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Genotypic variability and DNA diversity studies in sorghum (*Sorghum bicolor* L. Moench) varieties under contrasting moisture stress conditions

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ABSTRACT

Root morphology and genetic diversity were studied at vegetative growth stage in 14 Indian and Syrian sorghum varieties under well-watered (WW) and low-moisture stress (LMS) conditions. All the traits showed significant reduction except maximum root length and root to shoot length ratio were significantly increased when LMS was imposed. High heritability and genetic advance as percent of means were recorded for all the traits under WW. Heritability of all traits decreased from WW to LMS. Cluster analysis indicated three main clusters at a similarity level of 20%. The cluster I consisted of all Indian varieties except BP15881-3 genotype. Cluster II consisted of Syrian varieties (Rezenia and Azra3), while BP15881-3 was grouped alone in separate cluster. Varieties with good root system can go for yield evaluation in different locations in Libya for drought prone.

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INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) has a wide range of adaptation from sea level to an altitude of 3000 meters, a range of rainfall regimes (300-1800 mm), day lengths, soil conditions and varying biotic and abiotic factors depending upon the geographies [15]. Globally, Sorghum is the fifth important food and feed crop. It is largely grown for food under rain fed conditions in the semi-arid tropics. In the developed countries, grain Sorghum is a component of animal feeds. The variability in the weather factors, rainfall in particular, during the crop season between regions, locations, years, seasons frequently introduces an uncertainty factor. Rainfall fluctuations, aberrations and catastrophes are not uncommon. Hence, besides adaptation, adaptability and stability of performance assumes importance for successful sorghum cultivation [8].

Drought is the major important constraint on crop production in the world today. Drought tolerance is one of sorghum's most important traits, allowing it to be grown in harsh environments such as Slok region in Libya. Complexity of inheritance pattern of drought resistance encouraged breeders to adopt alternative strategies to improve stress resistance [2]. Fukai and Cooper [7] believed that under low-moisture stress, traits that help the plant gain access to additional reserves were more important than traits associated with reducing moisture losses. Among the several factors contributing to enhanced tolerance, root characters are believed to be a vital component of dehydration postponement mechanism since they contribute to regulation of plant growth and extraction of water and nutrients from deeper soil layers [1].

Molecular markers have been proved to be valuable tools in the characterization and evaluation of genetic diversity between sorghum genotypes. Of these techniques, RAPD has several advantages, such as simplicity of use, low cost, and the use of small amount of plant material, etc. The essential use of RAPDs is the detection of polymorphism through revealing differences in DNA that occurs between different individuals. In Sorghum RAPDs detect a greater number of genetic polymorphisms than isozyme analysis and thus reflect the true level of genetic variation [12].

The aim of this research was assessing the amount of genetic variation between Indian and Syrian sorghum varieties using morphological and molecular markers, and determining drought resistant varieties of sorghum.

MATERIALS AND METHODS

Plant materials:

A total of 14 sorghum genotypes, 12 from ICRISAT, India (296B, N13, IS9830, CSV-5, ICSV745, TX7078, IS22380, PB15881-3, IS18551, B-35, E-36-1, R16) and 2 from Syria (Rezenia and Azra3) were used

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in this research. The field experiment was laid out in completely randomized design with three replications for each condition at Faculty of Agriculture's farm (Slok zone), Benghazi University, Libya, during summer season 2013. Seeds were sown in poly vinyl chloride pipes measuring 1 meter length and 20 cm in diameter, which were filled with a mixture of sandy-clay loam and FYM in 4:1 proportion. After germination, one seedling was allowed to grow in each pipe. Two moisture regimes; well-watered and low-moisture stress conditions were imposed. In WW condition, all the entries were watered daily throughout the cropping period. In LMS treatment, moisture stress was imposed from 35 days after sowing (DAS) to 50 DAS by withholding irrigation. Pipes were removed carefully after 50 DAS and completely submerged in water. Observation were recorded for maximum root length (MRL) in cm , total root number (TRN), root volume (RV) in cc, root dry weight (RDW) in g, shoot dry weight (SDW) in g, and plant height (PHT) in cm. Root to shoot weight (R/SW) and root to shoot length (R/SL) ratios were computed.

