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Inter Simple Sequence Repeats Polymorphism in Sudanese *Sorghum bicolor* (L.) Moench Accessions

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Authors' contributions

This work was carried out in collaboration between all authors. Author NBH designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author HKAE managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: Investigate the degree of polymorphism using 41 ISSR (Inter-simple sequence repeat) markers in 50 sorghum accessions from 11 different regions in Sudan and Republic of South Sudan.
Study Design: UPGMA cluster analysis using STATISTCA- SPSS software Ver. 9.
Place and Duration of Study: Department of Molecular Biology, Commission for Biotechnology and Genetic Engineering, National Center for Research, Khartoum, Sudan (2010-2012).
Methodology: 50 sorghum accessions with important agronomic traits, representing 11 regions in Sudan and Republic of South Sudan were assayed for polymorphism using Inter-simple sequence repeats (ISSRs). Seven primers out of 41 tested (807, 808, 810, 814, 848, 872 and 879) showed high polymorphism among the Sorghum accessions.
Results: The results indicated 75 polymorphic bands out of 78 bands with percentage of polymorphic bands of 97%. UPGMA analysis showed ISSR distance matrix ranged between (0.04-0.47) which reflected high genetic diversity. The ISSR UPGMA dendrogram showed high molecular variance within regions. Based on the results of this

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study ISSR technique showed differences among closely related accessions of sorghum. Also it proved to be useful technique to study genetic variation among the Sudanese Sorghum accessions.
Conclusion: Sorghum accessions from Sudan exhibits high genetic variation within and among regions. ISSR marker technique used in this study proved that it is efficient and could be very useful for breeders and researchers community in various fields of sorghum improvement in Sudan.

Keywords: Sorghum; ISSR markers; genetic variation; cluster; UPGMA; Sudan

1. INTRODUCTION

Sorghum is an important stable food for millions of poor people [1]. It also provides forage, fiber, dextrose syrup, biofuels, alcohol, and other products [2]. Sorghum genome is relatively small size with 750 Mbp [3]. The sorghum genetic recombination map of 2512 loci spaced at average 0.4 cm (ca. 300 kb) which open opportunities to help advance genomics in the larger genomes of many other Poaceae members [4]. The DNA molecular markers become the choice for the crop genetic diversity studies, it has become routine, to revolutionize the plant biotechnology [5]. There are many DNA markers available, like randomly amplified polymorphic DNA (RAPD) [6], inter simple sequence repeats (ISSRs) [7], and amplified fragment length polymorphism (AFLP) [8]. The different types of DNA markers have potential to differentiate and detect differences among genotypes. In relation to cost, easiness of use, consistency and repeatability of the results [9], inter simple sequence repeat (ISSR) is a technique based on Polymerase Chain Reaction (PCR), which includes amplification of the DNA [10]. It is considered as a dominant marker [11]. Due to the long ISSR primers (16–25 mers) have high reproducibility possibly as compared to the RAPD primers (10 mers) which permits the subsequent use of high annealing temperature (45– 60ºC) leading to higher stringency [10]. ISSRs are efficient in reflecting the genetic variation among closely related crop/plant genotypes such as sorghum [12], Rice bean [13] and date palm [14].

The main objective of the study was to investigate the degree of polymorphism in 50 sorghum accessions from 11 regions in Sudan and Republic of South Sudan by using ISSR (Inter-simple sequence repeat) markers.

2. MATERIALS AND METHODS

2.1 Seed Material

The seeds of the 50 sorghum accessions used in study were kindly provided by the Germplasm Bank of the Genetic Resources Unit (Agricultural Research Corporation, Wad Madani) (Fig. 1). They were collected from ten different regions of Sudan namely River Nile, West Darfur, North Kordofan, Sinnar, Kassala, Blue Nile, South Kordofan, White Nile, Red Sea, North Darfur, and Bahr EL Jabel region of South Sudan (Table 1). Two millet accessions were included in the study as controls. Sorghum seeds were sown in pots containing equal volumes of sand and clay (1:1). Each pot contained about 20 seeds.

