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Phylogenetic Diversity of *Sorghum bicolor* (L.) Moench Accessions from Different Regions in Sudan

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ABSTRACT
Sorghum (*Sorghum bicolor* L.) is believed to be domesticated for the first time within the geographical range of Sudan, so, studying the genetic variation of sorghum genotypes collections attracts special interest. In this study, 17 sorghum accessions with important agronomic traits, representing four states in Sudan were assayed for polymorphism using Random Amplified Polymorphic DNA technique (RAPD). Ten primers out of 40 tested (A-1, B-20, C-20, D-18, OPE-04, UBC-101, UBC-103, UBC-127, UBC-155 and UBC-157) showed high polymorphism among the accessions. The results indicated 110 polymorphic bands out of 145 bands with percentage of polymorphic bands of 77.6%. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) result showed two major clusters, with a clear trend of grouping of each region. A cluster contained all accessions from eastern Sudan (Red Sea). However, accessions from north Sudan (River Nile), western Sudan (North Kordofan) and south Sudan (Blue Nile) were diverse in another cluster. Based on the results of this study RAPD technique proved to be useful to study genetic variation among the Sudanese sorghum accessions.

Key words: Sorghum, RAPD markers, genetic variation, cluster, UPGMA, Sudan

INTRODUCTION
In Africa, especially in Sudan *Sorghum bicolor* is a large subsistence food, forge and ethanol production crop for more than 75% of the population (Dawelbeit *et al*., 2010). Sorghum has attracted the attention of breeders not only in Sudan but around the world for his rich source of proteins, vitamins, carbohydrates and economical important (Shiringan, 2009). Sorghum is a diploid (2n = 20) and inter fertile (Curtis, 1968). About 2512 loci exist in its genetic map (Bowers *et al*., 2003). Sorghum has small genome (730 Mb); therefore was considered as model for functional genomics of C4 grasses, it also set a good example to understand the biology of weeds (Paterson, 2008). The Morphological variation doesn't express the genetic variation due to the genotype-environment interactions (Smith and Smith, 1992). Therefore, studying genetic variation is important (Bandyopadhyay, 2011). Wide spectrum of methods and techniques exist for the analysis of discrete morphological traits to molecular traits (Soller and Beckmann, 1983). Therefore, understanding the real genetic variation is crucial for breeding programs in order to help maintain genetic diversity (Pu *et al*., 2009).

Molecular markers proved to be powerful and reliable tools for studying the variation within crop genotypes compared with the biochemical markers (Gepts, 1993). Random Amplified Polymorphic DNA (RAPD) technique is one of the most used molecular methods, as it offers many advantages such as detect high polymorphism and relatively fast (Fahima *et al.*, 1999), RAPD could also reveal within accessions variation more efficiently (Patra *et al.*, 2011). The RAPD technique is based on the PCR amplification of discrete regions inside the genome with short oligonucleotide primers of arbitrary sequence (Thangaraj *et al.*, 2011). A minimal amount of DNA is required and no DNA sequence information is needed before carrying the technique (Subudhi and Huang, 1999). Although of the robustness of the method, its reproducibility can vary from low to extremely high variability (Fang and Roose, 1997; Nagaoka and Ogihara, 1997). From its advantages is that it is simple and fast method for detecting high amount of DNA markers (Younis *et al.*, 2007). Over 800 markers are mapped in sorghum, these maps are compared to those of rice (Paterson *et al.*, 1995, 2004), maize (Bowers *et al.*, 2003; Whitkus *et al.*, 1992), sugarcane (Ming *et al.*, 1998; Dufour *et al.*, 1997), millet (Jessup *et al.*, 2003), switch grass (Missaoui *et al.*, 2005) and Bermuda grass (Bethel *et al.*, 2006).

The main objectives of the study were to investigate the degree of polymorphism detected using 10 RAPD (Random Amplified Polymorphic DNA) markers in 17 sorghum accessions from four different regions in Sudan.

