypes were planted on July 10, 2007 and July 4, 2008 with experimental plots comprising of two rows, each 4 m long and 30 cm apart in 3 replications. At post-anthesis stage, in both the years, flag leaves of 10 randomly selected plants were used for measurement of various physiological parameters. Flag leaf area (FLA) of 10 randomly selected plants from each replication was measured during early morning hours (h) when leaves were fully turgid. FLA (cm^2) was measured by using leaf area meter (LI-3000/Lambda Instr. Corp. Lincolin, Nebraska, USA). The leaves were oven dried at 80 ºC for 48 h and specific flag leaf area (SFLA) was calculated as a ratio of FLA to the oven dry weight (DW) of the leaves. The specific flag leaf weight (SFLW) was calculated as SFLW = DWF/FLA. All the weights were measured in grams. For determination of excised leaf weight loss (ELWL), 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, also known as cuticlar transpiration, or the rate of water transpired at minimum stomatal aperture in total water limitation) was measured according to Clarke et al. (1991). For this purpose, leaves were excised and immediately brought to the laboratory and were placed in darkness for stomatal closure for 30 minutes under ambient room conditions. After 30 minutes the leaves were weighed (W_1 in g) and were placed at room temperature in the laboratory for 180 min, the leaves were again weighed (W_2 in g) along with measurement of leaf area. Residual transpiration on leaf area basis (g $H_2O/min/cm^2/10^5$) was determined as given below; RT = $(W_1 - W_2)/(LA.180)$. Relative water contents (RWC) were determined for detached leaves. The relative water contents (RWC) were calculated from flag leaf blades using the method devised by Mata & Lamattina (2001) with help of the following equation; RWC

Table 2. Simple coefficients of correlation among morph-physiological traits under drought stress in sorghum

	FRW	*FSW*	*DRW*	*DSW*	*RL*	*CL*	*SL*	*RL:SL*	*FLA*	*SFLA*
FRW	1.00									
FSW	0.42**	1.00								
DRW	0.01	-0.14	1.00							
DSW	0.45**	0.69**	0.26	1.00						
RL	-0.16	-0.35*	-0.19	-0.27	1.00					
CL	0.04	-0.07	0.11	-0.09	0.20	1.00				
SL	0.01	0.15	-0.06	0.24	-0.22	-0.12	1.00			
RL:SL	-0.14	-0.34*	-0.15	-0.31*	0.93**	0.21	-0.50**	1.00		
FLA	0.20	0.08	-0.16	0.10	0.30*	-0.16	0.02	0.27	1.00	
SFLA	0.00	0.20	-0.32*	0.03	0.19	-0.18	0.11	0.11	0.48**	1.00
SFLW	-0.21	0.05	0.28	0.07	-0.23	0.14	-0.04	-0.18	-0.53**	-0.62**
ELWL	-0.20	-0.06	0.28	0.08	-0.03	0.23	0.14	-0.10	-0.50**	-0.30*
LDM	0.20	0.04	-0.19	0.03	0.20	-0.27	-0.17	0.27	0.66**	0.25
RDW	0.22	0.01	0.14	0.01	0.04	-0.05	-0.11	0.06	-0.07	0.08
RWC	0.28	0.40**	0.03	0.29	-0.23	0.06	0.03	-0.19	0.13	-0.04
CMS	-0.18	0.22	0.11	0.11	-0.27	-0.09	0.06	-0.22	0.08	0.05
RT	0.10	-0.14	0.27	0.15	0.24	0.18	0.06	0.12	-0.13	-0.08
HL	0.14	0.09	-0.03	-0.01	0.01	-0.17	-0.38*	0.14	0.02	0.06
H WD	-0.35*	-0.31*	0.14	-0.36*	0.09	0.10	-0.37*	0.19	-0.02	-0.09
HW	-0.30*	-0.36*	0.18	-0.41**	-0.05	0.07	-0.38*	0.07	-0.28	-0.21
GY	-0.28	-0.38*	0.12	-0.44**	-0.05	0.04	-0.40**	0.08	-0.27	-0.25