Table 1. The names and regions of sorghum accessions used in the study

No.	Accessions name	Regions	No.	Accessions name	Regions
1	HSD 2790	River Nile	**27**	HSD 5194	Kassala
2	HSD 2791	River Nile	**28**	HSD 5640	Blue Nile
3	HSD 2792	River Nile	**29**	HSD 5641	Blue Nile
4	HSD 2793	River Nile	**30**	HSD 5642	Blue Nile
5	HSD 2795	River Nile	**31**	HSD 5643	Blue Nile
6	HSD 2939	Bahr EL Jabel	**32**	HSD 5650	Blue Nile
7	HSD 2941	Bahr EL Jabel	**33**	HSD 6001	South Kordofan
8	HSD 2945	Bahr EL Jabel	**34**	HSD 6002	South Kordofan
9	HSD 3220	West Darfur	**35**	HSD 6003	South Kordofan
10	HSD 3221	West Darfur	**36**	HSD 6006	South Kordofan
11	HSD 3222	West Darfur	**37**	HSD 6007	South Kordofan
12	HSD 3223	West Darfur	**38**	HSD 6541	White Nile
13	HSD 3226	West Darfur	**39**	HSD 6542	White Nile
14	HSD 3444	North Kordofan	**40**	HSD 6543	White Nile
15	HSD 3445	North Kordofan	**41**	HSD 6544	White Nile
16	HSD 3447	North Kordofan	**42**	HSD 6545	White Nile
17	HSD 3449	North Kordofan	**43**	HSD 6974	Red Sea
18	HSD 3901	Sinnar	**44**	HSD 6975	Red Sea
19	HSD 3903	Sinnar	**45**	HSD 6977	Red Sea
20	HSD 3905	Sinnar	**46**	HSD 6991	Red Sea
21	HSD 3906	Sinnar	**47**	HSD 7115	North Darfur
22	HSD 3907	Sinnar	**48**	HSD 7116	North Darfur
23	HSD 5190	Kassala	**49**	HSD 7117	North Darfur
24	HSD 5191	Kassala	**50**	HSD 7125	North Darfur
25	HSD 5192	Kassala	**A**	HSD 2369	South Kordofan
26	HSD 5193	Kassala	**B**	HSD 5564	Blue Nile

2.2 DNA Extraction

DNA was extracted from fresh leaf tissues of *Sorghum bicolor* accessions using modified CTAB method [15]. 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 60ºC with gentle shaking for 30 min and left to cool at room temperature for 10 min. Chloroform: Isoamylalcohol mixture (24:1) was added to each tube and the phases were mixed gently for 10 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: isoamylalcohol 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 -20ºC for further use.

Fig. 1. Variation in some sorghum accessions collected from different regions in Sudan

2.3 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 assessed using spectrophotometer following [16] method.

2.4 PCR of the ISSR Technique

Forty one ISSR primers were tested for amplification using the polymerase chain reaction (PCR) in a final volume of 25 µL containing 1.0 µL (25ng) DNA diluted, 0.5 µL (2.5U) Taq polymerase, 2.5 µL 10X buffer, 2.5 µL (2mM/µl) dNTPs, 1.5 µL (50 mM) MgCl2, 2.0 µL (10 pmol/ µl) ISSR primer and 15 µL ddH2O. The amplifications were performed in a thermal cycler following the program: 94ºC for 5 min, 40 cycles (1 min at 94ºC, 1 min at 43ºC and 1 min at 72ºC) and final elongation of 7 min at 72ºC.

2.5 DNA Documentation

In order to load the samples on the 2% agarose gel stained with 2µl (10mg/ml) Ethidium Bromide, 4 µl of PCR product of each sample were mixed with 2 µl of loading dye and loaded. 1.4 µl of 1 Kbp DNA ladder was used. Electrophoresis was done at 80 Volts. The separated fragments were visualized with an ultraviolet (UV) transilluminator (Fig. 2).

2.6 ISSR Data Analysis

The number 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: (polymorphic bands/total number of bands x 100). The tree diagram was produced by clustering the similarity data with the UPGMA method using *STATISTCA- SPSS* software *Ver. 9* following the method used by [17].

3. RESULTS AND DISCUSSION

To isolate high quality of DNA, the CTAB-based procedure optimized in the present study, yielded good quality DNA free of phenols, which may inhibit the activity of Taq polymerase.

Out of forty one primers tested for amplification of the 50 accessions (*Sorghum bicolor* (L.) Moench), seven primers showed high percentage of polymorphic bands (97%) (Fig.2). The seven informative primers were selected and used to evaluate the degree of polymorphism and genetic relationships among the genotypes under study. Total of 78 amplified fragments were distinguished across the selected primers and the statistical analysis showed 75 polymorphic bands among the accessions. The maximum number of bands were produced by primer 807 (19 bands) with 89.5% polymorphism, while the minimum number of fragments were produced by the primer 879 (8 bands) with 100%, polymorphism. ISSR fragments pattern produced by the seven primers is shown in Table 2.

