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Assessment of Genetic Variability in Sorghum Accessions (*Sorghum bicolor* L. Moench) at the National Centre for Genetic Resources and Biotechnology, Ibadan, Nigeria

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ABSTRACT: The determination of genetic variation using molecular markers has been found to facilitate the conservation of crops and ensure food security. Genetic diversity among 80 accessions of *S. bicolor* in the gene bank of National Centre for Genetic Resources and Biotechnology (NACGRAB) Ibadan, was studied using 5 pairs of simple sequence repeat (SSR) markers. The polymorphic information content (PIC) of individual primer ranged from 0.34 to 0.70 with a mean value of 0.54 indicating enough diversity or variability among the accessions studied. The binary matrix obtained from the gel profiles generated a dendrogram which was made up of 4 clusters and one ungrouped accession at 0.66 coefficients of similarity. From the clustering pattern, 7 pairs of accessions were found to be 100% similar. Each similar pairs were subsequently merged together and reduced to a total of 7 accessions. However, it was also observed that the geographical location of collection of accessions did not affect the clustering pattern. The information obtained from this study could serve as the basis for the improvement and breeding programs of *Sorghum* to achieve food security in the country, and by extension, worldwide. © JASEM

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***Keywords*:** Sorghum; Simple Sequence Repeat markers; Genetic variation; Polymorphic Information Content; Coefficient of similarity.

Sorghum bicolor L. Moench, commonly called Guinea corn is one of the most important staple food crops in Nigeria especially in Northern states that covers the guinea savannah ecological zone (FAO, 2004). It is a tropical plant belonging to the family Poaceae. Its usage as staple food is not limited to Nigeria alone as it is also a food crop of millions of the poor in semi-arid tropics of Africa, Asia and Latin America and it is the fifth in acreage among the world cereals (Anglani, 1998). *Sorghum* includes the cultivated grain races, and is a diploid, highly self-pollinated plant. It also possesses considerable diversity in morphological and agronomic traits, such as adaptive pest resistance (El-Awady *et al*., 2008). Upon this information, El-Awady *et al*., (2008) stated that the wide range of genetic diversity of *Sorghum* could be exploited as a possibility of improving its productivity as well as studying its diversity.

Many studies have been devoted to assessing the patterns of *Sorghum* genetic variation based on morphology (Djè *et al*., 1998) or pedigree (Jordan *et al*., 1998). However, Smith *et al*., (2000) reported that phenotypic variation does not reliably reflect genetic variation because of the role of environmental interaction in determining the phenotype. To enhance the breeding programs of any crop plant, the usage of modern techniques such as molecular markers is required. In recent years, the number of molecular assays available for application in area of breeding has increased dramatically, with each method differing in principles, applications, type and amount of polymorphism detected, as well as cost and time requirements (Karp *et al*., 1996). Some of these DNA-based techniques include Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeat (SSR), Amplified Fragment Length Polymorphism (AFLP) etc (Botstein *et al*., 1980).

According to Gupta and Varshney (2000), among these techniques, SSR (also known as microsatellites) represents an ideal marker system. This is due to its codominant inheritance, multi-allelic characters, occurrence in high frequency, locus specificity and distribution throughout the genomes of all higher plants and animals. Rakshit *et al*. (2012a) also stated that SSRs are most commonly used among different DNA markers because they are hypervariable, robust, chromosome specific and has widely been used for assessment of diversity in several cultivated crop species including *Sorghum*. Brown *et al*. (1996) had earlier reported that SSR also displays a high level of polymorphism, even among closely related accessions, and readily responds to simple and inexpensive polymerase chain reaction (PCR) assays. Therefore, SSR have been established as useful genetic markers in many plant species (Cregan *et al*., 1999) and in genetic mapping initiatives for cereals including *Sorghum* (Smith *et al*., 2000).

It is against this backdrop that this project was aimed at characterizing through SSR primers with a view to assessing the genetic diversity within and among clusters of accessions of *Sorghum* germplasm collected

across Nigeria and conserved at the National Centre for Genetic Resources and Biotechnology, Ibadan, Oyo State, Nigeria.