DNA extraction and PCR amplification:

Thirty days old seedlings were used for DNA extraction. DNA extraction was done as per modified CTAB (Cetyltrimethylammoniumbromide) method [4] the National Commission for Biotechnology, Damascus, Syria, in 2011. The concentration and quality of DNA was estimated using spectrophotometer at 260 nm and 280 nm wavelength. A total of 20-mer oligonucleotides of RAPD were used in genetic analysis (Table 1). The PCR reaction mixture consisted of 2 μL of template DNA, 20 ng of random decamer primer, 1 mM each of dNTPs, 0.3 units of *Taq* polymerase and 1X PCR buffer (10 mM Tris pH 8.0, 50 mM KCl, 1.8 mM MgCl2 and 0.01 mg/ml gelatin) in a volume of 20 μL. A negative control containing all reaction components except sorghum DNA was included each time PCR was performed. Amplification was carried out on PTC 100 thermocycler using several decamer primers. The amplification profile was as follows: initial denaturation temperature 94°C for 5 min, followed by 45 cycles of 94 °C for 1 min, 36 ºC for 1 min and 72 ºC for 1 min, with a final extension at 72 ºC for 7 min., followed by soak temperature for 4°C till removal. Agarose gel of 1% was prepared using electrophoresis grade agarose in a volume of electrophoresis buffer (1X TAE) sufficient for constructing a gel (300 ml for 18 x 30 cm gel). Ethidium bromide was added at concentration of 0.5 mg/ml of gel. The gel was allowed to set fully before removing the combs and loading the sample. 4 μL of loading dye (containing Bromophenol blue and Xylene cyanol) was added to 20 μL of PCR products and mixed well before loading in to the wells. Care was taken to prevent mixing of samples between the wells. A voltage of 1-5 v/cm was given for a time period of one hour for separation of PCR fragments. The gel was viewed under UV transilluminator and the DNA banding pattern was recorded directly or photographed using Polaroid camera. Size of bands was estimated using a 200 bp DNA ladders. The banding patterns obtained from RAPD were scored as present (1), absent (0) or as a missing observation (.), each of which was treated as an independent character.

Table 1: Primer names, sequences and percentage of polymorphism.

Sr. No.	primer name	Primer sequence 5'----------3'	Total number of loci	Number of polymorphic loci	% of polymorphic loci
1	AC09	5¢- AGAGCGTACC -3¢	7	6	85.71
2	AD15	5¢- TTTGCCCCGT -3¢	12	10	83.33
3	G11	5¢- TGCCCGTCGT -3¢	4	4	100.00
4	AB06	5¢- GTGGCTTGGA -3¢	6	4	66.67
5	AC02	5¢- GTCGTCGTCT -3¢	5	5	100.00
6	AA19	5¢- TGAGGCGTGT -3¢	9	7	77.78
7	AC05	5¢- GTTAGTGCGG -3¢	7	5	71.43
8	AB05	5¢- CCCGAAGCGA -3¢	8	6	75.00
9	G04	5¢- AGCGTGTCTG -3¢	6	5	83.33
10	A04	5¢- AATCGGGCTG -3¢	6	6	100.00
11	G06	5¢- GTGCCTAACC -3¢	5	4	80.00
12	F01	5¢- ACGGATCCTG -3¢	9	5	55.56
13	C04	5¢- CCGCATCTAC -3¢	7	6	85.71
14	J16	5¢- CTGCTTAGGG -3¢	10	8	80.00
15	N07	5¢- CAGCCCAGAG -3¢	11	9	81.82
16	O19	5¢- GGTGCACGTT -3¢	9	8	88.89
17	P1	5¢- ACACAGAGGG -3¢	7	7	100.00
18	P2	5¢- CCTCTCGACA -3¢	8	6	75.00
19	P3	5¢- GTGTGCCCCA -3¢	6	5	83.33
20	P4	5¢- CCACGGGAAG -3¢	7	5	71.43
Total			149	121	81.21

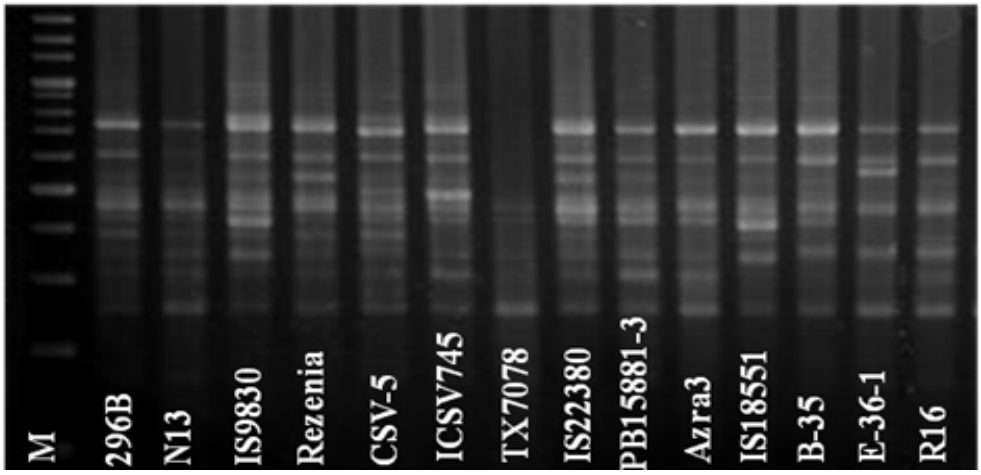

Fig. 1: An example of PCR amplification profile generated from genomic DNA of 14 sorghum varieties with OPAD15 RAPD primer resolved on 1% agarose gel. M-marker = 200bp.