MATERIALS AND METHODS

The DNA of 17 sorghum accessions was extracted from fresh leaf tissues (Table 1) using modified CTAB method (Porebski *et al.*, 1997). The modification was made in intention to improve the quantity and the quality of the DNA. 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 60°C with gentle shaking for 30 min and left to cool at room temperature for 5 min. Isoamylalcohol and chloroform mixture

Table 1: Sorghum genotypes list contained accessions number, accessions name, Area location of collection and the region of collection

Accessions No.	Accessions name	Area location	Region
1	Debeikri I	El-Nikhala area	River Nile
2	Abjaro	Balook area	River Nile
3	Abjaro Abu Soof	Balook area	River Nile
4	Debeikri II	Aldaroosa	River Nile
5	Zinari Ahmer	Abu Snoon	North Kordofan
6	Ankoleeb Ahmer I	Abu Haraz	North Kordofan
7	Sifaira	Abu Haraz	North Kordofan
8	Ankoleeb Ahmer II	Abu Haraz	North Kordofan
9	Dura Abied	Mengenza	Blue Nile
10	Kolom I	Mengenza	Blue Nile
11	Kolom II	Jebel El Nimir	Blue Nile
12	Arfaa gadamak I	Abu Sowailik	Blue Nile
13	Arfaa gadamak II	Abu Sowailik	Blue Nile
14	Baham Hazaz	Adramieb	Red Sea
15	Hazaz I	Adramieb	Red Sea
16	Hazaz II	Adramieb	Red Sea
17	Hazaz III	Adramieb	Red Sea

(1:24) was added to each tube and the phases were mixed gently for 5 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: isoamyl alcohol 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 after spinning with flash centrifugation. 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 -20°C for further use. 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 spectrophotometrically assessed following Sambrook *et al.* (1989) method.

Forty RAPD primers (Operon technologies and university of British Columbia (UBC)) were used in the Polymerase Chain Reaction (PCR) in a final volume of 25 μL containing 1.0 μL DNA diluted, 0.5 μL Taq polymerase, 2.5 μL 10x buffer, 2.5 μL (2 mM μL^{-1}) dNTPs, 1.5 μL (50 mM) $MgCl_2$, 2.0 μL (10 pmol μL^{-1}) RAPD primer and 15 μL dH_2O. The amplifications were performed in a thermal cycler following the program: 94°C for 5 min, 40 cycles (1 min at 94°C, 1 min at 33°C and 1 min at 72°C) and final elongation of 7 min at 72°C.

Four Microliter of PCR product was mixed with 2 μL of loading dye and 1.5 μL of 1 kbp DNA ladder were electrophoresed using 2% agarose gel at 73 volts followed by staining with ethidium bromide then the separated fragments were visualized with an Ultraviolet (UV) transilluminator.

The number of polymorphic and monomorphic bands was determined for each primer. Genotype 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 x100). The tree diagram was produced by clustering the similarity data with the unweighted pair group average method using statistical software package STATISTCA-SPSS following the method of El-Amin *et al.* (2011).

RESULTS

To isolate a good quality of DNA, the CTAB-based procedure optimized in the present study, yielded high quality DNA free of phenols which may inhibit the activity of Taq polymerase. Several primers were tested on the 17 accessions (*Sorghum bicolor* L.) and the results indicated that 10 primers out of the 40 tested showed a high polymorphic band percentage (75.9%). The 10 informative primers were selected and used to evaluate the degree of polymorphism and genetic relationships among the genotypes under study. Total of 145 amplified fragments were distinguished across the selected primers and the statistical analysis showed 110 polymorphic bands among the accessions with an average of 11 polymorphic bands per primer. The maximum number of bands were produced by the primer UBC-157 (21 bands) with 52.4% polymorphism respectively while the minimum numbers of fragments were produced by the primer C-20 (8 bands) with 62.5%, polymorphism, respectively. RAPD fragments pattern produced by the 10 primers is shown in Table 2.

The Tree Diagram reflects the accessions of the four regions which were divided into two clusters based on their genetic similarity. The first cluster contained 3 groups: River Nile group, North Kordofan group each grouped separately from each other and Blue Nile group. River Nile group have accessions Abjaro Abu Soof and Debeikri II from Aldaroosa area as sisters, Debeikri I

Table 2: Polymorphic bands percentages of 10 RAPD primers used to study the 17 sorghum accessions

Primer name	Sequence (5'-3')	Total No. of bands	No. of polymorphic bands	No. of monomorphic bands	Percentage of polymorphic bands
A-1	CAG GCC CTT C	9.0	8	1.0	88.8
B-20	GGA CCC TTA C	9.0	8	1.0	88.8
C-20	ACT TCG CCA C	8.0	5	3.0	62.5
D-18	GAG AGC CAA C	13.0	12	1.0	92.3
OPE-04	GTG ACA TGC C	17.0	14	3.0	82.4
UBC-101	GCG GCT GGA G	18.0	13	5.0	72.2
UBC-103	GTG ACG CCG C	19.0	13	6.0	68.4
UBC-127	ATC TGG CAG C	15.0	13	2.0	86.7
UBC-155	CTG GCG GCT G	16.0	13	3.0	81.3
UBC-157	CGT GGG CAG G	21.0	11	10.0	52.4
Total		145.0	110	35.0	775.8
Average		14.5	11	3.5	77.6

