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Comparative Analysis of Genetic Structure and Diversity of Sorghum (*Sorghum bicolor* L.) Local Farmer's Varieties from Sudan

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Authors' contributions

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

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Original Research Article

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ABSTRACT

Aims: Investigate the genetic diversity and structure of 50 Sorghum accessions from 10 different regions in Sudan and one from the county of Central Equatoria in the Republic of South Sudan, by screening 40 RAPD and 10 ISSR (Inter-simple sequence repeat) markers.
Study Design: UPGMA method using *STATISTCA- SPSS* software Ver. 9 and PCA using *GenAlEx* ver. 6.5
Place and Duration of Study: Department of Molecular Biology, Commission for Biotechnology and Genetic Engineering, National Center for Research, Khartoum, Sudan (2010-2012).
Methodology: 47 sorghum accessions with important agronomic traits, representing 10 states in Sudan and three sorghum accessions from the county of Central Equatoria of Republic of South Sudan were assayed for polymorphism using Random Amplified Polymorphic DNA (RAPD) and

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Inter-simple sequence repeats (ISSRs).
Results: Ten polymorphic RAPD primers distinguished 163 bands. 156 bands were polymorphic among the 50 accessions with 96.6% polymorphism. The seven polymorphic ISSR primers distinguished 78 bands, of which 75 bands were polymorphic with 97% polymorphism. The RAPD distance matrix ranged between 0.07-0.43 which proved wide range of variation, ISSR distance matrix ranged between 0.04-0.47 showing higher genetic variability among the sorghum accessions than the RAPD, whereas, combined data distance matrix for both RAPD and ISSR markers ranged between 0.08-0.39 which reflected more trusted result among Sudanese sorghum accessions. The White Nile state accessions showed the highest percentage of polymorphic loci with 39.75%, whereas lowest was given by Red Sea accessions with 17.99%. The molecular variance within states was 70% and 30% among states.
Conclusion: In conclusion, Results based on combined analysis of both RAPD and ISSR data were most accurate for covering large area inside the genome. White Nile state accessions was the highest in number of bands; number of private bands; percentage of polymorphic loci and heterozygosity (*He*) mean compared to accessions of other states.

Keywords: Sorghum; genetic variation; structure; RAPD; ISSR; markers; UPGMA.

1. INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is a monocot plant [1]. Both the cultivated and wild varieties of *Sorghum bicolor* have diploid chromosomes number (2n=20) and are completely inter fertile [2]. The genetic recombination map for Sorghum is 2512 loci [3]. It's first been domesticated in North Africa, possibly in the Nile or Ethiopian regions as recently as 1000 BC [4]. Morphology variation does not reliably reflect the real genetic variation because of genotype-environment interactions and the largely unknown genetic control of polygenically inherited morphological and agronomic traits [5]. The methods for detecting and assessing genetic diversity have extended from analysis of discrete morphological traits to molecular traits [6]. DNA markers showed powerful and reliable tools for variation within crop genotypes compared with the biochemical markers [7]. DNA markers are identifiable DNA sequences found at specific locations of the genome and transmitted by the standard laws of inheritance from one generation to the next. They are considered constant landmarks in the genome [8]. The most commonly used marker systems are restriction fragment length polymorphism (RFLP); random amplified polymorphic DNA (RAPD); amplified fragment length polymorphism (AFLP), inter simple sequence repeats (ISSRs) and microsatellites or simple sequence repeats (SSRs) [9]. [10]; used the SSRs technique for estimating genetic diversity and determining the genetic relationship among 96 Sudanese sorghum accessions, they found unique fingerprints and polymorphic markers, which could be very important for breeders in Sudan and around the world. Another study, eighty Sudanese sorghum accessions from Sudan were characterised using 15 genomic and 16 expressed sequence tag (EST) derived simple sequence repeat (SSR) markers, the results showed large genetic distances between clusters of Sudanese landraces [11].

The main objectives of the study were to:-

Determine the level of genetic diversity within and among 50 Sorghum accessions according to their regions of collection by using two different techniques: RAPD (Random Amplified Polymorphic DNA) and ISSR (Inter-Simple Sequence Repeat) markers. This information will very useful for Sorghum breeders working on improvement of Sorghum.

2. MATERIALS AND METHODS

2.1 Seed Materials

The seeds of the 50 sorghum accessions used in study (Table 1) were provided by the Gene Bank of the Genetic Resources Unit of the Agricultural Research Corporation, Wad Medani. They were collected from ten States 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-Central Equatoria county of Republic of South Sudan (Table 1). Two millet accessions were included in the study as control. Sorghum seeds were sown in pots containing equal volumes of sand and clay (1:1). Each pot contained about 20 seeds.

