



Genetic Variability and Drought Parameters among Some Grain Sorghum Genotypes (*Sorghum bicolor* L. Moench) Using Quantitative Traits and Molecular Markers

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Genetic diversity is one of the main element in the enhancement of many crops, including sorghum. For that, twenty-grain sorghum genotypes were evaluated at Shandaweel Agricultural Research Station, Sohag governorate, Egypt, during the summer season of 2023 in two experiments (normal irrigation 100% and severe water stress 40% of the optimum) for assessment of the variability among these genotypes, RAPD molecular markers and drawing the phylogenetic tree using cluster analysis. The results indicated highly significant differences among the genotypes, irrigation treatments and their interaction for all traits, suggesting that these genotypes were highly variable, therefore, would respond to selection, the genotypes G3, G7, G8, G13 and G16 gave the best performance for grain yield/plant under both environments and their combined data. These genotypes will be testing in a large scale. High genetic advance as a percentage of mean ($\Delta g\%$) was obtained for plant height and 1000 grain weight and moderate for days to 50% flowering and grain yield/plant. High GCV% and PCV% revealed for plant height, moderate for 1000-grain weight, and low for days to 50% flowering and grain yield/plant., this demonstrates that

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the genotypes have a diverse genetic background as well as the capacity to respond favorably to selection. The desirable genotypes that had high grain yield and tolerant to drought according to SSI, STI, HM, MPI, YI, SM, RP, YSI, TOL and YIX values, were genotypes G3 and G 13.

The Results of RAPD molecular markers showed that the percent of polymorphism (%P) were between 44.44 to 77.78 with an average of 59.78%. The number of polymorphic bands ranged from 4 to 12 with an average of 6.38 bands per primer. The bands size ranged from 259 bp to 2318 bp, generated by OPA-18 and OPH-01 primers, respectively. The Polymorphism information content (PIC) values varied from 0.10 to 0.28 with an average of 0.20. While marker index (MI) varied from 0.40 to 2.76 with an average of 1.31. In this trend the results revealed that the resolving power (Rp) varied from 1.10 (OPA-18 & OPAV-13) to 5.20 (OPG-09) with an average of 2.90. Single-marker analysis (SMA) indicated that three of the RAPD markers identified in this study showed significant association with the two traits viz., plant height and 1000-grain weight under normal and drought environments conditions. The cluster analysis based on RAPD and means of morphological data showed similarity coefficient values ranged from 0.64 to 0.92 with an average similarity index of 0.78. The Mantel test revealed, there was positive and non-significant correlation between the genetic distances based on phenotypic data and the similarity data based on RAPD markers, ($r=0.07$, $P<0.05$) and ($r=0.03$, $P<0.05$) under normal and drought conditions, respectively.

Keywords: Drought indices; molecular markers; phylogenetic tree; similarity coefficient; cluster analysis.

1. INTRODUCTION

Sorghum is an important crop that grown in many parts of the world, particularly in regions with limited water resources. The water deficiency problem is also most important production limitation in Egypt. But as climate change continues to intensify, drought conditions are increasingly becoming a challenge for sorghum farmers. Fortunately researchers are working hard to identify the genetic variability within sorghum that allows it to survive and thrive under harsh environment conditions. By examining quantitative traits accompanied with molecular markers, scientists become able to gain a better understanding of the genetic factors that contribute to sorghum's drought tolerance. There are many advantages of RAPD over other markers are its simplicity, rapidity, requirement for only a small quantity of DNA, and the ability to generate numerous polymorphisms with good coverage of the entire genome [1]. Using gene-marker will help the sorghum breeders for selecting their parent's materials, find out the diversity between genotypes, put the good explanation, interpretation, and answer the question for why these materials are tolerant and that are susceptible to drought [2]. Therefore, the present study the aim of this study was to determine the extent of diversity of twenty-grain sorghum genotypes (selected genotypes from the advanced generations F7 and F8, as well as 12 genotypes that formed sources of isolation from which these new genotypes were selected.) under normal and drought conditions.

2. MATERIALS AND METHODS

2.1 Plant Materials and Agronomic Traits

The experimental materials included 8 new grain sorghum genotypes selected from the advanced generations F7 and F8, as well as 12 genotypes which formed sources of isolation from which these new genotypes were selected. These genotypes planted in a randomized complete block design with three replications at Shandaweel Agricultural Research Station, Sohag governorate, Egypt, during the summer season of 2023 in two experiments (normal irrigation, 100% and severe water stress, 40% of the optimum). The plot consisted of three rows of 4.0-meter long; with 60 cm inter row spacing and 15 cm intra row spacing. All agricultural recommended practices followed in the proper time. The data recorded on four quantitative characters viz., days to 50% flowering, plant height (cm), 1000-grain weight (g), and grain yield per plant (g). The amount of used water calculated using [3] modified Penman equation for estimating evapotranspiration (ET). For each character, the mean data of five random plants in each plot used for analysis of variances. Table 1 lists the genotypes' names, pedigrees, and origins.

2.2 Genetics Components

The data was subjected to analysis of variance for each environment based on plot means in order to estimate the extent or magnitude of

Table 1. The pedigree and the origins of the plant materials

No.	Pedigree	Source	Origin
G1	F7 7019/2020	Dorado x G - 16	Sorghum Research department, FCRI, ARC, Egypt
G2	F7 7026/2020	LG - 8 x Dorado	Sorghum Research department, FCRI, ARC, Egypt
G3	F7 7030/2020	ICSR - 89016 x G - 15	Sorghum Research department, FCRI, ARC, Egypt
G4	F7 7035/2020	Dorado x R Sh - 8	Sorghum Research department, FCRI, ARC, Egypt
G5	F7 7043/2020	R Sh - 8 x ICSR - 89028	Sorghum Research department, FCRI, ARC, Egypt
G6	F8 8002/2020	ICSR - 89025 x G - 15	Sorghum Research department, FCRI, ARC, Egypt
G7	F8 8004/2020	ICSR - 92003 x G - 113	Sorghum Research department, FCRI, ARC, Egypt
G8	F8 8012/2020	MR - 812 x MR - 1	Sorghum Research department, FCRI, ARC, Egypt
G9	Giza - 16	Giza - 16	FCRI, ARC, Egypt
G10	Dorado	Dorado	ICRISAT, India
G11	L33\82	LG - 8	Local, Egypt
G12	ICSR - 89016	ICSR - 89016	ICRISAT, India
G13	Giza - 15	Giza - 15	FCRI, ARC, Egypt
G14	R Sh - 8	R Sh - 8	FCRI, ARC, Egypt
G15	ICSR - 89028	ICSR - 89028	ICRISAT, India
G16	ICSR - 89025	ICSR - 89025	ICRISAT, India
G17	ICSR - 92003	ICSR - 92003	ICRISAT, India
G18	Giza - 113	Giza - 113	FCRI, ARC, Egypt
G19	MR - 812	MR - 812	Texas, USA
G20	MR - 1	MR - 1	Texas, USA

variation among these genotypes. Once homogeneity of variance was detected, the data was then combined and analyzed across the tested environments (Normal and Drought) using the methods outlined by [4]. [5] Approaches which were used to evaluate the phenotypic and genotypic coefficient of variations (PCV % and GCV %). According to the techniques described by [6], genetic advance (Δg) and its percentage of the mean ($\Delta g\%$) were computed assuming selection of superior 5% of the genotypes.