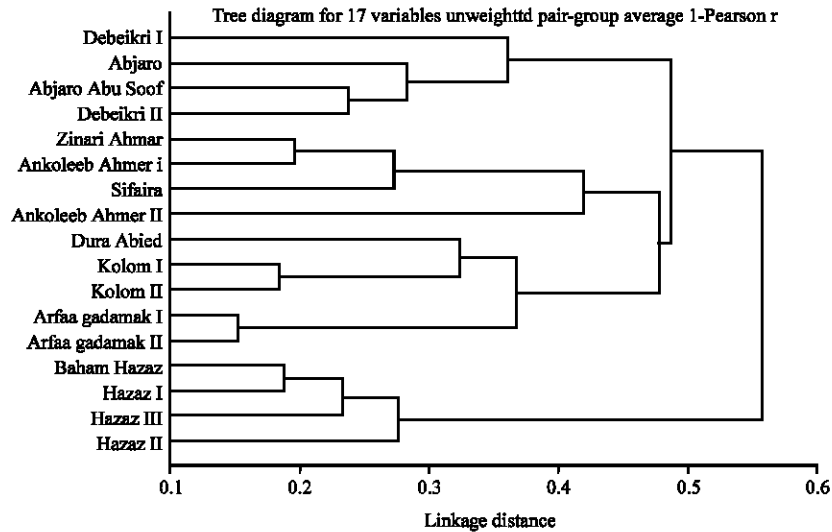

Fig. 1: Tree diagram constructed for 17 sorghum accessions based on their genetic distances using 10 RAPD primers

from El-Nikhala area and Abjaro in same group but Abjaro more genetically related than Debeikri from El-Nikhala area. North Kordofan group contained Zinari Ahmer and Ankoleeb Ahmer I as sisters, within the same group Sifaira closer to the sisters than Ankoleeb Ahmer II. Blue Nile group have two sisters (Kolom from Mengenza area, Kolom from Jebel El Nimir) and (Arfaa gadamak I, Arfaa gadamak II) beside Dura Abied. The second cluster have Red Sea group alone, accessions Baham Hazaz, Hazaz I appeared as sister where Hazaz III and Hazaz II were within the same group, with Hazaz II more genetically distant (Fig. 1).

Six out of the 17 sorghum accessions had unique bands that distinguished them from the others and could be useful as fingerprints. Kolom II had unique fragment that appear with the C-20, Ankoleeb Ahmer II accession had a unique fragment with UBC-101; Dura Abied accession had specific fragment with UBC-103; Baham Hazaz accession had a specific fragment with primer UBC-127; Abjaro Abu Soof accession had specific fragments with primer UBC-155 and Abjaro

Table 3: Distance matrix among the 17 sorghum accessions studied

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	0.00																
2	5.74	0.00															
3	5.92	4.47	0.00														
4	5.66	5.00	4.36	0.00													
5	7.28	6.32	6.16	6.08	0.00												
6	7.14	6.00	6.00	5.57	4.00	0.00											
7	6.71	6.00	5.48	5.57	4.69	4.69	0.00										
8	7.62	6.56	6.40	6.00	5.92	5.92	5.57	0.00									
9	6.71	6.63	6.93	6.08	5.83	5.48	6.00	6.40	0.00								
10	6.16	7.00	6.71	5.83	6.86	6.56	6.40	6.48	5.00	0.00							
11	6.24	6.93	6.63	5.57	6.63	6.63	6.32	6.56	5.29	3.87	0.00						
12	6.16	6.08	5.92	5.66	6.08	5.74	5.92	6.63	5.39	5.48	5.00	0.00					
13	6.56	6.32	6.32	6.08	6.16	6.00	6.32	6.24	5.83	5.92	5.66	3.61	0.00				
14	7.48	6.56	7.14	6.93	6.71	6.40	6.86	6.63	6.08	6.78	6.86	5.66	5.57	0.00			
15	7.28	6.63	7.35	7.14	6.78	6.78	6.63	6.71	6.16	6.86	7.07	5.74	5.66	3.87	0.00		
16	8.06	7.21	7.62	7.28	6.93	6.48	6.63	6.71	6.16	6.86	6.93	6.08	6.16	4.58	4.69	0.00	
17	7.00	6.93	7.21	7.14	6.93	6.93	6.78	6.86	6.63	6.56	6.93	5.92	6.16	4.36	4.24	4.69	0.00