	ELWL	*LDM*	*RDW*	*RWC*	*CMS*	*RT*	*HL*	*H WD*	*HW*	*GY*
ELWL	1.00									
LDM	-0.60**	1.00								
RDW	-0.03	0.04	1.00							
RWC	0.01	0.19	0.15	1.00						
CMS	0.11	0.16	-0.02	0.52**	1.00					
RT	0.46**	-0.25	0.12	-0.32*	-0.32*	1.00				
HL	-0.28	0.28	0.50**	0.11	-0.03	-0.06	1.00			
H WD	-0.10	-0.02	-0.22	-0.26	0.05	-0.19	0.01	1.00		
HW	0.00	-0.14	-0.12	-0.26	-0.05	-0.06	0.12	0.85**	1.00	
GY	-0.03	-0.09	-0.10	-0.25	-0.06	-0.06	0.14	0.80**	0.98**	1.00

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-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; HL- Head length; H WD- Head width; HW- Head weight and GY-Grain yield per plant, * = Significant at p≥5%, ** = Significant at p≥1%

(%) = (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 h at 4 ºC in darkness, and the dry weight (DW) after oven drying at 80 ºC during 48 h. Leaf dry matter (LDM) was determined by taking the average of dry weight in ELWL and dry weight in RWC. The relative dry weight of the leaves (RDW) was calculated following using the formula; RDW = DW/ (TW-DW). Cell membrane stability (CMS) was determined on the basis of amount of electrolyte leakage from the leaf cells following Ali et al. (2009a & b). In this procedure, 0.4 g of plant leaf material (0.5 cm diameter leaf discs) was washed with double distilled deionized water and placed in tubes with 20 ml of water. The material was incubated for 2 h at 25 °C. Subsequently, the electrolyte leakage in terms of electrical conductivity (L_1) of the solution containing leaf material was determined using conductivity meter (Model 145 A+, Thermo Electron USA). Samples were then autoclaved at 120 °C for 20 min and the final electrical conductivity (L_2) was measured after temperature equilibrium at 25°C. The CMS was the mean percentage of five leaf sample and was calculated as follows; CMS (%) = $[(1-(L_1/L_2)] \times 100$. At maturity heads from ten randomly selected plants were detached and placed in sunlight for 5 days. After 5 days data were recorded for head length (HL) in cm, and those of head width (HWD) and head weight (HW) in g. The heads were threshed to determine grain yield per plant (GY) in g.

Statistical analysis

The average data of both the years were subjected to basic statistics, correlation analysis, cluster analysis and principal component analysis (PCA) using statistical software packages of SPSS version 12 and STATISTICA version 5.0 (Sneath and Sokal, 1973). Cluster analysis was performed using K-means clustering while tree diagram based on elucidation distances was developed by Ward's method. The D^2 statistics was calculated according to Mahalnobis (1936) and Rao (1952). First two principal components were plotted against each other to find out the patterns of variability among genotypes and association between different clusters using SPSS version 12.

Results

Patterns of character correlation

The basic statistics of various morpho-physiological traits (data not shown) demonstrated considerable range and variability among 44 sorghum genotypes. Simple correlation

Table 3. Principle component analysis of various morpho-physiological traits in sorghum under water stress at different growth stages

