Asian Journal of Crop Science

ISSN 1994-7879

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Asian Journal of Crop Science 5 (2): 106-124, 2013
ISSN 1994-7879 / DOI: 10.3923/ajcs.2013.106.124

Genetic Diversity among Egyptian Sorghum (*Sorghum bicolor* L. Moench) Landraces in Agro-morphological Traits and Molecular Markers

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ABSTRACT

Genetic diversity in agro-morphological traits and molecular markers was assessed among sorghum landraces (*Sorghum bicolor* L.) collected from three agro-climatic regions in Upper Egypt. Days to 50% flowering, plant height (cm), panicle length (cm), panicle width (cm), thousand grain weight (g), number of grains panicle^{-1} and grain yield panicle^{-1} (g) were evaluated in two successive seasons. Molecular diversity was also assessed by RAPD and ISSR markers. The results indicated significant differences among genotypes for the agro-morphological traits and clustering based on these traits classified the genotypes into two single branches and three main clusters. A total of 109 DNA fragments were amplified by 10 primers from all landraces with an average 10.9 bands/primers. A total of 64 fragments (58.72%) showed polymorphism. Based on combined RAPD and ISSR data, the highest genetic similarity (0.963) was observed between Assuit 31-94 and Assuit 32-94 landraces and the lowest relatedness (0.81) was observed between Assuit 132-94 and Assuit 52-93. Cluster analysis based on a combined RAPD and SSR data set revealed four major distinct groups. Positive correlation was found between two molecular markers ($r = 0.267$, $p = 0.001$). A low correlation ($r = 0.083$, $p = 0.001$) was found between the morphological dissimilarity matrix and the matrices of genetic dissimilarity based on RAPD and ISSR markers. From the result of this study, it was found that combination of morphological and molecular markers may be useful in studying genetic diversity of sorghum for conservation, breeding and other crop improvement activities.

Key words: Sorghum landraces, yield and yield components, RAPD and ISSR markers

INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is one of the most important cereals crop in the world especially in arid and semiarid regions. And it can be grown in the environments where other crops cannot be able to grown successfully (Evan, 2009). Landraces are the most varied populations of cultivated crops (Frankel *et al*., 1995). The genetic variation among and within landraces makes them an important resource as potential donors of genes for the development of new crop varieties use by farmers (Qualset *et al*., 1997; Soleri and Smith, 1995). The earliest markers used to study diversity in germplasm were morphological traits (Stanton *et al*., 1994) but they have some

limitations such as low polymorphism, low heritability, late expression and influences by environmental conditions (Muthusamy *et al*., 2008). The genetic variations within and among sorghum landraces using morphological traits has been studied by Mehmood *et al*. (2008), Bucheyek *et al*. (2009), Reddy *et al*. (2012) and Rakshit *et al*. (2012).

Molecular markers are not influenced by the environmental conditions for this reason these markers are decisive and more efficient for selection in breeding programs as well as to assess genetic diversity amongst (Shehzad *et al*., 2009). Different molecular markers have been used in many studies of sorghum diversity (Mehmood *et al*., 2008; Shehzad *et al*., 2009) for example, SSRs (Nguni *et al*., 2011), RFLPs (Bhattramakki *et al*., 2000) and AFLPs (Ritter *et al*., 2007). Inter Simple Sequence Repeats (ISSR) (Chakraborty *et al*., 2011; Turki *et al*., 2011) and Random amplified polymorphism DNA (RAPD) (Mehmood *et al*., 2008; Iqbal *et al*., 2010; Sah and Khanna, 2010; Chakraborty *et al*., 2011; EL-Amin and Hamza, 2013) have been used successfully to assess plant genetic variations. This is due to the simplicity of these techniques and does not require previous knowledge of the sequence of the genome being tested. Molecular markers assay in complementary with agro-morphological traits analyses are very useful for germplasm collection, management and improvement because they increase the resolving power of genetic diversity analyses and provide complementary information (Singh *et al*., 1991).

The present study was, therefore, conducted with the following objectives: (1) to study the morphological and genetic diversity of Egyptian sorghum landrace accessions, (2) to classify sorghum landrace accessions into groups based on both agro-morphological traits and molecular profiles and (3) to assess the correlation between phenotypic and genotypic distances.

MATERIAL AND METHODS

Field evaluation: Twenty sorghum landraces that were collected from Assiut (14), Sohag (5) and Aswan (1) were grown at Arab El-Awamer research station, Assiut, Egypt in 2010 and 2011 seasons in a randomized complete block design with three replications. The two experiments were sown on the 15th of June with experimental unit being a five-row of 3.5 m long, spaced at 0.60 m apart and hill to hill distance of 0.20 m. Plots were thinned down to two plants per hill two weeks after crop emergence. Nitrogen, P and K were used at 100, 30 and 50 kg fad.$^{-1}$ which were added to all plots. All other cultural practices were carried out as recommended for sorghum production in both seasons.

Data were taken from the two inner rows to determined days to 50% flowering. Ten guarded plants for each plot were randomly chosen for measuring plant height (cm), panicle length (cm), panicle width (cm), thousand grain weight (g), number of grains panicle^{-1} and grain yield panicle^{-1} (g).

The homogeneity between the two years was ascertained using the method described by Snedecor *et al*. (1989) and then combine analysis was done. The correlation coefficients between all possible pairs of the characters were computed from the genotype means then test of significance was carried out with (n-2) degrees of freedom for phenotypic correlation by referring to the table given by Snedecor *et al*. (1989). Means were compared by using Revised LSD at 1% level of probability (Gomez and Gomez, 1984).

Genetic relationships between sorghum landraces based on morphological traits

Euclidean distance: The mean values of days to 50% flowering, plant height (cm), panicle length (cm), panicle width (cm), thousand grain weight (g), number of grains panicle^{-1} and grain yield panicle^{-1} (g), were calculated for all tested genotypes in order to detect patterns of genetic

relationship among the genotypes. Data analysis on the means of clearly defined seven traits was initially performed based on the Euclidean distance matrix. Firstly, the data was subjected to analysis to produce a matrix of dissimilarity values and the phenotypic distance between each pair of genotypes was estimated as Euclidean distance. Secondly, cluster analysis was conducted on the Euclidean distance matrix with un-weighted pair group method based on arithmetic averages (UPGAMA) to develop a dendrogram using computer program NTSYS-pc ver. 2.1 (Rohlf, 2000).

DNA extraction: Total genomic DNA was isolated from fresh leaves, bulked from 5 different plants per genotype using CTAB protocol for plants (Saghai-Maroof *et al*., 1984) with some modifications. RNA was removed from the DNA preparation by adding 10 μL of RNAase (10 mg mL^{-1}) and then incubated for 30 min at 37°C. DNA sample concentration was quantified by using a spectrophotometer (Genova 2138).

RAPD analysis: Six RAPD primers (Table 1), obtained from (metabion international AG), were tested in this experiment to amplify the templated DNA. The reaction conditions were optimized and mixtures (25 μL total volume) were composed of 11.0 μL dH_2O, 3.0 μL 10x reaction buffer, 3.0 μL dNTP's mix, 2.0 μL primer, 4.0 μL $MgCl_2$, 0.3 μL *Taq* DNA polymerase and 1 μL Template DNA. Amplification condition were carried out in a TECHNE thermocycler (Model FTGEN5D, TECHNE, Cambridge Ltd., Duxford and Cambridge, U.K.) with the following specification: initial denaturation for 3 min at 94°C (1st step), 40 cycles of 1 min at 92°C, 1 minutes at 33°C and 2 min at 72°C (2nd step), 10 minutes at 72°C (3rd step), then followed by a final hold at 4°C.

