

Full Length Research Paper

Genetic variation within a collection of Nigerian accessions of African yam bean (*Sphenostylis stenocarpa*) revealed by RAPD primers

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African yam bean (*Sphenostylis stenocarpa*, Hochst. ex A. Rich, Harms) an indigenous food crop legume in tropical Africa, is highly under-exploited. Very little information is available on the nature and extent of genetic diversity of Nigerian accession of African yam bean (AYB) particularly using molecular markers. In this study, random amplified polymorphic DNA (RAPD) primers were used to assess genetic diversity in twenty-four accessions of Nigerian collection of AYB. Eleven random decamer primers were used for PCR amplification, but only nine RAPD primers that gave distinct bands were considered for analysis. A total of Fifty-three RAPD bands were generated by the nine RAPD primers and analyzed using Numeric Taxonomy System of Statistic (NTSYS). The similarity indices ranged from 0.42 to 0.96; 8 distinct DNA cluster groups were identified at 0.80 similarity indexes. Results showed a high genetic diversity among Nigerian accession of African yam bean. Such genetic diversity is useful in facilitating the development of large number of new varieties through hybridization, transfer of useful genes, thus maximizing the use of such available germplasm as genetic resource materials for breeders.

Key words: Cluster groups, conservation, genetic diversity, genetic resource, *Sphenostylis stenocarpa*.

INTRODUCTION

So little is known about the African yam bean (AYB, *Sphenostylis stenocarpa*, Hochst. ex A. Rich, Harms), though it is an important food crop in tropical Africa. Okigbo (1973) first introduced African yam bean at a grain legume improvement workshop held at IITA, Ibadan. AYB is classified as minor grain legumes because it is under-exploited (Saka et al., 2004). It is an indigenous legume, usually cultivated in association with yam, cassava, maize and sorghum and usually, the other crops (cassava, maize and sorghum) or the same stake for yam, serves as support for the crop (Togun et al.,

1997). AYB belongs to the family Fabaceae and order Fabales (USDA Plants database). It is common in central and western Africa, especially Cameroon, Cote D'Ivoire, Ghana, Nigeria, and Togo (Porter, 1992).

The crop is cultivated for both tuber and seeds in tropical Africa. It produces small tuber that looks like elongated sweet potatoes but tastes more like Irish potatoes. It produces good yields of edible fruits above ground. AYB is known in Nigeria as "girigiri". It was popular in the eastern Nigeria, where it is cultivated for foods and income generation. However, it is being neglected in most Nigerian homes for consumption because of long hours of cooking, 4 - 6 h, after soaking in water and tedious manual removal of the skin coat (Thomas et al., 2005). In Ghana, AYB is used extensively in various dietary preparations and has a potential for supplementing the protein requirements of many family throughout

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the year (Klu et al., 2001).

The chemical composition and nutritional values of African yam bean has been addressed previously. The crude protein level and quality of tuberous roots of some legumes determined by chemical methods showed that African yam bean have crude protein levels ranging from 21 to 29%, which is lower than soybean (38%), but the amino acid analysis indicated high level of methionine and lysine, equal to or better than those of soybean and corresponding to WHO/FAO recommendations (Evans et al., 1997). The proximate analysis determination of nutritionally valuable minerals and the functional properties of the seed flour of African yam bean, as investigated by Oshodi et al. (1997), showed the average composition of the whole seeds as follows: 20.50% protein, 8.25% fat, 59.72% total carbohydrate, 3.26% total ash and 8.10% moisture. The results of the same study revealed further that the whole seeds were rich in potassium (649.49 mg/100 g) and phosphorus (241.21 mg/100 g).

Entomological studies at IITA, Ibadan indicated that the pods and seeds of this edible legume may be resistant to the major pests of cowpea, specifically cowpea pod borer, *Maruca vitrata*, (Omitogun et al., 1995, unpublished) and cowpea weevil, *Callosobruchus maculatus*. Lectin, an albumin believed to be responsible for this resistance, was extracted from African yam bean and applied to cowpea leaves in high concentration showed a considerable level of resistance to cowpea pod borer while it induced high larval mortality rate in low dosages in cowpea weevil (Machuka et al., 2000).