The obtained genetic distance values between the accessions are shown in Table 3 below. The highest similarity was between accessions (43 and 45); (44 and 46); and (45 and 46) where the genetic distance between each of the two mentioned was 0.04, all those accessions were from the Red Sea. Where as accessions 1(from River Nile, Sudan) and 7 (from Bahr EL Jabel, South Sudan) had the lowest similarity value of 0.47 (Table 3).

The tree diagram of the seven ISSR markers analyses showed accessions (1) and (39) as outgroup and genetically close to each others (sisters) (Fig. 4). The two accessions appear as genetically distant from all other sorghum accessions. Accession (22) was distant from both 1 & 39 and all others. Cluster A contained only three accessions (2), (4) from River Nile and accession (24) from Kassala region. Cluster B grouped the regions accessions appeared according to their region of sampling as shown in Fig. 3. Based on the distribution of the samples on the tree, the ISSR marker detected high polymorphism among accessions within the same region and among the different regions.

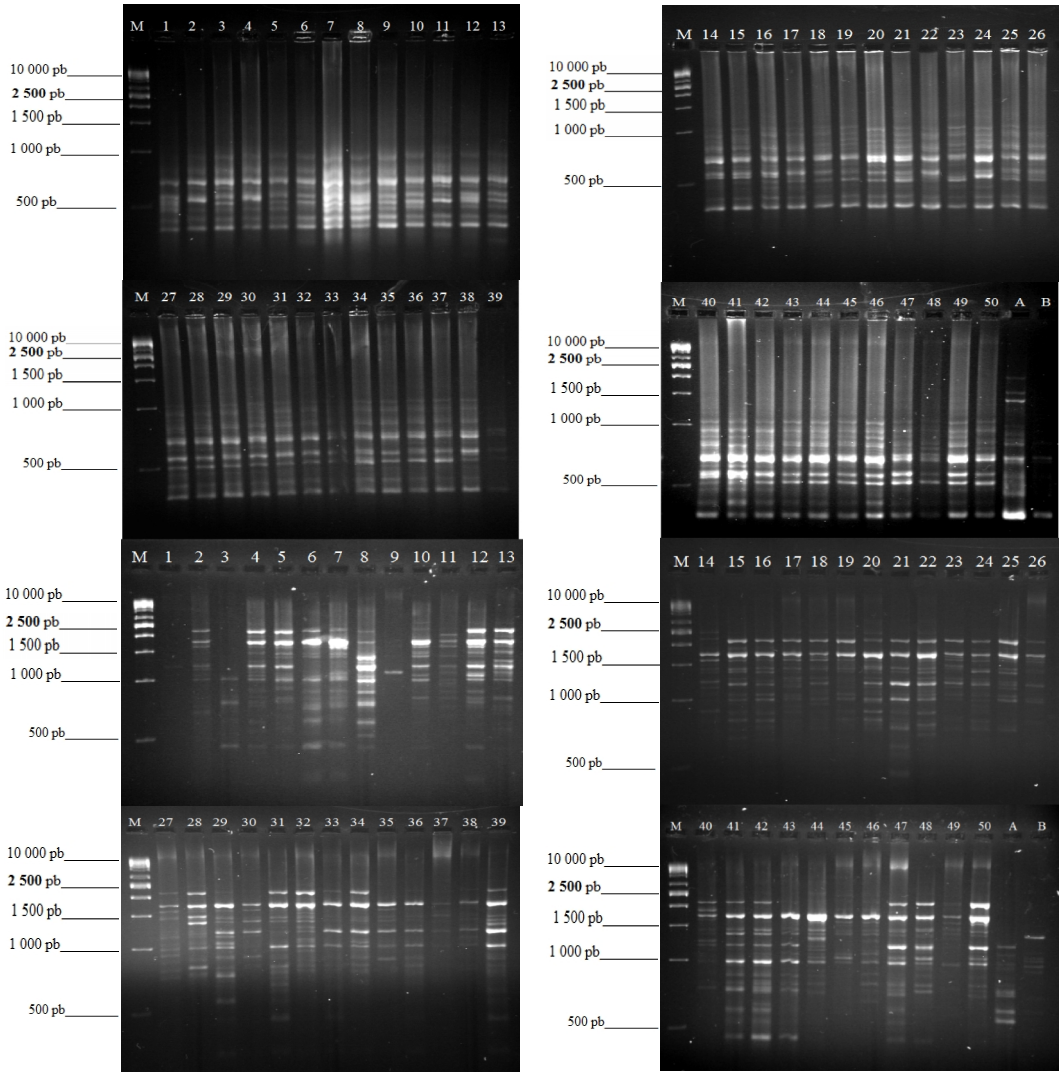

Fig. 2. ISSR amplification patterns of 50 sorghum accessions with ISSR primers 807 and 879

