academicJournals

Vol. 13(18), pp. 1850-1858, 30 April, 2014
DOI: 10.5897/AJB2014.13734
Article Number: 07224B844431
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Assessment of the genetic diversity of African yam bean *(Sphenostylis stenocarpa* Hochst ex. A Rich. Harms) accessions using amplified fragment length polymorphism (AFLP) markers

Omena B. Ojuederie[1,2]*, Morufat O. Balogun[2], Iyiola Fawole[3], David O. Igwe[4] and Mercy O. Olowolafe[5]

[1] Department of Biotechnology, Bells University of Technology, Ota, Nigeria.
[2]Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan Nigeria.
[3]Department of Biological Sciences, Bells University of Technology, Ota, Nigeria.
[4]Molecular Virology and Diagnostic Laboratory, International Institute of Tropical Agriculture, Ibadan Nigeria.
[5]Department of Biotechnology, Institute of Agricultural Research and Training, Moor Plantation, Ibadan, Nigeria.

Received 17 February, 2014; Accepted 29 April, 2014

The genetic diversity of 40 African yam bean (AYB) accessions was assessed using amplified fragment length polymorphism (AFLP) markers. Seeds of 40 accessions of AYB obtained from the International Institute of Tropical Agriculture (IITA) and Institute of Agricultural Research and Training (IAR&T) Ibadan, Nigeria, were grown in a greenhouse and young leaves from two weeks old plants collected for DNA extraction. The four primer combinations used generated a total of 1730 amplification fragments across the AYB accessions used in this study of which 1647 were polymorphic (95.20%). The number of amplified polymorphic AFLP bands per primer pair varied from 360 to 520 with an average percentage polymorphism of 95.6%. E-AGC/M-CAG produced the highest number of polymorphic bands (520). Polymorphic information content (PIC) values ranged from 0.9447 to 0.9626. The highest level of polymorphism (100%) was recorded for two primer combinations (E-AAC/M-CAG and E-ACT/M-CAG). The results of cluster analysis using UPGMA tree, grouped the 40 accessions of AYB into two major clusters with an overall similarity of 67.5%. The level of similarity between the accessions spanned 0.66 to 0.91. TSs 138 and TSs 139 were the most closely related accessions with high level of similarity index (0.91). Comparable results were obtained using Factorial Coordinate Analysis (FCO). The results from the present study confirm the robustness and the suitability of the AFLP as a molecular tool for the assessment of genetic diversity in AYB accessions.

Key words: Amplified fragment length polymorphism (AFLP), cluster analysis, genetic diversity, *Sphenostylis stenocarpa,* polymorphism.

INTRODUCTION

African yam bean (AYB) *Sphenostylis stenocarpa*, (Hochst. Ex. A. Rich) Harms is an important underutilized food crop in tropical Africa, the most economically important species in the genus *Sphenostylis* (Potter, 1992). It is an inexpensive source of protein (Apata and Ologhobo, 1990), high in sulphur containing amino acid

(Ezueh, 1977). The amino acid content in AYB seeds is higher than those in pigeon pea, cowpea, and bambara groundnut (Uguru and Madukaife, 2001, Adewale and Dumet, 2009). Despite the nutritional benefits of AYB, it is faced with several production constraints. The presence of high antinutritional factors such as tannins, saponins, oxalate, phytate, trypsin inhibitors and lectin, long cooking time, (Fasoyiro et al., 2006) and low seed yield (Saka et al., 2004) are the major constraints to its cultivation and utilization. The current germplasm base available to breeders for genetic improvement is also narrow. AYB is among the 400 under exploited legumenous crops of the lowland tropics (Rachie and Robert, 1974) and is currently susceptible to genetic erosion, which would undoubtedly reduce the genetic bases and ultimately in the future, it is in danger of extinction (Klu et al., 2001). Utilization of underutilized crops such as AYB could be one of the best strategies to buffer nutritional, environmental and financial vulnerability (Jaenicke and Pasiecznik, 2009).

In order to improve any crop species with valuable genetic attributes by either plant breeders or scientists, germplasm characterization and assessment of genetic diversity are essential prerequisites. The degree of success in the genetic improvement of any crop species to a large extent depends on the amount of genetic diversity existing among cultivated accessions as well as their wild relatives. Such variations would make up a valuable source of potential parents for hybridization and subsequent development of improved cultivars. Cultivars with a great amount of diversity, if collected and appropriately evaluated would provide a great chance for breeders to select and disseminate best performing genotypes (Bainiwal and Jatasra, 1980). Most AYB accessions are in the hands of farmers and little activity has been carried out so far to genetically characterize these accessions. Only a very small sector of the farmers appreciates its cultivation, hence, they are the holder of the crop's genetic resources (Adewale et al., 2012). Explorations to collect more landraces from farmers are necessary to increase the gene pool for crop improvement. The Genetic Resources Centre of the International Institute of Tropical Agriculture only conserves a few accessions. Research on understanding the genetic diversity of AYB accessions through morphological characterization has been reported (Akande, 2008; Popoola et al., 2011; Adewale et al., 2012; Aremu and Ibirinde, 2012). Accessions are clustered into distinct groups based on the morphological data. Characterizations of morphological traits are however, affected by environmental factors and need to be assessed at particular stages of growth of the crop. It also requires expertise on crop and/or species.