Traditionally, genetic variability analysis in AYB has been done with easily distinguishable phenotypic traits. However, the limitations of morphological and biochemical markers for estimating genetic diversity in plants have been amply demonstrated. The use of molecular markers is considered best for analysis of genetic diversity and variety identification because there is no effect of stage of development, environment or management practices. Currently, the commonly used molecular markers are PCR based markers which include Random Amplified Polymorphic DNA, (RAPD, Williams et al., 1990) otherwise known as Arbitrarily Primed PCR (AP-PCR, Welsh et al., 1991), Amplified Fragment Length Polymorphism (AFLP) (Vos et al., 1995), and Microsatellites/Simple Sequence Repeat (SSR) markers (Tautz, 1989). Among these various classes of PCR-based molecular markers, RAPD marker is still widely used because of its speed, simplicity and amenability to automation, despite some limitations (Penner 1996). RAPD is a non-specific marker and therefore, it is easier to use for minor crops and under-exploited species, or for small research teams (Lanaud and Lebot, 1997).

RAPD analysis has been used extensively for identification and characterization of plant accessions (*Arachis* genus, Santos et al., 2003; *Cassava*, Tonukari et al., 1997; *Cowpea*, Mignouna et al., 1998; *Passion fruit*, Aukar et al., 2002; *Pawpaw*, Huang et al., 2000; *Plantain*,

Ude et al., 2003; *Potato*, Del Rio et al., 1997 and Ghislain et al., 1999; *Prunus rootstock*, Casas et al., 1999; *Rice*, Virk et al., 1995a; *Sorghum*, Nkongolo et al., 2003; *Sweet potato*, Gichuki et al., 2003; *Triticum aestivum*, Börner et al., 2000); genotype identification and genetic contamination (Barley, Poulsen et al., 1996; *Banana*, Damasco et al., 1996); duplicate and redundancy identification (*Perennial Kale*, Zeven et al., 1998; *Wheat*, Cao et al., 1998; *Rice*, Verma et al., 1999; Virk et al., 1995b); development of core collection (common bean, Skroch et al., 1998); and genetic mapping (*Cassava*, Akinbo et al., 2007; *Mustard*, Sharma et al., 2002; *Passion fruit*, Carneiro et al., 2002; *stevia*, Yao et al., 1999; and *Tribolium castaneum*, Beeman et al., 1999).

There is very little information available on the nature and extent of genetic diversity of Nigerian accession of African yam bean particularly that based on molecular markers. This information would be valuable for the rationalization of African yam bean germplasm conservation and the utilization of African yam bean germplasm in a breeding programme. Hence, the present study was conducted to understand the pattern of genetic variability in a few African yam bean accessions based on RAPD markers. While it is recognized that RAPD are inherently difficult to transfer between studies, and better markers exist for this type of analysis, RAPD was deemed adequate for this preliminary study.

MATERIALS AND METHODS

Plant material

Twenty-four accessions of African yam bean leaves were collected from Agronomy Department, University of Ibadan and kept at 4°C during transportation. The names of the cultivars and their sources are listed in Table 1. 5 g of each sample leaves was weighed, quickly ground in liquid Nitrogen, and stored at -80°C until needed.

DNA extraction and polymerase chain reaction amplification

Genomic DNA was extracted from ground leaf tissues, using a modified CTAB procedure as described by Doyle and Doyle (1987). DNA pellets obtained were re-dissolved in 400 µl of Tris-EDTA buffer (pH 8.0). Eleven random decamer primers gotten from Operon Technologies Inc. (Alameda, CA, USA) were used for the amplification of genomic DNA from the 24 samples of African yam bean used in this study. The primers' names and their sequences are listed in Table 2. The PCR reactions were carried out in 25 µl volumes in a mixture containing 100 mM Tris-HCl (pH 9.0), 50 mM KCl, 25 mM MgCl₂, 1% Triton X-100, 2.5 mM of each dNTP (Promega), 5 pM of random decamer primer, 25 ng of genomic DNA, and 2 unit of DNA Taq polymerase from Promega Corporation (Madison, WI, USA). The mixture was overlaid with 20 µl of sterile mineral oil. The PCR amplification was performed in a Perkin Elmer-Cetus DNA cycler model 960. For each amplification process, a preheating denaturation of DNA at 94°C for 3 min was followed by 45 cycles consisting of 1 min at 94°C, 1 min at 35°C (annealing), and 2 min at 72°C (extension). A final incubation for 7 min at 72°C was performed. The Amplified DNA products were separated on 1.4% agarose gel in Tris-borate buffer at 100 volts for 2 h. The DNA products in gel were visualized by staining 0.5 µg/ml

Table 1. Names and sources of the 24 Nigerian accessions of African yam bean used for the study.

