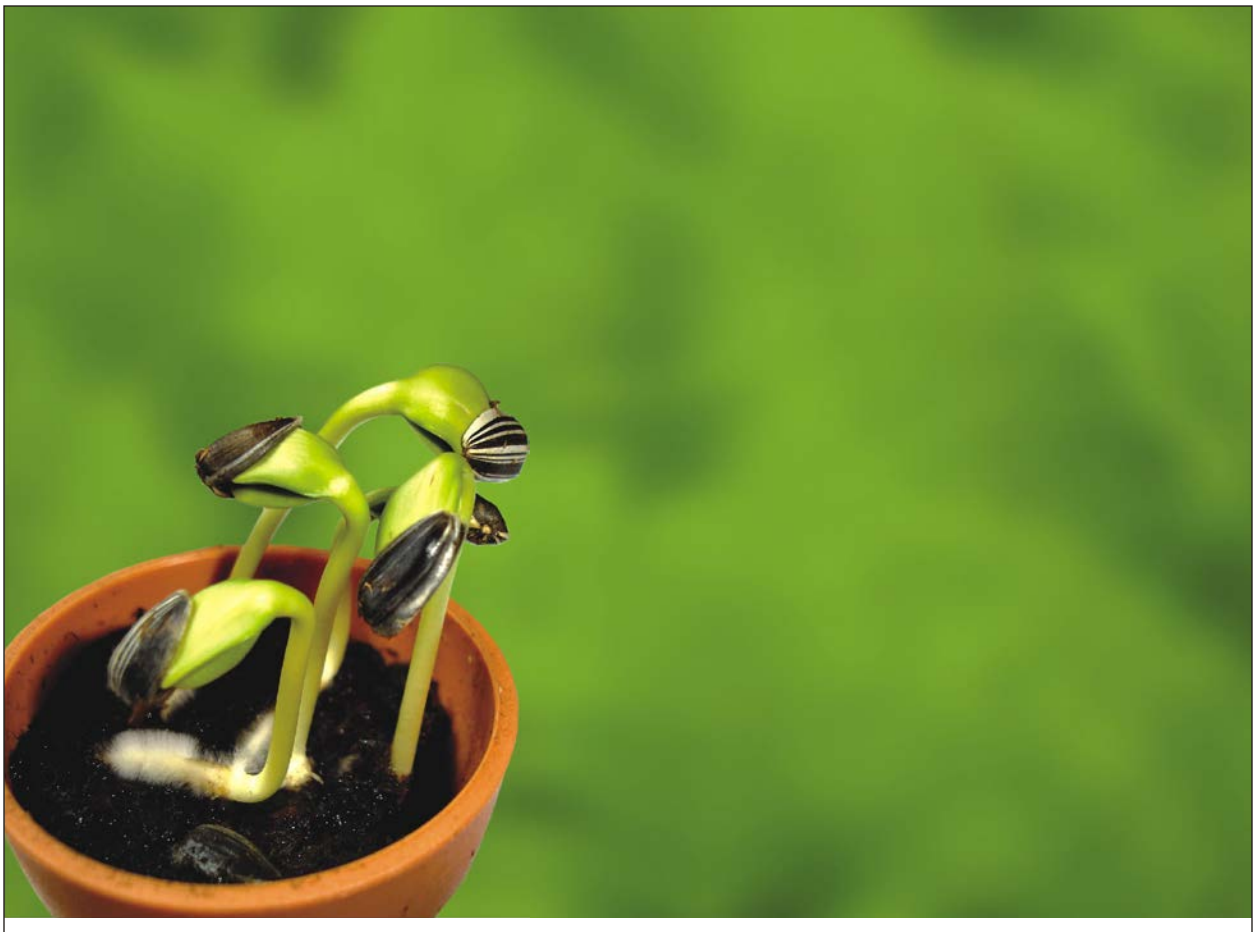

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SSR and SRAP Markers-based Genetic Diversity in Sorghum (*Sorghum bicolor* (L.) Moench) Accessions of Sudan

