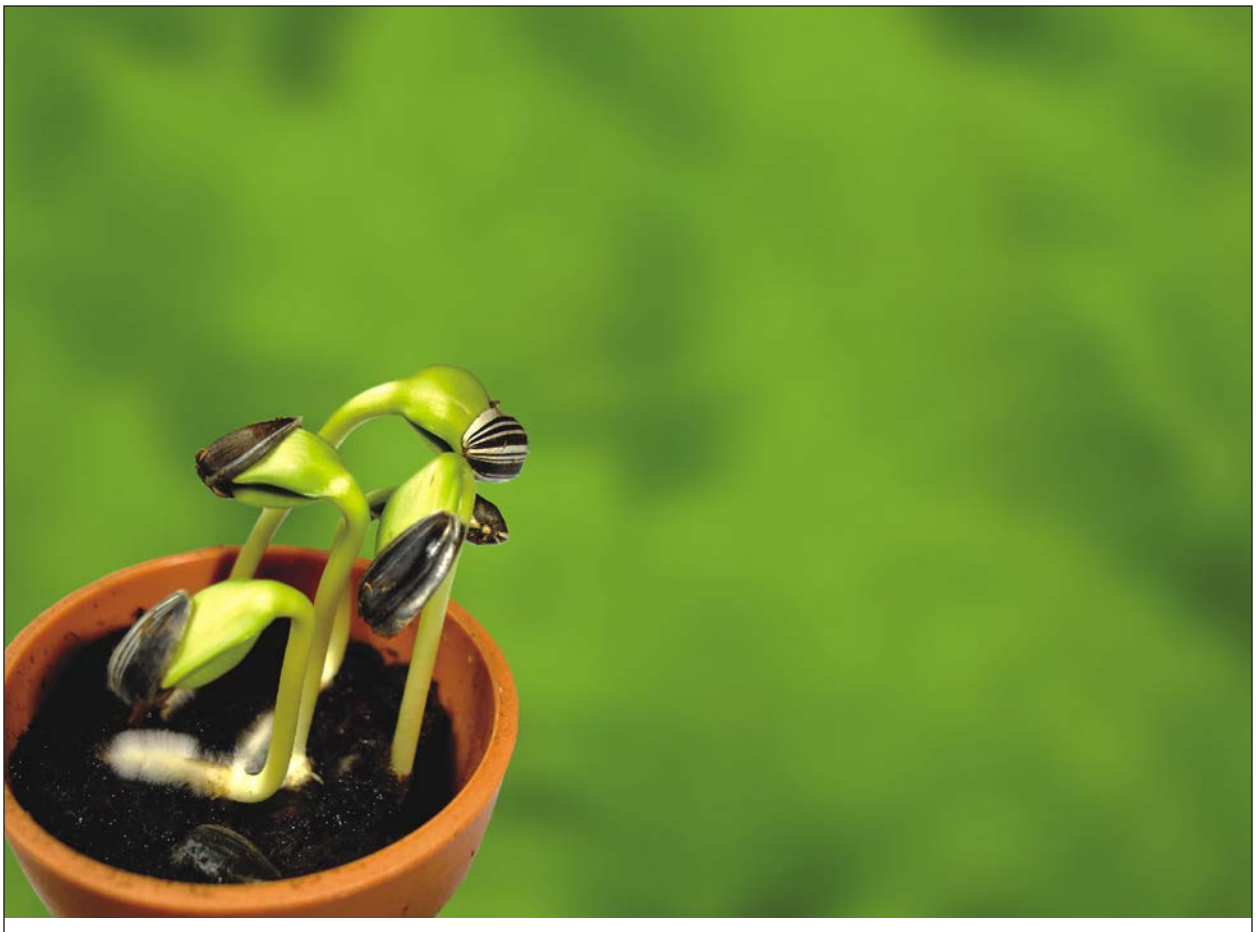

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Research Article
Drought Tolerance Assessment in Grain Sorghum (*Sorghum bicolor* [L.] Moench) Genotypes Using Agro-morphological Traits and DNA Markers