An index determination is a method of deriving "value-added" information linked to drought. Based on the behavior of tested genotypes under drought stress (D) and normal conditions (N), drought tolerance indices were estimated for grain yield/plant in Table (2) as follows

2.3 DNA Extraction and PCR Procedures

At the molecular genetics Laboratory, Genetics Department, Faculty of Agriculture, Sohag University, fresh young leaves of five sorghum

genotypes harvested, then immediately ground in extraction buffer using the cetyltrimethyl ammonium bromide (CTAB) protocol described by [16]. 0.2 ml of ground leaf tissue was suspended in 2 ml of extraction buffer (20 mM EDTA, 0.1 M Tris-HCL, 1.4 NaCl, 2% CTAB, 1% PVP). After that, the DNA pellet suspended in 100 μ l of TE buffer. Prior to 35 cycles of PCR amplification, genomic DNA was diluted 10-fold in water.

The RAPD marker PCR assays were carried out in a 20 μ l volume containing 0.2 μ l of Go Taq polymerase, 3.5 μ l of primer (8 pmol), 4 μ l 5X green buffer, 2 μ l MgCl₂, 2 μ l dNTPs (2.5mM), 5.3 μ l of free nuclease water, and 3 μ l (150-200 ng) of genomic DNA templates. The thermal Cycler 96-Labmet (USA) was programmed with the following parameters: 1 cycle (an initial denaturing step) of 5 minutes at 95 °C, 40 cycles of 30 seconds at 95 °C (denaturation step), 30 seconds at 49 °C to 56 °C (annealing step, optimized for each primer), 2 minutes 30 seconds at 72 °C (elongation step), and 10

minutes at 72 °C (final extension), then kept at 20 °C. The amplified products separated by electrophoresis in 1-1.5% agarose gel stained with 0.2 µl ethidium bromide. The amplified fragments photographed and visualized using the UVP Bio Doc-It imaging system (USA) [17]. The RAPD technique used with ten primer combinations (Table 3).

Table 2. Drought tolerance indices based on grain yield/Plant under drought and normal conditions

Parameters	References
Stress susceptibility index (SSI)	[7]
Stress Tolerance Index (STI)	[8]
Harmonic mean (HM)	[9]
Mean Productivity Index (MPI)	[10]
Tolerance (Tol)	
Yield injury % (YI)	[11]
Superiority measure (SM)	[12]
Relative performance (RP)	[13]
Yield Stability Index (YSI)	[14]
Yield Index (YI)	[15]

2.4 Data of Molecular Markers Analysis

Gene Profiler software used to analyze the DNA banding patterns generated by the RAPD technique (version 4.03). For each genotype, the presence (1) or absence (0) of each band recorded for all primers studied. Genetic distance calculated according to Jaccard [18]. To assess the informativeness of the RAPD markers in distinguishing between sorghum genotypes, the polymorphic information content (PIC) was calculated using [19] formula as $PIC = 1 - [(p)^2 + (q)^2]$, where p is the frequency of allele band present and q is the frequency of allele band absent across the tested genotypes.

The marker index (MI) was calculated for each RAPD primer combination as $MI = PIC \times \eta \beta$, where PIC is the mean PIC value, η the number of bands, and β is the proportion of polymorphism [20]. Analysis of variance (ANOVA) was conducted using the 0-1 data. The association analysis conducted using simple linear regression. For this, data on individual phenotypic traits were regressed on whole 0-1 binary marker data for each individual phenotypic marker using Excel programme. The coefficient of determination (R^2) was calculated as $R^2 = 1 - (SSE/SST)$, where SSE is the sum of squares of error and SST is the total sum of squares.

For each RAPD primer combination, the marker index (MI) was calculated as $MI = PIC \times \eta \beta$, where PIC is the mean PIC value, η the number of bands, and β is the proportion of polymorphism [21]. The 0-1 data used for analysis of variance (ANOVA). Simple linear regression used to perform the association analysis. Individual phenotypic trait data were regressed on whole 0-1 binary marker data for each individual phenotypic marker using Excel.

2.5 Dendrogram Construction

Using the computational package MVSP version 3.1, the genetic similarities among the tested genotypes computed, and a UPGMA-dendrogram was generated using Jaccard's coefficient [18]. By comparing the matrices using the Mantel test, a cophenetic matrix derived from each matrix to test the goodness of fit of the clusters [21]. Finally, using NTSYS-pc version 2.20, the correlation between RAPD molecular markers and morphological traits was calculated [22].

Table 3. Code and sequence and TM of RAPD primers used in this study

Primer Name	Primer Sequence (5'.....3')	TM
OPA-10	AAGTGACCGG	32.00
OPA-18	AGGTGACCGT	32.00
OPAV-13	CTGACTTCCC	32.00
OPBB-01	ACACTGGCTG	32.00
OPG-09	CTGACGTCAC	32.00
OPH-01	GGTCGGAGAA	32.00
OPJO-01	AGGAGTCGGA	32.00
OPP-05	CCCCGGTAAC	32.00
OPW-13	CACAGCGACA	32.00

3. RESULTS AND DISCUSSION

3.1 Variances and Mean performance

For all variables under consideration, the individual and combined analyses (Table 4) revealed highly significant differences among the genotypes, demonstrating the existence of genotypic variances among the genotypes. Additionally, for every variable under study, highly significant differences identified between the two irrigation treatments, demonstrating that responses of genotypes were differently to the amount of irrigation applied. The interactions between genotypes and irrigation were highly significant in the same direction for all studied traits, indicating that differences among genotypes were not the same under irrigation treatments. This emphasizes the value of assessing genotypes to determine their stability for use as trustworthy genetic resources for crop improvement in drought-prone environments. [23-25] all are described similar findings when testing grain sorghum under different environments.

The average performance of 20 genotypes of grain sorghum under normal (N), drought (D), and combination (C) data shown in (Table 5). For days to 50% flowering, the drought environment mean 75.79 days was higher than normal environment mean 70.40 days, indicating that drought environment delayed the flowering. The desirable genotypes for earliness under normal (N), drought (D) and combined data (C) were G2, G5, G6, G8 and G12. For Plant height, the mean of (N) 211.95 cm. was higher than the mean of (D) 196.10 cm. indicating that the drought environment decreased plant height. The desirable genotypes compared to grand mean of genotypes under N, D and C were G1, G2, G3, G4, G5, G6, G8, G10, G12, G14, G15, G16, G17, G19 and G20. For 1000-grain weight, the mean of N and D were 28.21 g and 23.15 g, respectively, indicating that the drought environment decreased the 1000-grain weight. The higher genotypes under N, D and C were G2, G3, G5, G13 and G18. For grain yield/plant, mean of (N) 65.95 g was greater than mean of (D) 56.01 g, meaning that the drought environment cause decreased in grain yield/plant. The better genotypes under normal environment were G3, G6, G7, G8, G13 and G16, also under drought environment were G3, G4, G7, G5, G7, G8, G11, G13, G14, G16 and 17. Meanwhile, genotypes G3, G7, G13 and G16 had the highest grain yield/plant under normal,

drought and combined data. These genotypes will be test in advanced experiments.

3.2 Genetic Components

The means, phenotypic variance (σ^2p), genotypic variance (σ^2g), environmental variance (σ^2e), the expected genetic advance (Δg), and the expected genetic advance as a percentage of the mean ($\Delta g\%$), for studied traits across environments are shown in Table 6. The environments less influenced the expression of all the studied traits because the genotypic variance (σ^2g) was greater in magnitude than the environmental variance (σ^2e), which suggests that the expression of the traits caused by genetic variance that can be taken advantage of through breeding. The findings of [23, 24 and 26] are in agreement with these findings. Due to environmental influences, the GCV% has a lower value than the PCV%. [27] defined PCV% and GCV% values as high if they are greater than 20%, low if they are less than 10%, and medium if they are between 10% and 20%. High GCV% and PCV% were obtained for plant height, moderate for 1000 grain weight, and low for days to 50% flowering and grain yield/plant. These findings concur with those of Endalamaw et al. [28-29].