ISSR analysis: Four ISSR primers (Table 1), obtained from (metabion international AG) were tested in the present experiment, to amplify the templated DNA of the selected lines and their parents. The ISSR-PCR method was carried out, according to Nagaoka and Ogihara (1997). Amplification reactions were carried out in 25 μL volumes, containing (11.0 μL dH_2O, 3 μL of 10x buffer, 3.0 μL of dNTPs (2.5 mM) 4 μL of $MgCl_2$ (25 mM), 3.0 μL primer (2.5 μL), 0.3 μL of *Taq* polymerase (5 IU μL^{-1}) and 2.0 μL of genomic DNA (50 ng μL^{-1}). Amplification was performed in a TECHNE thermocycler (Model FTGEN5D, TECHNE, Cambridge Ltd., Duxford and Cambridge, U.K.). Programmed for an initial denaturation at 94°C 5 min, 45 cycles of 1 min denaturation at 94°C, 1 min annealing at 40°C and 2 min extension at 72°C followed by final extension for 10 min at 72°C. Amplification products were separated by horizontal gel electrophoresis unit using 1.4%

Table 1: Primer sequences and codes used

Serial No.	Primer codes	Sequence (5' to 3')
1	OPA01	5'-CAGGCCCTTC-3'
2	OPI09	5'-GAGTCAGCAG-3'
3	OPA08	5'-GTGACGTAGG-3'
4	OPA06	5'-GGTCCCTGAC-3'
5	UBC01	5'-CCTGGGCTTC-3'
6	OPA09	5'-GGGTAACGCC-3'
7	HB	5'-CCTGCTCATC-3'
8	HB11	5'-TGTGTGTGTGTCC-3'
9	HB12	5'-CACCACCACGC-3'
10	HB15	5'-GTGGTGGTGGC-3'

(for RAPD analysis) or 2% for (ISSR analysis) agarose gel. Electrophoresis was carried out under constant voltage of around 80 V for approximately 3-3.5 h. The banding patterns were visualized on a Transilluminator (Ultra-Violet Product, Upland, CA, USA).

Data analyses: RAPD and ISSR-based molecular markers were scored visually using the software package MVSP (Multi-Variate Statistical Package) and DNA bands were scored as present (1) or absent (0). The pairwise comparisons between the tested isolates were used to calculate the coefficient of genetic similarity matrix (Gs) according to Nei and Li (1979). Cluster analysis was presented as the dendrogram based on similarity estimates using the unweighted pair-group method with arithmetic average (UPGMA).

The relationships between the Euclidean distance matrix based on morphology and the Nie and Li distance matrices obtained with RAPD and ISSR markers were analyzed using the approach developed by Mantel (1967). All the data analyses were performed using computer program NTSYS-pc ver. 2.1 (Rohlf, 2000).

RESULTS AND DISCUSSION

From the results of this study, it was found that combination of morphological and molecular markers may be useful in studying genetic diversity of sorghum for conservation, breeding and other crop improvement activities.

Yield and yield attributes: The means of the agro-morphological traits of the twenty different landraces over two years are presented in Table 2. The analyses revealed that all the characters

Table 2: Means of grain yield/plant and other attributes over two year

Genotypes	No. of days to 50% flowering	Plant height (cm)	Panicle length (cm)	Panicle width (cm)	No. of kernel head^{-1}	1000 kernel weight (g)	Grain yield plant^{-1} (g)
Assuit 51-93	83.17	269.29	21.25	10.63	1553.00	36.83	65.52
Assuit 52-93	83.84	315.25	18.58	8.60	1266.00	34.32	47.49
Assuit 53-93	82.83	309.64	19.62	6.65	721.00	31.77	54.37
Assuit 63-93	84.83	261.10	20.40	7.90	962.50	35.87	40.35
Assuit 73-93	85.17	270.15	20.08	6.67	765.00	35.80	40.73
Assuit 11-94	84.67	229.72	17.73	8.05	893.00	31.77	53.85
Assuit 12-94	86.00	223.43	18.05	7.58	506.50	33.22	36.03
Assuit 31-94	84.67	247.75	18.22	7.42	973.50	35.25	44.65
Assuit 32-94	84.50	263.44	22.40	6.62	1088.00	35.17	45.00
Assuit 111-94	84.67	276.29	20.74	9.48	710.00	33.37	45.99
Assuit 112-94	84.17	263.62	20.77	9.43	929.50	34.05	50.50
Assuit 113-94	85.34	228.14	15.12	7.60	662.00	35.98	35.99
Assuit 131-94	95.17	244.62	17.62	7.45	791.50	35.53	21.89
Assuit 132-94	82.67	228.85	20.63	8.13	1048.00	35.43	61.07
Sohag 1-93	84.50	266.55	19.25	8.63	1154.50	34.48	51.84
Sohag 2-93	84.50	180.38	20.65	8.97	1843.00	32.98	56.45
Sohag 4-93	84.84	188.88	22.82	9.13	1154.00	34.72	50.52
Sohag 51-93	84.84	236.77	21.67	8.45	794.50	32.33	36.85
Sohag 52-93	83.84	252.97	19.85	7.68	1522.00	16.13	21.00
Aswan 1-93	83.17	261.97	20.28	5.73	1100.00	32.22	44.24
Revised LSD 0.01	3.09	11.81	2.38	0.54	229.51	1.93	3.74

studied showed highly significant (p = 0.01) variation between genotypes (Table 3). The results exhibited in Table 2 revealed that the flowering time extended from 82.67 days (Assuit 132-94) to 95.17 days (Assuit 131-94). With respect to plant height, results showed that the landrace Assuit 52-93 was the tallest plant height with 315.25 cm while, the shortest was that of Sohag 2-93 with a mean 180.38 cm. The differences between landraces studied in Panicle length among the twenty landraces ranged from 15.12 cm (Assuit 113-94) to 22.82 cm (Sohag 4-93). Moreover, landrace Assuit 51-93 gave the highest mean value of panicle width (10.63 cm) while the lowest one (5.73 cm) was obtained by landrace Aswan 1-93. Here too, the highest mean value of number of kernel head^{-1} (1843) was exhibited by landrace Sohag 2-93 while the lowest mean value (506.50) was displayed by landrace Assuit 12-94. The 1000 kernel weight ranged from 16.13 g (Sohag 52-93) to 36.38 g (Assuit 51-93). Grain yield panicle^{-1} ranged from 21 g (Sohag 52-93) to 65.52 g (Assuit 51-93). Evidently, the superiority of landrace Assuit 51-93 in grain yield could be attributed to its superior 1000 kernel weight as well as in number of kernel panicle^{-1}, which are considered the most important yield components affecting grain yield panicle^{-1}. Similar results were found by Tesso *et al.* (2011).