(M: DNA ladder (1 kbp), 1: HSD 2790, 2: HSD 2791, 3: HSD 2792, 4: HSD 2793, 5: HSD 2795, 6: HSD 2939, 7: HSD 2941, 8: HSD 2945, 9: HSD 3220, 10: HSD 3221, 11: HSD 3222, 12: HSD 3223, 13: HSD 3226, 14: HSD 3444, 15: HSD 3445, 16: HSD 3447, 17: HSD 3449, 18: HSD 3901, 19: HSD 3903, 20: HSD 3905, 21: HSD 3906, 22: HSD 3907, 23: HSD 5190, 24: HSD 5191, 25: HSD 5192, 26: HSD 5193, 27: HSD 5194, 28: HSD 5640, 29: HSD 5641, 30: HSD 5642, 31: HSD 5643, 32: HSD 5650, 33: HSD 6001, 34: HSD 6002, 35: HSD 6003, 36: HSD 6006, 37: HSD 6007, 38: HSD 6541, 39: HSD 6542, 40: HSD 6543, 41: HSD 6544, 42: HSD 6545, 43: HSD 6974, 44: HSD 6975, 45: HSD 6977, 46: HSD 6991, 47: HSD 7115, 48: HSD 7116, 49: HSD 7117, 50: HSD 7125)

Table 2. Results of polymorphism and percentages of polymorphism detected by the use of seven ISSR primers on 50 sorghum accessions

Primer name	Sequence (5'-3')	Total number of bands	Number of polymorphic bands	Number of monomorphic bands	Percentage of polymorphic bands
807	(AG)8 T	19	17	2	89.5%
808	(AG)8 C	10	9	1	90%
810	(GA)8 T	9	9	0	100%
814	(CT)8 A	9	9	0	100%
848	(CA)8 RG	9	9	0	100%
872	(GATA)4	14	14	0	100%
879	(CTTCA)3	8	8	0	100%
Total		78	75	3	
Average		11.1	10.7	0.4	97%

Table 3. The Genetic Distance Matrix of 50 sorghum accessions obtained by analysis of seven ISSR primers