Johnson et al. [6] classified genetic advance as a percentage of mean ($\Delta g\%$) as 0–10% is Low, 10–20% is Moderate, and 20% and above is High; hence, high genetic advance as a percentage of mean ($\Delta g\%$) was shown for plant height and 1000 grain weight and moderate genetic advance for days to 50% flowering and grain yield/plant. This demonstrates that the genotypes have a diverse genetic background as well as the capacity to respond favorably to selection. [24 and 30] all observed similar findings. The success of selection determined by the character's genetic advancement. Control of additive gene effects and early selection may be useful for these qualities, according to Jafar [26,28].

3.3 Drought Tolerance Indices

The data in Table (7) represent stress tolerance indices for 20 grain sorghum genotypes evaluated under normal and drought water stress condition for grain yield/plant. The results revealed that the stress susceptibility index (SSI) values were divided the genotypes into two groups, the first group included the genotypes had values less than 1 and represented the most

Table 4. Analysis of variances for four traits under Normal (N), drought (D) environments and their combined data (C)

SOV	df		Days to 50% Flowering (days)			Plant Height (cm)			1000 Grain Weight (g)			Grain Yield / Plant (g)		
	S	C	N	D	C	N	D	C	N	D	C	N	D	C
Irrigation (I)	---	1	---	---	929.63**	---	---	7,536.68**	---	---	960.90**	---	---	2,965.99**
Rep/I	2	4	7.35	6.22	6.78	72.20	2.60	37.40	0.09	1.60	0.84	0.58	4.59	2.58
Genotypes (G)	19	19	144.72**	140.77**	279.37**	14,702.36**	9,559.55**	23,953.87**	33.36**	43.15**	74.12**	100.23**	129.78**	211.79**
G x I	---	19	---	---	6.13**	---	---	308.04**	---	---	2.39**	---	---	18.21**
Poled Error	38	76	1.89	2.50	2.20	9.41	9.86	9.64	0.83	1.27	1.05	4.002	3.02	3.51
CV %			1.95	2.08	2.02	1.45	1.60	1.52	3.16	4.87	3.94	3.03	3.11	3.07

* = significant at $P < 0.05$, and ** = significant at $P < 0.01$ **Table 5. Means performance for 20 grain sorghum genotypes under Normal (N), drought (D) environments and their combined (C) data**

No.	Genotypes	Days to 50% Flowering (days)			Plant Height (cm)			1000 Grain Weight (g)			Grain Yield / Plant (g)		
		N	D	C	N	D	C	N	D	C	N	D	C
1	(G1)	72.33	78.67	75.50	166.00**	154.67**	160.33**	30.34*	23.91	27.13	63.37	49.60	56.48
2	(G2)	61.67**	68.00**	64.83**	190.00**	177.00**	183.50**	30.48*	26.95**	28.71**	68.00	49.76	58.88
3	(G3)	73.67	78.00	75.83	180.67**	169.33**	175.00**	31.47**	26.50**	28.99**	74.40**	65.91**	70.16**
4	(G4)	70.33	74.00	72.17	182.67**	176.00**	179.33**	25.36	19.68	22.52	68.17	60.31**	64.24**
5	(G5)	57.33**	63.67**	60.50**	187.67**	179.33**	183.50**	33.01**	28.69**	30.85**	67.20	59.22*	63.21*
6	(G6)	65.33**	71.67**	68.50**	172.67**	164.67**	168.67**	32.03**	24.02	28.03**	72.97**	54.93	63.95**
7	(G7)	74.33	80.67	77.50	202.33**	193.67	198.00**	27.02	23.58	25.30	70.37**	61.60**	65.98**
8	(G8)	56.00**	59.67**	57.83**	155.00**	145.33**	150.17**	29.08	25.02*	27.05	69.48*	60.02**	64.75**
9	(G9)	80.33	87.33	83.83	300.00	275.67	287.83	23.77	15.88	19.83	53.20	44.13	48.67
10	(G10)	65.00**	77.33	71.17*	156.67**	148.67**	152.67**	23.51	16.80	20.16	61.07	46.51	53.79
11	(G11)	79.67	85.33	82.50	345.00	305.00	325.00	31.77**	24.81	28.29**	66.67	59.48*	63.07
12	(G12)	64.33**	71.33**	67.83**	184.33**	178.67**	181.50**	24.43	18.64	21.54	57.93	48.61	53.27
13	(G13)	73.00	78.33	75.67	371.00	315.00	343.00	34.44**	28.62**	31.53**	70.47**	62.55**	66.51**
14	(G14)	75.67	78.67	77.17	180.67**	171.67**	176.17**	29.51	23.36	26.43	67.40	60.05**	63.72*
15	(G15)	69.00	73.00*	71.00*	182.67**	172.00**	177.33**	27.51	21.80	24.65	66.03	54.85	60.44
16	(G16)	72.00	78.00	75.00	183.67**	174.00**	178.83**	26.04	19.53	22.79	73.67**	64.79**	69.23**
17	(G17)	70.00	73.67	71.83	169.00**	156.00**	162.50**	29.11	24.18	26.65	67.50	60.91**	64.21**
18	(G18)	72.33	77.33	74.83	361.67	315.67	338.67	33.76**	28.62**	31.19**	61.73	54.06	57.90
19	(G19)	76.67	81.67	79.17	175.00**	166.67**	170.83**	26.63	21.44	24.03	55.63	45.90	50.77
20	(G20)	79.00	83.00	81.00	192.33**	183.00**	187.67**	26.92	20.96	23.94	63.73	56.92	60.33
	Means	70.40	75.97	73.18	211.95	196.10	204.03	28.81	23.15	25.98	65.95	56.01	60.98
	LSD _{0.05}	2.27	2.61	1.70	5.06	5.18	3.57	1.50	1.86	1.18	3.30	2.87	2.15
	LSD _{0.01}	3.04	3.49	2.26	6.77	6.93	4.73	2.01	2.49	1.56	4.42	3.84	2.86

* = significant at $P < 0.05$, and ** = significant at $P < 0.01$

tolerant ones (12 genotypes), While the second group included the genotypes had values greater than 1 and represented the genotypes that are less tolerant to drought (8 genotypes). The best genotypes that had high grain yield and tolerant to drought according to SSI were G3 and G13. In the same context, high stress tolerance index (STI) values indicate tolerance to moisture stress was in four genotypes (>1) (G3, G16, G13 and G7, respectively). In addition, the results of the harmonic mean (HM) and mean Productivity index (MPI) indices were consistent with the same results of STI completely with regard to the same four genotypes.