AYB is one of the underutilized crops with a wide genetic base. So far only few studies have been carried out to assess the genetic diversity of AYB using molecular markers. Moyib et al. (2008) used random amplified polymorphic DNA (RAPD) markers to understand the pattern of genetic variability in a few Nigerian AYB accessions. Eleven RAPD primers were used for PCR amplification, but only nine of them produced scorable bands. The results of the study revealed high genetic diversity among accessions of AYB. Adewale (2011) assessed the genetic diversity in 80 AYB accessions using amplified fragment length polymorphism (AFLP) markers with five primer combinations. Very few bands were generated (227) of which only 59 were polymorphic. He obtained an average percentage polymorphism of 25.6% which is extremely low because majority of the bands were monomorphic. The potential of AFLP marker to detect high level of polymorphisms by single reaction has made AFLP as one of the popular molecular tools in understanding the pattern of genetic variability (Vos et al., 1995). AFLP technique can be used to assess genetic variations within a species or among closely related species.

Therefore, the major objective of this research is to assess the genetic diversity in forty (40) accessions of AYB mainly collected from different regions of Nigeria using AFLP molecular markers.

MATERIALS AND METHODS

Plant material

27 accessions of AYB were obtained from the Genetic Resources Center of the International Institute of Tropical Agriculture (IITA) Ibadan which consists of 2 accessions from Ghana, 1 from Bangladesh and 24 from Nigeria, with 8 of them collected from Enugu State in South East Nigeria, while 13 accessions were from the Institute of Agricultural Research and Training (IAR&T) Obafemi Awolowo University, Moor plantation Ibadan, all of Nigerian origin (Table 1). Most of the accessions from IAR&T were collected from South West Nigeria except AYB 34, which is from Kaduna State in North West Nigeria (Figure 1). Seeds of each accession were grown in the greenhouse and young leaves from two weeks old plants were collected for DNA extraction.

DNA extraction

DNA samples were extracted from fresh leaf samples using a ZYMO ZR plant/seed DNA Miniprep extraction kit No. D6020

*Corresponding author. E–mail: omenabernojus@gmail.com. Tel : 2348075927894 or 2348185183876.

Abbreviation: AFLP, Amplified fragment length polymorphism; **AYB**, African yam bean; **FCO,** factorial coordinate analysis**; NUS**, neglected underutilized species; **PIC**, polymorphic information content; **TSs**, tropical *sphenostylis stenocarpa*; **UPGMA**, unweighted paired group method using arithmetic average.

Table 1. List of passport data of accessions used in this study.

Accession Code	Country	Region	Collection site	Collector Name
TSs 5	Nigeria	NA	NA	C.N. Aniagu
TSs 19	Nigeria	NA	NA	Kanti Rawal
TSs 26	Nigeria	NA	NA	Kanti Rawal
TSs 41	Nigeria	NA	NA	Kanti Rawal
TSs 42	Nigeria	NA	NA	Kanti Rawal
TSs 45	Nigeria	NA	NA	Kanti Rawal
TSs 51	Nigeria	NA	NA	L. Igbokwe
TSs 52	Nigeria	NA	NA	L. Igbokwe
TSs 66	Bangladesh	NA	NA	Dr N. Haq
TSs 68	Ghana	NA	NA	W.M. Steele
TSs 78	Nigeria	NA	NA	Unknown
TSs 88	Nigeria	NA	NA	Badra
TSs 107	Nigeria	NA	NA	Dr. N. Q. Ng
TSs 123	Ghana	NA	NA	Eastwood/Holloway
TSs 133	Nigeria	NA	NA	Unknown
TSs 134	Nigeria	NA	NA	Unknown
TSs 137	Nigeria	NA	NA	Unknown
TSs 138	Nigeria	NA	NA	Unknown
TSs 139	Nigeria	NA	NA	Unknown
TSs 140	Nigeria	South East	Agbani	Dr. J. Machuka
TSs 148	Nigeria	South East	Enugu	Dr. J. Machuka
TSs 150	Nigeria	South East	Enugu	Dr. J. Machuka
TSs 152	Nigeria	South East	Enugu	Dr. J. Machuka
TSs 153	Nigeria	South East	Enugu	Dr. J. Machuka
TSs 154	Nigeria	South East	Enugu	Dr. J. Machuka
TSs 156	Nigeria	South East	Umueze	Dr. J. Machuka
TSs 157	Nigeria	South East	Umueze	Dr. J. Machuka
AYB 1	Nigeria	NA	NA	Dr (Mrs.) R. Akande
AYB 4	Nigeria	South West	Ado-Ekiti	Dr (Mrs.) R. Akande
AYB 9	Nigeria	NA	NA	Dr (Mrs.) R. Akande
AYB 23	Nigeria	South West	Itaogbolu	Dr (Mrs.) R. Akande
AYB 26	Nigeria	South West	Ilesha	Dr (Mrs.) R. Akande
AYB 34	Nigeria	North West	Kaduna	Dr (Mrs.) R. Akande
AYB 45	Nigeria	South West	Itaogbolu	Dr (Mrs.) R. Akande
AYB 50	Nigeria	South West	Omi-Adio	Dr (Mrs.) R. Akande
AYB 56	Nigeria	South West	Itaogbolu	Dr (Mrs.) R. Akande
AYB 57	Nigeria	South West	Akure	Dr (Mrs.) R. Akande
AYB 61	Nigeria	South West	Serafu	Dr (Mrs.) R. Akande
AYB 70B	Nigeria	NA	NA	Dr (Mrs.) R. Akande
AYB IFE	Nigeria	South West	Ife	Dr (Mrs.) R. Akande