	PC I	PC II	PC III	PC IV	PC V	PC VI	PC VII
Eigen value	4.248	3.661	2.457	1.915	1.655	1.269	1.101
% of total variance	20.230	17.435	11.698	9.121	7.882	6.045	5.241
Cumulative variance %	20.230	37.665	49.363	58.484	66.366	72.411	77.653
Factor loading by various traits							
Fresh root weight	0.532	0.053	-0.003	0.422	-0.276	0.381	-0.266
Fresh shoot weight	0.671	-0.188	0.258	0.115	-0.041	0.243	-0.227
Dry root weight	-0.161	-0.425	0.008	0.367	-0.061	0.293	0.582
Dry shoot weight	0.647	-0.301	0.015	0.222	-0.101	0.438	0.161
Root length	-0.246	0.582	-0.565	0.116	0.401	0.034	0.039
Coleoptile length	-0.219	-0.167	-0.328	0.195	0.296	0.332	-0.399
Shoot length	0.444	-0.312	-0.183	-0.534	-0.157	-0.089	0.180
Root/shoot length ratio	-0.340	0.618	-0.401	0.270	0.437	0.051	-0.038
Flag leaf area	0.376	0.679	0.074	-0.124	0.195	0.313	0.282
Specific flag leaf area	0.339	0.543	-0.025	-0.262	-0.089	-0.061	0.200
Specific flag leaf weigh	-0.201	-0.740	0.117	0.151	0.446	-0.073	-0.029
Excise leaf weight loss	-0.181	-0.702	-0.409	0.072	0.216	-0.084	0.146
Leaf dry matter	0.273	0.665	0.325	0.111	0.202	0.110	0.190
Relative dry weight	0.151	0.099	-0.044	0.651	-0.193	-0.486	0.152
Relative water content	0.468	-0.193	0.412	0.283	0.486	-0.027	-0.109
Cell membrane stability	0.189	-0.245	0.532	-0.047	0.567	-0.126	0.312
Residual transpiration	-0.089	-0.188	-0.687	0.266	-0.211	0.168	0.316
Head length	0.015	0.323	0.286	0.645	-0.232	-0.338	-0.005
Head width	-0.755	0.148	0.395	-0.072	-0.003	0.306	0.091
Head weight	-0.837	-0.007	0.391	0.051	-0.194	0.180	0.032
Grain yield per plant	-0.829	0.014	0.399	0.070	-0.190	0.133	0.001

PC= Principal component

Table 4. Cluster analysis of different morpho-physiological traits in sorghum under water stress at different growth stages along with D^2 statistics among four clusters

	Cluster 1		Cluster 2		Cluster 3		Cluster 4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
FRW	0.372	0.107	0.348	0.080	0.309	0.080	0.333	0.113
FSW	2.780	1.202	3.538	0.845	2.862	1.146	3.445	1.254
DRW	0.063	0.047	0.042	0.040	0.056	0.046	0.050	0.041
DSW	0.533	0.214	0.569	0.129	0.471	0.188	0.517	0.138
RL	4.053	1.807	3.916	1.129	3.247	0.908	3.352	0.907
CL	1.886	0.355	1.687	0.164	1.796	0.360	1.949	0.558
SL	16.470	2.437	18.106	1.754	16.420	1.617	17.784	2.403
RL:SL	0.259	0.159	0.221	0.075	0.199	0.062	0.193	0.060
FLA	85.835	9.585	97.706	13.849	59.530	6.028	55.425	8.367
SFLA	31.665	5.830	62.307	18.362	31.180	5.054	34.479	7.377
SFLW	0.032	0.008	0.019	0.008	0.037	0.007	0.040	0.017
ELWL	15.851	4.446	12.809	2.471	18.950	6.563	24.346	11.426
LDM	0.602	0.044	0.610	0.046	0.549	0.036	0.522	0.059
RDW	0.467	0.052	0.506	0.156	0.465	0.091	0.485	0.042
RWC	47.632	9.521	51.372	7.877	44.001	8.988	51.249	13.269
CMS	60.419	8.717	64.210	9.459	60.282	8.423	62.698	8.417
RT	0.009	0.004	0.0091	0.0021	0.010	0.004	0.0088	0.0038
HL	16.913	3.576	18.432	8.768	19.911	7.484	15.304	2.764
H WD	4.440	0.749	4.088	0.769	4.942	0.708	3.867	0.392
HW	18.421	7.323	15.371	6.027	30.210	6.146	15.890	4.055
GY	13.753	6.527	11.282	4.905	23.482	4.756	11.892	2.934

D^2 statistics among different clusters

	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Cluster 1	0.000			
Cluster 2	54.220	0.000		
Cluster 3	45.643	138.547	0.000	
Cluster 4	49.459	128.978	22.821	0.000

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-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 contents; RT-Residual transpiration; CMS-Cell membrane stability; HL-Head length; H WD- Head width; HW- Head weight and GY-Grain yield per plant.