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ABSTRACT

To evaluate the genetic diversity among the 33 sorghum accessions of Sudan, 70 Simple Sequence Repeats (SSR) and 23 Sequence Related Amplified Polymorphism (SRAP) primer sets were utilized. Results indicated that, of the SSR markers used, 50 (71.4%) were polymorphic, producing 88 (53.0%) polymorphic alleles of PIC value ranging from 0.06 to 0.96 with an average of 0.58. Among the SRAP markers, 8 (34.8%) produced 33 alleles with 48.5% marker polymorphism and PIC value ranging from 0.23 to 0.93. The similarity coefficients based on SSRs were in the range of 0.15 to 0.78 with an average of 0.45, while those based on SRAP markers were in the range of 0.13 to 0.80 with an average of 0.50. Coefficients of similarity generated with SSRs grouped the sorghum accessions into five clusters. In contrast, grouping according to the similarity coefficients of the SRAPs resulted in another completely different five clusters. When data obtained from both SSRs and SRAPs were combined and utilized to generate a dendrogram in which the 33 sorghum accessions were again grouped into five clusters. Cluster 1 and 5 were typical to those of the SSR-based grouping. The present study indicates that fast, accurate and high throughput fingerprinting could be obtained using those markers, from the combined analysis, which revealed the existence of significant variation among the 33 accessions. The most distant accessions can be used by breeders to develop improved sorghum genotypes.

Key words: Molecular markers, diversity, sorghum, polymorphism, PIC

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important cereal crop worldwide and together with maize and pearl millet, form the most important dry land cereal crops for the semi-arid tropics. It is grown worldwide on a total area of 47 million ha (FAOSTAT, 2010). Sudan is within the geographical range where sorghum is believed to be domesticated and where the largest genetic variation for both cultivated and wild sorghum is found (Dawelbeit *et al*., 2010). Sorghum is the main staple food in the Sudan and it ranks first among other crops in allocated area and production. Sudan's average annual production is estimated as 3.7×10^5 MT (FAOSTAT, 2010). However, 90% of the cultivated sorghum is grown under rainfed conditions mainly in Gadarif, Damazin, Kordufan, Darfur and Nuba mountains. Gadarif State (Eastern Sudan) is the most

important region for sorghum production where about 2.1-2.5 million ha are annually cultivated. Large mechanized schemes, of about 600 ha, characterize this region where average yield accounts 2.4 MT ha^{-1} (FAOSTAT, 2010).

Sorghum yield is limited by inadequate and/or erratic rainfall, poor soil fertility, pest and disease invasions and high temperatures (FAOSTAT, 2005). It is important to increase sorghum yield to meet the worldwide increasing demand due to expanding population (Prakash *et al*., 2006). Information on various aspects of sorghum diversity will result in the formation of a sorghum core collection that contains a maximum amount of variation (Maqbool *et al*., 2001). This information can be used as a tool for mining germplasm collections for genomic regions associated with adaptive or agronomically important traits (Casa *et al*., 2005). In addition, assessment of genetic variation among sorghum accessions is a gateway to the study of evolutionary forces that influence the domestication process and has strong impact on conservation and breeding.

DNA molecular markers such as Restriction Fragment Length Polymorphism (RFLP) (Vierling *et al*., 1994; Ahnert *et al*., 1996), Random Amplified Polymorphic DNA (RAPD) (Uptmoor *et al*., 2003; Huang, 2004), Simple Sequence Repeats (SSRs) (Ghebru *et al*., 2002; Menz *et al*., 2004) and Sequence-Related Amplified Polymorphism (SRAP) (Li and Quiros, 2001) have been successfully used to estimate genetic diversity in sorghum. SSR markers have been found to be very efficient in sorghum diversity studies as they revealed more diversity in sorghum compared with other markers (Kudadjie, 2006). SRAP markers were considered as new and useful molecular markers that target coding sequences in the plant genome (Li and Quiros, 2001). They are based on two primer amplifications that preferentially amplify Open Reading Frames (ORFs) or coding regions resulting in a number of dominant and codominant markers. The forward primer amplifies the exon regions while the reverse primer amplifies the intron and promoter regions. Their polymorphisms result from the variation in length of these exons, introns, promoters and spacers both among individuals as well as species (Li and Quiros, 2001; Zhao *et al*., 2009). SRAP markers have been used in a wide range of plant species such as *Medicago sativa* (Ariss and Vandemark, 2007), *Buchloe dactyloides* (Budak *et al*., 2004), *Gossypium* (Lin *et al*., 2004), *Cucurbita* (Ferriol *et al*., 2003), *Paeonia suffruticosa* (Han *et al*., 2008) and *Triticum* spp. (Fufa *et al*., 2005; Zaefizadeh and Goliev, 2009).

The objective of this study was to examine the genetic variability within some sorghum accessions from different regions of Sudan and to group them based on SSR and/or SRAP markers fingerprinting.