TSs, Tropical (*Sphenostylis stenocarpa*) obtained from the Genetic Resources Centre, International Institute of Tropical Agriculture, Ibadan; **AYB**, African yam beans obtained from the Institute of Agricultural Research and Training, Obafemi Awolowo University, Moor Plantation, Ibadan.

(Inqaba Biotech, South Africa) following the manufacturer's instructions. The qualities of the extracted DNA samples were checked using agarose gel electrophoresis (1.5%) stained with GR Green (Excellgen, Inqaba Biotechnical, South Africa). The DNA purity and integrity was assessed using Nanodrop spectrophotometer (ND-1000).

Amplified Fragment Length Polymorphism (AFLP) analysis

AFLP analysis was conducted according to Vos et al. (1995). Five AFLP primer combinations were screened and four were selected for PCR amplification (Table 2). Genomic DNA (100 ng/µl) were digested with 1 µL of EcoR1/MseI unit enzymes (Invitrogen AFLP

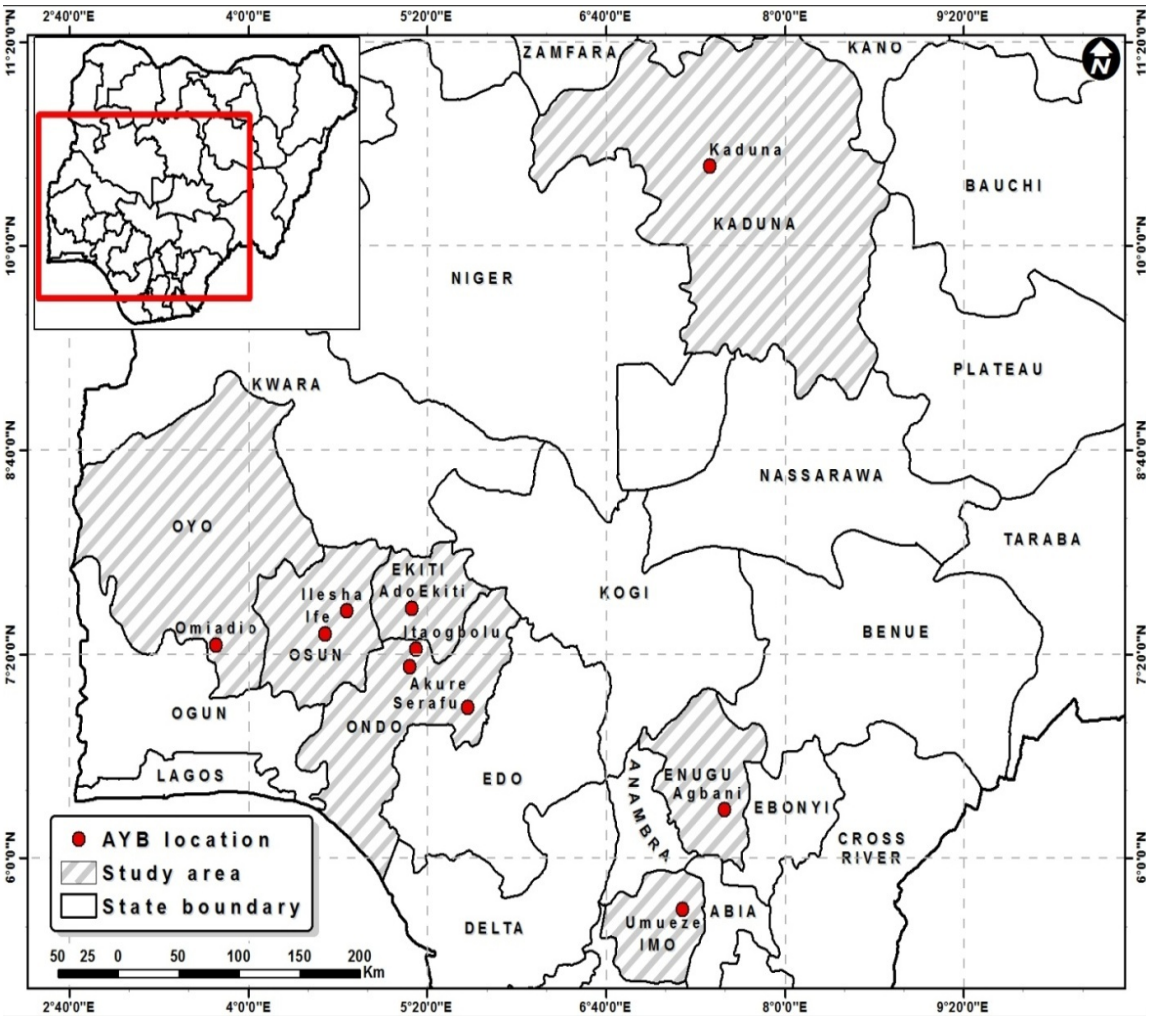

Figure 1. African yam bean accession collection sites in Nigeria.