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	A	B
1	0.00																																																			
2	0.30	0.00																																																			
3	0.28	0.16	0.00																																																	
4	0.42	0.17	0.20	0.00																																																
5	0.34	0.22	0.14	0.13	0.00																																															
6	0.38	0.24	0.24	0.25	0.14	0.00																																															
7	0.47	0.30	0.25	0.24	0.18	0.17	0.00																																													
8	0.42	0.28	0.22	0.21	0.13	0.17	0.16	0.00																																												
9	0.34	0.22	0.09	0.18	0.13	0.20	0.16	0.18	0.00																																											
10	0.34	0.20	0.14	0.16	0.08	0.17	0.13	0.18	0.11	0.00																																										
11	0.36	0.21	0.16	0.14	0.12	0.21	0.14	0.20	0.12	0.07	0.00																																									
12	0.36	0.21	0.24	0.25	0.17	0.16	0.14	0.25	0.20	0.12	0.13	0.00																																								
13	0.33	0.26	0.21	0.20	0.14	0.24	0.17	0.20	0.17	0.12	0.11	0.16	0.00																																							
14	0.38	0.18	0.16	0.17	0.09	0.18	0.20	0.17	0.12	0.12	0.16	0.21	0.21	0.00																																						
15	0.41	0.21	0.24	0.17	0.12	0.16	0.20	0.20	0.17	0.12	0.13	0.18	0.18	0.11	0.00																																						
16	0.38	0.18	0.18	0.12	0.07	0.16	0.17	0.14	0.17	0.12	0.13	0.18	0.18	0.13	0.13	0.00																																						
17	0.42	0.22	0.20	0.18	0.08	0.17	0.18	0.18	0.16	0.11	0.14	0.17	0.20	0.12	0.09	0.09	0.00																																					
18	0.42	0.20	0.22	0.21	0.16	0.20	0.16	0.18	0.16	0.13	0.12	0.17	0.20	0.12	0.12	0.14	0.11	0.00																																					
19	0.38	0.26	0.24	0.20	0.09	0.18	0.17	0.12	0.20	0.14	0.16	0.21	0.18	0.13	0.13	0.08	0.12	0.14	0.00																																					
20	0.43	0.26	0.24	0.22	0.14	0.24	0.20	0.14	0.20	0.20	0.18	0.24	0.24	0.13	0.16	0.13	0.14	0.12	0.11	0.00																																					
21	0.39	0.20	0.25	0.26	0.21	0.20	0.18	0.21	0.21	0.18	0.17	0.14	0.25	0.17	0.20	0.22	0.18	0.11	0.22	0.17	0.00																																				
22	0.34	0.30	0.30	0.29	0.21	0.25	0.26	0.26	0.26	0.24	0.28	0.30	0.33	0.17	0.17	0.22	0.16	0.18	0.22	0.22	0.26	0.00																																				
23	0.39	0.20	0.17	0.18	0.13	0.22	0.16	0.21	0.11	0.08	0.09	0.14	0.14	0.09	0.12	0.14	0.13	0.08	0.17	0.12	0.16	0.24	0.00																																				
24	0.39	0.17	0.20	0.13	0.13	0.25	0.24	0.18	0.16	0.18	0.20	0.28	0.20	0.12	0.20	0.09	0.18	0.18	0.14	0.17	0.26	0.26	0.16	0.00																																				
25	0.43	0.21	0.18	0.20	0.14	0.24	0.17	0.20	0.14	0.14	0.16	0.18	0.21	0.13	0.13	0.13	0.09	0.09	0.18	0.13	0.20	0.20	0.07	0.17	0.00																																				
26	0.45	0.22	0.17	0.18	0.13	0.22	0.18	0.21	0.13	0.16	0.20	0.25	0.25	0.12	0.14	0.17	0.11	0.13	0.20	0.17	0.24	0.18	0.11	0.18	0.07	0.00																																				