Where; 1: Debeikri I, 2: Abjaro, 3: Abjaro Abu Soof, 4: Debeikri II, 5: Zinari Ahmer, 6: Ankoleeb Ahmer I, 7: Sifaira, 8: Ankoleeb Ahmer II, 9: Dura Abied, 10: Kolom I, 11: Kolom II, 12: Arfaa gadamak I, 13: Arfaa gadamak II, 14: Baham Hazaz, 15: Hazaz I, 16: Hazaz II and 17: Hazaz III

accession had specific fragment with primer UBC-157. Accessions Arfaa gadamak I and Arfaa gadamak II from the Blue Nile region were the closest to each other, where the genetic distance matrix between them was (3.61), according to the genetic distance matrix shown in Table 3. Whereas, accessions Debeikri I and Hazaz II were the most distant among all (8.06).

DISCUSSION

In this study, the average number of amplification products produced per primer was 11 which is higher than finding in earlier reports (Iqbal *et al.*, 2010; Amrapali *et al.*, 2008; Mohamed *et al.*, 2008). Accessions from Red sea region formed a distinct cluster by itself. According to Kachapur *et al.* (2009), it may be due to the presence of unique alleles inside those accessions and such alleles are important because, they may be diagnostic for particular regions with a genome specific to a particular type of sorghum. However, using these primers, access on specific DNA fingerprints were found for six accessions out of 17 studied. This will be of high value for sorghum breeders working in the improvement of the crop. Also, the primers (C-20, UBC-101, UBC-103, UBC-127, UBC-155 and UBC-157) that showed specific fingerprints can be used in between to screen longer spectrum of Sudanese sorghum accessions. An interesting finding from this study, are those accessions having the same name as classified earlier based on their morphological characteristics, appeared to be genetically different. This is clear for accessions Debeikri I and Debeikri II, although they clustered within one cluster, there were considerable genetic distance among them (Fig. 1, Table 3), the same was for Ankoleeb Ahmer I and Ankoleeb Ahmer II, although from the same region. Hazaz I, Hazaz II and Hazaz III similarly. Our results are in agreement with reports of Ayana *et al.* (2000) and Iqbal *et al.* (2010) who observed no genetic relationship with quantitative agro-morphological traits which shows no correlation between molecular markers and morphological traits. Ezzat *et al.* (2010) mentioned that different weather

patterns, soil type and the location might cause agronomic differences among genotypes. Likewise, an insufficient relationship between RAPD markers and agronomic descriptors was also reported by Dahlberg *et al.* (2002). Different environmental conditions might affect the phenotypic traits, leading to clear differences even among identical genotypes (Shehzad *et al.*, 2009). So, the indigenous or local names given by farmers of a region cannot be considered a good guide to the presence of diversity (Chakauya *et al.*, 2006).

Agrama and Tuinstra (2003) reported that despite the sorghum is predominantly a self pollinated cultivars it has unusual amount of genetic diversity. Minkir *et al.* (1997) also concluded that the multiple origin theory of sorghum is also considered to be a factor contributing to the extensive genetic diversity observed in grain sorghum.

It may be suggested that RAPD marker have important implications for accessions identification and determination of hybridity and phylogenetic diversity of sorghum because of a requirement of simple agarose gel electrophoresis. Eventually, our study agrees with previous studies that RAPD analysis can be utilized with confidence in such applications (Udupa *et al.*, 1998; Agrama and Tuinstra, 2003). So, it is concluded that RAPD technique proved to be useful to study genetic variation among the Sudanese sorghum accessions.

REFERENCES

Agrama, H.A. and M.R. Tuinstra, 2003. Phylogenetic diversity and relationships among sorghum accessions using SSRs and RAPDs. Afr. J. Biotechnol., 2: 334-340.

Amrapali, A.A., S.B. Sakhare, P.L. Kulwal and D.B. Dhumale, 2008. RAPD profile studies in sorghum for identification of hybrids and their parents. Inter. J. Integ. Bio., 3: 18-24.