2.2 DNA Extraction

Sorghum grains were germinated in the greenhouse of the Commission for Biotechnology & Genetic Engineering, National Center for Research, Sudan. DNA was extracted from 50 sorghum accessions as described in [12].

2.3 DNA Quality and Quantity

Nanodrop 1000 spectrophotometer (Thermo Scientific, Wilmington) was used to assess the quality and concentration of the extracted DNA. The obtained DNA extractions concentrations for all samples were above 20 ng/µL. The DNA was diluted 6x for the analysis.

2.4 PCR Protocol for RAPD and ISSR Techniques

Each reaction contained 25 ul total volume of reaction. For each of the primers, a master mix was done separately for the 50 samples plus the two control samples. The PCR reactions were carried out in 25 µl volume containing 15µl sterile distilled water, 2.5 µl 10X *taq* buffer, 2.5 µl (2 mM/µl) dNTPs, 1.5 µl (50 mM) MgCl2, 2 µl (10 pmol/µl) primer, 0.5 µl (5u/ µl) Taq-polymerase and 1 µl (10 to 30 ng/µl) template DNA, for each sample. The PCR amplification protocol was programmed for 5 min at 94℃ for initial denaturation, followed by 40 cycles of 1min at 94℃, 1 min at (34-36℃ for RAPD and 42℃ for ISSR) and 1 min at 72℃, final extension was programmed for 7min at 72℃ followed by hold time at 4℃ until samples were collected.

2.5 DNA Visualization

4 µl of PCR product were mixed with 2 µl of loading dye and 1.4 µl of 1 Kbp DNA ladder, then were electrophoresed using 2% agarose gel at 80 Volts followed by staining with Ethidium Bromide then the separated fragments were visualized with an ultraviolet (UV) transilluminator (Fig. 1).

2.6 RAPD and ISSR Combined Data Analysis

Data was scored as Zero (0) for absence of a fragment and one (1) for presence of a fragment in an excel file with binary data. The file was run with the software *STATISTICA* ver. 9 to reflect the genetic relationships among the 50 studied sorghum accessions based on 10 RAPD primers, 7 ISSR primers data and combined RAPD and ISSR primers datasets obtained. The software *GenAlEx* ver. 6.5 was used for Principal Coordinates Analysis, Band Frequencies,

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

No.	Accessions name	Region	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

Estimated Allele Frequencies and Estimated Heterozygosity by State, Band Patterns and Analysis of Molecular Variance, following the method as used by [12].

3. RESULTS AND DISCUSSION

3.1 Amplification of RAPD and ISSR Primers

Forty RAPD primers & Ten ISSR primers were tested on the 50 accessions (*Sorghum bicolor* L.) and the results indicated that seventeen informative primers were selected and used to evaluate the degree of polymorphism and genetic relationships among the genotypes under study, ten RAPD primers (A-1, B-20, C-20, D-18, OPE-04, UBC-101, UBC-103, UBC-127, UBC-155 and UBC-157 and seven ISSR primers A-1, B-20, C-20, D-18, UBC-103, UBC-127 and UBC-155) showed high polymorphic bands percentages (96.6%) and (97%) respectively. The average number of polymorphic bands produced per RAPD primer was 15.6, which is higher than the findings in earlier reports such as [13] who reported an average of 7; [14] obtained an average of 6.5; [15] had an average of 5.8. The average number of polymorphic bands produced per ISSR primer was 10.7, which is equally higher than the previous studies (Table 2).

3.2 Distance Matrix

The distance matrix was obtained by analysis of combined data of both RAPD and ISSR showed the highest similarity among accessions (11, 13) and (43, 44) with 0.08, whereas, the lowest similarity was found between (1, 45) with 0.39 (Table 3). This could be considered as a more trusted result as the data used from two different DNA markers.

3.3 Combined data of RAPD-ISSR UPGMA Dendrogram

The tree diagram of RAPD and ISSR markers analyses (Fig. 1) showed two main clusters, Cluster A had two groups, group I included accessions (1-5), from River Nile. Group II contained two subgroups, the first from River Nile and west Darfur. Cluster B had two groups, group 1 contained accessions from North Kordofan, Sinnar, and Kassala. Group 2 contained two subgroups, first subgroup contained accessions from Kassala (27), Blue Nile and South Kordofan. Subgroup 2 contained accessions from White Nile State, Red sea and North Darfur. Accession 39 from White Nile appeared as out-group to all accessions with high genetic distance. The combined data of RAPD-ISSR UPGMA Dendrogram showed that accession 37 from South Kordofan emerged as single accession outside clusters distant from individuals from the same State. According to [16] this may be due to the presence of unique alleles and such alleles are important because they may be diagnostic for particular regions with a genome specific to a particular type of sorghum. The River Nile accessions appeared as one group. The North Kordofan also appeared as one group. The remaining accessions overlapped among the states, where the molecular variance within states was higher than among states, this might be affected by human activities in agriculture.