The phenotypic correlations between agro-morphological traits studied are presented in Table 4. Negative and significant correlation were obtained between days to 50% flowering and number of kernel panicle^{-1} (-0.2870, p<0.05), panicle length (-0.3515, p<0.05) as well as grain yield panicle^{-1} (-0.5958, p<0.05) such negative association might be due to the low temperature that prevailed late in the season which caused an inhibition of pollination and fertilization that consequently reduced number of grains panicle^{-1} and grain yield. Abdalla and Gamar (2011) reported highly significant differences between sorghum genotypes in days to 50% flowering. Similar results were earlier reported by Mahajan *et al.* (2011) who found negative correlation between days to 50% flowering and grain yield. Furthermore, thousand kernel weight and number of kernel panicle^{-1} showed positive and highly significant correlation with grain yield panicle^{-1} (0.4480 and 0.3135 for thousand kernel weight and number of kernel panicle^{-1}, respectively)

Table 3: Combined analysis of variance of different characters

SOV	df	Days to 50% flowering	Plant height	Panicle length	Panicle width	1000 kernel weight	No. of kernel head^{-1}	Grain yield plant^{-1}
Year	1	4.03	1342.68*	18487*	2.852**	1561.18**	9651	345.78**
Error a	4	18.083	79.865	1.985	0.0828	4.496	22471.5	4.38
Genotype	19	39.66**	6635.79**	20.116**	8.2411	112.388**	662315**	779.64**
Year×genotype	19	3.875	33.67	0.0669	0.064	1.306	15400	2.31
Error b	76	3.999	40.57	1.417	0.0871	1.081	15334	4.065

*,**Significant at 5 and 1% level of probabilities, respectively

Table 4: Phenotypic correlations of yield and yield contributing characters in sorghum

Characters	Days to 50% flowering	Grain yield panicle^{-1}	1000 kernel weight	Number of kernel panicle^{-1}	Plant height	Panicle length	Panicle width
Days to 50% flowering							
Grain yield panicle^{-1}	-0.5958**						
1000 kernel weight	0.1613	0.4480**					
Number of kernel panicle^{-1}	-0.2870*	0.3135**	-0.2862*				
Plant height	-0.1736	0.0171	-0.0092	-0.1849			
Panicle length	-0.3515**	0.3456**	-0.0212	0.3745**	-0.0519		
Panicle width	-0.1137	0.4269**	0.1374	0.3669**	-0.1610	0.2565*	

*,**Significant at 5 and 1% level of probabilities, respectively

whereas, the two characters were negatively correlated (-0.2862). This might be due to the distribution of assimilates on greater number of grains which led to corky grains. Also, The correlation between panicle length and grain yield panicle^{-1} was positive and highly significant (0.3456) which agrees closely with the results obtained by Mahajan *et al.* (2011).

There were positive and highly significant ($p \leq 0.01$) correlations between grains yield panicle^{-1} and panicle length, panicle width, thousand kernel weight and number of kernel panicle-1 (Table 4). Significant and positive correlations between grain yield and other yield attributes are quite desirable in plant breeding, because it facilitates the selection process. Similar observations were reported by Aba and Obilana (1994), Bello *et al.* (2001) and Izge *et al.* (2006).

Morphological distance and cluster analysis: Estimates of the genetic distances matrix based on Agro-morphological traits for all pair-wise combinations of (20×19)/2 = 190 for the 20 sorghum landraces are presented in Table 5. Genetic distances from 1.26 to 7.323 were observed in the pair-wise combinations, indicating that the landraces were diverse for the Agro-morphological traits measured.

The minimum genetic distance (1.26) was recorded between the two landraces Assuit 73-93 and Assuit 63-93. On the other hand, the maximum genetic distance of 7.323 was recorded between landraces Assuit 51-93 and Assuit 131-94, indicating a high genetic diversity between the landraces.

Cluster analysis: Cluster analysis of the twenty sorghum landraces based on the standardized value of morphological traits was performed by UPGMA method and a dendrogram was constructed as depicted in Fig. 1.

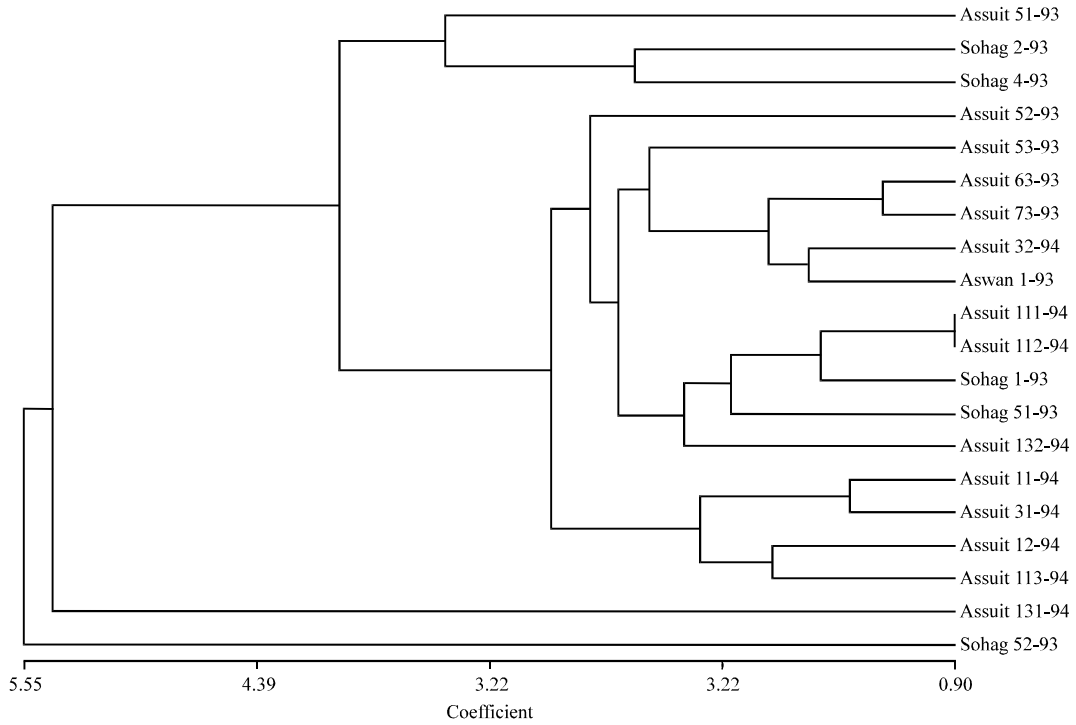

Fig. 1: Cluster analysis of twenty sorghum landraces derived by UPGMA from Euclidian dissimilarity matrix of agro-morphological data

Table 5: Euclidean distance matrix of the twenty sorghum landraces using agro-morphological traits