MATERIALS AND METHODS

Eighty (80) sorghum accessions used for this study were collected from gene bank of National Centre for Genetic Resources and Biotechnology (NACGRAB) Moor Plantation Ibadan (Table 1). The accession numbers were well written out on each accession, and this was carefully followed during analysis to avoid any mix-up.

*Deoxyribonucleic acid (DNA) Extraction***:** Total genomic DNA was extracted from the dry seeds of sorghum accessions following a modified Cetyltrimethyl Ammonium Bromide (CTAB) protocol of Doyle and Doyle (1990). The extracted DNA concentration and purity level was estimated through Nanodrop spectrophotometer (Thermo Scientific NanoDrop™ 2000) and on 1% agarose gel electrophoresis.

Polymerase Chain Reaction (PCR) Amplification: Five primer pairs of Microsatellite markers were used (Table 2) to amplify the genomic DNA of all accessions. The polymerase chain reaction (PCR) was performed in a 13µl mixture containing 1 µl of template DNA, 5µl of ready to use master mix, 5µl of double distilled water, 1µl forward primer and 1µl reverse primer. Amplification was performed on the thermal cycler (Eppendorf AG Mastercycler Nexus Gradient, 22331, Hamburg) using initial denaturation temperature of 94 ºC for 1min; primer annealing temperature 51ºc for 1min; extension 72 ºC for 20 s; and a final extension at 72 ºC for 10 mins.

Thereafter, the amplicon were checked on 1.5% agarose gel electrophoresis, viewed and photographed using Gel Documentation and Analysis Systems (UVdoc, GA-9000/9010 Version 12).

Data Analysis: Genetic diversity was estimated by scoring distinct and clear DNA bands on the photographs of gel profile (Figure 1) obtained from electrophoresis, to generate binary data tables allocating a 1 point where the band is present and 0 point when the band is absent. The scores were then exported to the NTSYS-pc 2.02j software package (Rohlf, 1996). The dendrogram generated from the matrix grouped the test lines using Unweighted Pair Group Method with Mathematic Average (UPGMA) on the basis of genetic similarity and Jaccard's coefficient. Data analysis to estimate the genetic distance among accessions was achieved by cluster analysis, which is an example of multivariate method, as described by Mohammadi and Prasanna (2003). So also the polymorphic information content (PIC) was calculated for individual primer using GenStat Discovery Edition 4 software

RESULTS AND DISCUSSION

The PIC values for individual primer ranged from 0.34 to 0.70 with a mean value of 0.54 (Table 3). An estimation of genetic coefficients of similarity, with values ranging from 0.5 to 0.94 revealed a range of variability in this collection of sorghum accessions. The dendrogram generated from the gel profiles revealed that at a coefficient of similarity of 0.66 (66%), four clusters and one ungrouped (U) accession were distinguishable (Figure 2).

Table 1: The accession number and location of collection of sample as documented in the NACGRAB Gene Bank.