Ayana, A., E. Bekele and T. Bryngelsson, 2000. Genetic variation in wild sorghum (*Sorghum bicolor* ssp. *verticilliflorum* (L.) Moench) germplasm from Ethiopia assessed by random amplified polymorphic DNA (RAPD) Hereditas, 132: 249-254.

Bandyopadhyay, T., 2011. Molecular marker technology in genetic improvement of tea. Int. J. Plant Breed. Genet., 5: 23-33.

Bethel, C.M., E.B. Sciara, J.C. Estill, J.E. Bowers, W. Hanna and A.H. Paterson, 2006. A framework linkage map of bermudagrass (*Cynodon dactylon x transvaalensis*) based on single-dose restriction fragments. Theor. Applied Genet., 112: 727-737.

Bowers, J.E., C. Abbey and S. Anderson, 2003. A high-density genetic recombination map of sequence-tagged sites for Sorghum, as a framework for comparative structural and evolutionary genomics of tropical grains and grasses. Genetics, 165: 367-386.

Chakauya, E., P. Tongoona, E.A. Matibiri and M. Grum, 2006. Genetic diversity assessment of sorghum landraces in Zimbabwe using microsatellites and indigenous local names. Int. J. Bot., 2: 29-35.

Curtis, D.L., 1968. The relationship between the date of heading of Nigerian sorghums and the duration of growing season. J. Applied Ecol., 5: 215-226.

Dahlberg, J.A., X. Zhang, G.E. Hart and J.E. Mullet, 2002. Comparative assessment of variation among sorghum germplasm accessions using seed morphology and RAPD measurements. Crop Sci., 42: 291-296.

Dawelbeit, S.E., F.M. Salih, O.A. Dahab and E.H. Ahmed, 2010. Status of fertilization and crop nutrition in irrigated agriculture in Sudan 2: Main crops consuming fertilizers and the role of education in optimizing fertilizer use. Research Findings: e-ifc No. 23. http://www.ipipotash.org/en/eifc/2010/23/2

Dufour, P., M. Deu, L. Grivet, A. D'Hont and F. Paulet *et al.*, 1997. Construction of a composite sorghum genome map and comparison with sugarcane, a related complex polyploid. Theor. Applied Gen., 94: 409-418.

El-Amin, H.K.A., N.B. Hamza and A.I. Abuali, 2011. Molecular and agronomical assessment of six wheat (*Triticum aestivum* L.) cultivars under salt-stress conditions. Int. J. Agric. Res., 6: 163-171.

Ezzat, E.M., M.A. Ali and A.M. Mahmoud, 2010. Agronomic performance, genotype X environment interactions and stability analysis of grain sorghum (*Sorghum bicolor* L. Moench). Asian J. Crop Sci., 2: 250-260.

Fahima, T., G. Sun, A. Beharav, T. Krugman, A. Beiles and E. Nevo, 1999. RAPD polymorphism of wild emmer wheat populations, *Triticum dicoccoides*, in Israel. Theor. Applied Genet., 98: 434-447.

Fang, D.Q. and M.L. Roose, 1997. Identification of closely relative citrus cultivars with inter simple sequence repeat markers. Theor. Applied Genet., 95: 408-417.

Gepts, P., 1993. The Use of Molecular and Biochemical Markers in Crop Evolution Studies. In: Evolutionary Biology, Hecht, M.K. (Edn.). Plenum Press, New York, pp: 51-94.

Iqbal, A., B. Sadia, A.I. Khan, F.S. Awan, R.A. Kainth and H.A. Sadaqat, 2010. Biodiversity in the sorghum (*Sorghum bicolor* L. Moench) germplasm of Pakistan. Genet. Mol. Res., 9: 756-764.

Jessup, R.W., B.L. Burson, Y.W. Wang, C. Chang and Z. Li *et al.*, 2003. Segmental allotetraploidy and allelic interactions in buffelgrass (*Pennisetum ciliare* (L.) Link syn. *Cenchrus ciliaris* L.) as revealed by genome mapping. Genome, 46: 304-313.

Kachapur, R.M., P.M. Salimath and B.V.S. Reddy, 2009. Genetic diversity within sweet sorghum (*Sorghum bicolor* (L.) Monech) accessions as revealed by RAPD markers. J. Maharashtra Agric. Univ., 34: 38-42.