	Assuit 51-93	Assuit 52-93	Assuit 53-93	Assuit 63-93	Assuit 73-93	Assuit 11-94	Assuit 12-94	Assuit 31-94	Assuit 32-94	Assuit 111-94	Assuit 112-94	Assuit 113-94	Assuit 131-94	Assuit 132-94	Sohag 1-93	Sohag 2-93	Sohag 4-93	Sohag 51-93	Sohag 52-93	Aswan 1-93
Assuit 51-93	0																			
Assuit 52-93	3.27	0																		
Assuit 53-93	4.736	2.59	0																	
Assuit 63-93	3.771	2.35	2.65	0																
Assuit 73-93	4.782	2.87	2.15	1.26	0															
Assuit 11-94	4.082	3.01	3.01	2.32	2.63	0														
Assuit 12-94	5.501	3.93	3.57	2.37	2.25	2.1	0													
Assuit 31-94	4.188	2.48	2.64	1.38	1.57	1.42	1.9	0												
Assuit 32-94	4.215	3.17	2.68	1.66	1.68	3.26	3.46	2.45	0											
Assuit 111-94	3.382	2.51	2.9	1.8	2.55	2.66	2.95	2.56	2.9	0										
Assuit 112-94	2.641	2.34	3	1.67	2.65	2.37	3.15	2.35	2.66	0.9	0									
Assuit 113-94	5.838	4	4.15	3.22	3.14	2.48	1.81	2.19	4.48	3.91	3.97	0								
Assuit 131-94	7.323	5.69	6.12	4.66	4.55	5.08	3.97	4.59	5.46	5.32	5.57	4.29	0							
Assuit 132-94	2.959	3.21	3.14	2.25	2.84	2.11	3.41	2.27	2.49	2.64	1.94	4.07	6.27	0						
Sohag 1-93	2.749	1.61	2.67	1.51	2.35	1.79	3.01	1.56	2.52	1.84	1.29	3.4	5.26	1.829	0					
Sohag 2-93	3.424	4.65	5.59	4.03	4.92	3.7	4.98	3.98	4.2	4.58	3.79	5.39	6.7	3.059	3.46	0				
Sohag 4-93	3.472	4.51	4.86	2.96	3.85	3.36	3.92	3.49	3.16	3.22	2.63	4.97	6.05	2.303	3.08	2.5	0			
Sohag 51-93	4.185	3.42	3.39	1.5	2.2	2.67	2.37	2.38	2.23	1.79	1.85	3.78	4.91	2.603	2.4	4.04	2.39	0		
Sohag 52-93	6.735	5.32	5.59	5.18	5.49	5.19	5.4	5.25	5.33	5.46	5.44	6.09	6.78	5.944	5.25	5.66	5.87	4.75	0	
Aswan 1-93	4.932	3.17	2.22	2.2	1.77	2.87	3.21	2.12	1.63	3.51	3.31	3.91	5.62	2.833	2.74	4.49	4.03	2.87	4.75	0

The dendrogram divided the landraces into two single branches and three main clusters. The latest flowering landrace Assuit 131-94 was separated in a single branch from the other landraces at distance of about 5.33. While the landrace Sohag 52-93 which was the lowest in 1000 kernel weight and grain yield per plant was separated in a single branch at distance of about 5.55. The first main cluster was produced at a genetic distance of (2.176) and created two sub-clusters, the first sub-cluster included Assuit 11-94 and Assuit 31-94 and the second sub-cluster included Assuit 12-94 and Assuit 113-94. The second main cluster was formed at a genetic distance of (2.872) and comprised eight landraces from Assuit, two landraces from Sohag and the one from Aswan. The third cluster consisted of three landraces Sohag 2-94, Sohag 4-93 and Assuit 51-93.

The range of Euclidean distance among all genotypes (1.26-7.323) is relatively wide and the average Euclidean distance (4.29) was high too. This indicated that the amount of agro-morphological variation among the genotypes is relatively high. These values, which are assumed to reflect the genetic diversity of the loci controlling these traits, indicated the possibility of selecting varieties that have a diverse genetic background and the prospect of obtaining broad segregation for the characters. Similar finding were obtained by Mehmood *et al.* (2008), Bucheyek *et al.* (2009), Reddy *et al.* (2012) and Rakshit *et al.* (2012).

RAPD analysis: The RAPD profiles of the amplification products of the six random primers are shown in Fig. 2-3 and the number of bands generated by each primer is given in Table 6. Six primers were used to screen twenty sorghum landraces produced a total number of 67 amplified DNA products were generated across twenty sorghum landraces with an average of 11.17 bands/primer. Out of the total bands, 42 were polymorphic and the percentage of polymorphic amplified products ranged from 76.92% for primer OPA06 to 50% for primer OPA09 and OPI09. The total number of the amplified RAPD produced by each primer varied from a minimum number of 9 amplified products by primer OPA01 to a maximum of 13 amplified products by primer OPA-06. The size of amplified bands also varied with different primers. The largest 1069 bp band was amplified by primer OPA06, while the smallest size 95 bp was amplified by primer OPI09. Unique DNA fragments with different sizes were detected in particular genotypes but not in the others using different primers. The presence of a unique band for a given genotype is referred as positive marker while the absence of a common band served as negative marker. Such bands could be used as DNA markers for genotype identification and discrimination. In this respect, one DNA fragment in the landrace Assuit 112-94 [173 bp (OPA09)], one band in Sohag 51-93 [752 bp (OPA06)], one band in Assuit 131-94 [696 bp (UBC01)] and one band in Assuit 51-93 [343 bp (UBC01)] were genotype-specific positive markers (Fig. 2, 3). Varieties-specific negative markers were also recorded for Assuit 52-93 [398 bp, 334 bp and 302 bp (OPA01)] and Sohag 52-93 [211 bp (OPA01)].

Such a level of polymorphism was consistent with some reports based on RAPD marker (Mehmood *et al.*, 2008; Iqbal *et al.*, 2010; Sah and Khanna, 2010; Chakraborty *et al.*, 2011; EL-Amin and Hamza, 2013; Dalvi *et al.*, 2012).

In order to compare the extent of agreement between dendrograms derived from RAPD marker and morphological traits, a distance matrix was constructed for each assay and compared using Mantel matrix correspondence test (Table 7). Positive correlation was found between RAPD marker and morphological traits ($r = 0.161$, $p = 0.001$). The RAPD technique used here was found to be quite effective in determining the genetic variation among sorghum landraces. By knowing about the diversity of sorghum landraces, a plant breeder can use the highly diverse lines in further breeding programs (Quagliaro *et al.*, 2001).