Table 2. Adapters and primers used for pre-amplification and selective amplification of AFLP procedure.

Name of Primer / adapter	Sequence (5'- 3')
***EcoRI* adapter**	CTCGTAGACTGCGTACC CATCTGACGCATGGTTAA
***MseI* adapter**	GACGATGAGTCCTGAG TACTCAGGACTCAT
***EcoRI* primer**	
E- AAC	GACTGCGTACCAATTCAAC
E-ACG	GACTGCGTACCAATTCACG
E-ACT	GACTGCGTACCAATTCACT
***MseI* primer**	
M-CAT	GATGAGTCCTGAGTAACAT
M-CAG	GATGAGTCCTGAGTAACAG
M-CTG	GATGAGTCCTGAGTAACTG

Core reagent kit) incubated at 37ºC for 5 h after which the enzymes were inactivated at 70°C for 15 min. Pre selective amplification was carried out by ligating digested fragments with adapters following manufacturer's instruction. 10 µL of ligation reaction master mix was added to each of the tubes containing the digested genomic DNAs. PCR amplification was carried out using diluted ligations (1:10) mix. The PCR program in the thermal cycler (GeneAmp PCR system 9700, Applied Biosystems, USA) was denatured for 2 min at 94°C, annealing at 56°C for 60 s and extension at 72°C for 60 s for a total of 25 cycles. Pre-selective PCR products were stored at 4°C. For selective amplification, pre-selective products were diluted (1:50) and then selective amplification was run using a touchdown programme {94°C for 30 min, 94°C for 30 s, 65°C for 30 s (-0.7 per cycle), 72°C for 60 s for a total of 12 cycles; 94°C for 30 s, 56°C for 30 s and 72°C for 60 s and 72°C for 2 min for a total of 23 cycles}.

Data collection and Analysis

Amplified DNA fragments were separated on a 6% denaturing polyacrylamide gel. The presence/absence of unequivocally scorable bands was transformed into a binary character matrix (1 for presence and 0 for absence of a band at a particular position). For cluster analysis, pair wise distance matrices were compiled by the NTYSYSpc 2.1 software packages (Rohlf, 2000), using the

Table 3. Polymorphism obtained from four primer combinations on 40 AYB accessions.

Primer combination	Number of band	Number of monomorphic bands	Number of polymorphic band	Percentage of polymorphism (%)	PIC
E-AAC/M-CAG	360	0	360	100	0.9447
E-ACT/M-CAG	383	0	383	100	0.9579
E-AGC/M-CAG	521	1	520	99.8	0.9573
E-ACG/M-CAT	466	82	384	82.4	0.9626
Total	1730	83	1647	382.2	3.8225
Average	432.5	20.75	411.75	95.55	0.9556

PIC, Polymorphic information content.

Jaccard's similarity coefficient (Jaccard, 1908). A dendrogram was constructed by unweighted paired group method using arithmetic average (UPGMA). For Factorial Coordinate Analysis (FCO) DARwin software package Version 5.0.158 (Perrier and Jacquemoud-Collet, 2006) was used. Polymorphic information content (PIC) was also calculated using the method described by Anderson et al. (1993) Thus:

$PIC = 1- \sum p_i^2$

Where; p_i is the frequency of the i^{th} allele.

RESULTS

Polymorphism as detected by amplified Fragment Length Polymorphisms (AFLPs)

The four informative primer combinations generated a total of 1730 amplification fragments across all AYB accessions of which 1647 were polymorphic (95.20%). The number of amplified polymorphic AFLP bands per primer pair varied from 360 to 520 with an average of 411.75. An average percentage polymorphism of 95.6% was also generated. E-AGC/M-CAG produced the highest number of polymorphic bands 520, followed by E-ACG/M-CAT (384), E-ACT/M-CAG (383 bands), and E-AAC/M-CAG (360). Primer combination E-ACG/M-CAT produced the highest number of monomorphic bands (82) (Table 3). Primer combinations E-AAC/M-CAG and E-ACT/M-CAG displayed the highest percentage polymorphism (100%). Polymorphic information content (PIC) values ranged from 0.9447 to 0.9626.

Cluster analysis of AYB accessions

The dendrogram grouped the accessions into two major clusters with an overall similarity of 67.5% (Figure 2). The level of similarity between the accessions ranged from 0.66 to 0.91. TSs 138 and TSs 139 were the most closely related accessions with a similarity index of 0.91. Cluster 1 was further grouped into two sub clusters 1.1 and 1.2. Twenty seven accessions were found to cluster together in sub cluster 1.1, while six accessions were grouped in sub cluster 1.2. Out of these six accessions, a pair of accessions; TSs 68 (from Ghana) and TSs 66 (from Bangladesh), and TSs 107 and TSs 88 (both from Nigeria) were found to be genetically close to each other compared to TSs 78 and TSs 123 which displayed some degree of genetic distinctness. The pattern of clustering of accessions appears to be based on their geographical location. Thus, for example, some of the accessions that cluster together in sub cluster 1.1 were from South West Nigeria (AYB 4, Ado-Ekiti (Ekiti State), AYB 50, Omi-adio (Oyo State), AYB 26, Ilesha (Osun State), AYB IFE, Ife (Osun State) and AYB 57, Akure; AYB 23, AYB 45 and AYB 56, Itaogbolu (Ondo State)) and some were also from South East Nigeria (TSs 140, TSs 148, TSs 150, TSs 152, TSs 153, TSs 154, all from Enugu State and TSs 156 and TSs 157 from Imo State). In Cluster II, two sub clusters were identified one containing six accessions (TSs 19, TSs 26, TSs 41, TSs 42, TSs 51 and TSs 52) while the other only with a single accession TSs 45.