27	0.39	0.22	0.17	0.21	0.11	0.14	0.16	0.16	0.16	0.13	0.12	0.14	0.20	0.12	0.12	0.09	0.11	0.11	0.09	0.12	0.18	0.21	0.13	0.18	0.14	0.16	0.00																																				
28	0.38	0.18	0.16	0.14	0.09	0.18	0.14	0.14	0.09	0.09	0.11	0.18	0.18	0.05	0.13	0.08	0.12	0.09	0.11	0.11	0.17	0.20	0.07	0.09	0.11	0.12	0.09	0.00																																				
29	0.42	0.25	0.22	0.21	0.11	0.17	0.13	0.13	0.16	0.13	0.14	0.17	0.20	0.09	0.12	0.09	0.11	0.11	0.09	0.12	0.16	0.18	0.13	0.16	0.16	0.14	0.16	0.08	0.07	0.00																																		
30	0.37	0.22	0.20	0.21	0.16	0.20	0.18	0.18	0.16	0.18	0.17	0.20	0.22	0.17	0.17	0.12	0.18	0.16	0.14	0.17	0.21	0.26	0.18	0.18	0.20	0.21	0.13	0.12	0.13	0.00																																		
31	0.41	0.21	0.24	0.22	0.20	0.26	0.17	0.22	0.17	0.17	0.18	0.21	0.18	0.18	0.21	0.18	0.20	0.17	0.21	0.21	0.20	0.30	0.12	0.14	0.13	0.14	0.22	0.13	0.20	0.25	0.00																																		
32	0.43	0.21	0.21	0.17	0.14	0.26	0.12	0.20	0.14	0.12	0.13	0.18	0.16	0.13	0.18	0.13	0.14	0.12	0.18	0.18	0.20	0.25	0.09	0.14	0.11	0.12	0.17	0.08	0.12	0.17	0.08	0.00																																		
33	0.41	0.24	0.18	0.20	0.17	0.21	0.14	0.25	0.12	0.12	0.16	0.18	0.16	0.13	0.18	0.18	0.20	0.17	0.24	0.21	0.20	0.30	0.09	0.20	0.16	0.14	0.20	0.13	0.17	0.20	0.16	0.11	0.00																																		
34	0.36	0.21	0.13	0.20	0.12	0.18	0.17	0.20	0.12	0.12	0.13	0.16	0.13	0.11	0.16	0.13	0.14	0.14	0.16	0.16	0.22	0.25	0.09	0.17	0.13	0.14	0.09	0.08	0.12	0.12	0.18	0.11	0.11	0.00																																		
35	0.37	0.25	0.20	0.21	0.13	0.25	0.24	0.18	0.18	0.18	0.14	0.22	0.17	0.14	0.17	0.14	0.16	0.16	0.14	0.09	0.21	0.26	0.13	0.18	0.17	0.18	0.13	0.12	0.13	0.13	0.20	0.14	0.17	0.09	0.00																																		
36	0.39	0.20	0.28	0.29	0.29	0.25	0.26	0.34	0.24	0.24	0.20	0.17	0.25	0.28	0.25	0.25	0.29	0.21	0.28	0.28	0.24	0.39	0.18	0.26	0.25	0.26	0.21	0.22	0.26	0.18	0.22	0.20	0.17	0.17	0.18	0.00																																		
37	0.46	0.29	0.24	0.28	0.22	0.29	0.20	0.28	0.17	0.17	0.18	0.24	0.24	0.18	0.21	0.24	0.20	0.17	0.24	0.18	0.22	0.30	0.14	0.25	0.21	0.17	0.17	0.16	0.17	0.20	0.18	0.13	0.13	0.16	0.12	0.17	0.00																																		
38	0.39	0.22	0.22	0.29	0.24	0.28	0.18	0.26	0.18	0.18	0.17	0.20	0.22	0.20	0.22	0.25	0.24	0.16	0.25	0.20	0.21	0.32	0.13	0.26	0.17	0.16	0.18	0.17	0.18	0.18	0.17	0.12	0.14	0.14	0.13	0.13	0.09	0.00																																		
39	0.32	0.36	0.41	0.39	0.34	0.38	0.34	0.42	0.37	0.34	0.33	0.33	0.36	0.33	0.33	0.36	0.32	0.26	0.38	0.30	0.26	0.29	0.26	0.37	0.30	0.32	0.34	0.30	0.32	0.37	0.25	0.25	0.30	0.30	0.26	0.32	0.28	0.29	0.00																																		
40	0.45	0.22	0.25	0.21	0.16	0.17	0.13	0.24	0.18	0.16	0.14	0.17	0.20	0.17	0.12	0.14	0.13	0.11	0.17	0.20	0.16	0.24	0.16	0.21	0.17	0.16	0.13	0.14	0.11	0.16	0.17	0.12	0.17	0.17	0.21	0.21	0.17	0.18	0.26	0.00																																		
41	0.41	0.21	0.29	0.30	0.22	0.18	0.22	0.25	0.22	0.17	0.24	0.16	0.29	0.18	0.16	0.24	0.17	0.14	0.24	0.18	0.09	0.25	0.14	0.28	0.18	0.20	0.22	0.18	0.17	0.22	0.18	0.21	0.21	0.24	0.22	0.22	0.18	0.17	0.28	0.17	0.00																																		