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Abstract

Background: The traditional crop varieties and modern cultivars grown by farmers are important source for food security and support the livelihoods of the majority in Africa. Sorghum improvement can be achieved by the utilization of the wide range of diversity existing in sorghum, which can lead to improvement of people's sustainable livelihoods, agricultural productivity and economic development. **Methodology:** The performance of 19 grain sorghum (*Sorghum bicolor* L.) genotypes was assessed under water stress conditions using agro morphological and Inter Simple Sequence Repeats (ISSRs) markers. Two field experiments were executed during the summers of 2012 and 2013 at Shambat Experimental Farm, Sudan. A split-plot design with three replications were used to layout the field experiment. The sorghum genotypes were evaluated in the field under normal irrigation and water stress conditions. Data was collected to determine genotypic variability among the grain sorghum genotypes and to estimate the phenotypic correlation coefficients between different traits. In addition, the genetic diversity of the genotypes was determined by ISSRs. **Results:** The results showed that, drought stress caused significant reduction in most of the studied characters. Significant differences among the genotypes were found for all characters studied. The sorghum genotypes showed differential yield response to drought stress. High grain yield (t ha^{-1}) was exhibited by genotypes HSD7511 and HSD8849 which gave 2.3 and 3.7 t ha^{-1}, respectively. Grain yield (t ha^{-1}) was significantly and positively correlated with grain yield per plant and other yield components. However, it had no significance and negative association with plant height and days to maturity. Based on DNA markers analysis (ISSR), high level of polymorphism was detected among the 19 sorghum genotypes. **Conclusion:** From the 19 grain sorghum, accessions HSD7511 and HSD8849 gave the highest yield under drought stress. Drought stress affected significantly yield and yield components than the vegetative growth. The ISSR technique was efficient in determining the genetic diversity among the 19 sorghum genotypes and thus can be exploited further to establish consistent heterotic group between sorghum genotypes.

Key words: Sorghum, drought, agro-morphological, genetic diversity, ISSR markers

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sorghum (*Sorghum bicolor* [L.] Moench) is one of the most important cereal crops in the world especially in arid and semi arid regions. It can be grown in the environments, where other cereal crops cannot be grown successfully[1,2]. In Africa, especially in Sudan, sorghum is the most important staple cereal crop for more than 500 million people in more than 30 countries worldwide[3]. Landraces from Sudan has been extensively used in sorghum breeding programs worldwide[3,4]. Average yield per unit area in the Sudan is very low (540 kg ha^{-1}) compared to the world average (1300 kg ha^{-1})[5]. However, genetic variation among these landraces makes them important resources as potential donor of genes for development of new varieties used by farmer[2,6,7]. Morphological traits were earliest markers used to study genetic diversity in germplasm[2] but they have some limitations such as low polymorphism, low heritability, late expression and influences by environmental conditions[2,8]. Molecular markers are not influenced by environmental conditions for this reasons these markers are decisive and more efficient for selection in breeding programs as well as to assess genetic diversity amongst[2,9]. The objectives of this study were to assess 19 grain sorghum genotypes under drought stress conditions using agro-morphological traits, also to study their genetic diversity using Inter Simple Sequence Repeats (ISSRs) technique.

MATERIALS AND METHODS

Seed material: Nineteen grain sorghum genotypes obtained from the GenBank of the Agricultural Plant Genetic Resources Conservation and Research Centre, Agricultural Research Corporation, Sudan (Table 1). Seeds of all genotypes were sown at the experimental farm, Sudan University of Science and Technology, College of Agricultural Studies, Shambat. The leaf samples of sorghum genotypes were collected into labeled bags and stored in dry ice until DNA extraction was done.

DNA extraction: The DNA was extracted from fresh leaf tissue of the 19 sorghum genotypes using modified CTAB method[10]. The modification was made in intention to improve the DNA quantity and the quality. In this method, the fine powdered plant materials were immediately transferred into 15 mL falcon tubes containing 5 mL of pre-warmed lysis solution. Tubes containing the samples were then incubated in a water bath at 60°C with gentle shaking for 60 min and left to cool at room temperature for 10 min. Chloroform: Isoamyl alcohol mixture (24:1) was added to each tube and the phases were mixed gently for 5 min at room temperature to make a homogenous mixture. The cell debris was removed by centrifugation at 4000 rpm for 15 min and the resulted clear aqueous phases (containing DNA) were transferred to new sterile tubes. The step of the chloroform: Isoamyl alcohol extraction was repeated twice. The nucleic acids in the aqueous phase were precipitated by adding equal volume of deep cooled isopropanol. The contents were mixed gently and collected by centrifugation at 4000 rpm for 10 min. The formed DNA pellet was washed twice with 70% ethanol and the ethanol was discarded. The remained ethanol was removed by leaving the pellet to dry at room temperature. The pellet was dissolved in TE buffer (10 mM tris, 1 mM EDTA, pH 8) and stored at -20°C for further use.