Observation No.	Genotypes	Source	Observation No	Genotypes	Source
g1	AYB9501	Markurdi	g13	AYB9718	Onitsha
g2	AYB9502	Markurdi	g14	AYB9716	Onitsha
g3	AYB9503	Markurdi	g15	AYB9526	Ibadan
g4	AYB9623	Iseyin	g16	AYB9612	Ibadan
g5	AYB9514	Markurdi	g17	AYB9520	Markurdi
g6	AYB9608	Iseyin	g18	AYB9614	Markurdi
g7	AYB9512	Markurdi	g19	AYB9507	Iseyin
g8	AYB9613	Iseyin	g20	AYB9509	Markurdi
g9	AYB9602	Iseyin	g21	AYB9619	Iseyin
g10	AYB9711	Onitsha	g22	AYB9710	Onitsha
g11	AYB9706	Onitsha	g23	AYB9806	Umudike
g12	AYB9504	Markurdi	g24	AYB9815	Umudike

Table 2. Names and sequences of RAPD primers used for the African yam bean DNA amplification and number of alleles generated by each RAPD primer.

S/No	Primer name	Primer sequence 5' → 3'	No of RAPD alleles generated
1	OPA-12	TCGGCGATAG	10
2	OPB-09	TGGGGGACTC	8
3	OPD-08	GTGTGCCCCA	12
4	OPE-03	CCAGATGCAC	1
5	OPE-06	AAGACCCCTC	7
6	OPF-02	GGTCTAGAGG	6
7	OPG-03	GAGCCCTCCA	4
8	OPL-03	CCAGCAGCTT	3
9	OPY-20	AAGCGGCCTC	2

of ethidium bromide for 2 min and visualized under UV light.

Genetic diversity study

Nine polymorphic RAPD primers that showed distinct and scorable DNA bands were considered for analysis from the eleven primers used for the amplification process. Amplified DNA bands detected after electrophoresis separation in each accession were scored using binary number, 1 for the presence of a RAPD product band and 0 for the absence for a product band of similar length. Only distinct bands were considered for analysis, faints bands were omitted. A total of Fifty-three scorable bands were generated and analysed using Numeric Taxonomy System of Statistic (NTSYS, Roulph, 2000). A binary data matrix was generated; coefficient of similarity was determined between each pair of accessions using similarity coefficient method of Jaccard 1908 in NTSYS. The similarity index of Jaccard between plant *i* and *j* is given by

$$S_{ij} = a / a + b + c$$

$$D_{ij} = 1 - S_{ij}$$

Where *a* = the number of DNA band(s) present in both plants *i* and *j*, *b* = the number of DNA band(s) present in *i* and not in *j*, *c* = the number of DNA band(s) present in *j* and not in *i*, *D* = the distant coefficient.

The similarity data matrix was then used for clustering of the genotypes based on unweighted pair grouping method using the arithmetic average (UPGMA) in the NTSYS software package, which does not consider the joint absence of a DNA band as an indication of similarity. The clustering was used to obtain a dendrogram that distributed the 24 African yam beans into DNA cluster groups using tree plot option in the same software package.

A three-dimension scatter plot of the 24 accessions of African yam bean was drawn using the first three principal components scores to reveal further the ordination or grouping among them based on Principal Component Analysis (PCA) as generated from Statistical Analysis System (SAS), version 8, 1999.