Table 5. Germplasm accessions related to various clusters based on various morpho-physiological traits

Clusters	No. of accessions	Accessions
Cluster 1	12	BMR-14, Chakwal sorghum, FJSS-1, FJSS-4, H-18, S-58, YSS-10, YSS-18, YSS-3, YSS-8, YSS-9 and YSS-98
Cluster 2	10	FJSS-11, FJSS-15, FJSS-16, FJSS-17, FJSS-20, FJSS-3, FJSS-8, G-160, PARC-SS-1 and YSS-2
Cluster 3	11	FJSS-10, FJSS-21, FJSS-6, H-118, PGRI-141, PGRI-191, PGRI-35, S-149, YSS-1, YSS-5, and YSS-6
Cluster 4	11	FJSS-2, FJSS-5, FJSS-7, FJSS-9, JS-2002, GS-61, PGRI-29, S-1563, S-171, YSS-7 and YSS-4

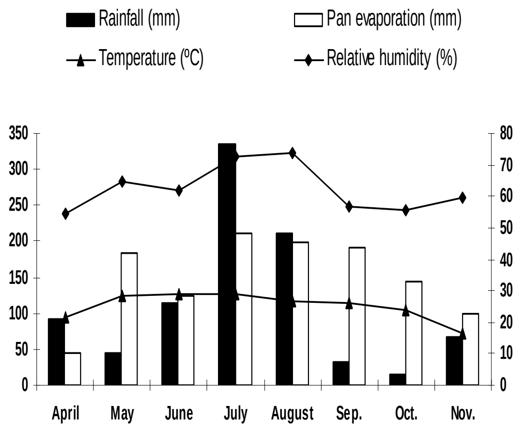
coefficients among 21 traits revealed a lot of significant and practical associations (Table II). Fresh root weight showed positive correlation with fresh and dry shoot weight while negative association with head width and head weight. Similarly, fresh shoot weight displayed significant positive relationship with dry shoot weight and relative water content where as negative one with root length, root/shoot ratio, head width, head weight and grain yield per plant. There was a negative association between dry root weight and specific flag leaf area. Likewise heavier dry shoots resulted in lower values of root/shoot ratio, head width, head weight and grain yield. However, lengthy roots caused elevated root shoot ratios and flag leaf area. Coleoptile length, shoot length and root/shoot ratio did not show any significant correlation with all the leaf related traits. Shoot length was negatively correlated with root/shoot ratio, head length, width and weight along with grain yield. Both flag leaf area as well as specific flag leaf area demonstrated negative correlation with specific flag leaf weight and excised leaf weight loss, however, flag leaf area revealed positive relationship with specific flag leaf area and leaf dry matter. Excised leaf weight loss was positively associated with residual transpiration while negatively correlated with leaf dry matter. High value of relative dry weight resulted in longer heads due to positive and significant correlation coefficient. There was positive correlation between relative water contents and cell membrane stability while both these traits revealed negative association with residual transpiration. Among the yield components, head width, head weight and grain yield per plant were positive and strongly inter-correlated with each other.

Principal component analysis

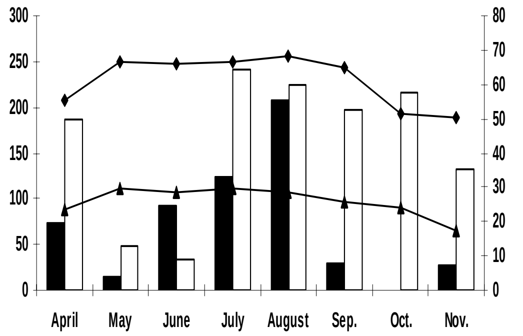
In this study, out of total 21, seven principal components (PCs) were extracted having Eigen value >1. These seven PCs contributed 77.653% of the total variability amongst the sorghum genotypes assessed for various morph-physiological traits (Table III). However, the remaining 14 components contributed only 22.347% towards the total morph-physiological diversity for this set of sorghum genotypes. The PC I contributed maximum towards the variability (20.23%) followed by PC II (17.435%), PC III (11.698%), PC IV (9.121%), PC V (7.882%), PC VI (6.045%) and PC VII (5.241%) respectively. The PC I was due to variations among the accessions mainly for yield attributes like head width, head weight and grain yield per plant. However, most of the seedling traits like fresh root and shoot weight, dry shoot weight and shoot length along with relative water contents had considerable positive factor loadings on PC I. The 2nd PC was related to diversity among sorghum genotypes due to specific leaf weight, excised leaf weight loss, root length and root/shoot ratios, flag leaf area and leaf dry matter. The PC III was explained by variation among genotypes due to residual transpiration, cell membrane stability and root length. Similarly PC IV was explicated by variation in relative dry weight and head length with their considerable positive factor loadings and shoot length with negative loadings. The PC V was elucidated by diversity among the genotypes for cell membrane stability with some considerable loadings of relative water contents and specific leaf weight. Similarly, dry shoot weight had the considerable positive weight on PC VI while PC VII was associated with dry root weight.