MATERIALS AND METHODS

Plant material: Seeds of 33 sorghum accessions collected from different regions of Sudan were used in this study (Table 1). In the 33 accessions, 10 are landraces (from Eastern Sudan, Gadarif area), 13 are inbred lines (Central Sudan), 6 are local cultivars (1 from Eastern Sudan and 5 from Western Sudan) and 4 are standard commercial cultivars from Central Sudan.

DNA extraction: Genomic DNA was extracted following a sap-extraction method from 100 mg of fresh tissue. Leaves of 2-week-old seedlings were placed between the two rollers of a sap-extraction apparatus (Ravenel Specialities, Seneca, S.C.) and 1 mL of extraction buffer (50 mM Tris-HCl, 25 mM EDTA, 1 M NaCl, 1% CTAB, 1 mM 1, 10-phenathroline and 0.15% 2-mercaptoethanol) was slowly added to the rollers, immediately mixing with the sap for collection in 1.5 mL microcentrifuge tubes. The extract was incubated at 60°C for 1 h, then mixed with equal volume

Table 1: Sorghum accessions used in the study, their collection codes, types and collection location

Locality	Type	Collection No.
Blue Nile State		
South-Eastern Sudan	Land race	ShSc 871
South-Eastern Sudan	Land race	ShSc 872
South-Eastern Sudan	Land race	ShSc 875
South-Eastern Sudan	Land race	ShSc 876
South-Eastern Sudan	Land race	ShSc 877
South-Eastern Sudan	Land race	ShSc 879
South-Eastern Sudan	Land race	ShSc 880
South-Eastern Sudan	Local variety "Karamaca"	ShSc 881
South-Eastern Sudan	Land race	ShSc 882
South-Eastern Sudan	Land race	ShSc 887
South-Eastern Sudan	Land race	ShSc 888
Gezira State		
Central Sudan	Standard commercial variety "Wadahmed"	ShSc 1
Central Sudan	Standard commercial variety "Dabar"	ShSc 3
Central Sudan	Standard commercial variety "Tabat"	ShSc 4
Central Sudan	Standard commercial variety "Teteron"	ShSc 5
Central Sudan	Inbred line	L 07-08
Central Sudan	Inbred line	L 09-08
Central Sudan	Inbred line	L 12-08
Central Sudan	Inbred line	L 13-08
Central Sudan	Inbred line	L 15-08
Central Sudan	Inbred line	L 16-08
Central Sudan	Inbred line	L 20-08
Central Sudan	Inbred line	L 23-08
Central Sudan	Inbred line	L 29-08
Central Sudan	Inbred line	L 35-08
Central Sudan	Inbred line	L 45-08
Central Sudan	Inbred line	L 52-08
Central Sudan	Inbred line	L 61-08
North Kordufan State		
Western Sudan	Local variety "Batanya Hamra"	Bot-1
Western Sudan	Local variety "Ajab Seedo"	Bot-2
Western Sudan	Local variety "Taferi Hamra"	Bot-3
Western Sudan	Local variety "Taferi Beida"	Bot-4
Western Sudan	Local variety "Batigy"	Bot-5

of chloroform-isoamyl alcohol (24:1). After centrifugation at 12,000 rpm, the supernatant was transferred to a new tube. To precipitate the DNA, isopropanol was added and the contents were incubated for 30 min. The pellet was dried, resuspended in 200 mL of TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) plus 20 μg of RNase and then incubated overnight at room temperature. The DNA solution was mixed with 20 μL of 8 M ammonium acetate and 400 μL of cold absolute ethanol for 30 min, centrifuged for 10 min and air dried at room temperature. The DNA was then resuspended in 200 μL of TE buffer and DNA concentration was quantified by spectrophotometry (TKO100 Fluorometer, Hoefer Scientific Instruments, San Francisco).

Markers analysis: A total of 93 oligonucleotide primer pairs that included 70 sorghum SSRs (Schloss *et al*., 2002) and 23 SRAP combinations (Li and Quiros, 2001) were used. The marker assays were conducted following the procedure of Kuleung *et al*. (2004). A 25 μL total

volume/reaction was used, consisting of 75 ng genomic DNA, 100 ng primer pair, 125 μM dNTPs, 50 mM KCl and 10 mM Tris-HCl, 25 mM $MgCl_2$ and 1 unit Taq polymerase. The amplification procedure consisted of one cycle at 94°C for 3 min, followed by 35 cycles of 1 min at 94°C, 1 min at 55°C for SSRs but 47°C for SRAPs, 1 min at 72°C and final extension step at 72°C for 5 min. The reaction was then cooled to a resting temperature of 4°C and resolved by electrophoresis in a 12% non-denatured polyacrylamide gel (37:1 acrylamide:bis-acrylamide). The gel was stained in 1 μg mL^{-1} ethidium bromide for 10 min, destained in deionized water for 15 min and photographed using the Gel Doc2000 (Bio-Rad, Hercules, CA.).