Accordingly, for yield injury (YI), four genotypes recorded the highest deficiency in grain yield (G2, G6, G10 and G1, respectively), while six genotypes (G17, G20, G11, G14, G13 and G3, respectively) had recorded the lowest deficiency in grain yield. The best genotypes that had high grain yield and tolerant to drought according to (YI) were G3 and G13. With regard to superiority measure (SM) and relative performance (RP), 12 genotypes have scored the higher means, while 4 genotypes (G2, G6, G10 and G1, respectively) has scored the low means of SM and RP. Low-value genotypes for (TOL) are more stable under two different environments and are suitable for drought tolerance screening of breeding materials. The lower TOL values were found in eight genotypes (G17, G20, G11, G14, G18, G4 and G5, respectively), whereas the higher TOL values were found in 12 genotypes, the best genotype that had high grain yield and tolerant to drought according (TOL) was G3 for drought conditions. Drought-tolerant genotypes with high Yield index (YIX) values were discovered. Tolerance is defined as a genotype with a value greater than one, while susceptibility is defined as a genotype with a value less than one. Hence, 11 genotypes have higher values. These genotypes are drought-resistant. While the results showed according to YIX genotypes has lower values that were susceptible to drought. The desirable genotypes that had high grain yield and tolerant to drought according to (YIX) were G3, G7, G8, G13 and G16. Similar results were recorded by Badran [31, 32] they found that STI, MP, GMP, and YI are appropriate indices for identifying genotypes that produce greater yields under both stress and non-stress environments (drought tolerant genotypes).

3.4 RAPD Molecular Markers

In the present study, nine out of twenty primers revealed different degrees of percentage of polymorphism (%P) among genotypes (Fig. 1 A, Fig. 1 B, Fig. 2 C, Fig. 2 D, Fig. 3 E, Fig. 3 F, Fig. 4 G and Fig. 4 H). Out of 84 amplified bands 51 were polymorphic. The %P ranged from 44.44 (OPA-18) to 77.78 (OPJO-01) with an average of 59.78% (Table 8, Fig. 1 A and Fig. 3 F). The number of polymorphic bands ranged from 4 (OPA-18 & OPAV-13) to 12 (OPG-09) with an average of 6.38 bands per primer (Fig. 1 A, Fig. 3 F and Fig. 2 D). The bands size ranged from 259 bp to 2318 bp, generated by OPA-18 and OPH-01 primers, respectively (Fig. 1 A and Fig. 3 E). The RAPD primers showed the highest level of polymorphism. In this trend [33] found an 80% percent polymorphism (%P) between 11 genotypes of grain sorghum. Similarly, [34] found a 51.37% percent probability difference among 7 genotypes of grain sorghum. Whereas, [35] reported a maximum of 94% polymorphism within the adapted zone (Ethiopia) sorghum cultivars.

3.5 PIC and MI Analysis

In this investigation (Table 8), the Polymorphism information content (PIC) values for RAPD primers varied from 0.10 (OPA-18) to 0.28 (OPJO-01) with an average of 0.20. In this trend [36] showed that the higher the PIC value, the more informative is the RAPD marker. While marker index varied from 0.40 (OPA-18 & OPAV-13) to 2.76 (OPG-09) with an average of 1.31. Resolving power (Rp) used as a measure of the discriminatory power of molecular marker. In this work, RAPD primers showed a resolving power (Rp) varied from 1.10 (OPA-18 & OPAV-13) to 5.20 (OPG-09) with an average of 2.90. These findings concurred with those of Hadeer et al. [34,37-38] in the case of grain sorghum and sweet sorghum, respectively.

3.6 Single Marker Analysis

3.6.1 RAPD markers

Analysis of variances for simple regressions (Tables 9 and 10) under normal and drought conditions showed significant regression of days to 50%flowering, plant height and 1000 grain weight (g) traits on two or more of the total of 51 polymorphic RAPD. Three of the RAPD markers identified in this study showed significant

association with the two traits viz., plant height and 1000-grain weight under normal and drought environment conditions. Under normal environment the RAPD markers OPBB-01 1007bp (Fig. 2 C) regarded as candidate marker linked to plant height and 1000 grain weight (g). Two markers linked to plant height such as OPG-09 693bp, and OPH-01 748bp (Fig. 2 D and Fig. 3 E). While, under drought environment the two RAPD markers OPG-09 693bp and OPH-01

748bp linked to the agronomic trait plant height (Fig. 2 D and Fig. 3 E). The associated markers each explained a maximum regression of 17.99 to 47.71% for plant height trait under normal environment. Whereas, under drought environment, the associated markers each explained a maximum regression of 27.02% to 47.90% for plant height trait [35-36,39] reported the similar results in grain sorghum using RAPD molecular marker.

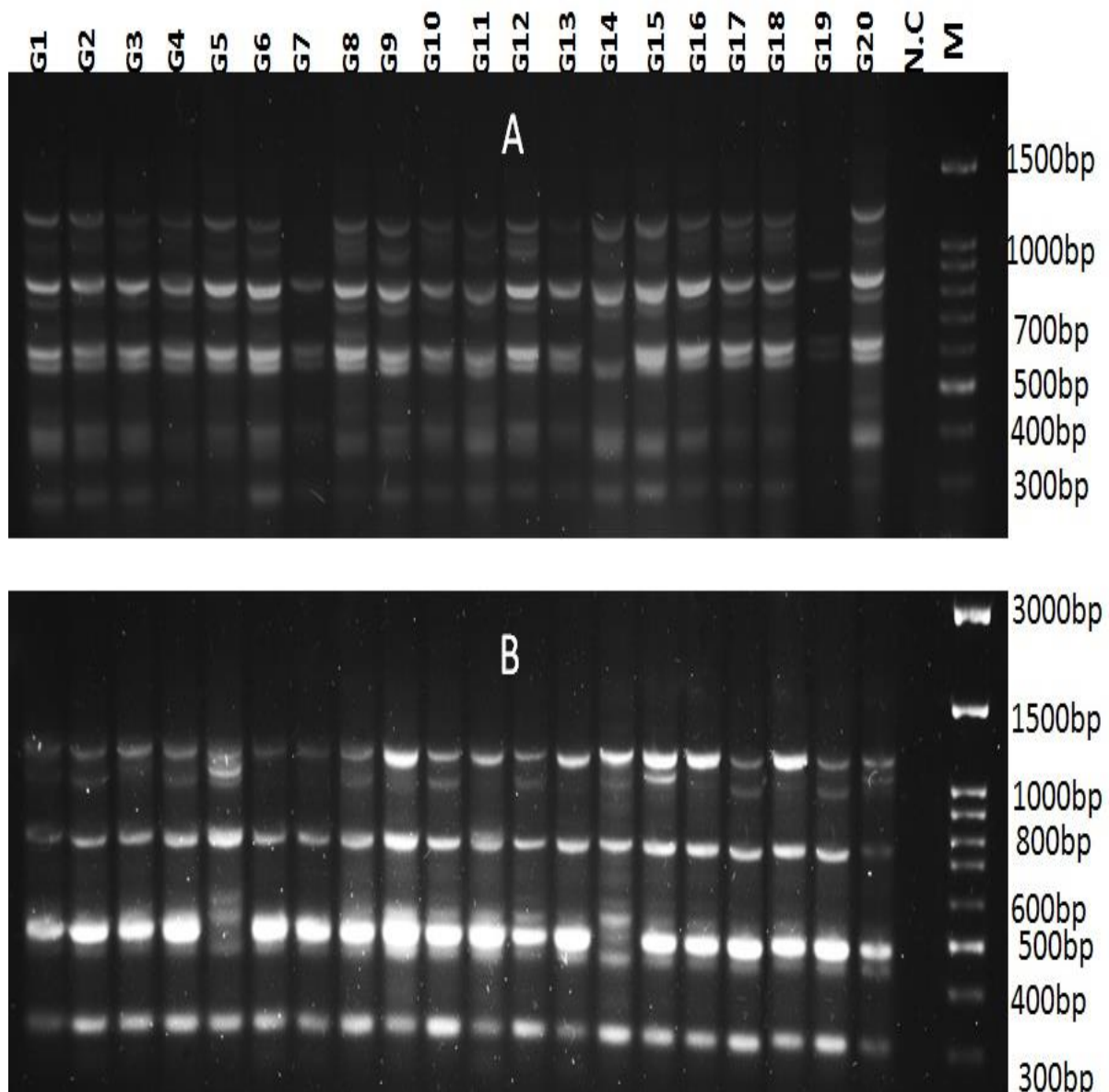


Fig. 1. RAPD profiles obtained for 20 Sorghum genotypes amplified with primers: (A) OPA-18 and (B) OPAV-13, M = 100bp ladder size marker