Cluster analysis

Forty four sorghum genotypes were grouped into 4 clusters based on various morph-physiological traits under water stress imposed on seedling stage and natural incidence of drought stress at post-anthesis stage of plant growth (Table IV). Cluster analysis showed that cluster 1 comprised of 12 genotypes, cluster 2 of 10 while both cluster 3 and 4 contained 11 genotypes (Table V). The genotypes in cluster 1 demonstrated heavier fresh root along with heavier dry roots and shoots. The members of 1st cluster were also associated with lengthy roots; higher root/shoot ratio. Similarly, the 2nd cluster comprised of genotypes with lengthy shoots at seedling stage, more flag leaf area, specific flag leaf area, lower specific flag leaf weight, high leaf dry matter and the lowest excised leaf weight loss, high relative water contents, more stable leaf cell membranes but with lower grain yield under water stress at post-anthesis stage (Table IV). The members of 3rd cluster were characterized by the lowest fresh root and shoot weights, lighter dry shoots, shorter roots as well as shoots and the least values for relative dry weight, relative water contents and cell membrane stability. On the other hand this cluster included genotypes with the highest values for grain yield and its attributes like head length, width and weight. The longest coleoptiles and the highest specific leaf weight and excised leaf weight loss were the characteristic features of fourth cluster along with the lowest values for residual transpiration, head length and head width. The pairwise Mahalanobis distances (D^2 statistics) among four clusters of 44 sorghum genotypes (Table IV) revealed that genotypes of cluster 2 showed maximum diversity against the members of cluster 3 followed by cluster 4 for most of the studied characters under drought stress at seedling as well as post-anthesis stage. On the contrary, minimum differences were found between the members of cluster 4 and cluster 5 due to the least value of D^2 statistics. The first two principal components contributing almost 38% towards the total variance were plotted to observe the relationships between different clusters with PC I on X-axis and PC II on Y-axis (Fig 2). Not a single cluster showed obvious separation. The tree diagram showed more or less similar results comprising of two main groups A and B each of which is further subdivided into two clusters (Fig 3).



(A)



(B)

Fig 1. Meteorological data regarding rainfall (mm), temperature (ºC), relative humidity (%) and pan evaporation (mm) during both sorghum growing seasons: a) 2007 and b) 2008

Discussion

The information about significant 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., 2009c). The results of correlation analysis revealed some important associations among the root traits as well as between the root and leaf related characters. Interestingly, most of the root characters demonstrated negative correlation with yield related traits which was opposite to the previous findings of Turner (1986) who found positive correlations between seed yield and root development in cereals, especially in barley, wheat and sorghum. However, Matsuura et al. (1996) reported a positive relation between drought tolerance and root length in sorghum and millet. Among the leaf traits, relative water contents displayed positive correlation with cell membrane stability while both of these traits revealed negative association with residual transpiration. This exposed a useful correlation because selection for elevated relative water contents and stable membranes could be carried out along with lower residual transpiration which also has positive association with excised leaf weight loss (Ali et al., 2009a).