RAPD analysis can be utilized efficiently in developing countries [17]; [18], also RAPD offers the simplest and fastest method for detecting a great number of genomic markers in a short period of time [19]. RAPD markers are highly effective for germplasm evaluation and certain molecular breeding approaches. It can be used without prior knowledge of the genome to rapidly and efficiently screen the genome [20]. ISSRs have high reproducibility and this was possibly due to the use of longer primers (16–25 mers) when compared to shorter RAPD primers (10-mers). The longer primers permits the subsequent use of high annealing temperatures (45– 60℃) leading to higher stringency [1]. Therefore, ISSR marker technique overcomes most of these limitations and it is rapidly being used by the breeders and researchers community in various fields of Sudanese sorghum improvement, because ISSR marker is a simple, quick, and efficient technique and it has high reproducibility [21].

RAPD and ISSR markers are significantly different because each technique amplifies different parts on the genome. Therefore, it is better to use the combined data of RAPD and ISSR techniques in order to cover more segment sites of the genome which increase the validity of the UPGMA Dendrogram and all the analysis. RAPD and ISSR have been extensively used in studying many crops and a comparison of both techniques concluded that ISSR would be better technique than RAPD for phylogenetic studies [1], whereas, RAPD would be better technique than ISSR for phylogenetic among the states in Sudan (Fig. 1).

3.4 Principal Coordinate Analysis

PCA diagram (Fig. 2) showed Red sea state accessions gather distantly from accessions of other states. Accessions from White Nile state and North Darfur state were genetically close (accessions 39 and 50). South Kordofan state accessions from 33 to 37 were related to White Nile state accessions from 38 to 42 as showed clearly from PCA figure and combined diagram. Sorghum accessions from Bahr el Jabel were genetically closer to 2 samples from river Nile state. North Kordofan state, Sinnar state and Kassala state were closer as appear in the Fig. 1. besides that they belong to the cluster b from combined diagram (Fig. 1). The principal coordinate's analysis showed that white Nile state and North Darfur state were close genetically (accessions 39 and 50), it may be accession 39 was brought to North Darfur by human agricultural activity. South Kordofan state accessions from 33 to 37 were related to white Nile state accessions from 38 to 42 as shown clearly from PCA diagram. Accessions 23 to 27 from Kassala state are genetically related to Blue Nile state accessions 28 to 32 as shown from the PCA analysis and combined data diagram probably due to the geographic distance between them. Sorghum from North Kordofan state, Sinnar state and Kassala state were related as showed in PCA diagram. The relatedness among the distribution of the accession on the PCA diagram can be justified by the human activity in agriculture that move the sorghum seeds from one state to another (Fig. 2).

3.5 Total Band Patterns

The highest total number of bands among the 11 studied regions was produced by sorghum accessions from Sinnar and White Nile States, as each produced 186 bands. The lowest number of bands was found in Blue Nile and North Darfur States where each produced 164 bands and that is because the performance of the molecular markers was the best in Sinnar and White Nile States. The remaining states each produced a number of bands falls in this range. Sinnar State had the highest number of private bands with 5 bands so this can be considered as a finger print for sorghum accessions from this State. Whereas, Bahr EL Jabel, West Darfur, North Kordofan, and South Kordofan had no private bands. The highest number of locally common bands found in 25% or less, Bahr EL Jabel and White Nile State was the highest with 3. The White Nile State had the highest number of locally common bands found in 50% or less with 17 followed by Sinnar. The expected heterozygosity (*He*) mean was highest in the White Nile with 0.152 and lowest was in Red Sea with 0.071. The performance of molecular markers depends on the availability of markers complementary in the genome and whenever the complementary sequence increase in genome and the band of the markers will increase as well (Fig. 3) (Table 4).

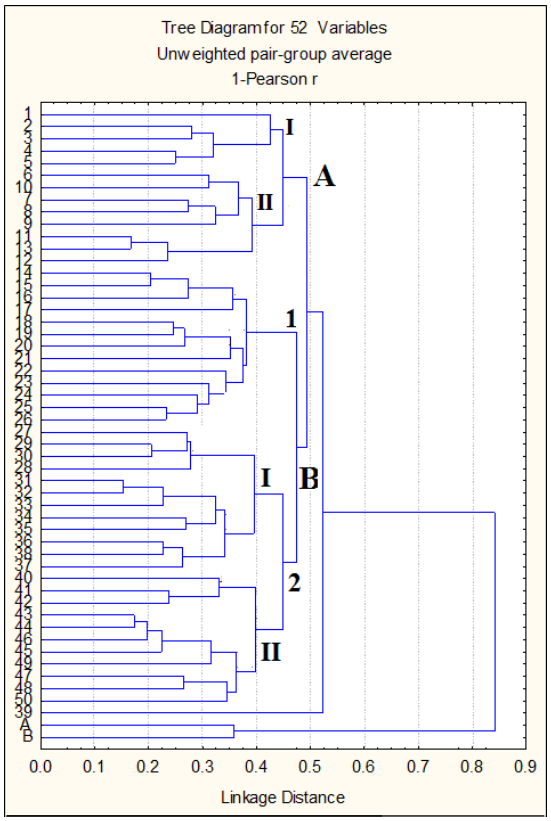

Fig. 1. UPGMA Dendrogram resulting from the combined data of 10 RAPD primers and seven ISSR primers reflecting the relationships among the 50 sorghum accessions with two control samples