42	0.43	0.26	0.32	0.30	0.20	0.18	0.20	0.25	0.22	0.17	0.21	0.16	0.26	0.18	0.13	0.21	0.14	0.14	0.21	0.18	0.09	0.22	0.14	0.28	0.18	0.20	0.20	0.18	0.14	0.22	0.18	0.21	0.21	0.24	0.22	0.25	0.18	0.22	0.28	0.14	0.05	0.00																																		
43	0.41	0.29	0.24	0.28	0.14	0.16	0.20	0.22	0.17	0.17	0.18	0.21	0.26	0.13	0.11	0.16	0.12	0.14	0.16	0.13	0.17	0.17	0.14	0.22	0.16	0.17	0.14	0.13	0.12	0.17	0.21	0.21	0.21	0.18	0.14	0.28	0.16	0.22	0.30	0.17	0.13	0.08	0.00																																		
44	0.37	0.25	0.28	0.32	0.18	0.17	0.24	0.26	0.21	0.21	0.22	0.17	0.28	0.17	0.14	0.20	0.16	0.18	0.20	0.17	0.18	0.21	0.18	0.26	0.20	0.21	0.18	0.17	0.13	0.16	0.25	0.22	0.22	0.20	0.16	0.21	0.17	0.18	0.32	0.16	0.12	0.09	0.07	0.00																																		
45	0.37	0.28	0.22	0.29	0.16	0.20	0.24	0.24	0.18	0.18	0.17	0.22	0.22	0.14	0.12	0.17	0.13	0.13	0.17	0.12	0.18	0.18	0.13	0.24	0.14	0.18	0.13	0.14	0.13	0.18	0.22	0.20	0.20	0.14	0.11	0.24	0.14	0.18	0.29	0.18	0.17	0.12	0.04	0.08	0.00																																		
46	0.33	0.24	0.26	0.33	0.20	0.18	0.28	0.28	0.22	0.22	0.21	0.18	0.26	0.18	0.16	0.21	0.17	0.17	0.21	0.16	0.17	0.22	0.17	0.28	0.18	0.22	0.17	0.18	0.17	0.20	0.24	0.24	0.24	0.18	0.14	0.20	0.18	0.17	0.30	0.20	0.13	0.11	0.08	0.04	0.04	0.00																																		
47	0.36	0.24	0.26	0.28	0.17	0.16	0.28	0.20	0.25	0.20	0.26	0.21	0.26	0.18	0.16	0.18	0.17	0.20	0.18	0.16	0.17	0.25	0.20	0.25	0.21	0.22	0.20	0.18	0.17	0.20	0.21	0.24	0.24	0.18	0.14	0.25	0.21	0.22	0.30	0.22	0.08	0.13	0.13	0.14	0.14	0.13	0.00																																		
48	0.32	0.20	0.22	0.24	0.16	0.17	0.26	0.21	0.21	0.16	0.22	0.20	0.22	0.17	0.14	0.14	0.18	0.18	0.17	0.17	0.24	0.26	0.16	0.21	0.17	0.21	0.18	0.14	0.18	0.16	0.20	0.20	0.20	0.14	0.16	0.21	0.22	0.21	0.32	0.21	0.14	0.20	0.17	0.16	0.16	0.14	0.07	0.00																																		
49	0.33	0.18	0.21	0.28	0.17	0.13	0.22	0.22	0.20	0.17	0.24	0.18	0.26	0.11	0.16	0.18	0.17	0.14	0.18	0.18	0.14	0.22	0.17	0.22	0.18	0.20	0.17	0.13	0.14	0.20	0.18	0.18	0.18	0.16	0.20	0.25	0.21	0.20	0.30	0.17	0.11	0.16	0.13	0.14	0.14	0.13	0.08	0.09	0.00																																		
50	0.34	0.17	0.25	0.26	0.21	0.17	0.21	0.24	0.18	0.16	0.17	0.12	0.20	0.17	0.17	0.20	0.18	0.11	0.22	0.20	0.13	0.26	0.11	0.24	0.14	0.21	0.18	0.14	0.18	0.18	0.17	0.14	0.17	0.14	0.21	0.16	0.22	0.16	0.29	0.16	0.12	0.14	0.20	0.16	0.16	0.12	0.17	0.13	0.12	0.00																																		
A	0.57	0.47	0.47	0.51	0.57	0.55	0.46	0.49	0.43	0.49	0.47	0.50	0.55	0.50	0.53	0.50	0.54	0.46	0.53	0.50	0.49	0.59	0.46	0.49	0.47	0.51	0.46	0.47	0.49	0.51	0.47	0.47	0.50	0.50	0.54	0.49	0.47	0.46	0.51	0.49	0.50	0.53	0.55	0.57	0.54	0.55	0.55	0.51	0.53	0.49	0.00																																		
B	0.38	0.45	0.50	0.51	0.57	0.55	0.49	0.51	0.46	0.54	0.53	0.50	0.55	0.53	0.53	0.50	0.57	0.51	0.53	0.47	0.54	0.49	0.49	0.49	0.47	0.51	0.51	0.50	0.51	0.46	0.50	0.50	0.50	0.50	0.49	0.46	0.50	0.41	0.38	0.51	0.50	0.55	0.55	0.49	0.54	0.50	0.53	0.46	0.53	0.49	0.29	0.00