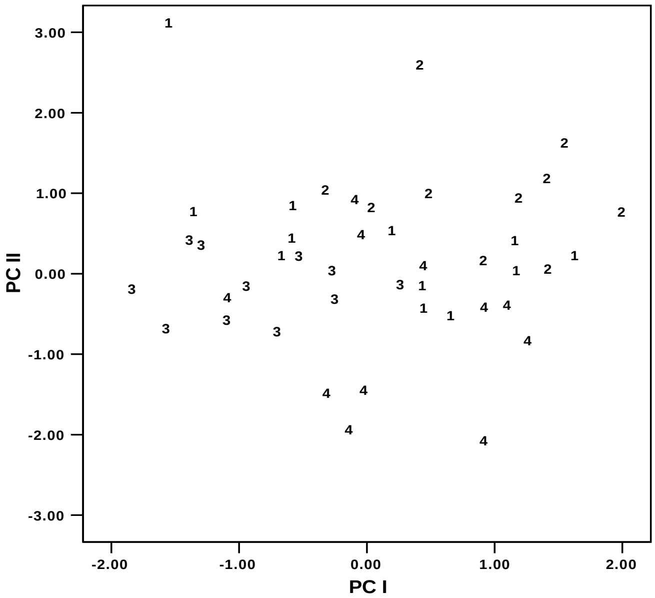


Fig 2. Scatter plot of wheat genotypes for first two Principal components contributing 37.43% to the total variability. The digits 1, 2, 3 and 4 indicate cluster number. None of the cluster displayed clear separation that revealed some degree of association between different clusters.

This suggested that these characters could be selected simultaneously with their positive effects on drought tolerance in different stages of crop growth in sorghum. Farooq and Azam (2002) also found a positive correlation of cell membrane stability and relative water contents among bread wheat (*Triticum aestivum* L.) drought stress, however, Dhanda and Sethi (2002) reported opposite results. All the flag leaf associated morph-physiological traits demonstrated no positive correlation with grain yield that might lead the breeders to evolve cultivars with reduced yield under stress condition (Fischer and Wood, 1979; Karamanos and Papatheohari, 1999). The positive correlation among the yield contributing traits like head width, weight and grain yield per plant suggested that these characters are important for direct selection of high yielding genotypes. Aruna and Audilakshm (2008) also reported the importance of these traits for selection of high yielding genotypes in sorghum. The partitioning of total variance into its components aids for conservation and practical exploitation of genetic resources. It also facilitates planning for utilization of appropriate germplasms in crop improvement for particular plant characters (Sneath and Sokal, 1973; Pecetti et al., 1996). Principal component (PC) analysis splits the total variance into different factors. In this experiment, the PC analysis divided the total variance into 7 PCs out of which first 4 PCs contributing main amout of diversity among the genotypes due to different characters studied. Ayana & Bekele (1999), Chozin (2007) and Mujaju and Chakuya (2008) working on different agro-morphological traits in sorghum also reported significance of first PCs in contributions towards the total variability. In our experiment, 1st PC was mainly due to variations in yield components and root traits but highest negative loadings of head width, head weight and grain yield per plant on PC I also suggested negative correlation of these yield contributing traits with fresh root and shoot weight, dry shoot weight, shoot length and relative water contents. This is also in agreement with the results of correlation analysis which showed negative association between yield compone-