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. Percentages of polymorphic bands detected by the use of 10 polymorphic RAPD primers and 7 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
	RAPD primers				
A-1	CAG GCC CTT C	10	10	0	100%
B-20	GGA CCC TTA C	10	10	0	100%
C-20	ACT TCG CCA C	8	8	0	100%
D-18	GAG AGC CAA C	17	17	0	100%
OPE-04	GTG ACA TGC C	17	16	1	94%
UBC-101	GCG GCT GGA G	23	21	2	91%
UBC-103	GTG ACG CCG C	21	21	0	100%
UBC-127	ATC TGG CAG C	20	20	0	100%
UBC-155	CTG GCG GCT G	16	16	0	100%
UBC-157	CGT GGG CAG G	21	17	4	81%
Total		163	156	7	
Average		16.3	15.6	0.7	96.6%
	ISSR primers				
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%

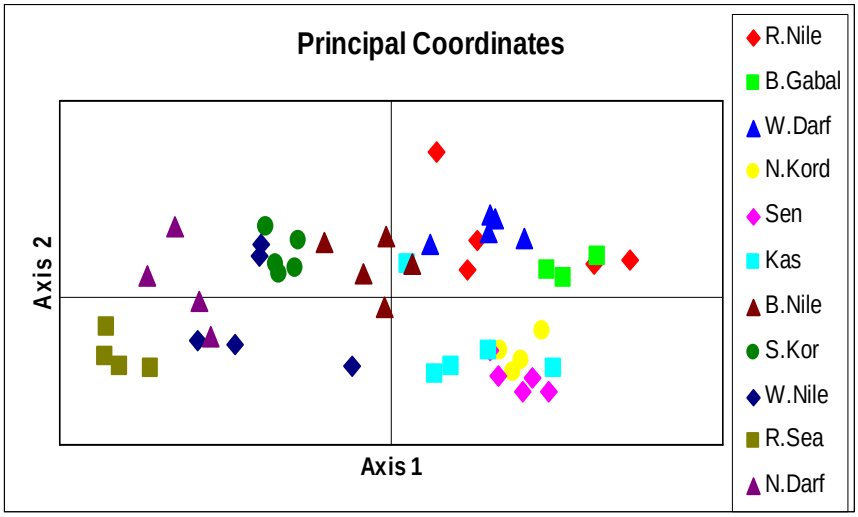

Fig. 2. Principal coordinates analysis from the combined analysis of 10 RAPD primers and seven ISSR primers analyzed jointly for the 11 regions

R. Nile: River Nile, B. Gabal: Bahr EL Jabel, W. Darf: West Darfur, N. Kord: North Kordofan, Sen: Sinnar, Kas: Kassala, B. Nile: Blue Nile, S. Kor: South Kordofan, W. Nile: White Nile, R. Sea: Red Sea, N. Darf: North Darfur

3.6 Percentage of Polymorphic Loci

The percentage of polymorphic loci for all studied sorghum is shown in Table (5) revealed the white Nile state as the highest in percentage of polymorphic loci with 39.75%, the lowest being red sea with 17.99%, and the mean of the percentage of polymorphic loci in all regions was 31.53%. The highest polymorphism in white Nile state is supported with the highest number of bands recorded among all regions.