Identification of minimum number of RAPD primers required

The polymorphic information content (PIC) value was calculated for each of the 9 DNA RAPD primers using the formula, $PIC = 1 - \sum (P_i)^2$, where *P* is the proportion of number of allele(s) present in the plant sample analyzed, and *n* is nth alleles present in a primer. Primers with PIC values above 0.50 were selected. Different combinations of the selected primers' data using simple progression combination method of arithmetic (2, 3, 4, and 5), in accordance with increasing PIC values were analyzed using the same NTSYS software package to identify the minimum number of primer(s) that could clustered the 24 accession into different groups;

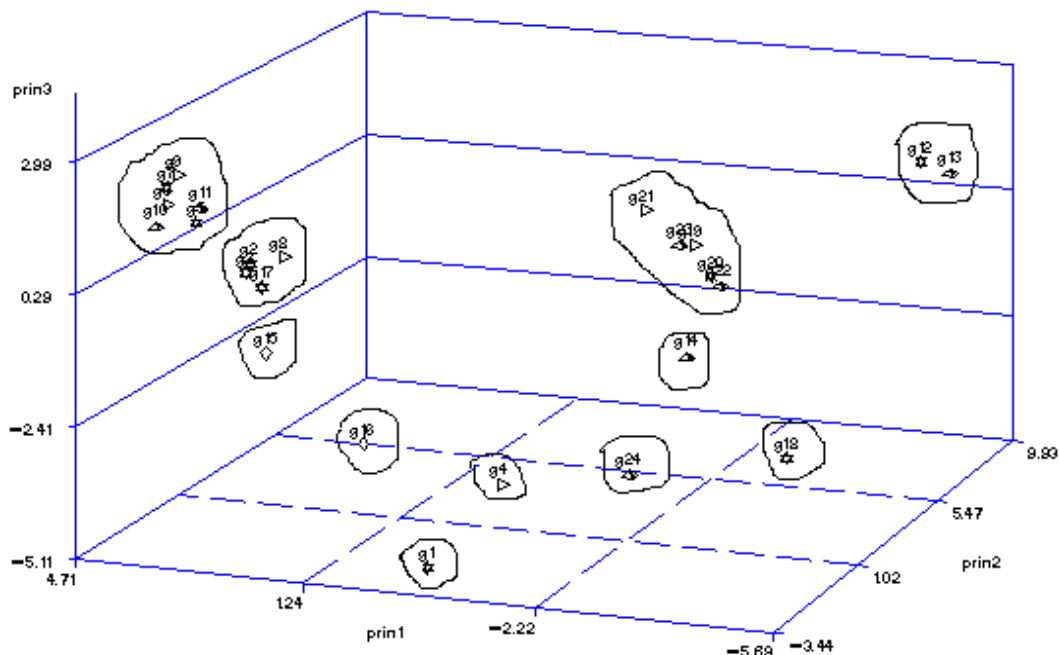


Figure 1. A 3-Dimension scatter plot of the 24 Nigerian accessions of African yam bean based on the scores of the first three principal components using SAS software package. The 24 African yam bean were clustered into eleven DNA groups which represented $\approx 60\%$ of the total genetic variation detected by the 9 RAPD primers. G1 to G24 represent the names of the cultivars according to Table 1. The sources of the cultivars are represented by shapes as follows: Markudi-star, Iseyin-flag, Onitsha-pyramid, Ibadan-diamond, and Umudike-oval.

and also distinguished the 24 African yam bean as distinct genotypes.

RESULTS

NTSYS analysis

Eleven random primers were used for amplification of genomic DNA from the 24 accessions of Nigerian collection of African yam bean for genetic diversity study. Two RAPD primers resulted in limited amplification and were visualized on gel as faint bands. The remaining 9 polymorphic RAPD primers were used to detect genetic variation among the 24 accessions of the African yam bean in this study. The nine random primers produced a total of 53 loci and were used for NTSYS analysis. The number of RAPD loci ranged from 1 for OPE-03 to 12 for OPD-08 with an average of ≈ 5.9 loci per RAPD primer (Table 2). The sizes of the amplified fragment length ranged from 300 to 2100 bp. None of the primers was specific for any of the accession. The similarity indices data matrix generated ranged from 0.42 to 0.96, the dendrogram revealed 8 distinct DNA cluster groups at similarity index of 0.80 (Figure 1 from left to right).

Principal component analysis (PCA)

The binary data generated from the 24 accessions of

AYB were also subjected to Principal component analysis (PCA) using SAS. The first three principal components contributed 30.20, 22.17, and 8.60, respectively, which equates 60.98% of the total variation observed among the 24 genotypes of AYB. The plot of the first three principal components scores generated a 3-dimension scatter graph that showed the relationship between the 24 accessions of AYB (Figure 2).