Data analysis: Each DNA fragment obtained by both marker types was scored as present (1) or absent (0), each of which was treated as an independent character. Similarity between the accessions was analyzed on the basis of the scores. Data were then used to create a matrix to analyze genetic relationship using the R software package (R Development Core Team, 2011). A dendrogram was constructed based on Jaccard's similarity coefficient (Jaccard, 1908) using the markers data for all sorghum accessions following the Unweighted Pair Group Method Analysis (UPGMA) as described by Sokal and Michener (1958). Polymorphism Information Content (PIC) values were calculated as in Anderson *et al.* (1993), who assumed homologous alleles. PIC for a locus is calculated as:

$$PIC = 1-\sum P_{ij}^2$$

where, P_{ij} is the relative frequency of the jth allele of the ith locus, summed over all the alleles for individual marker locus over all lines. A marker with a PIC value of more than 0.5 is considered as highly informative, between 0.25 and 0.5 as informative and less than 0.25 as slightly informative (Botstein *et al.*, 1980). The genetic diversity was estimated by similarity indices calculated from band sharing data of each pair of DNA fingerprints.

RESULTS AND DISCUSSION

Molecular markers analysis: A total of 70 SSRs and 23 SRAPs markers were used to characterize and evaluate the genetic diversity of 33 sorghum accessions. Out of the screened SSRs, 50 (71.4%) were polymorphic. The markers produced 166 alleles, 88 of them (53.0%) were polymorphic displying PIC values ranging from 0.06 to 0.96 with an average of 0.58. These values were higher than those of 0.40, 0.44 and 0.52 observed, for sorghum accessions, by Ali *et al.* (2008), Folkertsma *et al.* (2005) and Lekgari (2010), respectively. The differences may be attributed to the number of bands/alleles scored and the type of SSR markers used. For example, Ali *et al.* (2008) used only one type of SSR marker (xcup) while in this study four different SSR marker types (Xcup, Sam, Xsb and Drenhsbm) were used. In addition, the number of alleles/bands scored per marker differs between individual studies. The mean PIC value of 0.58 reported here indicates that the markers used were highly informative (Botstein *et al.*, 1980).

Similarity indices based on the polymorphic data obtained were used to estimate the genetic relatedness among the sorghum accessions. Results (Table 2) indicate that the genetic similarity coefficients for all accessions based on SSR markers ranged from 0.15-0.78 with an average of 0.45. It is also evident that accessions ShSc871 and ShSc875 are the most closely related genotypes as they showed the highest similarity index, while the genotypes ShSc879 and L15-08, with the lowest index, are the most distantly related. A dendrogram based on the similarity values produced

Table 2: Genetic similarity matrix for sorghum accessions as assessed by SSR (lower) and SRAP (upper) markers