Data analysis:

Statistical analysis on individual characters was carried out using standard biometrical procedure. The phenotypic coefficient of variation (PCV%) and genotypic coefficient of variation (GCV%) were computed by adopting the formula given by Burton and Devance [3]. Heritability and genetic advance as % of mean (GA%) were estimated as suggested by Hanson *et al*. [9] and Johnson *et al*. [10], respectively. The unweighted pair group method with arithmetic averages (UPGMA) and Squared Euclidean distances of the STATISTICA program were used to construct the matrices and the dendrograms [14]. This distance was computed in the program as Sneath and Sokal, [13].

RESULTS AND DISCUSSION

The analysis of variance revealed highly significant genotypic differences among the varieties for all the root and shoot morphological traits studied ($P<0.01$). Traits such as plant height, total root number, root volume, root dry weight, shoot dry weight and root to shoot weight ratio showed significant reduction under LMS (Table 2). Shoot dry weight, root dry weight, root number and root volume are biomass-related traits and indicated that plant produces lesser biomass to conserve of water and to increase water use efficiency [1]. Maximum root length and root to shoot length ratio showed significant increase when LMS was imposed. The increase in the root to shoot length ratio could be due to increase in the root length and decreasing in plant height under LMS condition. This is indicating the necessity of longer root length to go into the soil to extract water from deeper layers of soil [5].

Results the heritability (broad sense), GA as % of mean, PCV%, GCV%, and other parameters are presented in table 2. Heritability and GA were high for all the traits under WW condition. Hence, for these characters, scope for selection is amenable, as the influence of environment on these traits was at very low extent; more uniform condition is expected to show higher heritability for the traits [6] Heritability of all traits decreased from WW to LMS conditions as a result of increased environmental variance. Blum [1] has revealed similar pattern of heritability decrease.

GCV and PCV estimates were more in WW compared to LMS for most of the traits (except R/SW). Similar findings were reported by Kanbar *et al*. [11] in rice. Blum [1] reported reduction in genetic variance under LMS. PCV was significantly higher than GCV values for all the traits under LMS, indicating prominent rate of environment in creating variability.

The phenotypic correlation coefficients among the characters studied are presented in Table 3. Maximum root length showed significant positive association with root volume, root dry weight, shoot dry weight and root to shoot length ratio under well-watered condition. While this trait had positive non-significant association with plant height, number of roots, root volume, root dry weight and root to shoot weight ratio under LMS condition. The phenotype of a plant is the result of interaction of a large number of factors.

All root morphological characters showed positive correlation between each others. Blum, *et al*. [1] and Kanbar *et al*. [11] also reported strong interrelationship among root traits. Shoot dry weight recorded significant and positive correlation with plant height, maximum root length and root volume under both conditions. Under water deficit, varieties with dramatically reduced shoot biomass production also suffered reductions in root growth. No decline was observed in root growth in varieties that maintained better shoot biomass production. Furthermore, the root systems of tolerant varieties showed a redistribution of dry matter to deeper soil levels under stress [16].

Table 2: Descriptive statistics and genetic parameters of eight characters of 14 sorghum varieties.

Trait	Condition	Mean	SE±	Min.	Max.	h^2_{bs}	GCV (%)	PCV (%)	GA as % of mean
PHT	WW[$]	118.80 a	5.69	55.00	184.00	94.56	26.67	27.43	51.07
	LMS	86.76 b	4.75	35.00	159.00	30.76	12.82	23.12	14.65
TRN	WW	19.54 a	1.30	8.00	58.00	89.55	24.42	25.80	50.94
	LMS	11.40 b	0.48	5.00	20.00	78.15	12.80	14.47	22.97
MRL	WW	116.54 a	4.97	32.00	184.00	91.81	17.77	18.55	33.57
	LMS	132.35 b	7.05	35.00	240.00	91.80	14.82	15.46	27.74
RV	WW	72.17 a	3.78	20.00	140.00	83.15	16.42	18.01	29.67
	LMS	32.54 b	1.91	10.00	65.00	50.45	12.78	18.01	18.05
RDW	WW	16.10 a	1.03	3.70	32.50	97.35	37.33	37.84	75.75
	LMS	6.81 b	0.43	1.50	14.50	92.93	31.49	32.51	62.88
SDW	WW	28.06 a	1.77	10.00	60.50	96.73	32.51	33.06	66.98
	LMS	13.56 b	1.02	3.90	32.20	76.43	16.32	18.66	30.59
R/SW	WW	0.57 a	0.63	0.22	1.60	93.91	43.40	44.78	78.39
	LMS	0.50 b	0.05	0.17	1.16	88.96	62.97	89.05	55.25
R/SL	WW	0.98 a	1.05	0.28	2.27	94.48	35.31	35.32	66.32
	LMS	1.53 b	0.41	0.41	3.80	29.72	17.47	32.38	16.46

$: Means followed by the same letter are not significantly different.
PHT-Plant height, TRN-Total root number, MRL-Maximum root length, RV-Root volume, RDW-Root dry weight, SDW-Shoot dry weight, R/SW-Root to shoot weight ratio, R/SL-Root to shoot length ratio.