Table 3. The distance matrix of 50 sorghum accessions used in the study

	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	0.23	0.00																																																				
3	0.21	0.14	0.00																																																		
4	0.28	0.14	0.18	0.00																																																
5	0.24	0.18	0.14	0.12	0.00																																															
6	0.30	0.25	0.23	0.23	0.19	0.00																																															
7	0.34	0.26	0.22	0.23	0.16	0.19	0.00																																													
8	0.29	0.25	0.21	0.20	0.13	0.18	0.13	0.00																																												
9	0.26	0.23	0.17	0.20	0.15	0.21	0.15	0.16	0.00																																											
10	0.30	0.25	0.21	0.22	0.16	0.15	0.16	0.15	0.18	0.00																																									
11	0.27	0.26	0.23	0.21	0.19	0.22	0.20	0.22	0.15	0.17	0.00																																								
12	0.26	0.21	0.21	0.24	0.19	0.18	0.18	0.24	0.18	0.16	0.12	0.00																																							
13	0.26	0.25	0.24	0.25	0.20	0.22	0.20	0.22	0.16	0.18	0.08	0.11	0.00																																						
14	0.34	0.23	0.21	0.21	0.18	0.23	0.23	0.23	0.23	0.23	0.24	0.23	0.25	0.00																																						
15	0.34	0.22	0.21	0.21	0.17	0.23	0.23	0.22	0.24	0.21	0.21	0.22	0.23	0.10	0.00																																					
16	0.31	0.21	0.21	0.16	0.15	0.23	0.23	0.19	0.22	0.21	0.24	0.23	0.25	0.13	0.13	0.00																																					
17	0.38	0.25	0.26	0.23	0.18	0.23	0.19	0.20	0.24	0.18	0.26	0.23	0.26	0.18	0.18	0.16	0.00																																				
18	0.35	0.22	0.24	0.23	0.20	0.24	0.21	0.21	0.20	0.22	0.20	0.21	0.21	0.16	0.17	0.19	0.16	0.00																																				
19	0.36	0.26	0.24	0.23	0.18	0.23	0.21	0.19	0.20	0.23	0.23	0.23	0.23	0.16	0.17	0.17	0.17	0.12	0.00																																				
20	0.37	0.26	0.26	0.24	0.21	0.26	0.22	0.23	0.25	0.26	0.27	0.25	0.28	0.15	0.20	0.17	0.21	0.13	0.12	0.00																																			
21	0.34	0.25	0.25	0.26	0.21	0.25	0.21	0.22	0.23	0.24	0.23	0.19	0.25	0.22	0.23	0.22	0.22	0.16	0.18	0.16	0.00																																			
22	0.32	0.25	0.26	0.23	0.19	0.26	0.24	0.26	0.25	0.28	0.26	0.26	0.27	0.19	0.18	0.21	0.18	0.16	0.18	0.18	0.21	0.00																																			
23	0.36	0.23	0.21	0.23	0.19	0.27	0.23	0.23	0.21	0.24	0.22	0.21	0.24	0.18	0.17	0.19	0.21	0.14	0.15	0.19	0.16	0.17	0.00																																			
24	0.33	0.22	0.23	0.23	0.21	0.28	0.22	0.24	0.21	0.26	0.23	0.22	0.20	0.19	0.19	0.16	0.22	0.18	0.18	0.19	0.21	0.19	0.16	0.00																																			
25	0.34	0.24	0.23	0.21	0.18	0.25	0.21	0.23	0.21	0.24	0.22	0.21	0.22	0.16	0.16	0.15	0.15	0.13	0.18	0.16	0.20	0.14	0.15	0.13	0.00																																			
26	0.38	0.22	0.19	0.23	0.20	0.25	0.21	0.24	0.21	0.23	0.23	0.21	0.23	0.18	0.17	0.21	0.17	0.15	0.18	0.19	0.23	0.15	0.14	0.14	0.11	0.00																																			