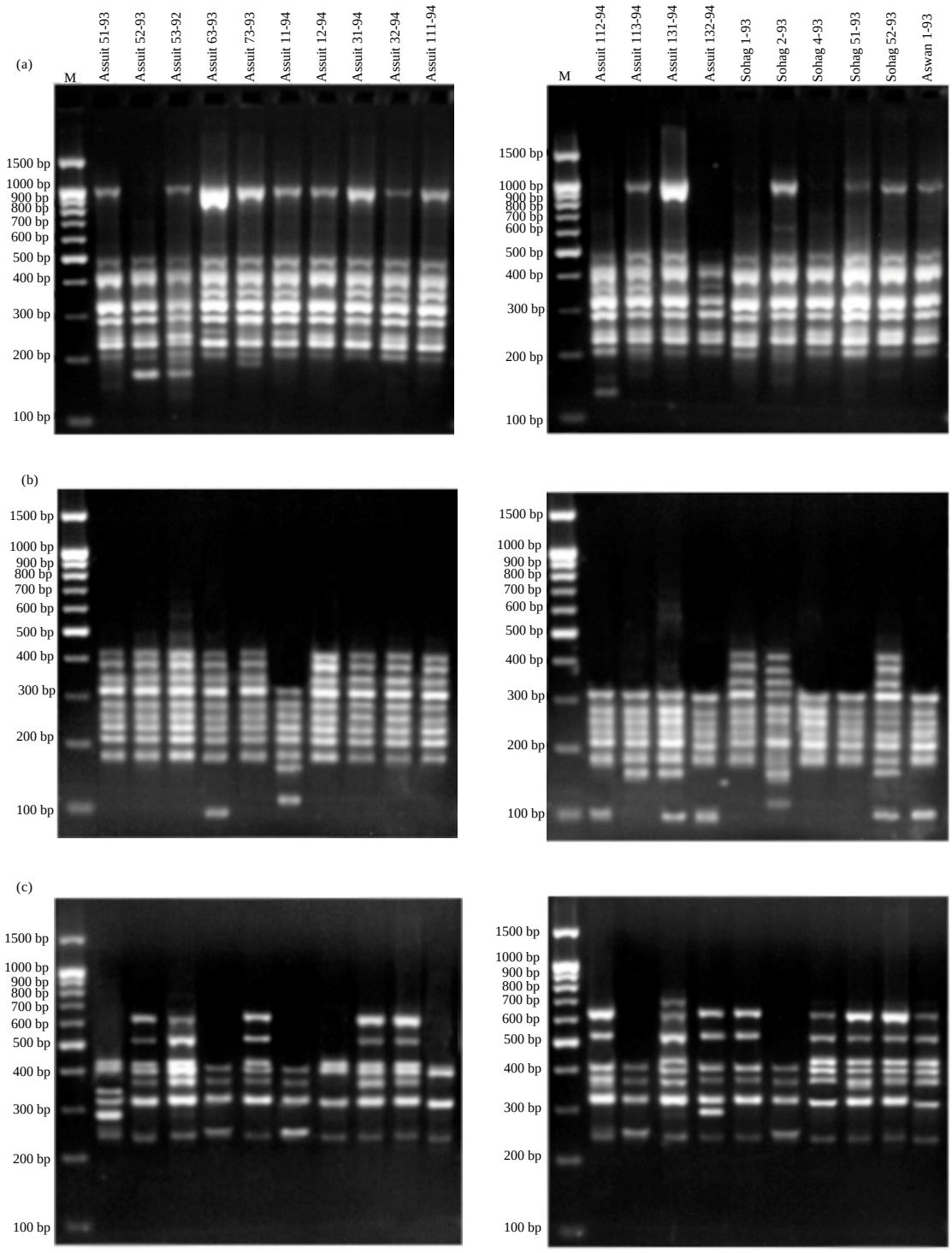

Fig. 2(a-c): Agarose gel electrophoresis of amplification products obtained with RAPD primers (a) OPA01, (b) OPA06 and (c) OPA08 from twenty sorghum landraces

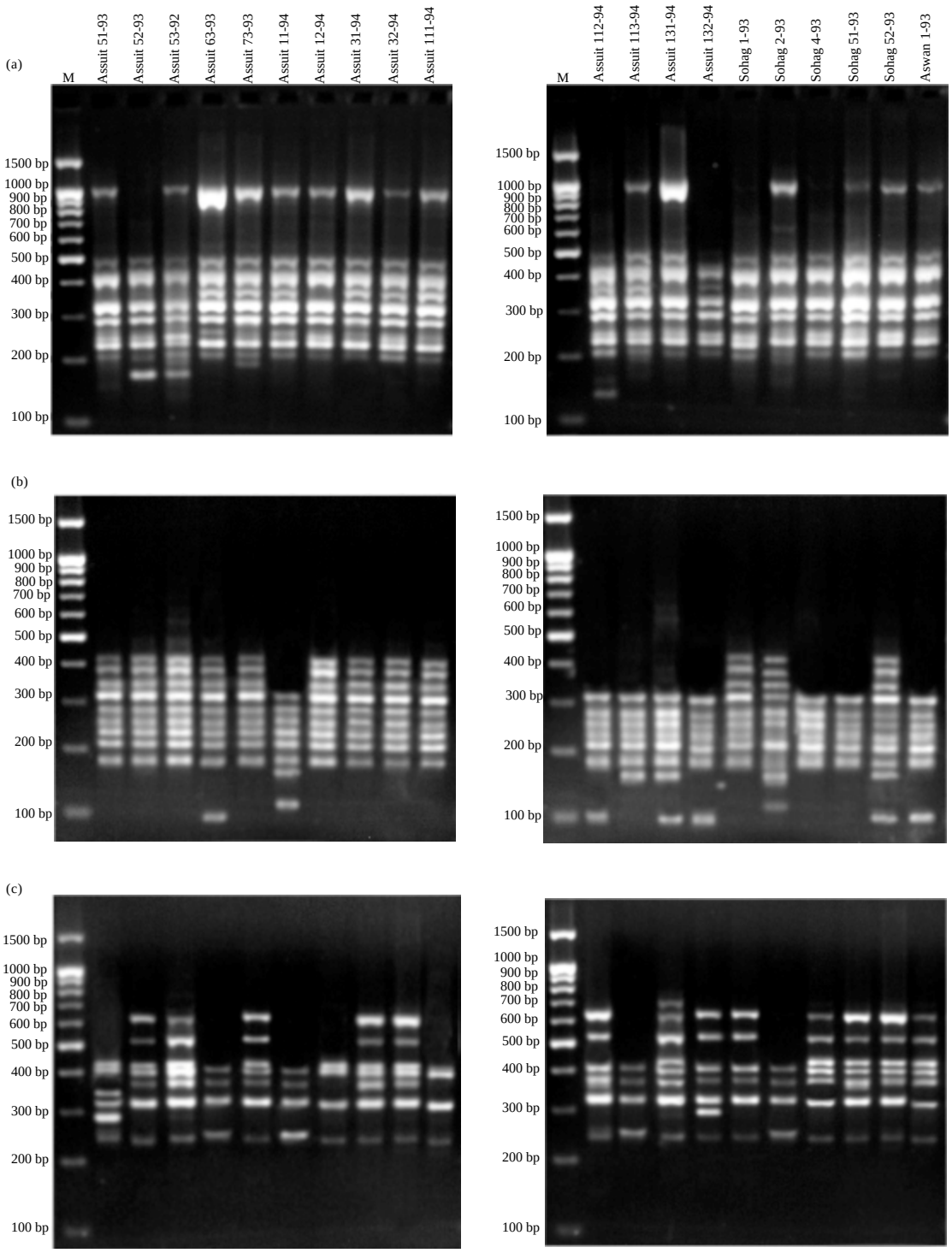

Fig. 3(a-c): Agarose gel electrophoresis of amplification products obtained with RAPD primers (a) OPA09, (b) OPI09 and (c) UBC01 from twenty sorghum landraces

Table 6: Number of amplified DNA-fragments and polymorphic bands in 20 sorghum lines investigated with RAPD and ISSR primers