S/N	ACCESSION NO.	LOCATION/ STATE
1	NG/SA /0095	Owena / ONDO
2	NG/SA /0093	Olode garage/ OSUN
3	NG/SA /0016	Olode garage/ OSUN
4	NG/SA /0098	Nuhubamali/ KADUNA
5	NG/SA /0071	Ife garage / OSUN
6	NG/SA /0083	Epini Akoko farm / ONDO
7	NG/AU/001	Ogbose/ ONDO
8	NG/AA/007	Modakeke / OSUN
9	NG/AA/010	Ede / OSUN
10	NG/AO /0001	Nuhubamali/ KADUNA
11	NG/AO/0004	Kangara town/ NIGER
12	NG/AA/0009	Ejigbo/ OSUN
13	NG/SA /0069	Olode garage/ OSUN
14	NG/AU/004	Ogbose/ ONDO
15	NG/SA /0074	Ife garage isale / OSUN
16	NG/AO/119	Owena / ONDO
17	NG/TO/005	Olode egarage/ OSUN
18	NG/SA /088	Olode garage/ OSUN
19	NG/AA/039	Ife garage isale / OSUN
20	NG/AO/113	Epini akoko farm l/ ONDO
21	NG/SA /0143	Owena / ONDO
22	NG/AU/003	Ogbose/ ONDO
23	NG/SA/0204	Owena / ONDO
24	NG/AA/158	Atakumosa / OSUN
25	NG/AO/0030	Gargaurawa / JIGAWA
26	NG/SA/0228	kwali area/ FCT
27	NG/AA/0011	Ile Ife / OSUN
28	NG/SA/078	Ile Ife / OSUN
29	NG/AA /159	Atakumosa / OSUN
30	NG/AO/0001	Ijaye / OYO
31	NG/AO/0001	Oja okoru / ONDO
32	NG/AU/002	Ogbose/ ONDO
33	NG/EO/013	Mokowa/ NIGER
34	NG/AA/163	Igede Ekiti/ EKITI
35	NG/OE/0002	Omi Adio/ OYO
36	NG/AT/020	Olodo/ OGUN
37	NG/SA/0077	Gargaurawat/ JIGAWA
38	NG/SA/0083	NA
39	NG/OE/001	Omi Adio / OYO
40	NG/AO/0015	NA
41	NG/AA/161	Oja Oba Ado/ EKITI
42	NG/OJ/009	Gargaurawa / JIGAWA
43	NG/SA/0081	Kwali area/ FCT
44	NG/AA/ 035	Ife Garage Isale / OSUN
45	NG/AA/ 0036	Ife Oja Titun / OSUN
46	NG/TO/ 008	Omi Adio / OYO
47	NG/AO/ 0114	NA
48	NG/SA/ 0083	Zuba Vill / FCT
49	NG/SA/ 0137	Zuba Vill/ FCT
50	NG/SA/ 0109	Dankaku Vill/ KANO
51	NGB/06/ 016	Dankaku Vill/ KANO
52	NG/SA/ 0185	Gargaurawa/ JIGAWA
53	NG/SA/ 0236	Kwali Area/ FCT
54	NG/SA/ 0130	Dankaku Vill/ KANO
55	NG/SA/ 0168	Danladi G Vill/ BAUCHI
56	NG/SA/ 0093	Danladi G Vill/ BAUCHI
57	NGB/06/ 015	Ife Oja Titun / OSUN
58	NG/SA/ 0016	Omi Adio / OYO
59	NGB/06/ 015	N/A
60	NG/SA/ 0183	Gwagwalada/FCT
61	NG/SA/ 0224	Gwagwalada/FCT
62	NG/SA/ 0144	Tsaida Vill/KANO
63	NG/SA/ 0055	Mokwa Kad Rd/ NIGER
64	NG/SA/ 0275	Okene/ KOGI
65	NG/SA/ 0213	Kasaranmi Vill/ KADUNA
66	NG/SA/ 0240	Oka Akoko/ ONDO
67	NG/SA/ 0218	Zuba Vill/ FCT
68	NG/SA/ 0137	Gadadi Vill, Duste/ JIGAWA
69	NG/SA/ 0025	Mile 5 Ncri/ MOKWA
70	NG/SA/ 0040	Zuba Vill/ FCT
71	NG/SA/ 0071	Babalomo/ MOKWA
72	NG/SA/ 0063	Mokwa/ NIGER
73	NG/SA/ 0126	Wasai Minijibri/ NIGER
74	NG/SA/ 0095	Nuhubamali/ KADUNA
75	NG/SA/ 0047	Kangara Town/ NIGER
76	NG/SA/ 0098	Nuhubamli/ KADUNA
77	NG/SA/ 0096	Kangara Town/ NIGER
78	NGB/06/ 020	Kangara Town/ NIGER
79	NG/SA/ 0004	Fasola Settlement/ OYO
80	NG/SA/ 10094	Mokwa/ NIGER

Cluster 1 has the highest number of accessions of 35 while cluster 2 has the lowest with 7 accessions. Clusters 3 and 4 have 30 and 8 accessions respectively. From the clustering pattern also, 14 accessions were found to be 100% similar. They are 23 and 24; 63 and 67; 51 and 52; 71 and 78; 76 and 80; 72 and 74 and, 41 and 42. However, it was also observed that the geographical location of collection of accessions did not affect the clustering pattern. This is evident as accessions from the same geographical area did not necessarily cluster together.