	L16-08	L09-09	ShSc 871	ShSc 872	ShSc 875	ShSc 876	ShSc 877	ShSc 879	ShSc 880	ShSc 881	ShSc 882	ShSc 1	ShSc 3	ShSc 887	ShSc 4	ShSc 5
L16-08		0.38	0.71	0.27	0.71	0.54	0.57	0.46	0.46	0.67	0.73	0.57	0.38	0.54	0.67	0.60
L09-09	0.60		0.25	0.25	0.43	0.45	0.29	0.50	0.25	0.45	0.25	0.38	0.56	0.33	0.60	0.54
ShSc871	0.73	0.67		0.23	0.73	0.47	0.71	0.40	0.50	0.57	0.62	0.60	0.43	0.69	0.47	0.63
ShSc872	0.60	0.54	0.65		0.23	0.33	0.17	0.38	0.22	0.33	0.38	0.27	0.25	0.33	0.20	0.14
ShSc875	0.67	0.67	**0.78**	0.63		0.38	0.60	0.50	0.50	0.57	0.50	0.60	0.54	0.57	0.69	0.73
ShSc876	0.60	0.56	0.67	0.58	0.67		0.43	0.55	0.21	0.38	0.42	0.67	0.33	0.50	0.29	0.38
ShSc877	0.50	0.45	0.49	0.59	0.43	0.35		0.46	0.58	0.67	0.58	0.47	0.29	0.54	0.43	0.50
ShSc879	0.37	0.37	0.39	0.41	0.32	0.29	0.60		0.33	0.55	0.33	0.46	0.36	0.55	0.55	0.40
ShSc880	0.66	0.66	0.73	0.64	0.75	0.64	0.50	0.37		0.70	0.60	0.36	0.25	0.42	0.42	0.50
ShSc881	0.64	0.46	0.63	0.53	0.62	0.64	0.36	0.25	0.58		0.70	0.43	0.33	0.50	0.64	0.57
ShSc882	0.53	0.63	0.66	0.52	0.59	0.57	0.39	0.28	0.61	0.47		0.46	0.25	0.55	0.42	0.40
ShSc1	0.63	0.51	0.64	0.47	0.62	0.64	0.36	0.31	0.61	0.58	0.57		0.50	0.54	0.33	0.60
ShSc3	0.62	0.54	0.63	0.60	0.61	0.64	0.51	0.44	0.64	0.53	0.46	0.57		0.45	0.45	0.54
ShSc887	0.54	0.53	0.61	0.56	0.56	0.71	0.34	0.30	0.54	0.49	0.51	0.50	0.62		0.38	0.38
ShSc4	0.55	0.46	0.58	0.64	0.48	0.49	0.54	0.54	0.57	0.40	0.44	0.43	0.54	0.47		0.69
ShSc5	0.46	0.54	0.59	0.63	0.57	0.50	0.53	0.47	0.60	0.47	0.45	0.44	0.60	0.51	0.61	
ShSc888	0.46	0.41	0.51	0.56	0.45	0.40	0.54	0.53	0.46	0.40	0.35	0.36	0.49	0.40	0.59	0.61
L29-08	0.53	0.42	0.62	0.52	0.47	0.47	0.47	0.36	0.51	0.47	0.46	0.44	0.54	0.58	0.50	0.56
L13-08	0.52	0.47	0.59	0.63	0.61	0.60	0.44	0.29	0.60	0.53	0.49	0.52	0.56	0.51	0.45	0.46
L12-08	0.54	0.54	0.66	0.56	0.64	0.61	0.45	0.34	0.67	0.53	0.57	0.57	0.58	0.60	0.48	0.54
L45-08	0.68	0.62	0.73	0.58	0.69	0.67	0.42	0.28	0.66	0.66	0.63	0.64	0.56	0.54	0.45	0.52
L23-08	0.56	0.52	0.61	0.58	0.61	0.54	0.52	0.39	0.60	0.58	0.54	0.51	0.54	0.45	0.50	0.54
L20-08	0.55	0.46	0.60	0.59	0.53	0.47	0.59	0.48	0.53	0.47	0.48	0.51	0.50	0.39	0.52	0.52
L52-08	0.57	0.51	0.60	0.55	0.60	0.53	0.46	0.33	0.61	0.56	0.55	0.58	0.51	0.41	0.45	0.48
L07-08	0.49	0.49	0.58	0.60	0.54	0.49	0.43	0.34	0.51	0.52	0.49	0.47	0.55	0.44	0.51	0.60
L61-08	0.39	0.46	0.44	0.43	0.45	0.37	0.42	0.30	0.41	0.39	0.45	0.32	0.44	0.31	0.41	0.47
L35-08	0.45	0.49	0.55	0.40	0.50	0.47	0.26	0.25	0.45	0.50	0.48	0.41	0.44	0.50	0.36	0.48
L15-08	0.33	0.31	0.31	0.23	0.32	0.29	0.20	**0.15**	0.29	0.38	0.32	0.31	0.24	0.25	0.25	0.26
Bot-1	0.56	0.60	0.67	0.51	0.67	0.62	0.38	0.38	0.64	0.59	0.49	0.56	0.55	0.53	0.45	0.51
Bot-2	0.56	0.50	0.62	0.56	0.73	0.61	0.39	0.28	0.61	0.67	0.52	0.57	0.54	0.47	0.38	0.47
Bot-3	0.54	0.49	0.68	0.56	0.65	0.58	0.40	0.40	0.60	0.57	0.51	0.59	0.60	0.53	0.49	0.55
Bot-4	0.53	0.48	0.58	0.52	0.51	0.51	0.34	0.28	0.49	0.56	0.52	0.43	0.48	0.49	0.42	0.47
Bot-5	0.50	0.60	0.67	0.51	0.63	0.58	0.38	0.28	0.54	0.57	0.60	0.50	0.49	0.53	0.47	0.49