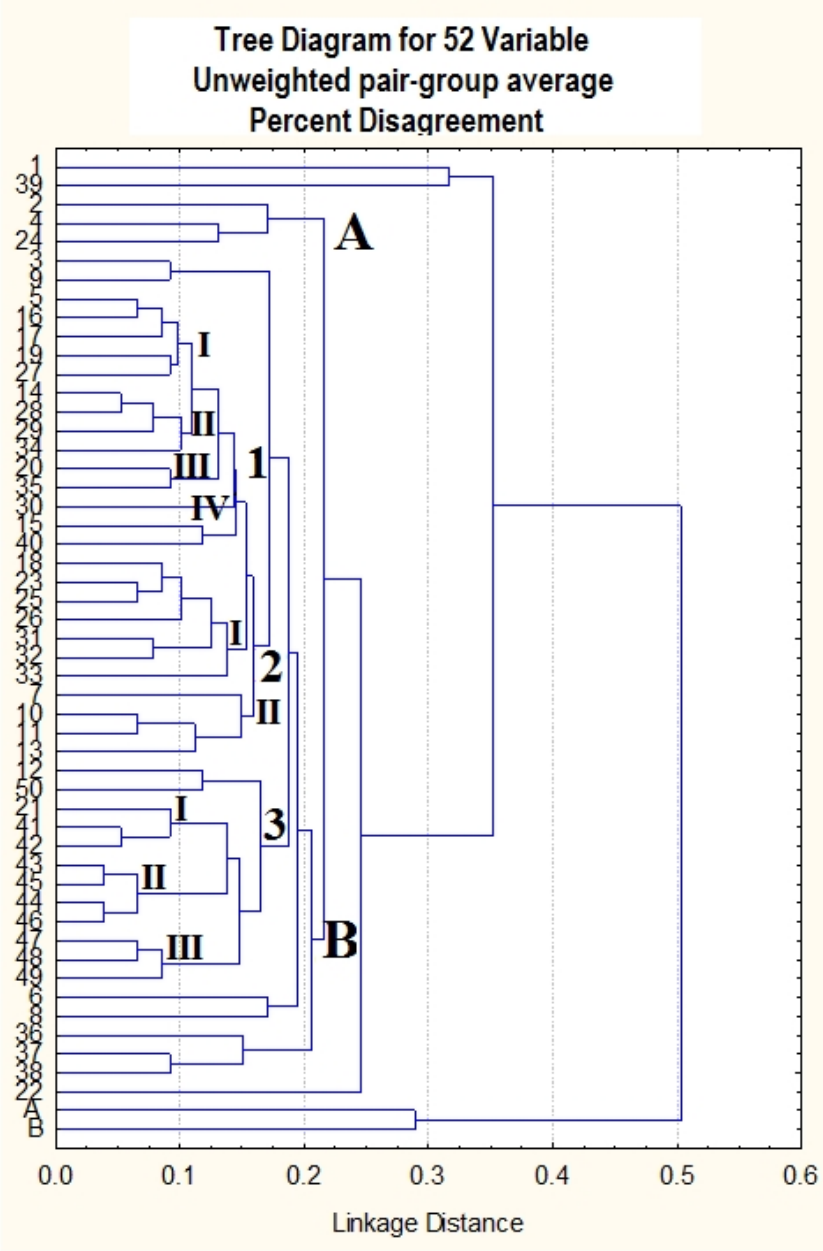

Fig. 3. UPGMA Tree resulting from the analyses of seven ISSR primers reflecting the relationships among the 50 sorghum accessions

1: HSD 2790, 2: HSD 2791, 3: HSD 2792, 4: HSD 2793, 5: HSD 2795, 6: HSD 2939, 7: HSD 2941, 8: HSD 2945, 9: HSD 3220, 10: HSD 3221, 11: HSD 3222, 12: HSD 3223, 13: HSD 3226, 14: HSD 3444, 15: HSD 3445, 16: HSD 3447, 17: HSD 3449, 18: HSD 3901, 19: HSD 3903, 20: HSD 3905, 21: HSD 3906, 22: HSD 3907, 23: HSD 5190, 24: HSD 5191, 25: HSD 5192, 26: HSD 5193, 27: HSD 5194, 28: HSD 5640, 29: HSD 5641, 30: HSD 5642, 31: HSD 5643, 32: HSD 5650, 33: HSD 6001, 34: HSD 6002, 35: HSD 6003, 36: HSD 6006, 37: HSD 6007, 38: HSD 6541, 39: HSD 6542, 40: HSD 6543, 41: HSD 6544, 42: HSD 6545, 43: HSD 6974, 44: HSD 6975, 45: HSD 6977, 46: HSD 6991, 47: HSD 7115, 48: HSD 7116, 49: HSD 7117, 50: HSD 7125

Fig. 4. Different seed shape and color of sorghum accessions that appeared in tree diagram as genetically close

4. CONCLUSION

In Sudan, sorghum is grown in different regions for multipurpose uses, which reflects the importance of this crop. As shown in this study, sorghum exhibited great variability in morphology and genetic characterization. The large number of unique fragments found for some sorghum accessions reflects the high genetic diversity of this crop. Sorghum accessions from Sudan showed high polymorphism within and among regions.

The tree diagram showed number of accessions as sisters, although seed morphology reflects the contrary, this may be due to the presence of unique alleles as such alleles are important because they can be used in diagnostic or to fingerprint accessions from particular regions in Sudan.

In this study, the ISSR marker was very reliable to study sorghum from Sudan. ISSR marker technique overcomes most of the limitations faced by using other techniques, and it is rapidly being used by the breeders and researchers community in various fields of improvement of sorghum landraces of Sudan, because ISSR marker is a simple, quick, and efficient technique and has high reproducibility.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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