Minimum number of RAPD primers required

Polymorphic information content (PIC) values ranged from 0.00 (for OPE-03) to 0.89 (for OPD-08) (Figure 3). Primers with PIC values above 0.5 were selected; they are OPD-08, OPB-09, OPF-20, OPG-03, and OPL-03. Two of the primers with highest PIC values, OPD 08 and OPD 09 (0.89 and 0.86, respectively) clustered the 24 African yam beans into 9 groups at 0.80 similarity coefficient but could not distinguish 4 AYB genotypes, AYB9514, AYB9608, AYB9512 and AYB9602 (Figure 4). Addition of primer OPF-20 and OPG-03 did not have much effect. When the primer with fifth highest PIC value, OPL-03 was added, the 24 African yam bean genotypes were clustered into 7 cluster groups at 0.80 similarity coefficient and could still not differentiate AYB9512 and AYB9602. This led to the addition of another RAPD primer OPY-20 with PIC value 0.38, which was the highest PIC of the remaining screened-out primers. The

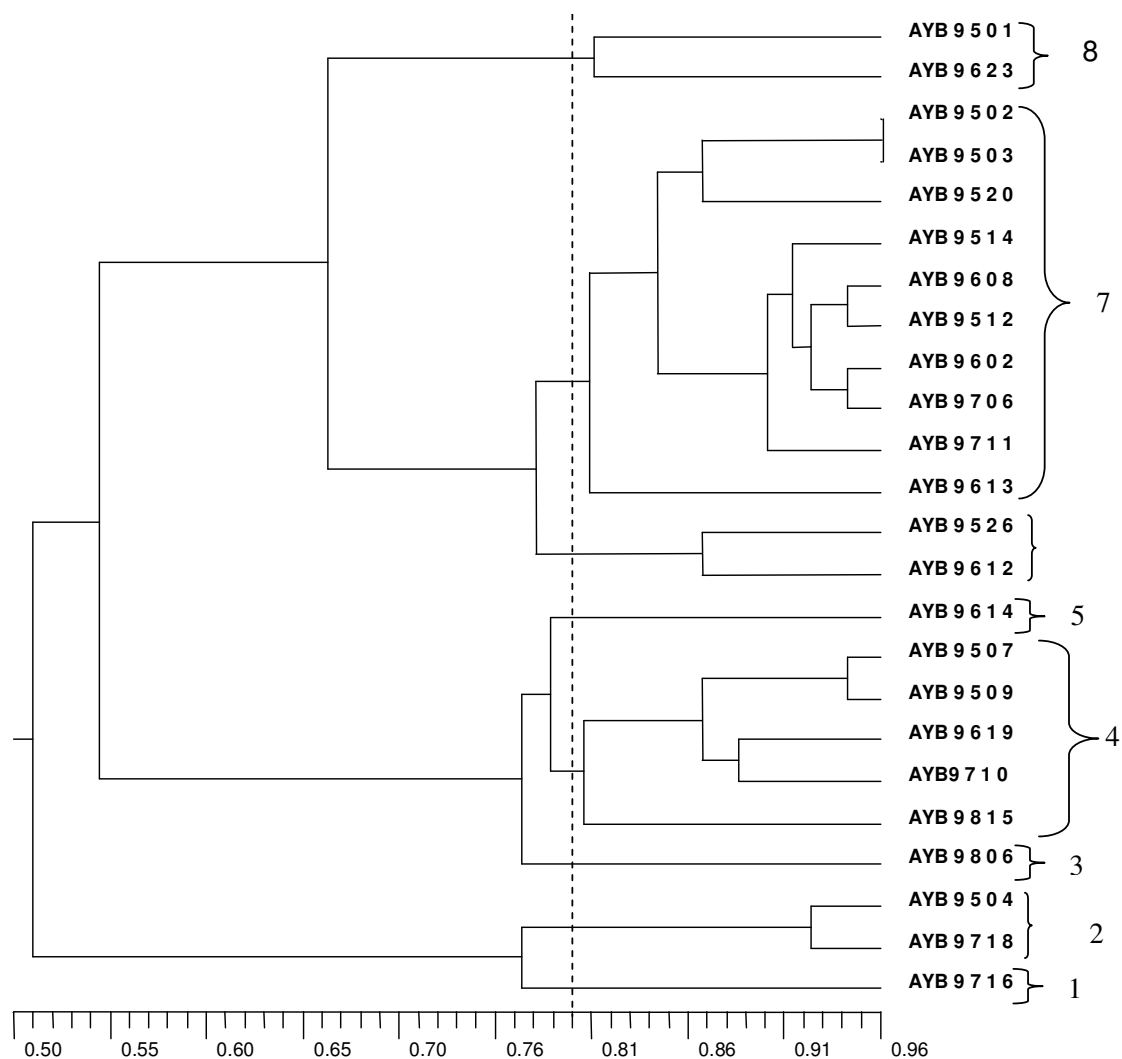


Figure 2. A dendrogram showing genetic diversity among the 24 accessions of African yam bean by 9 polymorphic RAPD primers.