Table 2: Continued

	ShSc 888	L29-08	L13-08	L12-08	L45-08	L23-08	L20-08	L52-08	L07-08	L61-08	L35-08	L15-08	Bot-1	Bot-2	Bot-3	Bot-4	Bot-5
L16-08	0.67	0.50	0.40	0.54	0.67	0.62	0.46	0.53	0.29	0.47	0.46	0.36	0.58	0.46	0.31	0.50	0.23
L09-09	0.50	0.42	0.31	0.23	0.23	0.31	0.36	0.27	0.40	0.38	0.50	0.22	0.36	0.36	0.30	0.31	0.20
ShSc871	0.69	0.53	0.53	0.69	0.69	0.64	0.40	0.79	0.43	0.60	0.50	0.31	0.62	0.50	0.46	0.53	0.38
ShSc872	0.21	0.30	0.18	0.33	0.33	0.30	0.22	0.25	0.25	0.27	0.38	0.40	0.38	0.22	0.29	0.30	0.14
ShSc875	0.80	0.53	0.53	0.69	0.57	0.53	0.40	0.67	0.43	0.60	0.50	0.31	0.40	0.50	0.36	0.64	0.38
ShSc876	0.53	0.36	0.46	0.29	0.38	0.46	0.55	0.50	0.33	0.54	0.42	0.44	0.55	0.31	0.36	0.46	0.17
ShSc877	0.67	0.50	0.50	0.43	0.54	0.62	0.46	0.77	0.29	0.57	0.46	0.36	0.58	0.46	0.31	0.31	0.33
ShSc879	0.47	0.38	0.38	0.42	0.42	0.64	0.45	0.54	0.25	0.58	0.45	0.33	0.45	0.23	0.27	0.29	0.18
ShSc880	0.57	0.80	0.38	0.42	0.42	0.50	0.33	0.54	0.25	0.46	0.60	0.20	0.45	0.60	0.27	0.29	0.30
ShSc881	0.64	0.73	0.36	0.50	0.64	0.73	0.42	0.62	0.33	0.54	0.70	0.30	0.70	0.55	0.36	0.27	0.27
ShSc882	0.57	0.64	0.38	0.42	0.55	0.50	0.45	0.54	0.25	0.46	0.45	0.33	0.60	0.60	0.27	0.38	0.18
ShSc1	0.79	0.50	0.50	0.54	0.54	0.40	0.36	0.64	0.50	0.57	0.46	0.36	0.36	0.36	0.55	0.62	0.33
ShSc3	0.50	0.31	0.42	0.45	0.33	0.21	0.25	0.36	0.56	0.29	0.50	0.22	0.36	0.36	0.63	0.42	0.50
ShSc887	0.53	0.46	0.36	0.50	0.38	0.46	0.31	0.62	0.33	0.67	0.55	0.44	0.55	0.42	0.36	0.36	0.27
ShSc4	0.53	0.46	0.36	0.50	0.50	0.58	0.42	0.40	0.33	0.43	0.42	0.18	0.42	0.42	0.25	0.36	0.27
ShSc5	0.80	0.64	0.53	0.57	0.57	0.53	0.40	0.56	0.54	0.50	0.50	**0.13**	0.40	0.50	0.46	0.53	0.38
ShSc888		0.71	0.60	0.53	0.53	0.50	0.47	0.73	0.50	0.67	0.57	0.29	0.47	0.57	0.43	0.60	0.36
L29-08	0.47		0.43	0.46	0.46	0.54	0.38	0.57	0.42	0.62	0.64	0.17	0.50	0.64	0.33	0.43	0.25
L13-08	0.36	0.41		0.36	0.36	0.43	**0.80**	0.69	0.42	0.50	0.38	0.17	0.38	0.64	0.45	0.54	0.36
L12-08	0.41	0.55	0.61		0.80	0.58	0.21	0.62	0.60	0.54	0.42	0.30	0.42	0.31	0.50	0.58	0.56
L45-08	0.38	0.51	0.60	0.61		0.73	0.31	0.62	0.45	0.43	0.42	0.30	0.55	0.31	0.50	0.46	0.40
L23-08	0.41	0.44	0.56	0.61	0.63		0.50	0.69	0.31	0.62	0.50	0.27	0.64	0.38	0.33	0.33	0.25
L20-08	0.49	0.48	0.54	0.54	0.59	0.64		0.54	0.25	0.46	0.33	0.20	0.45	0.60	0.27	0.38	0.18
L52-08	0.43	0.46	0.57	0.62	0.64	0.66	0.65		0.46	0.77	0.54	0.33	0.54	0.54	0.50	0.47	0.42
L07-08	0.50	0.51	0.50	0.41	0.58	0.53	0.49	0.51		0.50	0.36	0.22	0.36	0.36	0.63	0.55	0.71
L61-08	0.47	0.38	0.38	0.33	0.45	0.48	0.39	0.40	0.61		0.46	0.36	0.46	0.46	0.31	0.50	0.33
L35-08	0.32	0.46	0.43	0.42	0.51	0.44	0.32	0.35	0.54	0.41		0.33	0.60	0.60	0.56	0.29	0.30
L15-08	0.24	0.26	0.27	0.28	0.36	0.30	0.25	0.27	0.30	0.29	0.46		0.33	0.20	0.25	0.27	0.29
Bot-1	0.40	0.49	0.60	0.60	0.64	0.47	0.47	0.52	0.50	0.37	0.52	0.33		0.45	0.40	0.29	0.30
Bot-2	0.39	0.44	0.63	0.52	0.63	0.48	0.48	0.57	0.55	0.43	0.48	0.32	0.68		0.40	0.38	0.30
Bot-3	0.46	0.59	0.51	0.66	0.58	0.54	0.56	0.57	0.52	0.36	0.45	0.31	0.65	0.61		0.33	0.57
Bot-4	0.40	0.46	0.43	0.44	0.59	0.50	0.42	0.45	0.58	0.45	0.56	0.38	0.51	0.52	0.54		0.36
Bot-5	0.42	0.47	0.51	0.58	0.60	0.51	0.49	0.61	0.52	0.43	0.43	0.33	0.67	0.60	0.62	0.54	