Table 2: List of SSR Primers for *Sorghum* amplification

S/N	Primer I.D	Sequence	Molecular Weight	Tm (Min/Max)
1	A	**F-**GACAACTGTGTGGACCGATG	500.23	58.35/58.35
		R-CAGGGCTTTGAACCCAAATA	374.37	58.35/58.35
2	B	**F**-GCTGCGGAATCTTCTACTGG	357.74	62.45/62.45
		R-CAGGGCTTTGAACCCAAATA	461.69	58.35/58.35
3	C	**F**-GTGGACCGATGGGCTTACTA	615.24	62.45/62.45
		R-CAGGGCTTTGAACCCAAATA	363.96	58.35/58.35
4	D	**F-**GACAACTGTGTGGACCGATG	342.14	62.45/62.45
		R-GGGCTTTGAACCCAAATACA	478.74	58.35/58.35
5	E	**F**-CGATGGGCTTACTAGCCTTG	400.32	62.45/62.45
		R-CAGGGCTTTGAACCCAAATA	500.23	58.35/58.35

Legend: Tm = melting temperature (minimum/maximum) of the primer

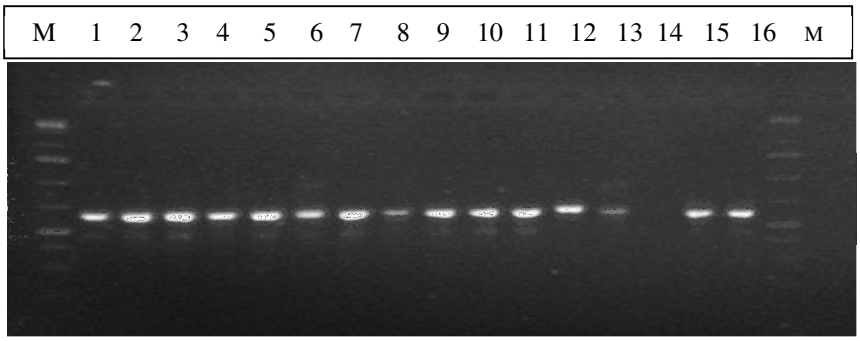

Fig 1: Gel profile of amplicon produced by primer pair A for 16 samples on 1.5 % Agarose gel where M = 100bp Marker; 1 – 16 are bands produced by amplicon from 16 samples. **NB**: The comb used can only take maximum of 16 samples at a time.

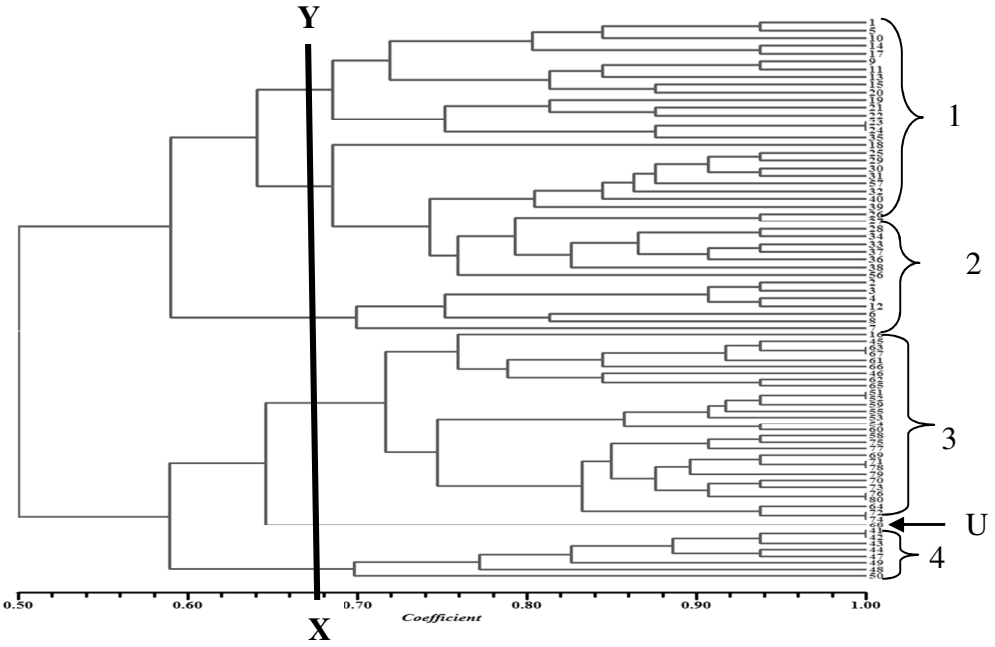

Fig 1: A UPGMA Dendrogram Showing the clustering pattern obtained from SSR primers among the 80 accessions of *Sorghum* studied.