Table 3: Phenotypic correlation coefficients among different shoot and root-related traits in sorghum.

Traits	Condition	PHT	NOR	MRL	RV	RDW	SDW	R/SW
NOR	WW	0.16	1.00					
	LMS	0.09	1.00					
MRL	WW	0.22	0.10	1.00				
	LMS	0.16	0.22	1.00				
RV	WW	0.22	0.17	0.51**	1.00			
	LMS	0.31*	0.63**	0.17	1.00			
RDW	WW	0.14	0.26	0.63**	0.53**	1.00		
	LMS	0.09	0.42**	0.27	0.25	1.00		
SDW	WW	0.50**	0.24	0.30*	0.37**	0.49**	1.00	
	LMS	0.44**	0.23	0.32*	0.30*	0.28	1.00	
R/SW	WW	-0.33*	-0.07	0.28	0.06	0.29*	-0.57**	1.00
	LMS	-0.17	0.18	0.04	0.03	0.55**	-0.50**	1.00
R/SL	WW	-0.63**	-0.12	0.50**	0.21	0.26	-0.17	0.41**
	LMS	-0.68**	0.02	0.56**	-0.12	0.09	-0.02	0.05

A dendrogram was constructed with the 14 varieties using Squared Euclidean distances (Table 4) by STATISTICA software to analyze the genetic distances. It indicated that all 14 sorghum varieties could be distinguished by RAPD markers. Cluster analysis indicated three main clusters at a similarity level of 16% (Fig. 2). The cluster I consisted of all Indian varieties except BP15881-3 genotype. Cluster II consisted of two Syrian varieties (Rezenia and Azra3), while BP15881-3 was grouped alone in separate cluster. Distribution pattern of all the genotypes into various clusters showed the presence of considerable genetic divergence among the genotypes.

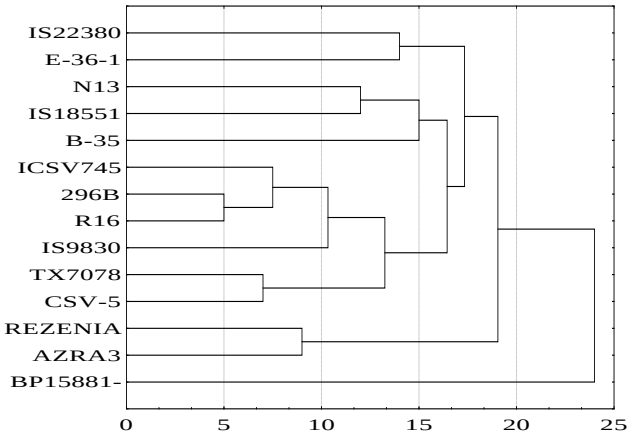

Fig. 2: Dendrogram showing grouping of 14 varieties of sorghum from Syria and India based on the genetic distance derived from RAPD markers using UPGMA analysis.

Table 4: Genetic distance estimated between 14 varieties of sorghum.

Varieties	N13	ICSV745	E-36-1	BP15881-3	TX7078	CSV-5	B-35	IS18551	296B	R16	Rezenia	Azra3	IS9830
IS22380	18	17	14	27	13	16	18	16	18	21	20	25	21
N13	0	11	20	17	15	18	16	12	14	17	18	19	19
ICSV745		0	17	24	10	13	15	15	7	8	13	20	10
E-36-1			0	27	13	16	18	20	16	19	20	21	15
BP15881-3				0	24	29	21	21	23	28	21	26	24
TX7078					0	7	19	13	11	14	19	24	14
CSV-5						0	16	18	12	15	20	27	17
B-35							0	14	14	17	14	21	17
IS18551									16	19	20	21	23
296B										5	12	19	11
R16										0	17	22	10
Rezenia											0	9	11
Azra3												0	16

The grouping of two Syrian varieties into same cluster indicated very diverse geographic structure in South India and Syria and the high degree of climatic heterogeneity of Syria (semi-dried area) compared to South India (Sub-tropical climate). Geographically isolated population accumulates genetic differences as they adapt to different environment.

In conclusion, varieties with good root system can go for yield evaluation in different locations in Libya for drought prone. And since, breeders have the task of combining them into a single genotype.

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