27	0.32	0.28	0.26	0.26	0.21	0.27	0.25	0.22	0.24	0.26	0.23	0.27	0.26	0.24	0.22	0.21	0.26	0.24	0.23	0.27	0.26	0.27	0.22	0.26	0.23	0.25	0.00																																			
28	0.31	0.24	0.23	0.25	0.18	0.28	0.21	0.22	0.21	0.21	0.21	0.23	0.25	0.16	0.17	0.18	0.21	0.17	0.20	0.20	0.24	0.23	0.18	0.19	0.19	0.18	0.14	0.00																																			
29	0.29	0.28	0.24	0.26	0.18	0.25	0.20	0.15	0.20	0.20	0.21	0.23	0.23	0.23	0.22	0.20	0.21	0.19	0.20	0.24	0.23	0.24	0.21	0.25	0.23	0.21	0.13	0.13	0.00																																			
30	0.28	0.27	0.23	0.25	0.18	0.26	0.21	0.19	0.19	0.23	0.22	0.24	0.24	0.24	0.24	0.21	0.24	0.22	0.22	0.24	0.25	0.27	0.25	0.25	0.23	0.23	0.14	0.15	0.10	0.00																																			
31	0.29	0.22	0.20	0.22	0.20	0.26	0.21	0.23	0.18	0.22	0.18	0.17	0.18	0.21	0.21	0.21	0.25	0.21	0.23	0.24	0.24	0.26	0.19	0.18	0.19	0.18	0.22	0.16	0.19	0.18	0.00																																			
32	0.32	0.23	0.21	0.22	0.20	0.26	0.18	0.24	0.16	0.21	0.16	0.16	0.16	0.20	0.21	0.21	0.21	0.18	0.21	0.23	0.24	0.22	0.18	0.18	0.18	0.15	0.24	0.17	0.18	0.19	0.08	0.00																																			
33	0.33	0.28	0.23	0.26	0.22	0.27	0.24	0.26	0.19	0.23	0.19	0.21	0.19	0.21	0.21	0.22	0.26	0.23	0.25	0.27	0.27	0.28	0.19	0.23	0.23	0.20	0.19	0.17	0.16	0.17	0.10	0.13	0.00																																			
34	0.31	0.24	0.21	0.25	0.21	0.30	0.23	0.24	0.19	0.25	0.22	0.21	0.23	0.22	0.23	0.21	0.23	0.23	0.20	0.25	0.24	0.26	0.20	0.21	0.23	0.22	0.22	0.21	0.20	0.18	0.18	0.17	0.17	0.00																																			
35	0.31	0.30	0.26	0.28	0.22	0.31	0.28	0.24	0.23	0.25	0.20	0.25	0.22	0.26	0.25	0.24	0.26	0.23	0.25	0.25	0.28	0.31	0.25	0.26	0.27	0.26	0.21	0.21	0.17	0.16	0.16	0.19	0.13	0.14	0.00																																			
36	0.30	0.21	0.21	0.28	0.22	0.27	0.24	0.26	0.21	0.26	0.23	0.20	0.25	0.26	0.22	0.25	0.27	0.21	0.23	0.24	0.23	0.28	0.21	0.22	0.24	0.22	0.25	0.20	0.24	0.19	0.17	0.17	0.18	0.18	0.18	0.00																																			
37	0.33	0.26	0.21	0.28	0.21	0.29	0.23	0.25	0.19	0.23	0.22	0.22	0.22	0.24	0.23	0.27	0.24	0.20	0.23	0.24	0.25	0.26	0.23	0.23	0.22	0.17	0.26	0.18	0.19	0.20	0.16	0.14	0.17	0.21	0.18	0.14	0.00																																			
38	0.28	0.19	0.19	0.26	0.22	0.28	0.23	0.27	0.20	0.25	0.22	0.22	0.25	0.23	0.23	0.25	0.28	0.23	0.24	0.25	0.25	0.30	0.21	0.26	0.24	0.22	0.23	0.19	0.24	0.21	0.15	0.15	0.18	0.15	0.19	0.11	0.13	0.00																																			
39	0.28	0.28	0.28	0.29	0.25	0.33	0.28	0.32	0.26	0.29	0.25	0.23	0.26	0.31	0.30	0.29	0.28	0.26	0.30	0.30	0.26	0.28	0.27	0.27	0.26	0.25	0.31	0.26	0.27	0.26	0.18	0.18	0.24	0.22	0.23	0.22	0.17	0.18	0.00																																			
40	0.38	0.22	0.25	0.23	0.21	0.24	0.21	0.24	0.22	0.25	0.24	0.23	0.26	0.21	0.20	0.19	0.19	0.15	0.17	0.21	0.22	0.22	0.20	0.23	0.21	0.17	0.23	0.18	0.19	0.22	0.21	0.18	0.23	0.22	0.28	0.22	0.23	0.23	0.26	0.00																																			
41	0.34	0.21	0.26	0.28	0.22	0.28	0.23	0.28	0.23	0.26	0.28	0.22	0.28	0.25	0.25	0.26	0.23	0.20	0.26	0.24	0.21	0.23	0.22	0.23	0.21	0.20	0.27	0.20	0.24	0.23	0.21	0.19	0.25	0.25	0.27	0.21	0.18	0.20	0.20	0.15	0.00																																		