Factorial coordinates analysis of 40 African yam bean accessions

Factorial coordinate analysis generated by DARwin software version 5.0 using dissimilarity coefficient matrix placed the 40 AYB accessions into four groups (Figure 3). Group 1 includes four accessions (TSs 134, TSs137, TSs 138 and TSs 139). These accessions also appeared as a subgroup in sub cluster 1.1 of the dendrogram. Group II contain eight accessions: TSs 140, TSs 148, TSs 150, TSs 152, TSs 153, TSs 154 (Enugu State) and TSs 156 and TSs 157 (Imo State) in South East Nigeria. These accessions were also clustered together in sub cluster 1.1 on the dendrogram (Figure 2). The accessions in group III belong to collections from South West Nigeria which was obtained from the Institute of Agricultural Research and Training (IAR&T) Ibadan. These accessions include AYB 1, AYB 4, AYB 9, AYB 23, AYB 26, AYB 34, AYB 45, AYB 50, AYB 56, AYB 57, AYB 61, AYB 70B and AYB IFE. In this group, AYB 23, AYB 26 and AYB 34 were more closely placed on the scatter plot. This was also reflected on the dendrogram in sub cluster 1.2 with AYB 23 and AYB 26 closely related

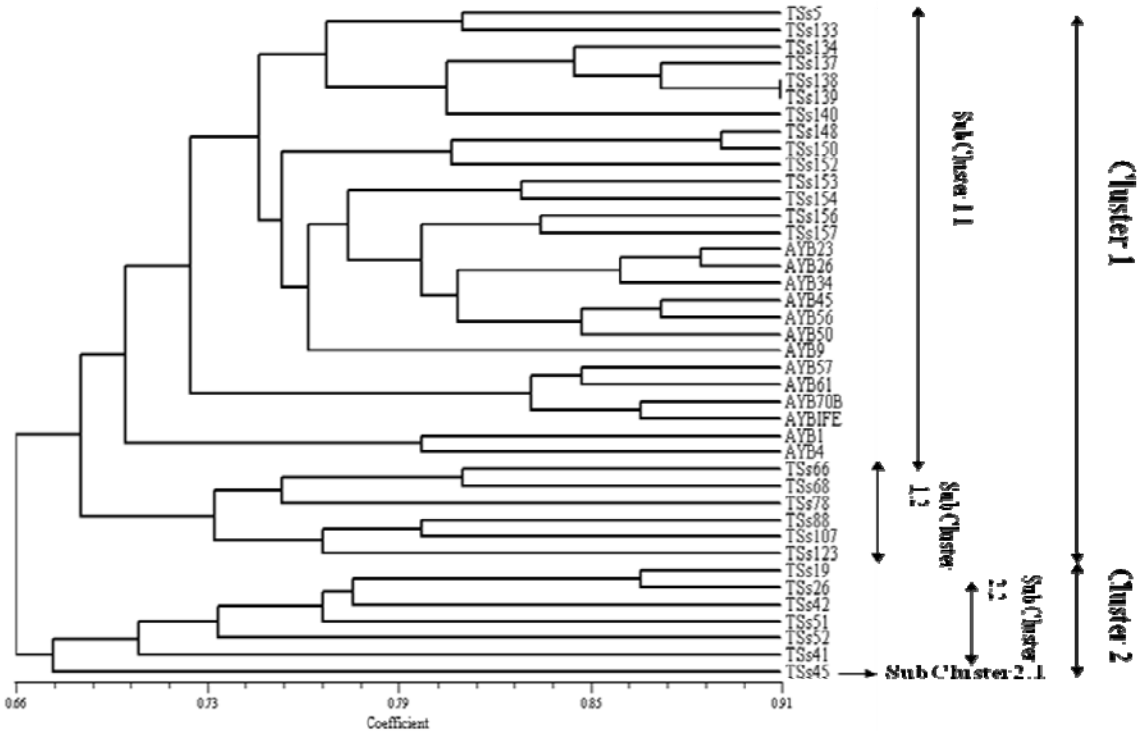

Figure 2. Dendrogram of 40 African yam bean accessions revealed by UPGMA cluster analysis using Jaccard's similarity coefficient.

and AYB 34 which was collected from Kaduna (North West Nigeria) displayed some degree of genetic isolation (Figure 2). The other fifteen accessions were found to be included in the 4th group. The results of both cluster and factorial coordinate analysis showed some resemblance in the pattern of grouping the 40 AYB accessions studied.