Figure 3: Tree diagram for 44 sorghum genotypes based on 21 traits

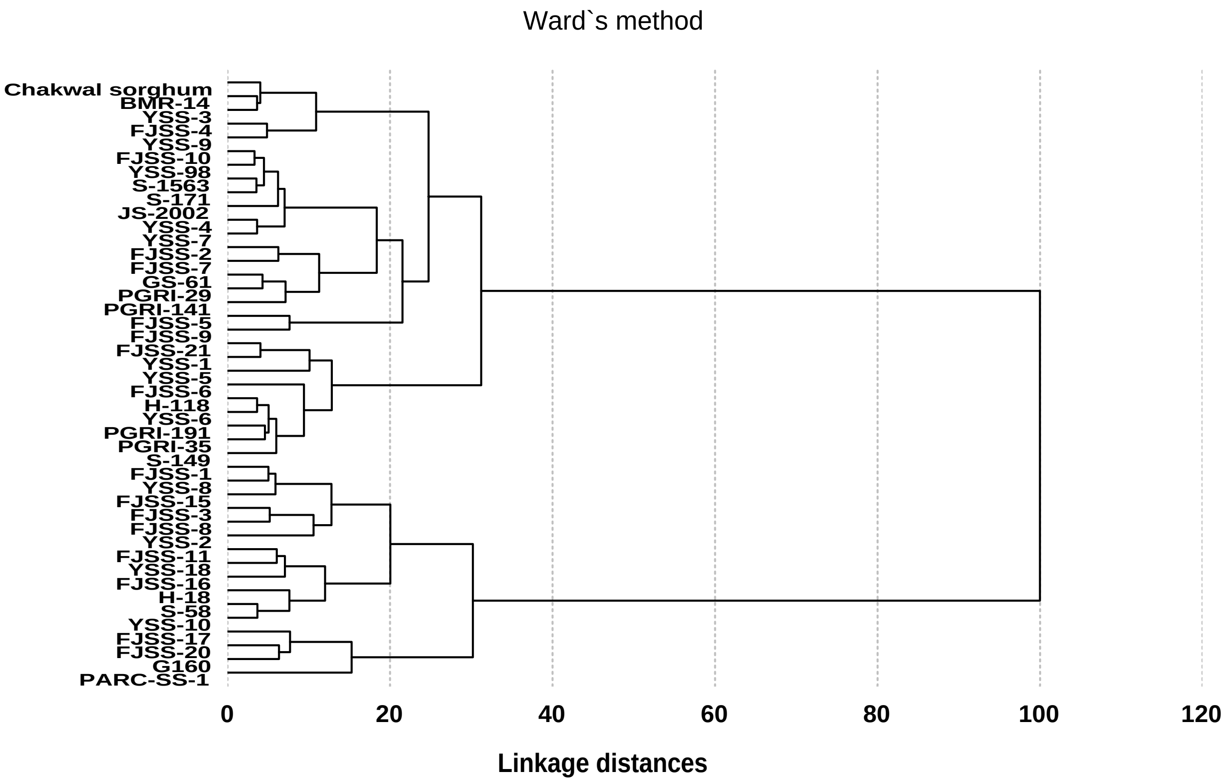


Fig 3. Tree diagram for sorghum genotypes for 21 morpho-physiological characters recorded under drought conditions.