Primers	Assuit 51-93	Assuit 52-93	Assuit 53-93	Assuit 63-93	Assuit 73-93	Assuit 11-94	Assuit 12-94	Assuit 31-94	Assuit 32-94	Assuit 111-94	Assuit 112-94	Assuit 113-94	Assuit 131-94	Assuit 132-94	Sohag 1-93	Sohag 2-93	Sohag 4-93	Sohag 51-93	Sohag 52-93	Aswan 1-93	Total amplified bands	No. of polymorphic bands	Polymorphic bands (%)
OPA09	8	7	10	9	9	9	8	8	9	8	9	9	8	8	7	7	7	7	8	7	12	6	50.00
OPA06	11	7	10	6	8	6	9	8	9	10	11	11	8	10	11	9	9	10	8	8	13	10	76.92
UBC01	6	7	7	4	7	4	4	7	7	3	6	4	8	7	6	4	7	7	7	7	10	7	70.00
OPI09	9	9	9	10	9	8	9	9	9	9	7	7	8	7	9	11	6	6	11	7	12	6	50.00
OPA01	9	4	9	9	9	9	9	8	9	9	8	8	8	9	9	8	7	8	8	9	9	7	77.78
OPA08	8	8	10	7	7	8	7	7	7	6	7	6	6	9	7	8	7	7	7	6	11	6	54.55
HB11	8	9	9	9	9	9	9	8	8	8	9	8	8	9	9	9	9	8	8	8	10	4	40.00
HB	8	7	10	9	9	9	9	9	8	7	6	9	9	9	9	9	9	9	9	9	13	10	76.92
HB15	7	7	7	4	7	7	7	7	7	7	4	7	7	4	7	7	7	7	7	7	7	3	42.86
HB12	11	10	10	10	10	10	10	10	10	10	10	10	10	11	11	11	10	8	10	10	12	5	41.67
Total	85	75	91	77	84	79	81	81	83	77	77	79	80	83	85	83	78	77	83	78	109	64	58.72

Table 7: Correlation between dissimilarity matrices of morphological traits and molecular markers analyzed according to Mantel test

Name	R
AGR+RAPD	0.161
AGR+ISSR	-0.086
AGR+ISSR+RAPD	-0.131
AGR+(ISSR+RAPD)	0.083
RAPD+ISSR	0.267
(ISSR+RAPD)+RAPD	0.906
(ISSR+RAPD)+ISSR	0.641

ISSR analysis: Four ISSR primers were used to analyze the twenty investigated sorghum landraces. ISSR marker profiles produced by the four primers are shown in Fig. 4-5. A total of 42 discernible and reproducible ISSR bands were generated with four selected primers across the twenty sorghum landraces, out of which 22 (52.38%) were polymorphic and 20 (47.62%) were common to all landraces studied (Table 6). The bands ranged in size from 732 bp for HB11 primer to 168 bp for HB primer. Number of bands produced per primer ranged between seven (HB15) and thirteen (HB) with an average of 10.5 and that of polymorphic bands per primer between three and ten with an average 5.5 (Table 6).

Unique DNA fragments with different sizes were detected in particular genotypes but not in the others using different primers. In this respect, one DNA fragment in the landrace Sohag 2-93 [236 bp (HB11)], one band in Assuit 111-94 [375 bp (HB)] and one band in Sohag 4-93 [320 bp (HB11)] were genotype-specific positive markers (Fig. 4). Genotype-specific negative markers were also recorded for Sohag 51-93 [550 bp and 610 bp (HB12)], one band in Aswan 1-93 [219 bp (HB11)], one band in Assuit 111-94 [595 bp (HB)], one band in Sohag 4-93 [354 bp (HB)], one band in Assuit 32-94 [236 bp (HB)] and one band in Assuit 113-94 [261 bp (HB12)] (Fig. 4). These results are in agreement with Turki *et al.* (2011) and Chakraborty *et al.* (2011) who indicated that ISSR markers could be realistically used to evaluate the genetic diversity and differentiation among sorghum genotype.

Mantel's test is presented in Table 7. The correlation between the Euclidean distance matrices based on morphological and ISSR data was low and non-significant ($r = -0.086$, $p = 0.3097$).

RAPD and ISSR analysis: The similarity coefficients of the twenty sorghum landraces based on RAPD and ISSR markers ranged from 0.963 to 0.818. Of the 190 pair wise combinations among the 20 landraces, Assuit 31-94 and Assuit 32-94 showed the highest similarity index (0.963), while the landraces Assuit 132-94 and Assuit 52-93 showed the lowest index (0.81) (Table 8).

Cluster analysis performed from combining data of both markers generated a dendrogram that separated the landraces into four distinct clusters. The first cluster was further divided into two sub-groups: the first regrouped seven landraces, Assuit 31-94, Assuit 32-94, Assuit 73-93, Assuit 53-93, Assuit 112-94, Assuit 51-93 and Sohag 1-93 with similarity ranged from 0.963 to 0.921 and the second contained four landraces, Assuit 131-94, Sohag 51-93, Sohag 52-93 and Aswan 1-93 similarity ranged from 0.944 to 0.921. The second cluster included five landraces, Assuit 63-93, Assuit 11-94, Assuit 113-94, Sohag 2-93 and Assuit 111-94. The third cluster contained three landraces, Assuit 112-94, Assuit 132-94 and Sohag 4-93. The fourth cluster was represent by one landraces Assuit 52-93 which separated in a single branch from the other landraces within 0.847% branched-off genetic similarity (Fig. 6).