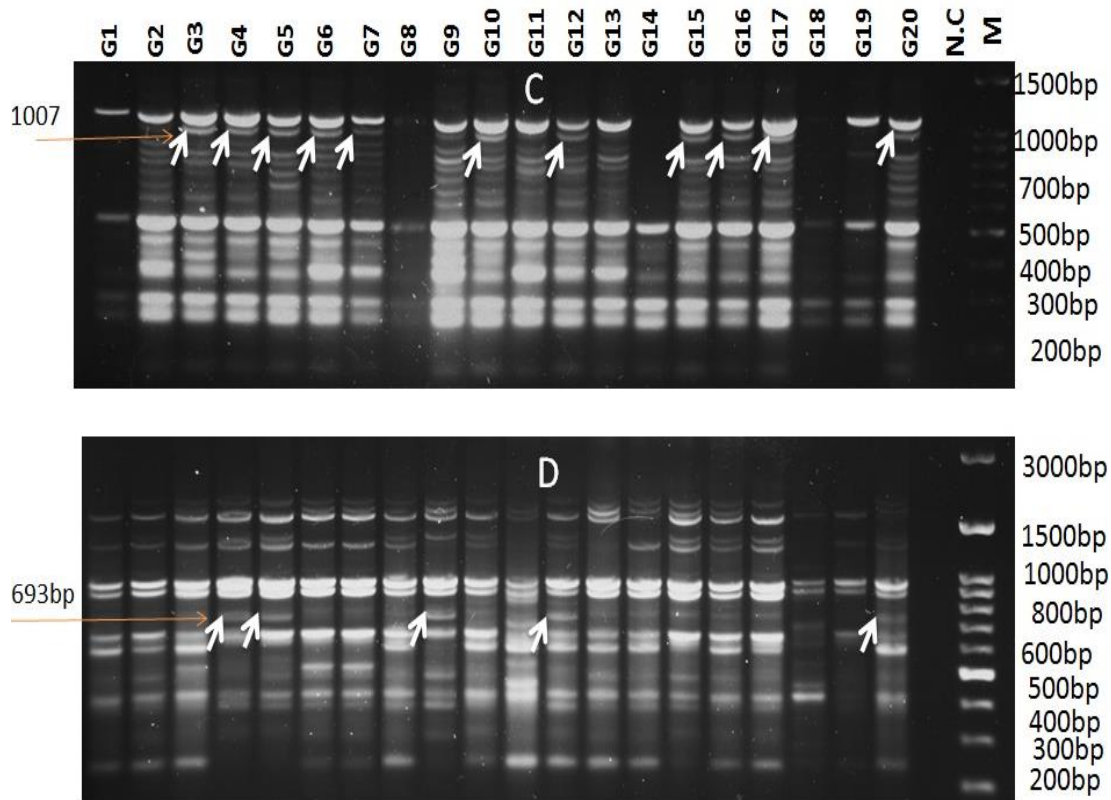


Fig. 2. RAPD profiles obtained for 20 Sorghum genotypes amplified with primers: (C) OPBB-01 and (D) OPG-09, M = 100bp ladder size marker

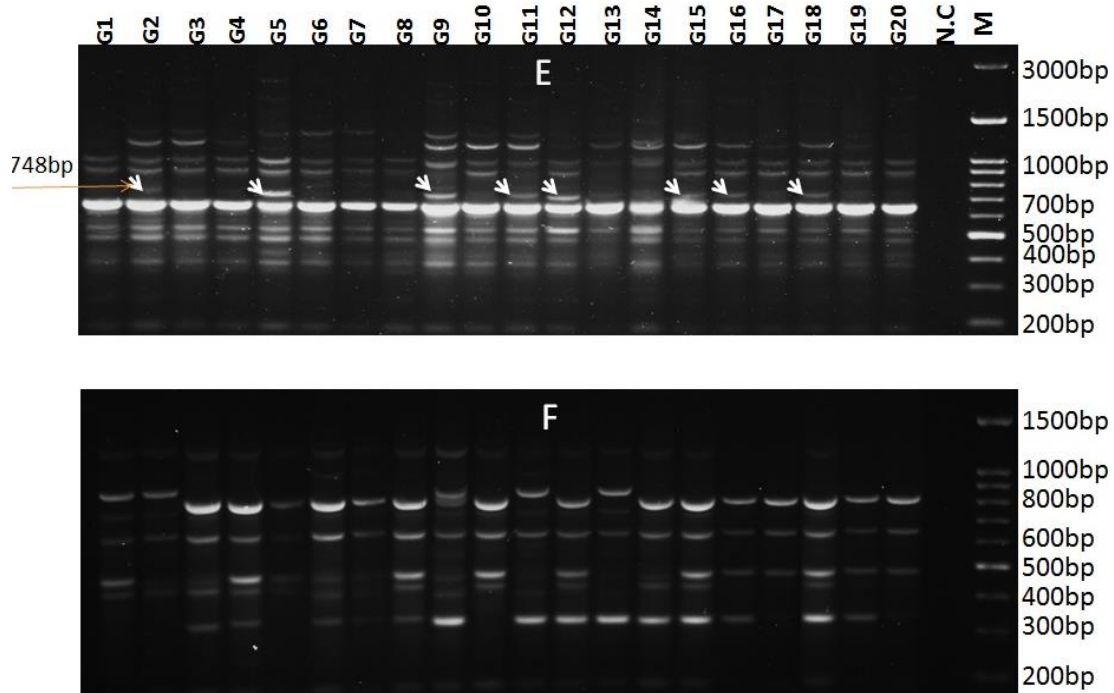


Fig. 3. RAPD profiles obtained for 20 Sorghum genotypes amplified with primers: (E) OPH-01 and (F) OPJO-01, M = 100bp ladder size marker

Table 6. Estimates of means, phenotypic (σ^2_p), genotypic (σ^2_g) and environmental (σ^2_e) variances components, phenotypic (PCV%) and genotypic (GCV%), expected genetic advance (Δg) and genetic advance as percentage of the mean ($\Delta g\%$) across normal and drought environments

Genetic Parameters	σ^2_g	σ^2_{gxe}	σ^2_e	σ^2_p	Means	GCV (%)	PCV (%)	Δg	$\Delta g\%$
Days to 50% flowering	45.54	1.31	2.20	46.56	73.18	9.22	9.32	13.75	18.79
Plant Height	3940.97	99.47	9.64	3992.31	204.03	30.77	30.97	128.48	62.97
1000 grain weight	11.96	0.44	1.05	12.35	25.98	13.31	13.53	7.01	26.97
Grain yield/plant	32.26	4.90	3.51	35.30	60.98	9.31	9.74	11.19	18.34

*, ** Significant at 0.05 and 0.01 probability levels, respectively

Table 7. Estimates of stress tolerance indices for 20 grain sorghum genotypes evaluated under normal and drought environment conditions for grain yield/plant