Table 1: Sorghum accessions used in the study

Code No.	Accession	Sources
1	HSD7507	Plant Genetic Resource Unit (ARC)
2	HSD7506	Plant Genetic Resource Unit (ARC)
3	HSD7584	Plant Genetic Resource Unit (ARC)
4	HSD7591	Plant Genetic Resource Unit (ARC)
5	HSD7601	Plant Genetic Resource Unit (ARC)
6	HSD7602	Plant Genetic Resource Unit (ARC)
7	HSD7606	Plant Genetic Resource Unit (ARC)
8	HSD7610	Plant Genetic Resource Unit (ARC)
9	HSD7616	Plant Genetic Resource Unit (ARC)
10	HSD8150	Plant Genetic Resource Unit (ARC)
11	HSD8176	Plant Genetic Resource Unit (ARC)
12	HSD8228	Plant Genetic Resource Unit (ARC)
13	HSD8231	Plant Genetic Resource Unit (ARC)
14	HSD7511	Plant Genetic Resource Unit (ARC)
15	HSD8653	Plant Genetic Resource Unit (ARC)
16	HSD8849	Plant Genetic Resource Unit (ARC)
17	HSD9566	Plant Genetic Resource Unit (ARC)
18	Wad Ahmed	Plant Genetic Resource Unit (ARC)
19	Tetron	Plant Genetic Resource Unit (ARC)

DNA quality and quantity: The extracted DNA samples were observed under UV illumination after staining with ethidium bromide and agarose gel electrophoresis. The purity and the concentrations of the DNA were then spectrophotometrically assessed following method[11].

PCR of the ISSR technique: Three ISSR primers were found polymorphic and used in the Polymerase Chain Reaction (PCR) in a final volume of 25 µL containing 1.0 µL DNA diluted, 0.5 µL Taq polymerase, 2.5 µL 10X buffer, 2.5 µL (2 mM μL^{-1}) dNTPs, 1.5 µL (50 mM) $MgCl_2$, 2.0 µL (10 pmol μL^{-1}) ISSR primer and 15 µL ddH_2O. The amplifications were performed in a thermal cycler following the program: 94°C for 5 min, 40 cycles (1 min at 94°C, 1 min at 43°C and 1 min at 72°C) and final elongation of 7 min at 72°C.

DNA visualization: For each reaction, 5 µL of PCR product were mixed with 2 µL of loading dye and 1.4 µL of 1 kbp DNA ladder was loaded, then were electrophoresed using 2% agarose gel at 80 V followed by staining with ethidium bromide. The separated fragments were visualized with an ultraviolet (UV) transilluminator.

ISSR data analysis: The numbers of polymorphic and monomorphic bands were determined for each primer. Genotypes were scored (1) for present band and (0) for absent band and then entered into a data matrix. Percentage of polymorphism was calculated as the following equation:

$$\text{Polymorphism (\%)} = \frac{\text{Polymorphic bands}}{\text{Total No. of bands}} \times 100$$

The tree diagram was produced by clustering the similarity data with the UPGMA method using STATISTCA/SPSS software ver. 9 following the method as used by Hamza[12].