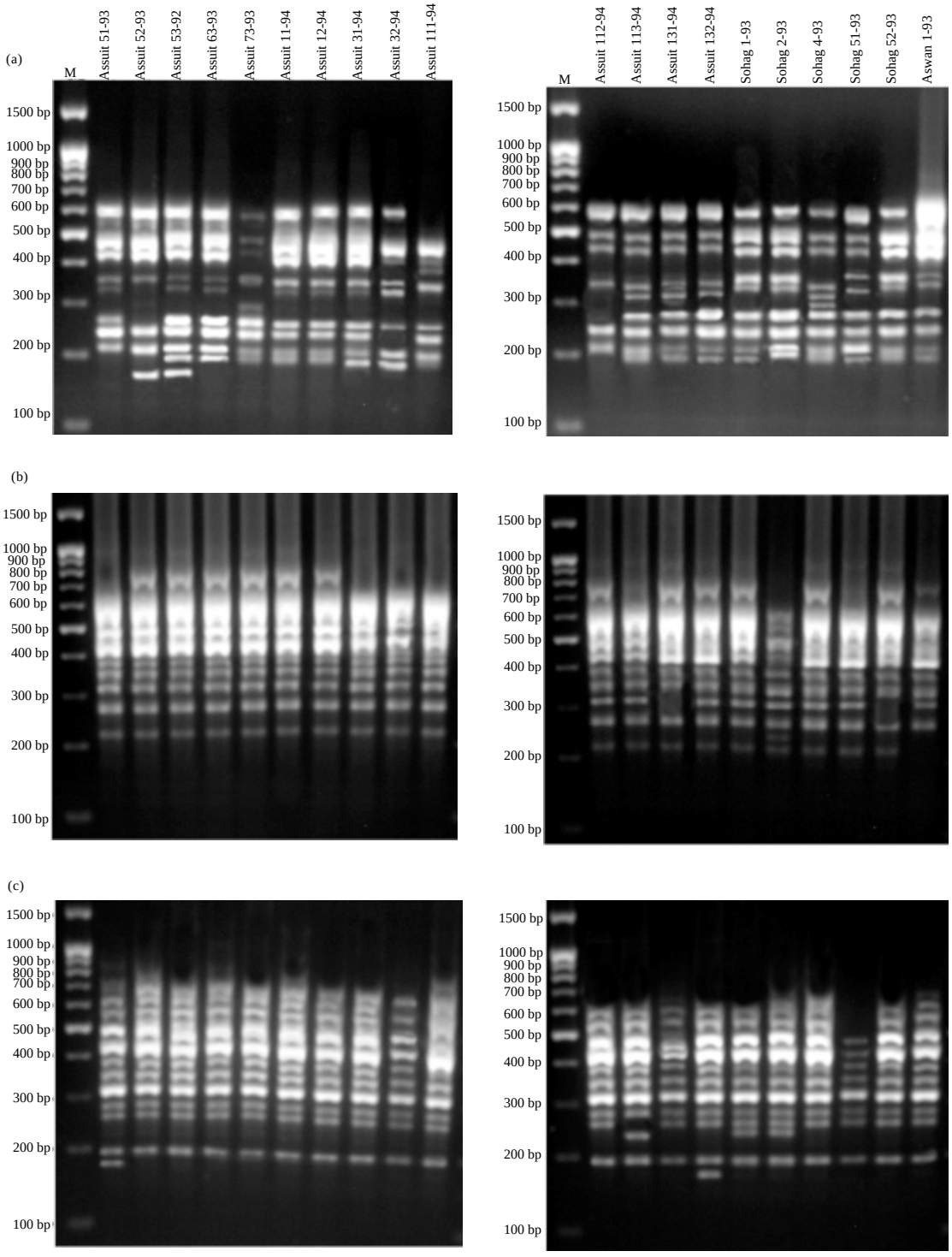

Fig. 4(a-c): Agarose gel electrophoresis of amplification products obtained with ISSR primers HB, HB11 and HB12 in twenty sorghum landraces

Table 8: Genetic similarity values calculated from 109 DNA fragments generated with 10 primers in twenty sorghum landraces

	Assuit 51-93	Assuit 52-93	Assuit 53-93	Assuit 63-93	Assuit 73-93	Assuit 11-94	Assuit 12-94	Assuit 31-94	Assuit 32-94	Assuit 111-94	Assuit 112-94	Assuit 113-94	Assuit 131-94	Assuit 132-94	Sohag 1-93	Sohag 2-93	Sohag 4-93	Sohag 51-93	Sohag 52-93	Aswan 1-93
Assuit 51-93	1																			
Assuit 52-93	0.825	1																		
Assuit 53-93	0.909	0.892	1																	
Assuit 63-93	0.877	0.829	0.881	1																
Assuit 73-93	0.888	0.868	0.926	0.907	1															
Assuit 11-94	0.878	0.818	0.882	0.936	0.896	1														
Assuit 12-94	0.94	0.846	0.93	0.924	0.933	0.913	1													
Assuit 31-94	0.904	0.885	0.93	0.911	0.945	0.9	0.938	1												
Assuit 32-94	0.917	0.861	0.943	0.913	0.946	0.901	0.939	0.963	1											
Assuit 111-94	0.914	0.855	0.869	0.896	0.907	0.885	0.924	0.911	0.913	1										
Assuit 112-94	0.84	0.829	0.869	0.87	0.857	0.859	0.848	0.861	0.875	0.844	1									
Assuit 113-94	0.902	0.831	0.882	0.897	0.883	0.937	0.925	0.913	0.914	0.923	0.885	1								
Assuit 131-94	0.861	0.839	0.901	0.879	0.89	0.906	0.907	0.919	0.908	0.841	0.892	0.918	1							
Assuit 132-94	0.881	0.81	0.874	0.913	0.874	0.901	0.878	0.878	0.892	0.863	0.925	0.901	0.883	1						
Sohag 1-93	0.918	0.875	0.932	0.901	0.911	0.902	0.94	0.928	0.929	0.914	0.889	0.927	0.897	0.917	1					
Sohag 2-93	0.893	0.823	0.885	0.888	0.886	0.914	0.915	0.915	0.892	0.9	0.825	0.914	0.871	0.855	0.929	1				
Sohag 4-93	0.859	0.85	0.876	0.839	0.889	0.879	0.868	0.868	0.882	0.839	0.877	0.892	0.886	0.882	0.908	0.87	1			
Sohag 51-93	0.877	0.842	0.893	0.87	0.907	0.897	0.911	0.937	0.913	0.87	0.857	0.923	0.93	0.875	0.901	0.888	0.89	1		
Sohag 52-93	0.893	0.861	0.92	0.925	0.922	0.914	0.939	0.927	0.94	0.888	0.85	0.901	0.933	0.892	0.929	0.892	0.87	0.9	1	
Aswan 1-93	0.883	0.863	0.899	0.903	0.914	0.917	0.918	0.918	0.919	0.89	0.865	0.917	0.924	0.907	0.92	0.87	0.897	0.929	0.944	1

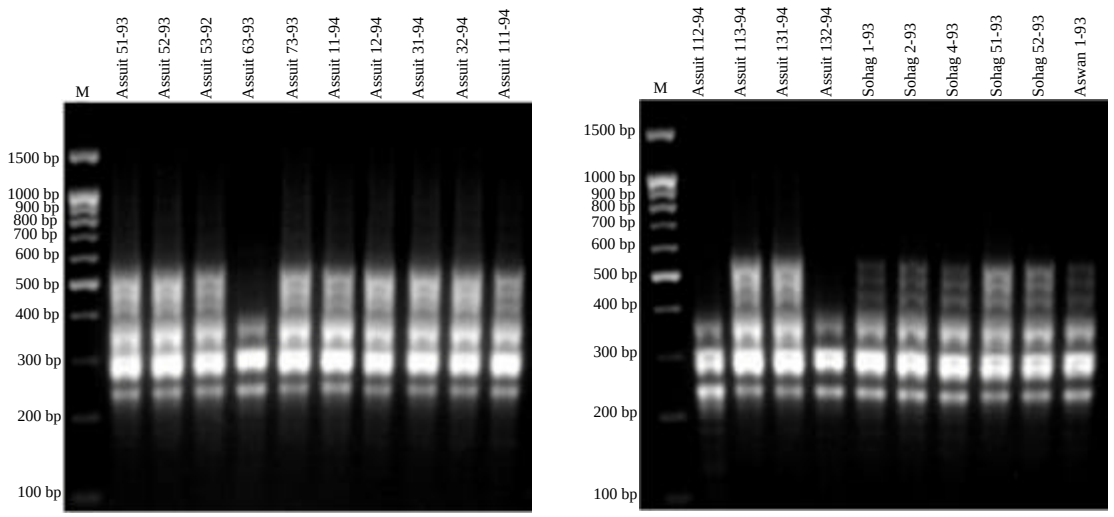

Fig. 5: Agarose gel electrophoresis of amplification products obtained with ISSR primer HB15 from twenty sorghum landraces