generated data was subjected to NTSYS analysis. The dendrogram generated has similarity coefficient that ranged from 0.36 to 0.96; clustered the 24 African yam bean into 6 groups; and also distinguished all the 24 accessions of African yam beans at 0.96 similarity coefficient (Figure 5).

DISCUSSION

Random amplified polymorphic DNA has been extensively used in genetic diversity and general genetic studies in species. Sarma and Bahar (2005) reported high genetic diversity among Bora rice of Assam with identification of duplicates using RAPD primers; Nkongolo and Nsapato (2003) detected variation among *Sorghum bicolor* (L.) and also, Santos et al. (2003) in the genus *Arachis Leguminosae* based on RAPD markers. In

this study, RAPD primers were also able to detect the genetic variation among 24 accessions of Nigerian collection of African yam bean.

Using the RAPD pattern generated by the 9 random primers, it was possible to detect genomic variation in African yam bean in Nigeria. Although, the cluster analysis assigned the cultivars into 8 groups at 0.80 similarity indexes, the 24 genotypes were differentiated more as we move to 1.00 similarity indexes. The highest genetic relationship observed was between AYB9502 and AYB9503 at similarity index of 0.96; the two genotypes were from the same source (Table 1). On the other hand, the lowest genetic relationships were found between AYB9501 and AYB9718; AYB9718 and AYB9512; and AYB9619 and AYB9718 which were from diverse sources. Generally, the genetic diversity observed among the 24 accessions of Nigerian African yam bean is considerably high. Therefore, large number of

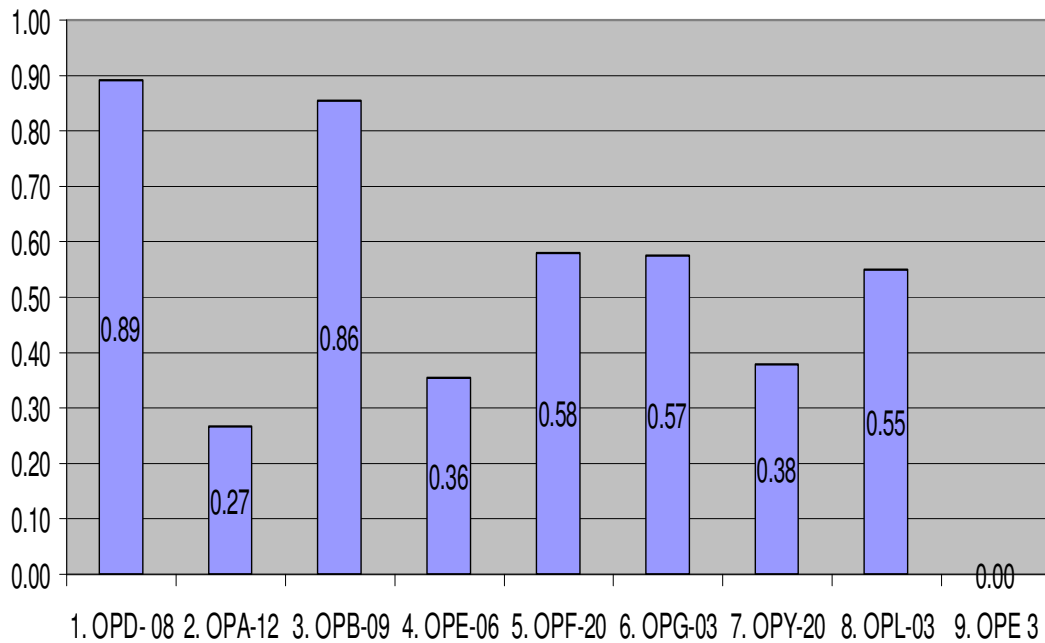


Figure 3. A chart of RAPD primers and their polymorphic information content values (PIC).