DISCUSSION

The present study revealed the extent and organization of genetic diversity within 37 selected accessions of AYB from Nigeria, 1 from Bangladesh and 2 from Ghana assessed with AFLP markers. High levels of genetic variation were observed. Both cluster and FCO tend to group the accessions based on their geographic origin and this might indicate considerable genetic divergence between the accessions reflecting different growing environments. TSs 68 and TSs 123 both from Ghana were clustered in sub cluster 1.2 along with three Nigerian accessions TSs 88 and TSs 107 which showed close resemblance and TSs 78 with some level of genetic distinctness just as TSs 123. TSs 66 from Bangladesh was closely linked with TSs 68; it may have originated from Ghana. From the dendrogram, AYB 1 was found to be closely related to AYB 4. This could suggest that this accession could also be collected from Ado-Ekiti (Ekiti State), South West Nigeria. The same is true for AYB 70B which was closely related to AYB IFE collected from Ile- Ife in Osun State. The level of polymorphisms ranged from 82.4% (E-ACG/M-CAT) to 100% (E-ACT/M-CAG and E-AAC/M-CAG). TSs 148, TSs 150, TSs 152, TSs 153, and TSs 154 originated from Enugu (Enugu State South East Nigeria) hence their grouping in sub cluster 1.1. TSs156 and TSs 157 both from Umueze (Imo State, South East Nigeria), showed close resemblance.

The accessions from Enugu were closely linked in group II of the scatter plot (Figure 3) while the two accessions from Umueze TSs156 and TSs 157 and TSs 140 from Agbani were clearly distinct. The FCO placed the accessions with known passport data into two geographically distinct regions South East (Group II) and (Group III) South West Nigeria. Accessions in Groups IV may have been from South South Nigeria where AYB is also widely cultivated and utilized.

Estimates of genetic distances based on differences in AFLP patterns are informative about genetic diversity (Greef et al*.,* 1997; Powell et al., 1996), phylogeny (Hill et al., 1996) and the geographic origins of genotypes and gene pools of plants (Beismann et al., 1997, Paul et al., 1997).

Moyib et al. (2008) also reported considerable genetic diversity among 24 AYB accessions of Nigerian origin from the Department of Agronomy, University of Ibadan, Nigeria, using random amplified polymorphic DNA (RAPD) markers which clustered the accessions into 8

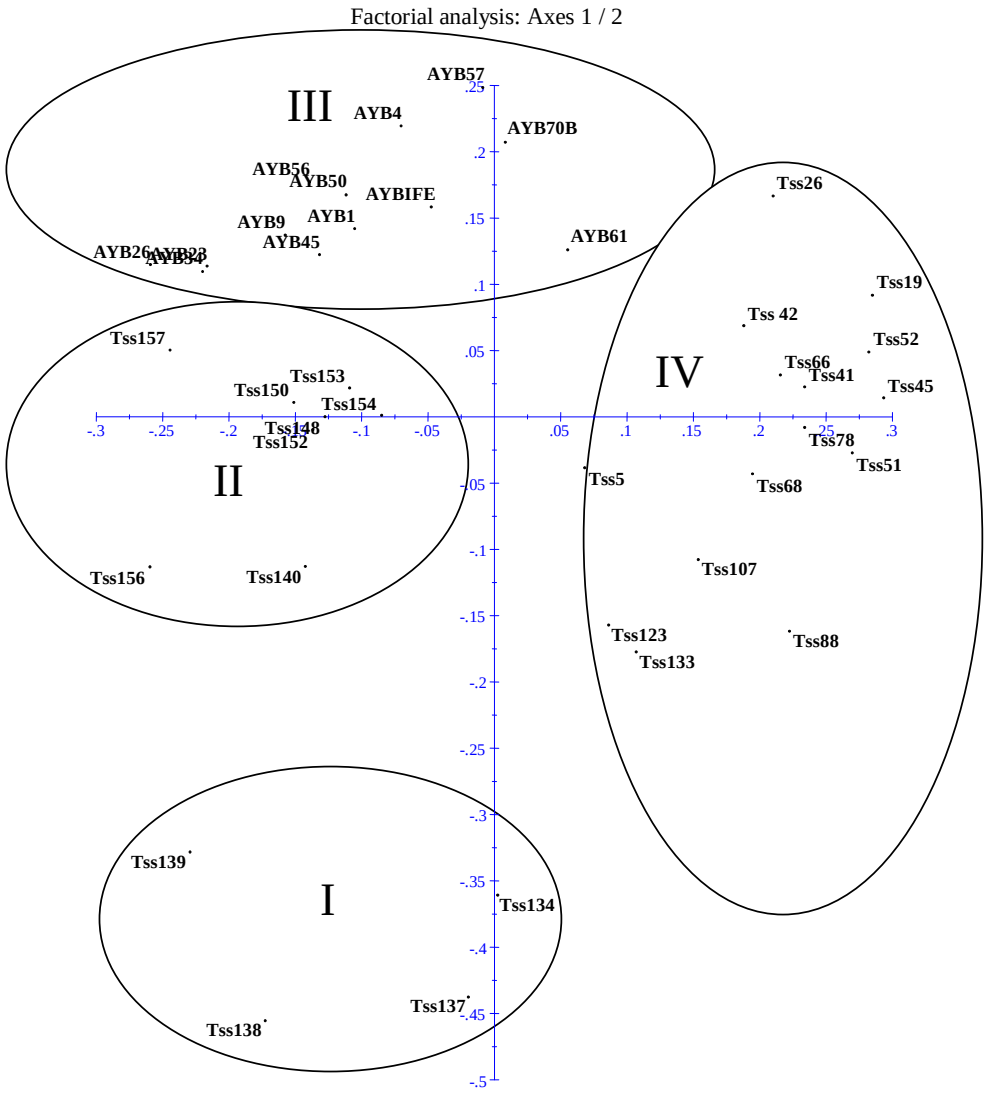

Figure 3. Factorial coordinate analysis for African yam bean accessions as generated by DARwin software version 5.0.0.158 using similarity coefficient matrix.