Ming, R., S.C. Liu, Y.R. Lin, J. da Silva and W. Wilson *et al.*, 1998. Detailed alignment of saccharum and sorghum chromosomes: Comparative organization of closely related diploid and polyploid genomes. Genetics, 150: 1663-1682.

Minkir, A., P. Goldsbrough and G. Ejeta, 1997. RAPD based assessment of Genetic diversity in cultivated races of sorghum. Crop Sci., 37: 564-569.

Missaoui, A.M., A.H. Paterson and J.H. Bouton, 2005. Investigation of genomic organization in switchgrass (*Panicum virgatum* L.) using DNA markers. Theor. Applied Genet., 110: 1372-1383.

Mohamed, E.A., S.S. Youssef, E.E.M. Selim and M.M. Ghonaim, 2008. Genetic diversity among *Sorghum bicolor* genotypes using Simple Sequence Repeats (SSRs) markers. Arab. J. Biotechnol., 11: 181-192.

Nagaoka, T. and Y. Ogihara, 1997. Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theor. Applied Genet., 94: 597-602.

Paterson, A.H., Y.R. Lin and Z. Li, 1995. Convergent domestication of cereal crops by independent mutations at corresponding genetic loci. Science, 269: 1714-1718.

Paterson, A.H., J.E. Bowers and B.A. Chapman, 2004. Ancient polyploidization predating divergence of the cereals and its consequences for comparative genomics. Proc. Natl. Acad. Sci. USA., 101: 9903-9908.

Paterson, H.A., 2008. Genomics of sorghum. Int. J. Plant Genomics, 10.1155/2008/362451.

Patra, A.P., A.K. Mukherjee and L. Acharya, 2011. Comparative study of RAPD and ISSR markers to assess the genetic diversity of betel vine (*Piper betle* L.) in Orissa, India. Am. J. Biochem. Mol. Biol., 1: 200-211.

Porebski, S., L.G. Bailey and B.R. Baum, 1997. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant Mol. Biol. Rep., 15: 8-15.

Pu, Z.E. Y.C. Hou, X.X. Xu, Z.H. Yan, Y.M. Wei, X.J. Lan and Y.L. Zheng, 2009. Genetic diversity among barley populations from West China based on RAMP and RAPD markers. Asian J. Plant Sci., 8: 111-119.

Sambrook, J., E.F. Fritsch and T. Maniatis, 1989. Molecular Cloning: A Laboratory Manual. 2nd Edn., Cold Spring Habour Laboratory Press, New York, USA., ISBN-13: 978-0879693091, pp: 1659.

Shehzad, T., H. Okuizumi, M. Kawase and K. Okuno, 2009. Development of SSR-based sorghum (*Sorghum bicolor* L. Moench) diversity research set of germplasm and its evaluation by morphological traits. Genet. Res. Crop Evol., 56: 809-827.

Shiringan, A.L., 2009. Identification of genomic regions of *Sorghum bicolor* (L.) Moench linked to biofuel-related traits in grain x sweet sorghum recombinant inbred lines. Ph.D. Thesis, Faculty of Agricultural Sciences, Nutritional Sciences and Environmental Management, Justus Liebig University, Giessen, Germany.

Smith, J.S.C. and O.S. Smith, 1992. Fingerprinting crop varieties. Adv. Agron., 47: 85-140.

Soller, M. and J.S. Beckmann, 1983. Genetic polymorphism in varietal identification and genetic improvement. Theor. Applied Genet., 67: 25-33.

Subudhi, P.K. and N. Huang, 1999. RAPD mapping in a doubled haploid population of rice (*Oryza sativa* L.). Hereditas, 130: 41-49.

Thangaraj, M., V. Prem, T. Ramesh and A.P. Lipton, 2011. RAPD fingerprinting and demonstration of genetic variation in three pathogens isolated from mangrove environment. Asian J. Biotechnol., 3: 269-274.

Udupa, S.M., F. Weigand, M.C. Saxena and G. Kahl, 1998. Genotyping with RAPD and microsatellite markers resolves pathotype diversity in the ascochyta blight pathogen of chickpea. Theor. Applied Genet., 97: 299-307.

Whitkus, R., J. Doebley and M. Lee, 1992. Comparative genome mapping of sorghum and maize. Genetics, 132: 1119-1130.

Younis, R.A.A., M.F. Ahmed and M.M. El-Menshawy, 2007. Molecular genetic markers associated with salt tolerance in grain sorghum. Arab J. Biotechnol., 10: 249-258.