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 https://dx.doi.org/10.4314/jasem.v21i6.25

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

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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 (NACCRAB) 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 amplicons 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 from electrophoresis, to generate binary data tables allocating a 1 point when 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 accessions into distinct and clear DNA bands on the photographs of gel profile (Figure 1). The dendrogram revealed that at a coefficient of similarity level was estimated through Nanodrop spectrophotometer (Thermo Scientific NanoDrop™ 2000) and on 1% agarose gel electrophoresis.

RESULTS AND DISCUSSION

| S/N | ACCESION NO. | LOCATION/ STATE |
|-----|--------------|----------------|
| 1   | NGSA/0095    | Osuna / Ondo   |
| 2   | NGSA/0094    | Osuna / Ondo   |
| 3   | NGSA/0093    | Osuna / Ondo   |
| 4   | NGSA/0098    | Osuna / Ondo   |
| 5   | NGSA/0071    | Osuna / Ondo   |
| 6   | NGSA/0083    | Osuna / Ondo   |
| 7   | NGAA/0001    | Osuna / Ondo   |
| 8   | NGAA/0007    | Osuna / Ondo   |
| 9   | NGAA/0010    | Osuna / Ondo   |
| 10  | NGAO/0001    | Osuna / Ondo   |
| 11  | NGAO/0004    | Osuna / Ondo   |
| 12  | NGAO/0009    | Osuna / Ondo   |
| 13  | NGSA/0069    | Osuna / Ondo   |
| 14  | NGAA/0004    | Osuna / Ondo   |
| 15  | NGAA/0014    | Osuna / Ondo   |
| 16  | NGAO/0019    | Osuna / Ondo   |
| 17  | NGAO/0005    | Osuna / Ondo   |
| 18  | NGAO/0014    | Osuna / Ondo   |
| 19  | NGAA/0009    | Osuna / Ondo   |
| 20  | NGAA/0013    | Osuna / Ondo   |
| 21  | NGAA/0015    | Osuna / Ondo   |
| 22  | NGAO/0001    | Osuna / Ondo   |
| 23  | NGAO/0003    | Osuna / Ondo   |
| 24  | NGAA/0015    | Osuna / Ondo   |
| 25  | NGAA/0003    | Osuna / Ondo   |
| 26  | NGAO/0022    | Osuna / Ondo   |
| 27  | NGAA/0001    | Osuna / Ondo   |
| 28  | NGAO/0009    | Osuna / Ondo   |
| 29  | NGAA/0009    | Osuna / Ondo   |
| 30  | NGAA/0008    | Osuna / Ondo   |
| 31  | NGAA/0007    | Osuna / Ondo   |
| 32  | NGAA/0006    | Osuna / Ondo   |
| 33  | NGAA/0005    | Osuna / Ondo   |
| 34  | NGAA/0004    | Osuna / Ondo   |
| 35  | NGAA/0003    | Osuna / Ondo   |
| 36  | NGAA/0002    | Osuna / Ondo   |
| 37  | NGAA/0014    | Osuna / Ondo   |
| 38  | NGAA/0015    | Osuna / Ondo   |
| 39  | NGAA/0016    | Osuna / Ondo   |
| 40  | NGAO/0015    | Osuna / Ondo   |
| 41  | NGAA/0016    | Osuna / Ondo   |
| 42  | NGAA/0009    | Osuna / Ondo   |
| 43  | NGAA/0008    | Osuna / Ondo   |
| 44  | NGAA/0007    | Osuna / Ondo   |
| 45  | NGAA/0006    | Osuna / Ondo   |
| 46  | NGAA/0005    | Osuna / Ondo   |
| 47  | NGAA/0004    | Osuna / Ondo   |
| 48  | NGAA/0003    | Osuna / Ondo   |
| 49  | NGAA/0002    | Osuna / Ondo   |
| 50  | NGAA/0001    | Osuna / Ondo   |
| 51  | NGAA/0010    | Osuna / Ondo   |
| 52  | NGAA/0009    | Osuna / Ondo   |
| 53  | NGAA/0008    | Osuna / Ondo   |
| 54  | NGAA/0007    | Osuna / Ondo   |
| 55  | NGAA/0006    | Osuna / Ondo   |
| 56  | NGAA/0005    | Osuna / Ondo   |
| 57  | NGAA/0004    | Osuna / Ondo   |
| 58  | NGAA/0003    | Osuna / Ondo   |
| 59  | NGAA/0002    | Osuna / Ondo   |
| 60  | NGAA/0001    | Osuna / Ondo   |
| 61  | NGAA/0000    | Osuna / Ondo   |
| 62  | NGAA/0009    | Osuna / Ondo   |
| 63  | NGAA/0008    | Osuna / Ondo   |
| 64  | NGAA/0007    | Osuna / Ondo   |
| 65  | NGAA/0006    | Osuna / Ondo   |
| 66  | NGAA/0005    | Osuna / Ondo   |
| 67  | NGAA/0004    | Osuna / Ondo   |
| 68  | NGAA/0003    | Osuna / Ondo   |
| 69  | NGAA/0002    | Osuna / Ondo   |
| 70  | NGAA/0001    | Osuna / Ondo   |
| 71  | NGAA/0000    | Osuna / Ondo   |
| 72  | NGAA/0009    | Osuna / Ondo   |
| 73  | NGAA/0008    | Osuna / Ondo   |
| 74  | NGAA/0007    | Osuna / Ondo   |
| 75  | NGAA/0006    | Osuna / Ondo   |
| 76  | NGAA/0005    | Osuna / Ondo   |
| 77  | NGAA/0004    | Osuna / Ondo   |
| 78  | NGAA/0003    | Osuna / Ondo   |
| 79  | NGAA/0002    | Osuna / Ondo   |
| 80  | NGAA/0001    | Osuna / Ondo   |

| TABLE 1: The accession number and location of collection of sample as documented in the NACCRAB Gene Bank. |
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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.

| S/N | Primer I.D | Sequence | Molecular Weight | Tm (Min/Max) |
|-----|------------|----------|-----------------|--------------|
| 1   | A          | F-GACAACTGTGGACCGATG | 500.23 | 58.35/58.35 |
|     |            | R-CAGGGCTTTGAACCCAAATA | 374.37 | 58.35/58.35 |
| 2   | B          | F-GCTGCGGAATCTTCTACTG | 357.74 | 62.45/62.45 |
|     |            | R-CAGGGCTTTGAACCCAAATA | 461.09 | 58.35/58.35 |
| 3   | C          | F-GTGGACCAGTGGGCTTACTA | 615.24 | 62.45/62.45 |
|     |            | R-CAGGGCTTTGAACCCAAATA | 363.96 | 58.35/58.35 |
| 4   | D          | F-GACAACTGTGGACCGATG | 342.14 | 62.45/62.45 |
|     |            | R-GGGCTTTGAACCCAAATA | 478.74 | 58.35/58.35 |
| 5   | E          | F-GTGGACCTGTGGGCTTACTA | 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.

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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).

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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