Legend: XY represents truncated line at a similarity co-efficient of 66%; 1 to 4 represents the four clusters and one ungrouped **(U)** that were distinguishable among the accessions at 66% co-efficient of similarity**.**

Molecular markers have been used because of their ability to give a clearer and better picture of genetic diversity, though a combination of molecular markers and phenotypic analyses have proved to be a more powerful tool in explaining genetic diversity and relationships among sorghum accessions (Burow *et al*., 2012). SSR marker is said to be the most variable component of the genome with a high rate of molecular evolution, and as such has been used in characterization of many organisms especially eukaryotes (El-Awady *et al*., 2008). Qureshi *et al*. (2004) had stated that slippage of the DNA polymerase that occurred during replication of unequal crossing over resulting in differences in the copy number of the core nucleotide sequence is believed to be responsible for the microsatellite variation.

The 80 sorghum accessions evaluated in this study were uniquely differentiated using the 5 pairs of SSR markers. The average PIC value of 0.54 for all the primers showed that they are polymorphic though with different levels of polymorphism, thus revealing a high level of diversity among the accessions studied. Previous studies have equally come up with findings in support of wide genetic variation in collections of indigenous sorghum as evident from this study. For instance, Folketsma *et al.* (2005) reported large genetic diversity as well as predominance of rare alleles among guinea race of sorghum collected from various parts of the world. Barnaud *et al.* (2007) had also documented the existence of high genetic variation in sorghum landraces from Burkina Faso and Cameroon respectively, even in areas of relatively small sorghum cultivation. However, moderate diversity was found by Menz *et al*. (2004) among the sorghum inbreds developed in the US, and also Burow *et al*. (2012), who worked on collection of Chinese sorghum landraces.

The fourteen accessions found to be 100% similar were subsequently merged together and reduced to 7 accessions. This result is in agreement with studies in other species such as soybeans, barley and corn (Powell *et al*., 1996; Wu and Tanksley, 1993).

Table 3: The polymorphic information content (PIC) of individual markers used to assess the diversity of 80 sorghum accessions

SSR Marker	Major Allele Freq.	Genotype No	No. of obs.	Allele No	Availa-bility	Gene Diversity	Hetero-zygosity	PIC
A	0.40	8.00	75.00	4.00	0.94	0.69	0.72	0.63
B	0.67	3.00	62.00	2.00	0.78	0.44	0.05	0.34
C	0.42	9.00	58.00	5.00	0.73	0.71	0.76	0.67
D	0.38	5.00	69.00	3.00	0.86	0.66	0.46	0.59
E	0.51	4.00	71.00	3.00	0.89	0.54	0.55	0.44
Mean	0.48	5.80	67.00	3.40	0.84	0.61	0.51	0.54

The clustering pattern was not affected by the location where samples were collected. This is evident because collections made from the same geo-political zone did not all occur in the same cluster; rather they are separated out in different clusters. This is in agreement with the findings of Singh *et al*., (2006).

The vast gene pool of Sorghum collection in NACGRAB has served several researchers and students from Research Institutes and tertiary institutions either for research and development or during their final year undergraduate projects respectively. The accessions that paired together at 1.0 coefficient of similarity could be having the same genotypes based on their 100 % similarity. Further studies need to be carried out on them to ascertain their true genotypes especially those that will combine their morphological and molecular data. Until that is done, the accessions may not be supplied to researchers and project students alike as variants or as the same unit.

*Conclusion***:** The SSR marker as used in this study was able to elucidate the genetic variation among the different sorghum accessions studied effectively. Insight into phylogenetic relationships among varieties and species of *Sorghum* could therefore be provided by the distribution and sequence of SSR markers. The information thereby obtained could be used in germplasm conservation and preservation for sustainable use of sorghum genotypes. It could also serve as baseline knowledge in *Sorghum* breeding works for crop improvement in Nigeria.

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