nts and the root character mentioned above. In contrast to our results, Brock and Galen (2005) reported that variations in the 1st PC was mainly due transpiration rate and relative water content of leaves in *Taraxacum ceratophorum* and *Taraxacum officinale* under drought stress. Specific leaf weight, excised leaf weight loss, root length and root/shoot ratios, flag leaf area and leaf dry matter were the main contributors of diversity in the PC II. However, Chozin found that variation in PC I was explained by leaf area while in PC II, it was due to dry root and leaf weight. The results from PC III and VI revealed that relative dry weight and head length are negatively associated with shoot length for this set of genotypes. PC analysis ultimately demonstrated the amount of diversity for the characters among the material in hand which could be exploited to execute a breeding program aimed at improving water stress tolerance as it is generally assumed that maximum variability yields maximum heterosis. The cluster analysis also substantiated considerable amount of morpho-physiological diversity for this group of genotypes under drought stress at various growth phases. The genotypes in 1st cluster were characterized by heavier fresh root along with heavier dry roots and shoots along with lengthy roots; higher root/shoot ratio and increased leaf dry matter. This revealed that this group of genotypes could be exploited for improvement in drought tolerance at seedling stage because selection of suitable genotypes on the basis of seedling traits has been considered a reliable technique for evaluating a large number of genotypes for drought tolerance (Dhanda et al., 2004; Khan et al., 2004; Ali et al., 2009a & b). Similarly, the 2nd cluster grouped most of the genotypes having drought tolerance at post-anthesis stage based on almost all the flag leaf related morpho-physiological characters. Hence the members of this group could be utilized as vital stuff for breeding sorghum genotypes with water stress tolerance at terminal growth stages like post-anthesis but this breeding program should include some high yielding genotypes to compensate the lower yield of drought tolerant lines. Fischer and Wood (1979) and Karamanos and Papatheohari (1999) reported that genotypes with significant drought contributing traits were lower in grain yield. First two clusters contained 9 local land races (with the name FJSS) out of 22 genotypes which suggested great scope of their utilization for improving drought tolerance. Brush (1999) and Ali et al. (2009b) also reported the importance of local land races for the improvement of crop plants against water stress. Although a massive amount of genetic diversity for yield contributing traits exists in the sorghum germplasm (Aruna and Audilakshm, 2008), but the natural character combinations in the landraces are largely unfavorable due to negative or non-significant correlation of yield with drought tolerance (Brush, 1999). The results of cluster 3 also confirmed the reports of Fischer and Wood (1979) and Karamanos and Papatheohari (1999) regarding negative correlation of grain yield with morpho-physiological characters contributing towards drought tolerance in bread wheat (*Trificum aestivum* L.), durum wheat (*T. turgidum* L.), triticale (*X. Tritosecale* Wittmack), barley (*Hordeum vulgare* L) and faba bean (*Vicia faba* L.). This suggested that a hybridization of genotypes of cluster 2 and cluster 3 would be the better option to balance the lower yield in cluster 2 and improvement of drought tolerance among the members of cluster 3. The longest coleoptiles and reduced residual transpiration were the features of members of 4th cluster and made these genotypes an asset to be used for improvement against drought stress in sorghum. Nevertheless, on the basis of the cluster analysis, it can be argued that sorghum dehydration avoidance could be linked with such an ideotype: long roots and coleoptiles, high root/shoot ratios, wider flag leaf area, high relative water contents, decreasing excised leaf weight loss and residual transpiration in combination with stable leaf cell membranes. Franca et al. (1998) carried out diversity analysis in rye grass under drought conditions and found that drought tolerance in this forage crop was associated with wide flag leaf, high seed production capability even with late flowering. However, Ayana and Bekele (1999) and Assefa et al. (1999) reported enormous diversity for morphological traits in sorghum and tef (*Eragrostis tef* (Zucc.) germplasm which showed that implication of that diversity could be exploited in the genetic improvement of the crop through hybridization and selection. The pairwise Mahalanobis distances (D^2 statistics) among four clusters revealed that genotypes of showed maximum distance between cluster 2 and 3 which suggested that the member of cluster 2 and 3 are contrasting for these characters and could be utilized in breeding program to develop transgressive segregants with a wide range of adaptability to water stress conditions in semi-arid regions. The scatter plot diagram showed association between different clusters which could be due to the mixture of genotypes with different degree of adaptation against water stress based on various morpho-physiological traits or some sort of homozygosity among the genotypes. However, cluster analysis assembled most of the genotypes together showing considerable tolerance against drought stress on the basis of characters studied here at both early as well as terminal growth stages. Amurrio et al. (1995) and Rabbani et al. (1998) also reported lack of association between various clusters based on agronomic traits and origins of genotype in peas (*Pisum sativum*) and mustard (*Brassica juncea*) respectively. The tree diagram comprised of four groups. The member of upper two clusters included most of the genotypes having considerable tolerance against drought stress. The occurrence of this obvious and wide differentiation between the clusters emerges to be of great genetic value in providing materials aimed at sorghum selection for adaptation to water stress conditions in rain-fed areas.

Conclusion

The results concluded positive correlation of root length, root/shoot ratio, leaf area, leaf dry matter, leaf water contents, cell membranes stability between each other and negative relationship of most of these traits with residual transpiration and excised leaf weight loss suggested considerable prospects for improvement in drought tolerance. PC analysis summarized most of the diversity among genotypes into 7 components with root and head traits being the main contributors towards diversity in PCI. Likewise clustering of drought tolerant (cluster 2) and drought susceptible (cluster 3) genotypes proposed the use of member of these clusters as parents to build up populations for selection of transgressive segregants against drought stress in subsequent generations and for the development of quantitative trait loci (QTLs) related with drought tolerance. The results suggested that utilization of genetic variability for various morpho-physiological markers contributing towards drought tolerance available in sorghum would be important for cultivar development with considerable drought tolerance at early and terminal growth stages.

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