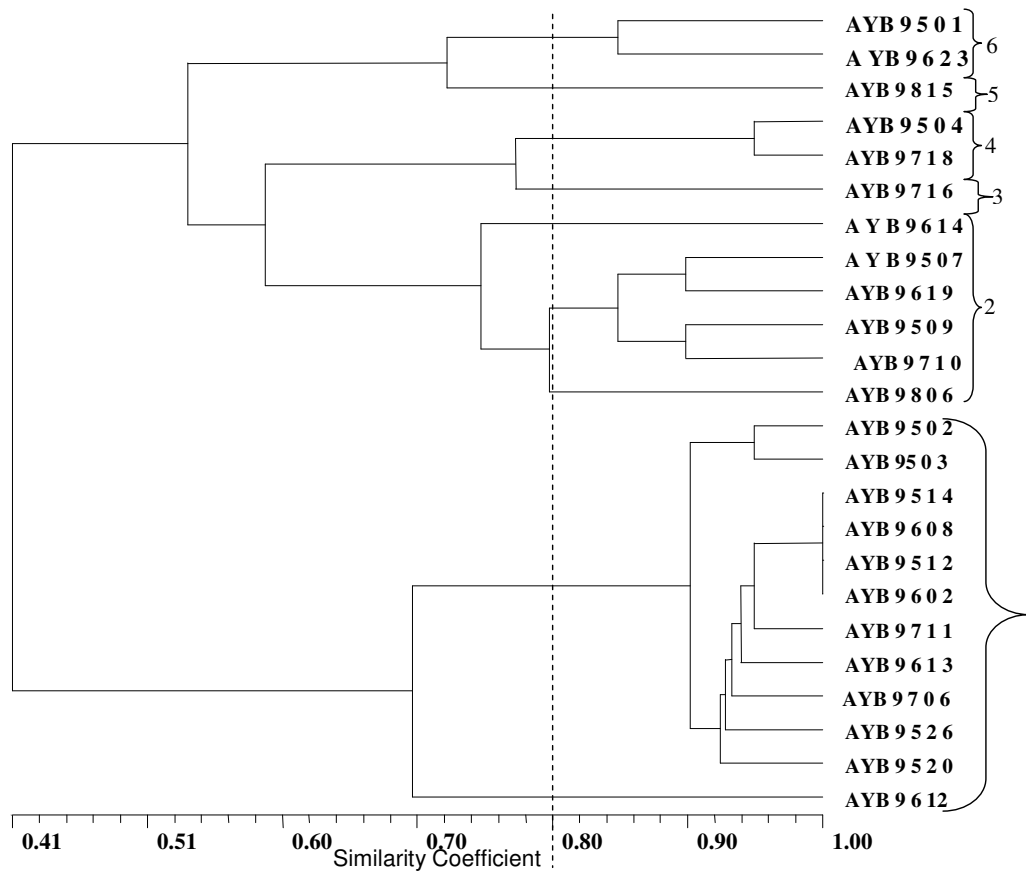


Figure 4. A Dendrogram showing genetic diversity and genotype identification by two highly polymorphic primers; OPD-08 and OPD-09.