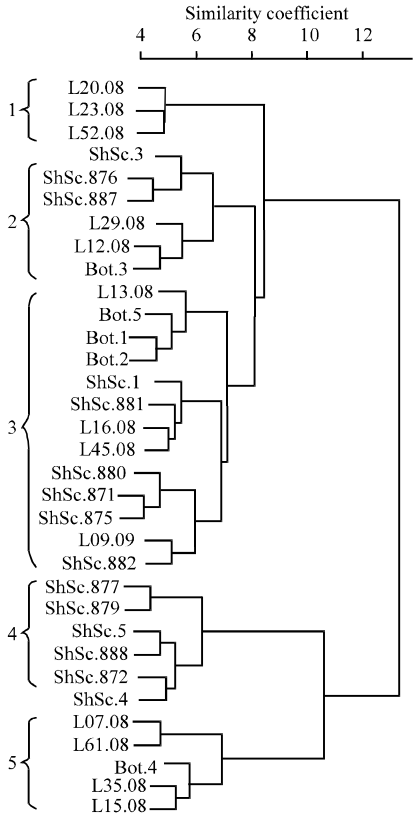

Fig. 1: Dendrogram of 33 sorghum accessions from Sudan based on SSR markers. Values along x-axis correspond to Jaccard's coefficients of dissimilarity

from the SSRs was constructed using the UPGMA cluster analysis (Fig. 1). Five major clusters were obtained; cluster 1 contained three accessions which were all collected from the Central Region of Sudan. Cluster 2 included accessions from Central (3), Eastern (2) and Western (1) regions. The largest group, cluster 3, has 13 members. Of these, five were from Central Sudan, five from Eastern Sudan and the remaining were from the Western region. The 67% of the accessions in cluster 4 and 80% of the accessions in cluster 5 are from Eastern and Central Sudan, respectively. It could also be noted in Fig. 1 that accession Bot.4, from Western Sudan, is distant from the other four accessions of the same region. The four standard cultivars of the Central Sudan were distributed in three different clusters, indicating that they are genetically different. This highlights the possibility of using these cultivars in breeding activities for introgression of desirable traits.

In SRAP analysis, the markers produced 33 alleles, the percentage of marker polymorphism is 48.5 and the PIC values ranged from 0.23 to 0.93. The similarity coefficients based on SRAP markers ranged from 0.13, in L15-08 and ShSc5, to 0.80, between L20-08 and L13-08 (Table 2). Grouping according to the coefficients of the SRAPs have also resulted in five clusters (Fig. 2) but they were completely different from those of SSRs. Of these, cluster 1 included four accessions, two from Central Sudan and two from Western Sudan. Three of these accessions are known cultivars, while the fourth is an inbred line. Cluster 2 included seven accessions five of them belong to the Central region, one from East while the other is from the West. In cluster 3 which contained six accessions, there was an equal number of accessions from Central and Eastern Sudan. The fourth cluster had accessions (n = 4) representing each of the regions studied. Being the largest group