distinct cluster groups at 80% similarity index. The similarity indices ranged from 0.42 to 0.96 which were considered to be useful in facilitating the development of large number of new varieties through hybridization and transfer of useful genes, thus maximizing the use of such available germplasm as genetic resource materials for breeders.

In the work of Adewale (2011), 80 AYB accessions were characterized using five AFLP primer combinations (E-AAC/M-CAG, E-ACT/M-CAG, E-AGC/M-CAG, E-ACG/M-CAT and E-ACG/M-CTG) which generated a low percentage polymorphism of 26% due to the many monomorphic fragments obtained (168). This revealed higher similarities across some of the genomic loci of the tested population of AYB, an indication of low genetic diversity among the collected accessions by Adewale (2011). In the present study using four of the primer combinations, E-AAC/M-CAG, E-AGC/M-CAG, E-ACT/M-CAG and E-ACG/M-CTG, a higher average polymorphic content of 0.96 and percentage polymorphism of 95.6% were obtained which indicates a higher genetic diversity across the genomic loci of the 40 AYB accessions studied. The study also revealed that the 37 AYB accessions collected from different places in Nigeria represent a genetically diverse population.

Genetic fingerprinting using AFLP markers has been reported in various crops; Dolichos Bean (Venkatesha et al. 2010); Velvet bean (Capo-chichi et al. 2003); and in assessment of genetic diversity in sesame (*Sesamum indicum* L.) (Ali et al. 2007). It has also been used successfully for analyzing genetic diversity in some other plant species such as peanut (Herselman, 2003) and soy-

bean (Ude et al. 2003). In these studies different primer combinations were used to assess genetic diversity. The AFLP primer combinations used for AYB in the present study are efficient for genetic diversity studies in the species. AFLP markers are the most ideal genetic markers for analysis of genetic diversity both at intraspecific and interspecific level particularly for closely related species and underutilized crops.

For selection of good parental material for heterosis breeding program, the diversity results obtained through AFLP can be used to correlate with the pedigree relationship and morphological traits for genetic improvement in AYB. Having a good understanding of the degree of genetic relationships among AYB accessions will be of paramount importance for genetic improvement of the crop.

Conclusion

The results obtained from the present study showed the robustness and the suitability of AFLP as a molecular tool for diversity analysis and for the assessment of genetic diversity among individuals of AYB species. The high percentage polymorphism obtained (95.6%), indicates the heterogeneity of the AYB accessions used. Primer combinations E-ACT/M-CAG and E-AAC/M-CAG which produced 100% level of polymorphism are the most useful primer combinations for diversity analysis of AYB accessions. The genetic relatedness among the accessions could provide useful information regarding selecting both potential cross parents and desirable phenotypic traits for breeding programs.

ACKNOWLEDGEMENTS

This research was supported by a grant from the International Foundation for Science (IFS) Sweden, No. C/5250-1 awarded to the first author. The authors are also grateful to Mr Ayodele Alonge, Mrs. Victoria Iwu, Mrs. Tope Owoeye, Mr Sunday Taiwo and Ms Ladejobi Funmilayo of the Bioscience Centre, International Institute of Tropical Agriculture Ibadan for their technical assistance as well as Mr. Taiwo Omodele of the Geospatial laboratory, Institute of Agricultural Research and Training for the construction of the map on AYB site location.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES

Adewale D, Dumet D (2009). African yam bean: A crop with food security potentials for Africa. Afri. Tech. Dev. Forum. 6(3/4):66-71.

Adewale BD (2011). Genetic diversity, stability and reproductive biology of African yam bean *Sphenostylis stenocarpa* (Hochst. Ex. A. Rich.) Harms. PhD thesis, Federal University of Agriculture, Abeokuta, Nigeria.

Adewale BD, Dumet DJ, Vroh-Bi I, Kehinde OB, Ojo DK, Adegbite AE, Franco J (2012). Morphological diversity analysis of African yam bean and prospects for utilization in germplasm conservation and breeding. Gen. Res. Crop Evo. 59(5):927-936.

Akande SR (2008) Germplasm characterization of African yam bean (*Sphenostylis stenocarpa*) from south-west Nigeria PGR News. 154:25-29.

Ali GM, Yasumoto S, Seki-Katsuta M (2007). Assessment of genetic diversity in sesame (*Sesamum indicum* L.) detected by Amplified Fragment Length Polymorphism markers. Elec. J. Biotech. 10(1):12-23.