No.	Genotypes	YN	YD	SSI	STI	HM	MPI	YI	SM	RP	YSI	TOL	YIX
1	(G1)	63.37	49.60	1.44	0.72	55.64	56.48	21.73	0.78	0.92	0.78	13.77	0.89
2	(G2)	68.00	49.76	1.78	0.78	57.47	58.88	26.82	0.73	0.86	0.73	18.24	0.89
3	(G3)	74.40	65.91	0.76	1.13	69.90	70.16	11.41	0.89	1.04	0.89	8.49	1.18
4	(G4)	68.17	60.31	0.76	0.95	64.00	64.24	11.52	0.88	1.04	0.88	7.85	1.08
5	(G5)	67.20	59.22	0.79	0.92	62.96	63.21	11.87	0.88	1.04	0.88	7.98	1.06
6	(G6)	72.97	54.93	1.64	0.92	62.68	63.95	24.71	0.75	0.89	0.75	18.03	0.98
7	(G7)	70.37	61.60	0.83	1.00	65.69	65.98	12.46	0.88	1.03	0.88	8.77	1.10
8	(G8)	69.48	60.02	0.90	0.96	64.41	64.75	13.61	0.86	1.02	0.86	9.46	1.07
9	(G9)	53.20	44.13	1.13	0.54	48.24	48.67	17.04	0.83	0.98	0.83	9.07	0.79
10	(G10)	61.07	46.51	1.58	0.65	52.80	53.79	23.84	0.76	0.90	0.76	14.56	0.83
11	(G11)	66.67	59.48	0.72	0.91	62.87	63.07	10.79	0.89	1.05	0.89	7.19	1.06
12	(G12)	57.93	48.61	1.07	0.65	52.86	53.27	16.09	0.84	0.99	0.84	9.32	0.87
13	(G13)	70.47	62.55	0.75	1.01	66.27	66.51	11.24	0.89	1.05	0.89	7.92	1.12
14	(G14)	67.40	60.05	0.72	0.93	63.51	63.72	10.91	0.89	1.05	0.89	7.35	1.07
15	(G15)	66.03	54.85	1.12	0.83	59.93	60.44	16.93	0.83	0.98	0.83	11.18	0.98
16	(G16)	73.67	64.79	0.80	1.10	68.94	69.23	12.05	0.88	1.04	0.88	8.88	1.16
17	(G17)	67.50	60.91	0.65	0.95	64.04	64.21	9.76	0.90	1.06	0.90	6.59	1.09
18	(G18)	61.73	54.06	0.82	0.77	57.64	57.90	12.43	0.88	1.03	0.88	7.67	0.97
19	(G19)	55.63	45.90	1.16	0.59	50.30	50.77	17.49	0.83	0.97	0.83	9.73	0.82
20	(G20)	63.73	56.92	0.71	0.83	60.14	60.33	10.69	0.89	1.05	0.89	6.81	1.02
Means		65.95	56.01	1.00	0.85	60.57	60.98	15.08	0.85	1.00	0.85	9.94	1.00

SSI: Stress susceptibility index, STI: Stress tolerance index, HM: Harmonic mean, MPI: Mean productivity Index, YI: Yield Injury, SM: Superiority measure, RP: Relative Performance, YSI: Yield stability index, TOL: Tolerance and YIX: Yield index

Table 8. Primers used for RAPD markers, total number of fragment detected by each primer, %P, PIC, MI and fragments sizes for twenty Sorghum bicolor genotypes

Primer combinations	Amplified bands		%P	PIC	MI	RP	Fragments size (bp)	
	Bands number	Polymorphic bands					Larger	Smallest
OPA-18	9	4	44.44	0.10	0.40	1.10	1145.00	259.00
OPAV-13	8	4	50.00	0.17	0.68	1.10	1208.00	361.00
OPBB-01	11	6	54.55	0.16	0.96	2.40	1214.00	280.00
OPG-09	16	12	75.00	0.23	2.76	5.20	1926.00	384.00
OPH-01	13	7	54.00	0.21	1.47	4.50	2318.00	465.00
OPJO-01	9	7	77.78	0.28	1.96	3.30	1126.00	325.00
OPP-05	10	6	60.00	0.20	1.20	2.90	1682.00	608.00
OPAW-13	8	5	62.50	0.21	1.05	2.70	638.00	282.00
Means	10.50	6.38	59.78	0.20	1.31	2.90		
Total	84	51						

%P, Percentage of polymorphism; PIC, Polymorphism information content; MI, Marker index;

Table 9. Details of variance (ANOVA) involving simple linear regression (R^2) for traits under normal environment using 51 RAPD polymorphic bands

Marker	Traits	S.V	df	SS	MS	R^2	P- value
OPBB-01 _{1007bp}	Plant height	Genotypes	1	16752.79	16752.79 [*]	17.99	0.05
		Error	18	76361.75	4242.32		
		Total	19	93114.54			
	1000 Grain weight	Genotypes	1	51.30	51.30 [*]	24.28	0.02
		Error	18	160.00	8.89		
		Total	19	211.30			
OPG-09 _{693bp}	Plant height	Genotypes	1	44420.97	44420.97 ^{**}	47.71	0.001
		Error	18	48693.57	2705.20		
		Total	19	93114.54			
OPH-01 _{748bp}	Plant height	Genotypes	1	24851.25	24851.25 ^{**}	26.69	0.01
		Error	18	68263.29	3792.40		
		Total	19	93114.54			

S.V : Source of variance.
DF : Degree of freedom.
R²%: Coefficient of determination.

SS : Sum square.
MS : Mean square.

Table 10. Details of variance (ANOVA) involving simple linear regression (R^2) for traits under drought environment using 51 RAPD polymorphic bands

Marker	Traits	S.V	df	SS	MS	R^2	P- value
OPG-09 _{693bp}	Plant height	Genotypes	1	28998.68	28998.68 ^{**}	47.90	0.001
		Error	18	31545.78	1752.54		
		Total	19	60544.46			
OPH-01 _{748bp}	Plant height	Genotypes	1	16359.20	16359.20 ^{**}	27.02	0.01
		Error	18	44185.26	2454.74		
		Total	19	60544.46			

S.V : Source of variance.
DF : Degree of freedom.
R²%: Coefficient of determination

SS : Sum square.
MS : Mean square.

Table 11. Similarity matrix for the 20 sorghum genotypes according to Jaccard's coefficient obtained from 84 RAPD fragments