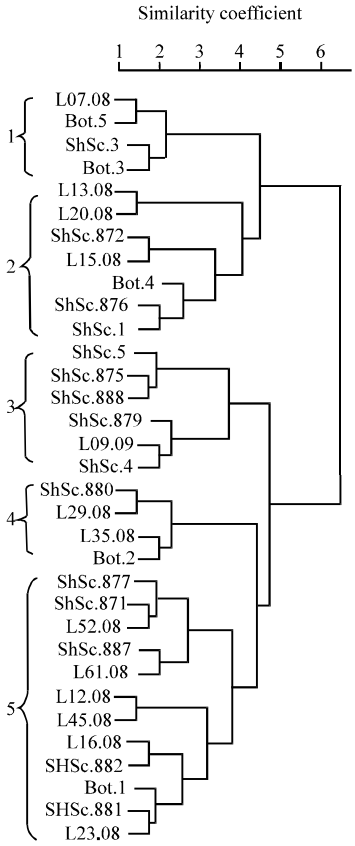

Fig. 2: Dendrogram of 33 sorghum accessions from Sudan based on SRAP markers. Values along x-axis correspond to Jaccard's coefficients of dissimilarity

(12 accessions), cluster 5 contained six accessions from the centre, five from East and only one accession from the West. This cluster included only two cultivars, one from Western Sudan (Bot.1) and the other from Eastern Sudan (ShSc881), the remaining were lines and landraces. With the exception of cluster 4, each of the other clusters contained at least one of the accessions collected from Western Sudan. This may indicate the diverse nature of the sorghum genotypes grown in that region.

Data obtained from both SSRs and SRAPs were combined and utilized to generate the diversity grouping. Based on the obtained results (Fig. 3), the 33 accessions were again grouped into five clusters. Cluster 1 and 5 were typical to those in the SSR-based grouping. In cluster 2 which had eight accessions, six were found in cluster 3 of the SSRs groups. Cluster 3 was the largest one, containing 11 accessions; six of them were local cultivars from all of the regions studied. Four of the six members of cluster 4 were similar to the members of cluster 3 in SRAPs based grouping.

Both marker types were efficient in elucidating the genetic diversity present in the tested sorghum accessions. Furthermore, clustering based on the combined data was found to be more informative than those derived from individual type of analysis. The results presented in this study reveal that the sorghum accessions grown in the potential parts in Sudan have wide genetic background and they are highly diverse.

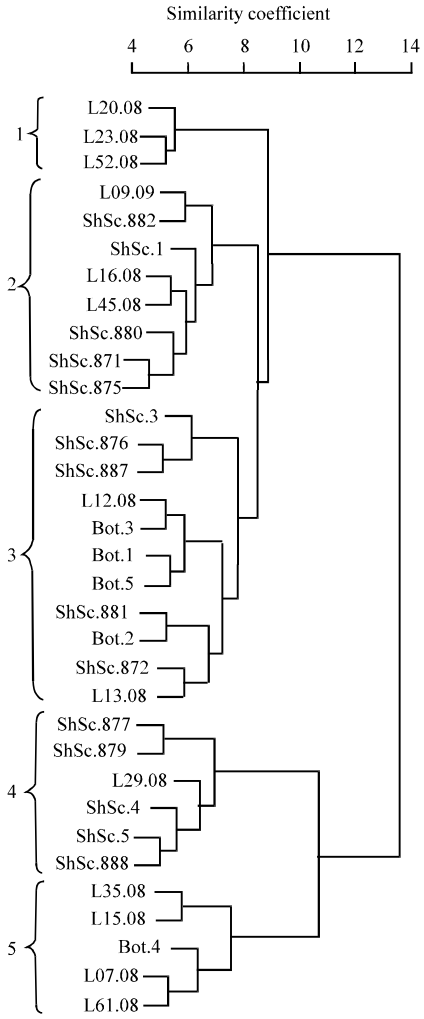

Fig. 3: Dendrogram of 33 sorghum accessions from Sudan based on both SSR and SRAP markers. Values along x-axis correspond to Jaccard's coefficients of dissimilarity

CONCLUSION

The present study indicates that fast, accurate and high throughput fingerprinting could be obtained using those markers, from the combined analysis, which revealed the existence of significant variation among the 33 accessions. The most distant accessions can be used by breeders to develop improved sorghum genotypes.

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