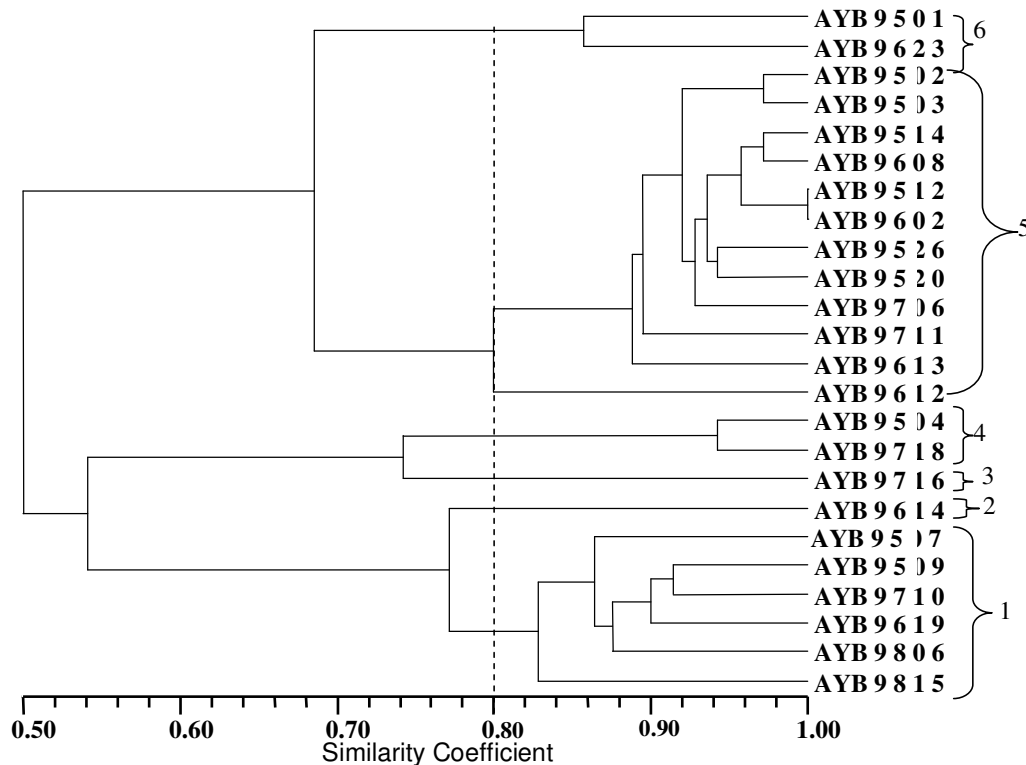


Figure 5. A Dendrogram showing genetic diversity and genotype identification by six highly polymorphic primers; OPD-08, OPD-09, OPF-20, OPL-03, OPG-03 and OPY-20.

new varieties, which incorporate multiple traits, could be obtained from Nigerian accessions of African yam bean. No clear-cut zonal demarcation was observed among the 24 accessions of AYB in the principal component analysis results, which indicates low environmental mutation among them. This demonstrated their ability to resist changes in environment and degree of adaptability to any environment. African yam bean is known to be resistant to cowpea borer (Machuka et al., 2000), therefore, genetic diversity between African yam bean and cowpea; and African yam bean and other legumes are necessary and pre-requisites for any genetic improvement of legumes using African yam bean as a genetic resource. This could also facilitate and maximize the use of African yam bean as a genetic resource for breeders and its conservation and management of its available germplasm.

In this study also, RAPD primers were able to detect high genetic diversity among a small collection of Nigerian accessions of African yam bean as a first step towards its genetic diversity study based on molecular markers. Minimum of two RAPD primers could be used conveniently for genetic diversity study in Nigerian accessions of African yam bean, though with low level of resolution while a minimum of 6 polymorphic primers was adequate for genotype identification studies. This result indicated that each accession is a distinct entity with no duplicate and therefore each genotype is capable of

generating varieties when crossed with one another. Sarma and Bahar, (2005) used RAPD primers for genetic diversity study in 23 accessions of Bora rice of Assam collection as a first step towards its genetic improvement. The results of their study revealed duplicates among the Assam rice accessions and were useful in removal of dormancy and development of a core germplasm.

Therefore, findings from this study support the proposal that RAPD markers are ideal for genetic diversity studies in plants. However, there is need for high level of standardization in RAPD to achieve reliable and comparable results (Penner, 1996) due to poor reproducibility of RAPD markers. Nevertheless, RAPD is still considered a quick and easy assay for estimation of genetic diversity in comparison with other classes of molecular markers (Sarma and Bahar, 2005). This is very useful, particularly, for under-exploited minor crops such as AYB. Furthermore, this study revealed the existence of considerable genetic variation in the AYB accessions of Nigerian (similarity coefficient of 0.42 to 0.96). With careful selection of PCR parameters and primers, RAPD analysis may help breeders to identify parents of AYB for hybridization to obtain new varieties. Knowledge of the genetic variation among AYB landraces would play a crucial role in their conservation. Our assessment here was limited to 24 accessions as the first time study of genetic variation/diversity based on molecular markers of Nigerian accessions of African yam bean. We plan to ex-

tend this study and analysis to accessions from other parts of Africa and larger number of accessions. This will likely revealed a wider genetic diversity among the African yam bean accessions.

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