Gen.	G ₁	G ₂	G ₃	G ₄	G ₅	G ₆	G ₇	G ₈	G ₉	G ₁₀	G ₁₁	G ₁₂	G ₁₃	G ₁₄	G ₁₅	G ₁₆	G ₁₇	G ₁₈	G ₁₉	G ₂₀
G1	1.00																			
G2	0.89	1.00																		
G3	0.82	0.83	1.00																	
G4	0.80	0.88	0.86	1.00																
G5	0.74	0.80	0.77	0.82	1.00															
G6	0.76	0.82	0.87	0.84	0.78	1.00														
G7	0.76	0.82	0.82	0.84	0.74	0.90	1.00													
G8	0.87	0.86	0.88	0.88	0.80	0.82	0.80	1.00												
G9	0.75	0.83	0.83	0.81	0.82	0.82	0.77	0.78	1.00											
G10	0.80	0.88	0.86	0.88	0.80	0.82	0.80	0.90	0.78	1.00										
G11	0.78	0.82	0.75	0.73	0.67	0.78	0.76	0.75	0.77	0.80	1.00									
G12	0.85	0.87	0.87	0.89	0.83	0.81	0.81	0.92	0.84	0.84	0.74	1.00								
G13	0.83	0.82	0.80	0.75	0.64	0.74	0.78	0.77	0.80	0.80	0.83	0.78	1.00							
G14	0.78	0.77	0.84	0.80	0.86	0.81	0.74	0.87	0.77	0.84	0.74	0.83	0.74	1.00						
G15	0.77	0.81	0.90	0.83	0.84	0.82	0.77	0.83	0.83	0.81	0.75	0.84	0.75	0.82	1.00					
G16	0.80	0.81	0.88	0.81	0.80	0.80	0.77	0.86	0.83	0.83	0.75	0.87	0.77	0.80	0.88	1.00				
G17	0.80	0.83	0.86	0.83	0.82	0.84	0.77	0.86	0.74	0.88	0.70	0.82	0.70	0.82	0.81	0.88	1.00			
G18	0.80	0.76	0.76	0.78	0.70	0.73	0.72	0.81	0.76	0.76	0.84	0.77	0.80	0.75	0.78	0.76	0.67	1.00		
G19	0.73	0.77	0.77	0.82	0.71	0.74	0.83	0.77	0.70	0.80	0.71	0.76	0.78	0.74	0.73	0.77	0.77	0.77	1.00	
G20	0.69	0.70	0.77	0.73	0.81	0.71	0.69	0.75	0.70	0.73	0.64	0.74	0.67	0.74	0.80	0.80	0.80	0.68	0.71	1.00

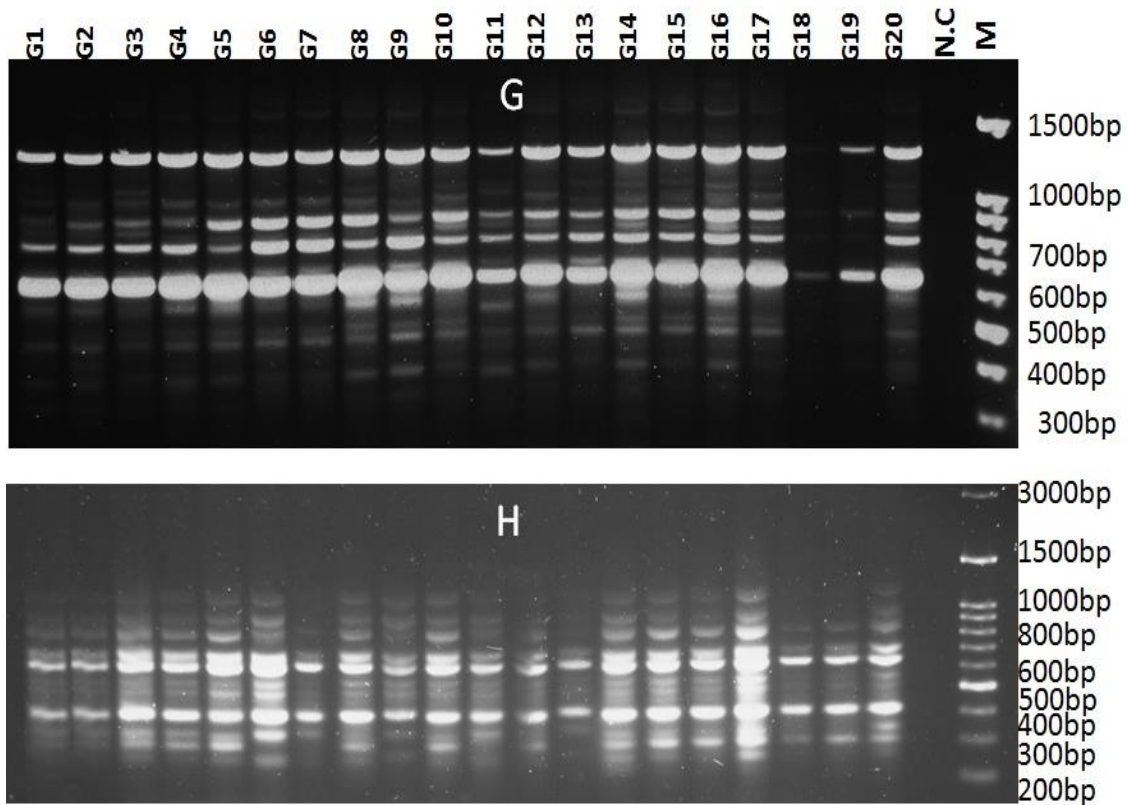


Fig. 4. RAPD profiles obtained for 20 Sorghum genotypes amplified with primers: (G) OPP-05 and (F) OPAW-13, M = 100bp ladder size marker

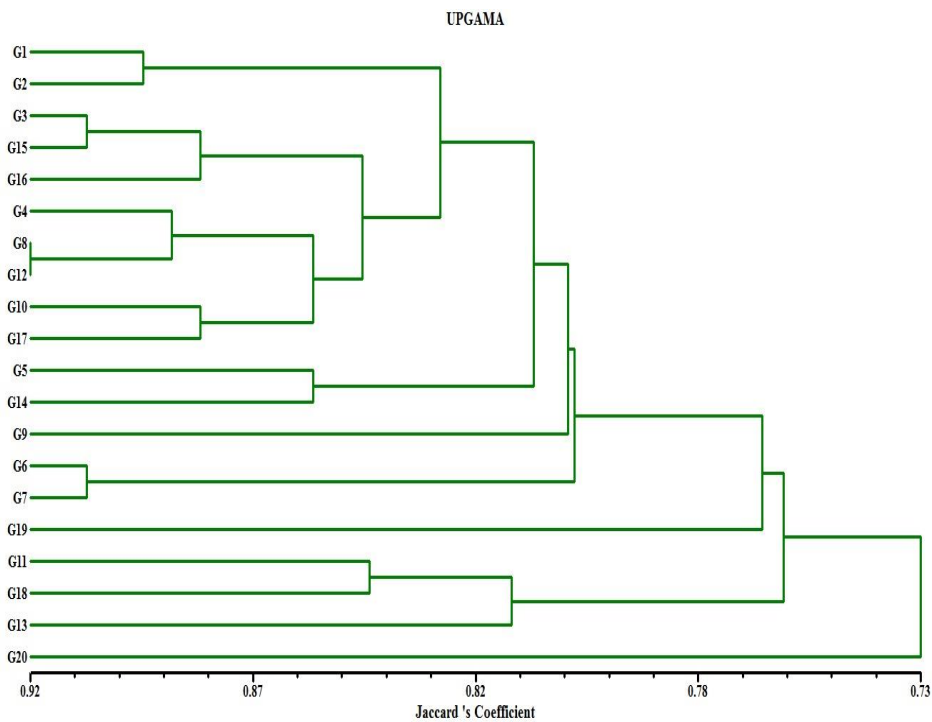


Fig. 5. Phylogenetic tree of the twenty sorghum genotypes obtained using 84 RAPD band