Field experiments: Nineteen sorghum genotypes (Table 1) were evaluated for two successive summer seasons of "2012 and 2013" under two levels of water treatments at Shambat Experimental Farm of the College of Agricultural Studies, Sudan University of Science and Technology (Longitude 32°32'E., latitude 15°40'N and 380 m a.s.l.). Drought stress was induced by applying the following watering regimes: The control treatment (D0) was watered every 7 days throughout the growing seasons and the water stressed treatment during both vegetative and reproductive stages which was watered every 21 days throughout the growing season (D1). The genotypes were evaluated using a split plot arrangements based on a randomized complete block design with three replications. The water regimes were assigned randomly as main plots and the genotypes were grown randomly as subplots, each genotype was shown on two ridges, each of 2 m length. All the cultural practices were done according to the recommendations. Ten randomly selected plants were used to record the data. Different plant characters were measured, which included plant height, stem diameter, No. of leaves, leaf area, plant dry weight, days to 50% flowering, days to maturity, panicle length, yield per plant and yield (t ha^{-1}). Data from each site was subjected to ANOVA separately to detect the significance of genotypic differences[13] before a combined ANOVA. Phenotypic correlation between yield and some vegetative traits were estimated according to the methods described by Burton and Devane[14] and Allard[15].

RESULTS AND DISCUSSION

ISSR markers analysis: Three ISSR primers were used to analyze the 19 investigated sorghum genotypes (Fig. 1). These primers were used to produce 21 polymorphic bands of 26 total amplified bands, with average of 7.0 bands per primer with percentage of polymorphism of 81% percentage of polymorphism. The ISSR primer 848 had the highest percentage of polymorphic bands (90%) (Table 2). These results are in agreement with Turki *et al.*[16] and

Table 2: Polymorphism detected in the 19 *Sorghum bicolor* genotypes by the use of three polymorphic ISSR primers

Primer names	Sequence (5-3)	Total No. of bands	No. of polymorphic bands	No. of monomorphic bands	Polymorphic bands (%)	Monomorphic bands (%)
810	(GA)8T	9	6	3	67	33
848	(CA)8RG	10	9	1	90	10
872	(GATA)4	7	6	1	68	14
Total		26	21	5		
Average					81	19

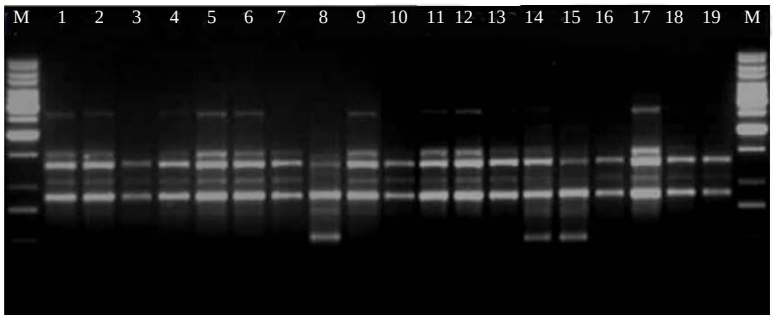

Fig. 1: ISSR amplification patterns of the 19 sorghum genotypes with ISSR primer (872)

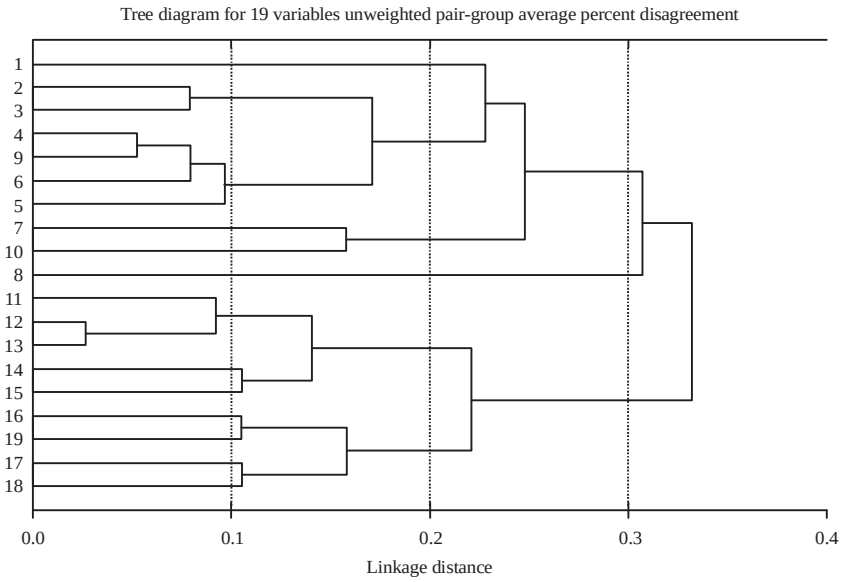

Fig. 2: Clustering diagram resulting from the analysis of ISSR data reflecting the relationship among the 19 sorghum genotypes