Anderson JA, Churchill JE, Autrique SD, Tanksley S, Sorrells ME (1993). Optimizing parental selection for genetic linkage maps. Genome 36:181–188.

Aremu CO, Ibirinde DB (2012). Biodiversity Studies on Accessions of African Yam Bean (*Sphenostylis stenocarpa*) Int. J. Agric. Res. 7:78-85.

Apata DF, Ologhobo AD (1990). Some aspects of the biochemistry and nutritive value of African yam bean seed (*Sphenostylis stenocarpa*). Food Chem. 36:271-280.

Bainiwal CR, Jatasra DS (1980). Genetic divergence in pigeon pea. Indian J, Genet. Plant Breed. 40(1):153-156.

Beismann H, Barker JH, Karp A, Speck T (1997). AFLP analysis sheds light on distribution of two Salix species and their hybrid along a natural gradient. Mol. Ecol. 6:989–993.

Capo-chichi LJA, Weaver DB, Morton CM (2003). The use of molecular markers in the study of genetic diversity in Mucuna Tropical and Subtrop. Agroecosys. 1**(**2-3):309-318.

Ezueh MI (1977). Cultivation and utilization of minor food legumes in Nigeria. 10**:**28-32.

Fasoyiro SB, Ajibade SR, Omole AJ, Adeniyan ON, Farinde EO (2006). Proximate, minerals and antinutritional factors of some under-utilized grain legumes in south-western Nigeria. Nutr. Food Sci. 36(1):18-23.

Greef JM, Deuter R, Jung C, Schondelmaier J (1997). Genetic diversity of European Miscanthus species revealed by AFLP fingerprinting. Gen. Res. Crop Evol. 44:185–195.

Herselman L (2003). Genetic variation among Southern African cultivated peanut (*Arachis hypogaea* L.) genotypes as revealed by AFLP analysis. Euphy. 133(3):319-327.

Hill M, Witsenboer H, Zabeau M, Vos P, Kesseli R, Michelmore R (1996). PCR-based fingerprinting using AFLP as a tool for studying genetic relationships in *Lettuca spp*. Theor. Appl. Genet. 93:1202–1210.

Jaccard P (1908). Nouvelles resecherches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat. 44: 22–270.

Jaenicke H, Pasiecznik N (2009). Making most of underutilized crops. LEISA Magazine, 25:11–12

KIu GYP, Amoatey HM, Bansa D, Kumaga FK (2001). Cultivation and use of African yam bean *(Sphenostylis stenocarpa*) in the Volta Region of Ghana. J. Food Tech. Afri. 6:74-77.

Moyib OK, Gbadegesin MA, Aina OO, Odunola OA (2008). Genetic variation within a collection of Nigerian accessions of African yam bean (*Sphenostylis stenocarp*a) revealed by RAPD primers Afr. J. Biotechnol. **7** (12): 1839-1846.

Paul S, Wachira FN, Powell W, Waugh R (1997). Diversity and genetic differentiation among populations of Indian and Kenyan tea (*Camellia sinensis* (L.) O. Kuntze) revealed by AFLP markers. Theort. Appl. Genetics 94:255–263.

Perrier X, Jacquemoud-Collet JP (2006). DARwin software http://darwin.cirad.fr/.

Popoola JO, Adegbite AE, Adewale BD, Odu BO (2011). Morphological intraspecific variabilities in African yam bean (AYB) (*Sphenostylis stenocarpa* Ex A. Rich) Harms. Sci. Res. Essay 6(3): 507-515.

Potter D (1992). Economic botany of *Sphenostylis* (Leguminosae). Econ. Bot. 46: 262-275.

Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol. Breeding 2:225–238.

Rachie KO, Roberts LM (1974). Grain legumes of the lowland tropics, Advan. Agron, 26:1-132.

Rohlf FJ (2000). NTSYS-pc numerical taxonomy and multivariate

system (v.2.1). User guide Exeter Software, Setauket, New York USA 31pp.
Saka JO, Ajibade SR, Adeniyan ON, Olowoyo RB, Ogunbodede BA (2004). Survey of underutilized grain legume production systems in south-west agricultural zone of Nig. J. Agric. Food Inform. 6(2/3): 93-108.
Ude GN, Kenworthy WJ, Costa JM, Cregan PB, Alvernaz J (2003). Genetic diversity of soybean cultivars from China, Japan, North America, and North American ancestral lines determined by amplified fragment length polymorphism. Crop Sci. 43 (5):1858-1867.
Uguru MI, Madukaife SO (2001). Studies on the variability in agronomic and nutritive characteristics of African yam bean *(Sphenostylis stenocarpa* Hochst ex. A. Rich. Harms). Pl. Prod. Res. J. 6(2):10-19.
Venkatesha SC, Gowda PH, Ganapathy KN, Byre Gowda M, Ramachandra R. *et al.* (2010). Genetic Fingerprinting in *Dolichos Bean* using AFLP Markers and Morphological Traits. Int. J. Biotech. Biochem. 6(3):395–404.
Vos P, Rene H, Marjo B, Martin R, Theo van de L, Miranda H, Adrie F, Jerina P, Johan P, Martin K, Marc Z (1995). AFLP: a new technique for DNA fingerprinting. Nuc. Acid Res. 23(21): 4407-4414.