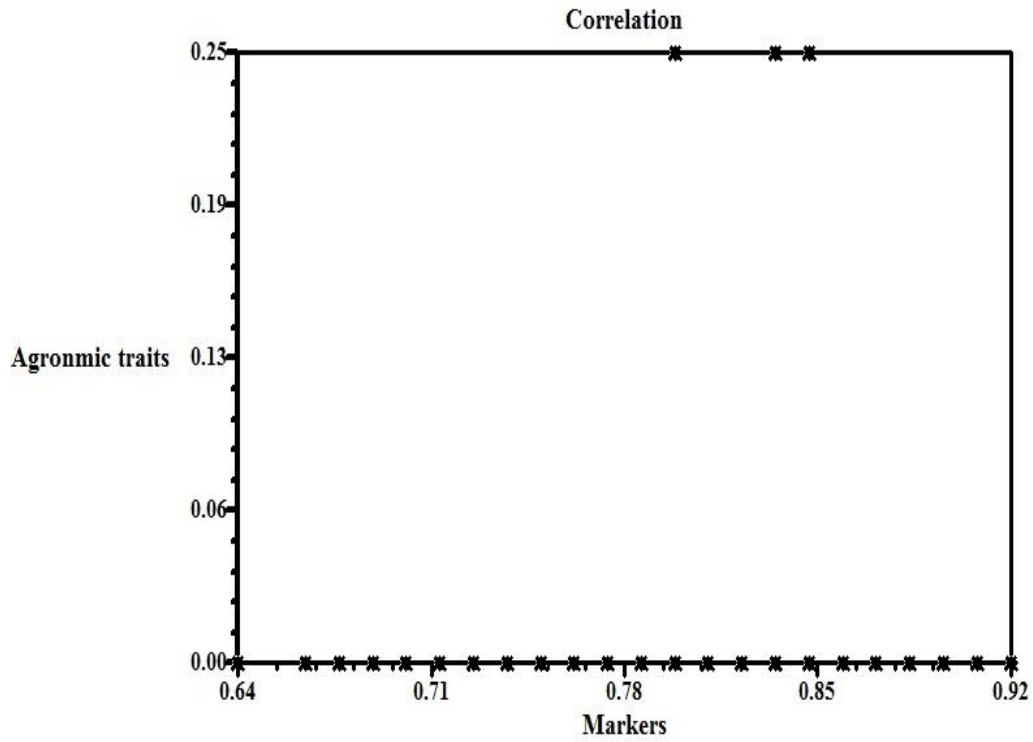


Fig. 6. Correlation between similarities percent obtained from markers and studied traits under normal conditions for the 20 Sorghum genotypes

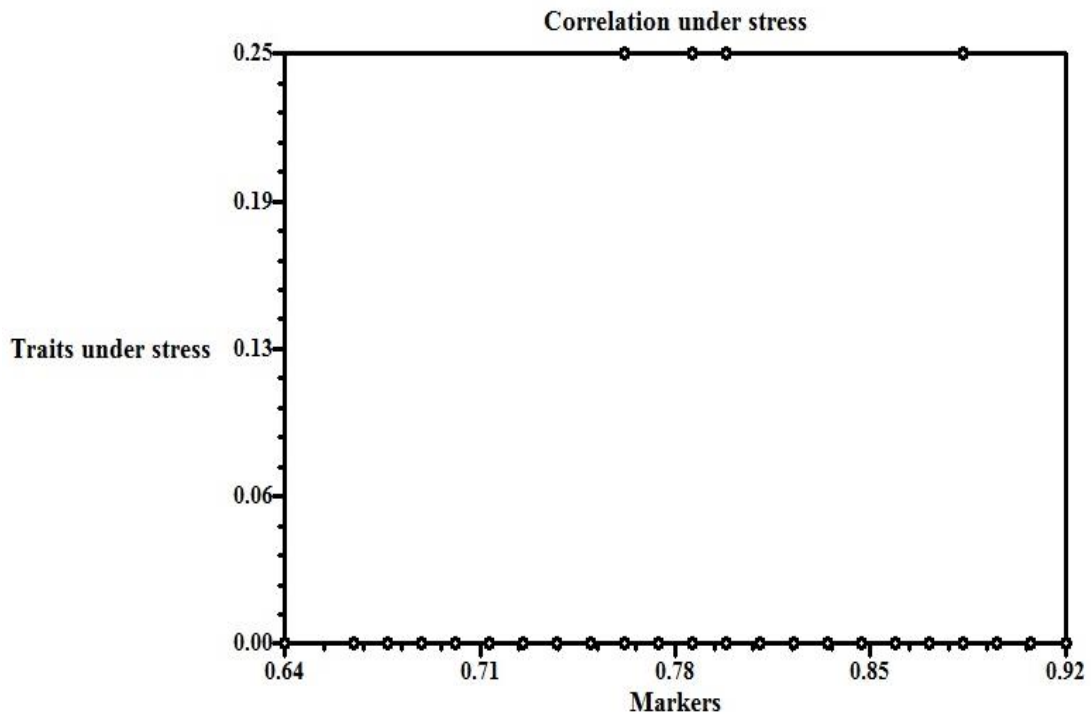


Fig. 7. Correlation between similarities percent obtained from markers and studied traits under stress conditions for the 20 Sorghum genotypes

3.7 Cluster Analysis

3.7.1 RAPD marker

Cluster analysis based on Jaccard's similarity coefficient using UPGMA grouped the seven flax genotypes into two main clusters and ranged from 0.64 to 0.92 with an average similarity index of 0.78 (Table 11). Similarity coefficient used to generate a dendrogram of sorghum genotypes based on UPGMA analysis (Fig. 5). The genetic tree divided the twenty genotypes into five groups. The first group formed four clusters, which contains ten genotypes. First cluster contain two genotypes branched at 85 % percent of similarity. While, the second cluster contains three genotypes branched at 86.5 % of similarity with the first cluster. The third cluster contains three genotypes branched at 86 % of similarity. The fourth cluster branched at 86.5 % with two genotypes. [39 and 40] illustrated a clear picture about classification and genetic diversity in sorghum-inbred lines.

3.8 Combined Molecular Markers and Morphological Markers

Correlation between the two distance matrices generated by the data of agronomic traits and molecular markers was calculated (Fig. 6 and Fig. 7). The Mantel test revealed that there were positive and non-significant correlation between the genetic distances based on phenotypic data and the similarity data based on RAPD markers, ($r= 0.07$, $P< 0.05$) and ($r= 0.03$, $P< 0.05$) under normal and drought conditions, respectively. Similar results were obtained by Shaikh et al. [40 and 41], which showed a non-significant positive correlation ($r= 0.07961$, $p = 0.7594$) between data of the morphological traits and RAPDs data in grain sorghum.

The results of this study demonstrated the effectiveness of the RAPD method as a useful DNA marker for identifying and estimating genetic variation among various genotypes of sorghum. Among the 20 genotypes, RAPD analysis shown good potential for determining genetic diversity under normal and drought environments. The genetic separations between the genotypes of sorghum may be useful in identifying parents for heterotic crosses and maximizing heterotic in hybridization projects. The polymorphic RAPD markers found in this work could be used to infer the evolutionary relationships between different cultivars. It is hypothesize that cultivars with the most unique

DNA profiles had the highest concentration of novel genes.

4. CONCLUSIONS

In conclusion, this study revealed that the genotypes G3, G7, G13 and G16 had the highest grain yield/plant under normal, drought and combined data. Theses genotypes will be test in advanced experiments. Traits with high genetic advance as a percent of mean are important traits, which should give attention in order to bring an effective response of grain improvement of the tested varieties. In addition, drought indices for harsh environmental conditions compared to optimal conditions, are useful in classifying the tested genotypes into groups that are associated with specific conditions. The RAPD analysis shown good potential for determining genetic diversity under normal and drought environments. The polymorphic RAPD markers found in this work could be used to infer the evolutionary relationships between different cultivars.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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