Table 3: Genetic distance matrix for 19 grain sorghum genotypes

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	0.00																		
2	0.18	0.00																	
3	0.26	0.08	0.00																
4	0.29	0.16	0.13	0.00															
5	0.18	0.16	0.13	0.11	0.00														
6	0.21	0.18	0.21	0.08	0.08	0.00													
7	0.29	0.21	0.18	0.21	0.16	0.13	0.00												
8	0.34	0.32	0.29	0.32	0.26	0.24	0.26	0.00											
9	0.24	0.21	0.18	0.05	0.11	0.08	0.21	0.32	0.00										
10	0.39	0.32	0.29	0.26	0.26	0.24	0.16	0.42	0.32	0.00									
11	0.26	0.24	0.26	0.24	0.24	0.21	0.24	0.39	0.18	0.39	0.00								
12	0.24	0.26	0.29	0.26	0.26	0.24	0.26	0.37	0.21	0.42	0.08	0.00							
13	0.26	0.29	0.26	0.29	0.29	0.26	0.24	0.34	0.24	0.39	0.11	0.03	0.00						
14	0.34	0.37	0.34	0.32	0.37	0.29	0.32	0.32	0.26	0.47	0.18	0.11	0.08	0.00					
15	0.39	0.37	0.39	0.32	0.37	0.29	0.37	0.37	0.26	0.53	0.13	0.16	0.18	0.11	0.00				
16	0.39	0.42	0.39	0.42	0.37	0.34	0.32	0.37	0.37	0.47	0.18	0.21	0.18	0.21	0.16	0.00			
17	0.34	0.37	0.34	0.42	0.42	0.39	0.37	0.42	0.42	0.42	0.29	0.26	0.24	0.26	0.32	0.21	0.00		
18	0.29	0.32	0.34	0.37	0.32	0.29	0.32	0.37	0.32	0.47	0.18	0.21	0.24	0.26	0.21	0.16	0.11	0.00	
19	0.34	0.37	0.34	0.37	0.32	0.29	0.26	0.26	0.32	0.42	0.18	0.21	0.18	0.21	0.21	0.11	0.16	0.11	0.00

Chakraborty *et al.*[17] who indicated that ISSR markers could be realistically used to evaluate the genetic diversity and differentiation among sorghum genotypes. The similarity coefficient of 19 sorghum genotypes based on ISSR markers ranged from 0.03-0.53. Genotypes: HSD8228 and HSD8231 showed the highest similarity index (0.03), while the genotypes HSD8150 and HSD8653 showed the lowest index (Table 3). The obtained value in this study was slightly less than the values previously obtained by Idris *et al.*[18] who used ISSR markers for assessment the genetic diversity between some of grain maize genotypes. Cluster analysis performed by analysis of all markers generated a dendrogram that separated the genotypes into two distinct clusters. The first cluster was further divided in to sub-groups. The first cluster contained sex genotypes as sisters which are HSD7506 and HSD7584 genotypes: HSD7591 and HSD 7616 together. Also, HSD7606 and 7616 genotypes were closely related (Fig. 2). Moreover, in the second cluster there are four sisters (Fig. 2). These results are in agreement with reports of Chakraborty *et al.*[17] and Turki *et al.*[16] who observed genetic diversity among the grain sorghum genotypes. For this study, it is concluded that, diversity existed among the genotypes of sorghum studied. The information obtained from this study will help the breeders in further sorghum breeding programs.