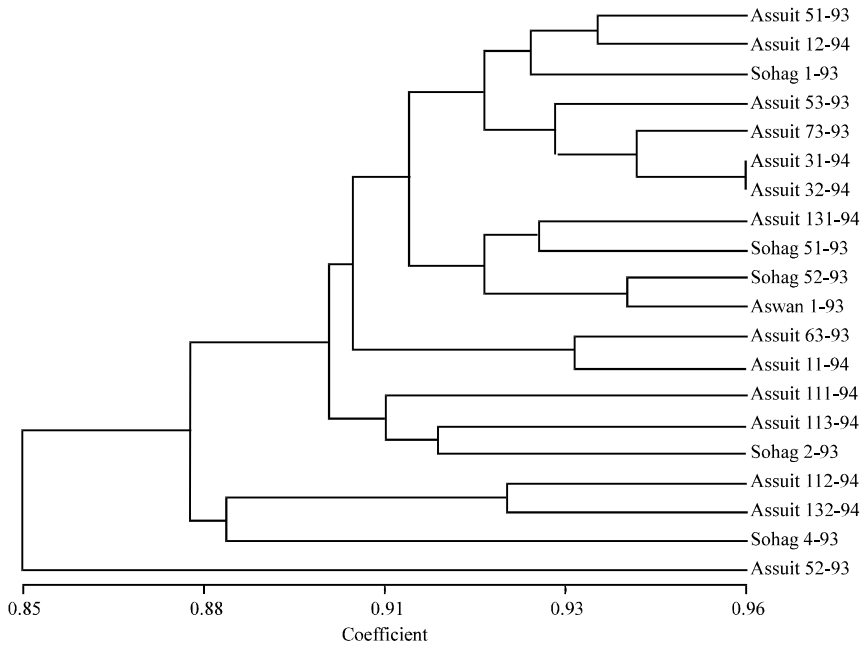

Fig. 6: Dendrogram of twenty sorghum landraces developed from RAPD and ISSR data using UPGMA analysis, The scale is based on Nei and Li coefficients of similarity

From the two molecular marker systems (RAPD and ISSR primers), a total of 109 DNA fragments were amplified by 10 primers from all landraces with an average 10.9 bands/primers. Out of these fragments 64 (58.72%) showed polymorphism and 45 (41.28%) bands were common

in all studied genotypes. The results showed that landrace Assuit 53-93 displayed the highest number of DNA fragments (91 bands), while the landrace Assuit 52-93 showed the lowest number 121 bands (Table 6).

Fragments at molecular size 508 bp and 428 bp generated by OPA08 primer, the 195 bp generated by OPA09 primer and the 195 bp generated by HB primer appeared in two landraces Assuit 52-93 and Assuit 53-93 which were the tallest but did not appear in the other landraces. Such fragments may be related to this trait and could be used as positive markers for plant height using such tested primers. On the other hand the absent fragment at molecular size 596 (OPA01) may be related to the lowest plant height in Sohag 2-93 and Sohag 4-93 landraces. Fragments at molecular size 231 bp, 294 bp and 171 bp generated by OPA08, UBC01 and HB12 primers respectively appeared in two landraces namely, Assuit 51-93 and Assuit 132-94 which were the highest in grain yield but were not observed in the other landraces. These fragments could be used as positive markers for high grain yield trait using such tested primers. while the absent fragment at molecular size 305 bp generated by HB11 primer in two landraces Assuit 131-94 and Sohag 52-93 which had the lowest average for grain yield, may be related to such trait. Fragment at molecular size 343 bp (UBC01) appeared only in landrace Assuit 51-93 which had the highest mean for panicle width, Fragment at molecular size 696 bp (UBC01) appeared only in landrace Assuit 131-94 which had the highest mean for number of days to flowering and Fragment at molecular size 236 bp (HB11) appeared only in landrace Sohag 2-93 which had the highest average for 1000 kernel weight. Such fragments could be used as positive markers for these traits using such tested primers. These results were in agreement with the general characteristics of the studied breeding genotypes.

Relationships among the twenty sorghum landraces based on combined RAPD and ISSR data are presented in Table 8. Positive and significant correlation was found between two molecular markers ($r = 0.267$, $p = 0.001$).

The utility of these markers for detection of genetic differences agrees with previous reports in sorghum and other species (Medraoui *et al*., 2007; Naik *et al*., 2010; Wangsomnuk *et al*., 2011). These results suggested that RAPD and ISSR approaches showed considerable potential for identifying and discriminating sorghum landraces.

Combined dendrogram and correlation dissimilarity matrix between morphological traits and molecular markers: In order to compare the extent of agreement between dendrograms derived from agro-morphological traits and two molecular markers (RAPD and ISSR) (Fig. 7), a distance matrix was constructed for each assay and compared using Mantel matrix correspondence test (Table 7). The Mantel test showed low correlation between the morphological traits and the molecular markers data ($r = 0.083$, $p = 0.001$).

Similar to these results, low correlation between molecular markers and morphological traits have been reported previously in wheat (Lage *et al*., 2003) Yucca (Demey *et al*., 2003) and Mandarins (Campos *et al*., 2005), All the above authors have suggested that molecular and morphological differences are apparently independent, due to divers pressure and evolutionary factors, because the former is invisible and therefore unselected by breeders, while the latter is subject to selection. For these reasons, the molecular diversity analysis should not be used to replace traditional morphological characterization but rather as complement of it (Lage *et al*., 2003).

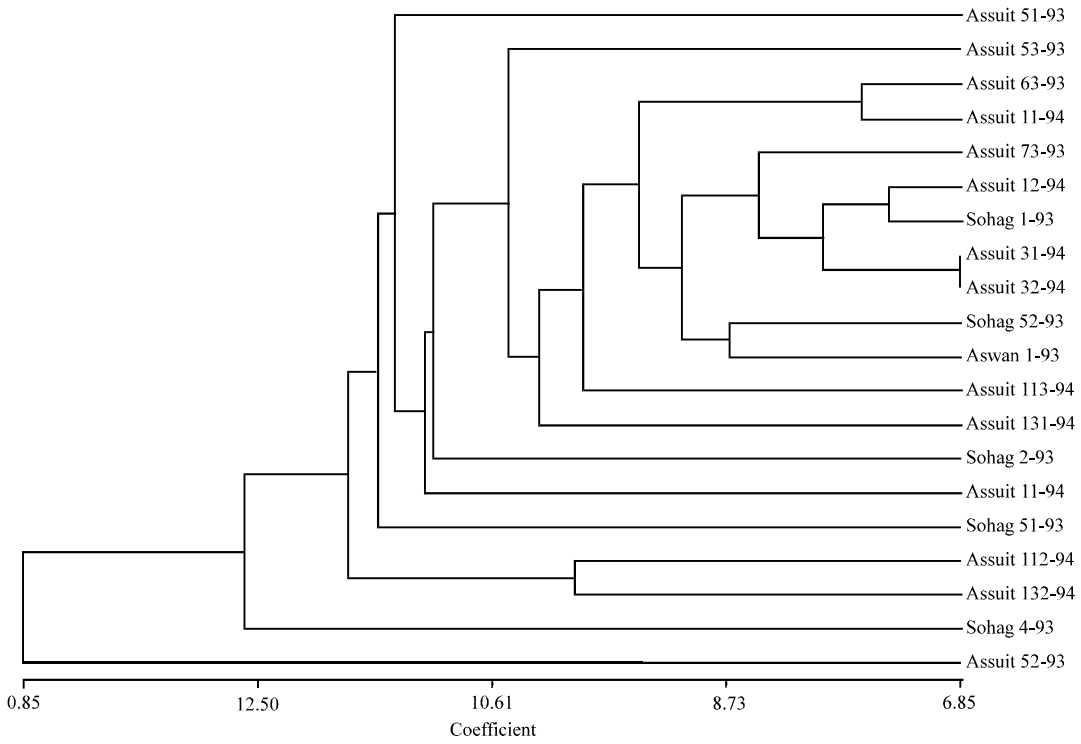

Fig. 7: Dendrogram of twenty sorghum landraces developed from combined agro-morphological and molecular markers data (RAPD and ISSR) using UPGMA analysis

From this study, we concluded that diversity existed among the genotypes of the sorghum germplasm studied. The information obtained from this study will help the breeders in future sorghum breeding programs.

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