42	0.36	0.25	0.25	0.31	0.24	0.26	0.26	0.29	0.27	0.23	0.28	0.22	0.28	0.28	0.25	0.26	0.26	0.23	0.26	0.25	0.23	0.27	0.24	0.25	0.25	0.23	0.28	0.22	0.23	0.23	0.23	0.22	0.25	0.26	0.28	0.23	0.20	0.22	0.24	0.16	0.11	0.00																																	
43	0.36	0.27	0.23	0.30	0.25	0.26	0.26	0.28	0.23	0.28	0.26	0.24	0.27	0.23	0.21	0.25	0.22	0.22	0.21	0.24	0.24	0.23	0.21	0.23	0.24	0.18	0.27	0.20	0.23	0.25	0.20	0.20	0.23	0.22	0.25	0.22	0.18	0.21	0.24	0.16	0.16	0.15	0.00																																	
44	0.34	0.26	0.26	0.33	0.27	0.27	0.28	0.31	0.25	0.28	0.26	0.22	0.28	0.25	0.24	0.25	0.24	0.22	0.24	0.25	0.26	0.25	0.21	0.22	0.25	0.20	0.29	0.21	0.24	0.26	0.22	0.21	0.23	0.20	0.23	0.20	0.20	0.21	0.25	0.19	0.17	0.14	0.08	0.00																																	
45	0.39	0.31	0.27	0.33	0.27	0.33	0.29	0.31	0.26	0.28	0.28	0.27	0.29	0.25	0.21	0.24	0.23	0.23	0.23	0.24	0.27	0.26	0.21	0.24	0.25	0.19	0.25	0.21	0.24	0.26	0.23	0.22	0.21	0.18	0.19	0.22	0.22	0.23	0.26	0.21	0.21	0.20	0.10	0.12	0.00																																	
46	0.31	0.28	0.26	0.32	0.28	0.27	0.29	0.28	0.26	0.28	0.26	0.22	0.26	0.26	0.25	0.26	0.25	0.25	0.27	0.26	0.25	0.28	0.23	0.26	0.27	0.23	0.27	0.24	0.23	0.26	0.22	0.23	0.24	0.21	0.23	0.22	0.23	0.23	0.27	0.21	0.20	0.18	0.10	0.09	0.10	0.00																																	
47	0.31	0.26	0.27	0.28	0.24	0.26	0.27	0.24	0.22	0.23	0.28	0.23	0.25	0.26	0.25	0.24	0.23	0.26	0.26	0.28	0.28	0.28	0.26	0.27	0.26	0.24	0.21	0.21	0.21	0.20	0.18	0.21	0.19	0.19	0.19	0.24	0.23	0.21	0.24	0.21	0.16	0.19	0.17	0.17	0.16	0.14	0.00																																	
48	0.28	0.28	0.27	0.31	0.29	0.31	0.34	0.30	0.29	0.27	0.29	0.26	0.28	0.26	0.26	0.27	0.33	0.31	0.32	0.33	0.36	0.38	0.31	0.32	0.33	0.31	0.26	0.24	0.26	0.24	0.23	0.28	0.23	0.25	0.23	0.26	0.27	0.25	0.31	0.30	0.28	0.27	0.25	0.24	0.24	0.21	0.14	0.00																																	
49	0.34	0.23	0.25	0.29	0.25	0.26	0.24	0.26	0.23	0.23	0.24	0.22	0.25	0.21	0.21	0.23	0.21	0.18	0.19	0.25	0.23	0.24	0.22	0.23	0.25	0.20	0.26	0.21	0.22	0.24	0.21	0.20	0.23	0.17	0.22	0.21	0.22	0.21	0.24	0.18	0.17	0.20	0.15	0.13	0.15	0.18	0.13	0.21	0.00																																	
50	0.34	0.21	0.24	0.29	0.27	0.28	0.28	0.28	0.26	0.24	0.28	0.20	0.25	0.24	0.25	0.26	0.27	0.23	0.26	0.28	0.25	0.29	0.21	0.27	0.26	0.23	0.24	0.22	0.25	0.23	0.19	0.21	0.21	0.20	0.28	0.23	0.26	0.21	0.27	0.20	0.21	0.19	0.19	0.18	0.22	0.18	0.16	0.20	0.16	0.00																																	
A	0.41	0.44	0.44	0.46	0.46	0.49	0.45	0.47	0.41	0.44	0.39	0.40	0.44	0.43	0.46	0.47	0.49	0.43	0.48	0.47	0.45	0.46	0.44	0.46	0.45	0.47	0.44	0.41	0.41	0.43	0.41	0.44	0.42	0.42	0.45	0.46	0.45	0.44	0.44	0.48	0.46	0.50	0.49	0.46	0.47	0.46	0.45	0.40	0.45	0.44	0.00																																	
B	0.35	0.44	0.45	0.45	0.46	0.52	0.48	0.47	0.44	0.47	0.44	0.43	0.48	0.43	0.46	0.44	0.51	0.49	0.50	0.46	0.46	0.47	0.46	0.47	0.48	0.50	0.44	0.41	0.42	0.40	0.41	0.46	0.42	0.42	0.43	0.44	0.46	0.41	0.41	0.49	0.46	0.49	0.49	0.42	0.47	0.43	0.43	0.35	0.46	0.41	0.17	0.00																																	