Table 4: Mean squares from the analysis of variance for different characters in 19 sorghum genotypes (*Sorghum bicolor*) evaluated in seasons 2012/2013 combined

	Season	Stress	Stress × season	Genotype	Genotype × season	Genotype × stress	Season × genotype × stress
D.F	1	1	1	18	18	18	18
Plant height	16747.8**	27999.8**	6.8^{ns}	7671.4**	1198.0**	447.9^{ns}	336.0^{ns}
Stem diameter	489.86**	2.30^{ns}	145.90**	40.00**	16.10**	1.68^{ns}	4.43^{ns}
Number of leaves	1.07^{ns}	5.37^{ns}	2.32^{ns}	32.00**	5.14**	1.89^{ns}	2.81^{ns}
Leaf area	217129.76**	12637.0**	14959.0**	14423.0**	6545.0^{ns}	6633.0^{ns}	4845.0^{ns}
Plant dry weight	2060.6^{ns}	21051.5**	1967.9*	4052.9**	1256.5**	1548.2**	1540.0**
Days to 50% flowering	5115.79**	687.79**	67.44^{ns}	1578.43**	279.54**	31.10^{ns}	31.69^{ns}
Days to maturity	19415.86**	620.07**	904.02**	1069.50**	304.26**	42.75^{ns}	0.428^{ns}
Panicle length	3.50^{ns}	602.12**	0.912^{ns}	156.30**	4.90^{ns}	13.80**	2.47^{ns}
Yield per plant	0.11^{ns}	5102.91**	82.20^{ns}	272.77**	50.55^{ns}	74.82^{ns}	23.06^{ns}
Yield (t ha^{-1})	0.32**	9.08**	0.326^{ns}	0.782**	0.091^{ns}	0.233*	0.065^{ns}

**Significant at the 0.01 level of probability, *Significant at the 0.05 level of probability and ns: None significant at the 0.05 level of probability

Table 5: Means of sorghum genotypes, averaged over two watering treatments (normal (D_0) and water-stress (D_1)) across two seasons (2012 and 2013) at Shambat Experimental Farm

Genotypes	Plant height	Stem diameter	No. of leaves	Leaf area	Bio dry	Days to 50% flowering	Days to maturity	Panicle length	Yield per plant	Yield (t ha^{-1})
HSD7507	146.50	15.50	11.30	374.80	58.535	65.10	88.20	25.32	23.30	1.70
HSD7506	160.90	16.90	11.90	392.60	76.805	71.20	114.50	19.75	15.00	1.10
HSD7584	140.10	17.90	13.70	406.60	75.620	83.80	116.80	13.31	16.40	1.10
HSD7591	138.00	17.30	13.30	412.40	77.440	80.20	108.30	22.68	19.90	1.40
HSD7601	110.10	18.10	13.10	336.60	64.085	69.10	105.00	18.64	26.80	1.90
HSD7602	68.80	19.80	10.90	374.00	67.275	63.50	88.10	17.48	16.80	1.20
HSD7606	107.80	19.30	13.30	392.00	82.950	76.80	100.40	18.37	19.10	1.40
HSD7610	70.50	18.20	11.00	338.00	47.240	61.10	93.40	17.5	25.80	1.80
HSD7616	116.00	17.90	11.00	354.10	77.105	59.80	95.10	19.015	20.70	1.50
HSD8150	104.00	17.80	11.30	381.00	60.785	72.20	100.80	20.94	15.10	1.10
HSD8176	133.90	16.80	11.70	373.20	57.925	65.30	94.90	27.33	26.40	1.90
HSD8228	145.70	19.30	12.40	371.10	82.275	81.20	111.80	15.55	15.60	1.10
HSD8231	114.40	18.00	13.60	438.50	71.180	77.10	108.80	18.385	19.10	1.40
HSD7511	113.10	20.70	13.00	449.00	71.820	74.80	109.50	24.085	32.90	2.30
HSD8653	98.50	19.20	14.90	381.50	79.195	87.70	116.80	20.665	24.00	1.70
HSD8849	124.10	18.90	12.90	406.10	59.635	77.40	104.50	23.945	40.90	2.70
HSD9566	91.60	18.80	13.20	398.40	62.435	83.30	111.10	14.965	20.10	1.40
Wad Ahmed	111.90	18.70	14.30	506.30	70.205	93.80	109.60	19.16	26.50	1.90
Tetron	139.90	16.00	13.60	395.30	70.490	104.30	117.00	20.82	26.00	1.90
Mean	117.67	18.16	12.65	393.76	69.110	76.19	104.98	19.89	22.65	1.61
LSD	31.07	2.69	2.32	109.10	43.980	8.56	11.80	2.15	12.98	0.62

In further efforts for selecting suitable sorghum genotypes for improvement, recently Hamza *et al.*[19] investigated the effect of drought stress on the expression levels of selected miRNAs across 11 elite sorghum accessions of African sorghum, high variation was reported.