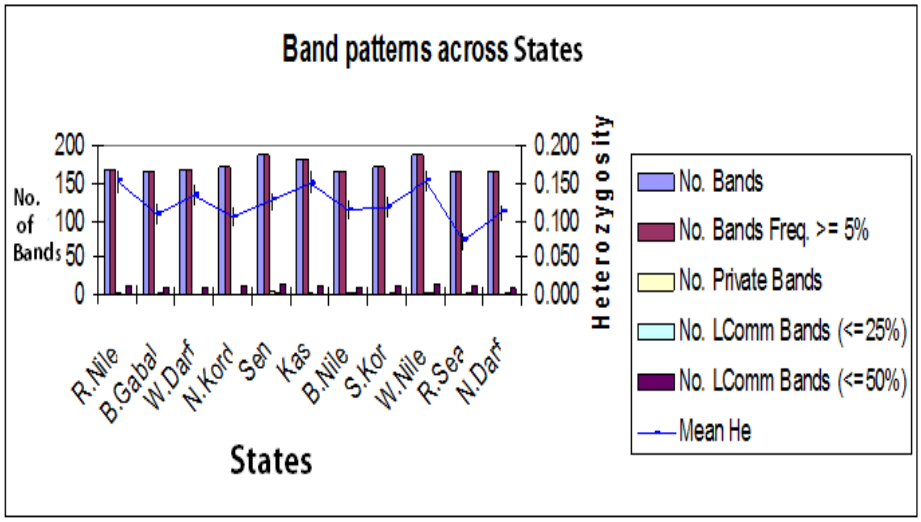

Fig. 3. Total Band Patterns for binary data by regions

Table 4. Total band patterns for binary data for each studied region

States bands	R. Nile	B. Gabal	W. Darf	N. Kord	Sin	Kas	B. Nile	S. Kor	W. Nile	R. Sea	N. Darf
No. bands	168	166	167	173	186	181	164	170	186	165	164
No. bands freq. >= 5%	168	166	167	173	186	181	164	170	186	165	164
No. private bands	2	0	0	0	5	2	2	0	3	1	1
No. LComm bands (<=25%)	1	3	1	1	2	1	2	2	3	2	2
No. LComm bands (<=50%)	14	10	10	14	15	14	8	14	17	12	11
Mean He	0.151	0.107	0.132	0.105	0.126	0.151	0.115	0.118	0.152	0.071	0.110
SE of Mean He	0.013	0.012	0.013	0.012	0.012	0.014	0.012	0.012	0.013	0.010	0.011

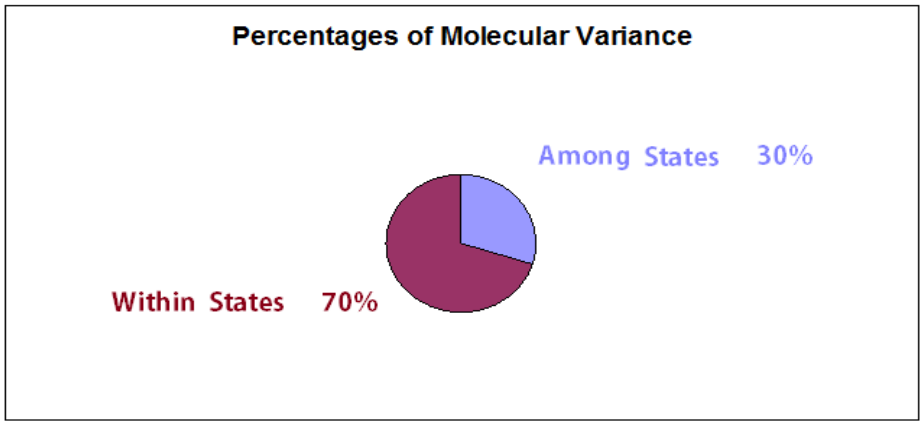

Fig. 4. Percentage of molecular variance within and among the 11 states regions based on analysis of 239 RAPD and ISSR loci

3.7 Molecular Variance

The results of molecular variance analysis showed that the percentage of molecular variance within States was 70%, which is higher than that among States (30%), this was proven also in other results found in this study (Fig. 4).

Table 5. Percentages of polymorphic loci of 10 states in Sudan and Bahr Al Gabel for sorghum

No.	States	Percentage of polymorphic loci
1	River Nile	38.08%
2	Bahr EL Jabel	25.52%
3	West Darfur	33.05%
4	North Kordofan	28.03%
5	Sinnar	33.05%
6	Kassala	37.24%
7	Blue Nile	30.54%
8	South Kordofan	32.64%
9	White Nile	39.75%
10	Red Sea	17.99%
11	North Darfur	30.96%
	Mean	31.53%
	SE	1.86%

4. CONCLUSIONS

Sorghum has a small genome size compared to its close relatives, maize and sugarcane but according to the present study, sorghum from Sudan exhibits great phenotypic variability and larger number of unique fragments, which reflects the high genetic diversity of this crop. So using both markers covers wider area in the genome, which means more accurate results.

The percentage of molecular variance found in Sorghum within States is higher than that among them, that means the genetic diversity among the Sorghum accessions inside the states is higher than Sorghum accessions among States of Sudan and Republic of South Sudan, which can be justified by the human activity in agriculture where farmers move the sorghum seeds from one place to another.

Sorghum from the White Nile state had the highest number of bands; number of private bands; percentage of polymorphic loci and heterozygosity (*He*) mean from accessions of other states.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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