Agronomic traits: The combined analysis of variance revealed that significant ($p<0.05$) differences were detected among the grain sorghum genotypes at both seasons. In addition, drought stress had highly significant effect on yield and yield components compared with other vegetative growth for the studied genotypes (Table 4). Similar results were observed by Eldikhery[20], Osmanzai[21] and Vanderlip[22]. The water treatment × genotype interaction revealed significant difference between some characters such as, plant dry weight per plant, panicle length and grain yield (t ha^{-1}) (Table 4). The results indicated some of the genotypes under this study were not stable under drought stress conditions. On the other hand, the analysis of variance showed highly significant differences ($p<0.01$) for all the traits measured among sorghum genotypes (Table 4). The morphological data showed a wide range of flowering among the genotypes (59.8-104.3 days from sowing date) (Table 5). Plant height ranged from 70.5-160.9 cm and grain yield (t ha^{-1}) ranged from 1.1 t ha^{-1} for HSD7506 and HSD7584 to 2.7 t ha^{-1} for HSD8849 (Table 5).

There was positive and significant correlation of grain yield (t ha^{-1}) with leaf area panicle length, thousand seed weight and grain yield per plant (Table 6). The results agreed with the findings of Abdel-Fatah *et al.*[2] who found highly significant and positive difference between panicle length and grain yield. Therefore, selection for these traits can simultaneously improve potential grain yield and accumulate the desirable genes. However, the significant negative

Table 6: Phenotypic correlation between different characters averaged over two watering treatments (normal (D_0) and water-stress (D_1)) and across two seasons (2012 and 2013) at Shambat Experimental Farm

	Plant height	Stem diameter	No. of leaves	Leaf area	Plant dry weight	Days to 50% flowering	Days to maturity	Panicle length	Yield per plant	Yield (t ha^{-1})
Plant height	1.00									
Stem diameter	-0.47*	1.00								
No. of leaves	0.15	0.20	1.00							
Leaf area	0.15	0.12	0.60**	1.00						
Plant dry weight	0.14	0.15	0.62**	1.00**	1.00					
Days to 50% flowering	0.21	-0.02	0.79**	0.65**	0.66**	1.00				
Days to maturity	0.40	0.12	0.79**	0.41*	0.42*	0.71**	1.00			
Panicle length	0.18	-0.28	-0.09	0.34	0.33	-0.03	-0.33	1.00		
Yield per plant	-0.09	0.12	0.10	0.39*	0.89*	0.18	-0.16	0.57**	1.00	
Yield (t ha^{-1})	-0.08	0.15	0.11	0.37*	0.37*	0.15	-0.13	0.57**	0.99**	1.00

**Significant at the 0.01 level and *Significant at the 0.05 level

association of grain yield with flowering is indicative that delay in flowering is correlated with lowest grain yield. Similar conclusion was reported by Ribaut *et al.*[23]. Therefore, sorghum improvement will benefit from this wide range of diversity and genotypes exchange will be the key to the success of improving sorghum genotypes as a source for grain. This study has also strengthened the point that the use of molecular markers is essential and beneficial to plant breeders. The molecular markers were useful to compliment the agronomic data when pedigree information is limited or unavailable. Moreover, based on their positive association with grain yield, the characters, plant height and No. of leaves would be the best selection criteria for sorghum improvement.

CONCLUSION

It can be concluded that, the evaluated 19 sorghum genotypes showed high genetic variability for all of the characters studied. Drought stress had highly significant effect on yield and yield components. The significant and positive correlation for panicle length and grain yield was found in this study can be very useful traits for selection for sorghum breeders in Sudan and in the region. The high variation among the sorghum genotypes was detected by using ISSR markers. Indicating that, the technique was efficient in determining the genetic diversity among sorghum genotypes and thus could be exploited further to establish consistent heterotic group between sorghum genotypes.

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