Archives of Current Research International

17(4): 1-10, 2019; Article no.ACRI.49178
ISSN: 2454-7077

Evaluation of the Genetic Diversity of African Yam Bean (*Sphenostylis stenocarpa* (Hoechst. ex. A Rich.) Harms.) Using Seed Protein Marker

Josephine Imeh Usoroh[1], Effiom E. Eneobong[1], Solomon Okon Abraham[1] and Anthony John Umoyen[1*]

[1]Department of Genetics and Biotechnology, Faculty of Biological Sciences, University of Calabar, Calabar, Nigeria.

Authors' contributions

This work was carried out in collaboration between all the authors. Author JIU was the principal investigator, collected samples and carried out laboratory work for the research. Author EEE conceived, designed the original work plan and supervised the research. Author SOA conducted data analysis, interpreted the results. Author AJU assisted in data analysis, managed the literature searches, read the first draft of the manuscript and finalized the manuscript for publication. All authors read and approved the final manuscript.

Article Information

DOI:10.9734/ACRI/2019/v17i430117
Editor(s):
(1) Dr. Amal Hegazi Ahmed Elrefaei, Division of Radioisotope Production, Hot Lab and Waste Management Center, Atomic Energy Authority, Egypt.
Reviewers:
(1) Schirley Costalonga, Universidade Federal do Espírito Santo, Brazil.
(2) Paul Benyamin Timotiwu, University of Lampung, Indonesia.
(3) Dr. R. K. Lal, Central Institute of Medicinal and Aromatic Plants, India.
Complete Peer review History: http://www.sdiarticle3.com/review-history/49178

Original Research Article

Received 11 March 2019
Accepted 24 May 2019
Published 31 May 2019

ABSTRACT

Aims: The present research seeks to assess the genetic diversity of African yam bean accessions in Nigeria using seed protein marker; geared towards its genetic improvement and conservation.
Introduction: African yam bean is an "Orphan Crop" highly nutritious and has been listed to be among the underutilized legumes despite its immense nutritional advantages in human diets. African yam bean has been reported as a good source of essential amino acids including cysteine, lysine, methionine, phenylalanine and Proline.
Study Design: Fifteen (15) accessions of African yam bean were collected from the International Institute for Tropical Agriculture (IITA), Ibadan and 25 local accessions were collected from

**Corresponding author: Email: anthonyumoyen4000@gmail.com, anthonyumoyen2015@yahoo.com;*

different parts of Nigeria including Enugu.
Results: This study evaluated the genetic diversity on forty (40) accessions of African yam bean (*Sphenostylis stenocarpa*) obtained from IITA germplasm bank and other parts of Nigeria. In this study, the genetic differences among the accessions were detected by the presence or absence of bands, which could be used as a basis for variation among accessions. The results from this present study revealed high polymorphism (≈93%), indicating high intra-specific genetic variation with potential linkage to traits desired by breeders.
Conclusion: The study demonstrated that seed storage protein using SDS-PAGE is an important tool for genetic diversity study in African yam bean, providing baseline information. This baseline information will be useful in further molecular studies geared toward efficient breeding and conservation strategies for this crop.

Keywords: Diversity; Sphenostylis stenocarpa; protein marker; Nigeria.

1. INTRODUCTION

Grain legume constitutes the main source of protein in the diets of every home in Africa and Asia; some important ones are cowpea (*Vigna unguiculata*), groundnut (*Arachis hypogaea*), lima bean *(Phaseolus lunatus)* and Soya bean *(Glycine max)* [1-4]. There are other plants that could meet these dietary needs but are cultivated only in localized areas and are not commonly utilized. These under exploited legumes include Bambara groundnut (*Vigna subterranean*), Africa yam bean *(Sphenostylis stenocarpa)* and pigeon pea *(Cajanus cajan)* as reported by researchers [5-8]. African yam bean is a legume that is indigenous to Africa and widely eaten [9]. It belongs to the family *Fabaceae*, sub-family *Papilionoideae* and is a vigorous, herbaceous climbing vine reaching 2-3 m in height and widely cultivated for its seeds and edible tubers [7].

African yam bean is an "Orphan Crop" highly nutritious and has been listed to be among the underutilized legumes despite its immense nutritional advantages in human diets [6,7]. African yam bean has been reported as a good source of essential amino acids including cysteine, lysine, methionine, phenylalanine and Proline [10]. It is predominantly grown by the older, local farmers and leaving the bulk of the genetic resources of this crop in the hands of these farmers threatens its survival [7]. The crop has a dual advantage in that it produces both seeds and tubers. Seed and tuber yield are inversely related as reported [11]. Africa yam bean is widely grown in West and Central Africa but its place of origin within African country is not known because its domestication cannot be traced to any locality [8,9]. African yam bean is also believed to have originated from Ethiopia and spread across many areas of tropical Africa where it is found growing in wild. A study was conducted to trace the origin of African yam bean, but the results favoured two independent domestications as shown by both cDNA analysis and linguistic data; although not very conclusive [9]. The study concluded that African yam bean has two regions or areas of domestication (West and Central Africa) where it is grown for seed and tuberous roots [9]. The center of diversity affirms that African yam bean spreads from the West through the East and Southern parts of Africa according to GRIN [12]. These areas host the genetic resources of African yam bean. The utilization of African yam bean has links with the socio-cultural values of some ethnic groups within the African society [13]. For instance, the Avatimes in Ghana prepare a special meal from it during the celebration of the puberty rites of adolescent girls [8,14]. Likewise a special meal from it features during the marriage ceremony among the Ekiti's in Nigeria [15], pointing that different forms of local recipes are prepared from the crop to meet the dietary needs of the people. African yam bean is grown in other African countries like Cameron, Cote d'Ivoire, Togo, Ghana and Nigeria [8-9,15]. In Nigeria, African yam bean is grown mostly in the western and eastern parts of the country by peasant farmers.

In crop improvement, genetic diversity between parents confers higher heterosis in the resultant progenies [16,17,18]. Genetic diversity study is important in providing information for population monitoring, assessment; useful in breeding and conservation planning [19]. SDS-PAGE is an important biotechnological tool used to estimate the molecular weights of proteins; in order to examine taxonomic differences among plants [20-22]. Seed proteins may be comparatively less affected by environmental factors [23]. Plant breeders use genetic information to improve

existing plants, create new varieties and reduce unfavorable inherited traits [17,18,23]. Characterization of germplasm is essential for obtaining information on the traits of accessions and also assures maximum utilization of the germplasm collected. The compilation of data on the important characteristics allows for simple grouping of accessions, distinguishing accessions within species; enabling easy identification of differences among phenotypes [16-18]. Molecular characterization in conjugation with morphometric data helps in determining the breeding pattern of species, individual reproductive success and the existence of gene flow within and between populations of the same related species [19-22, 24-29]. There is paucity of documented research on genetic diversity of African yam bean in Nigeria especially using protein marker. Therefore, this research seeks to assess the genetic diversity of African yam bean accessions in Nigeria using seed protein marker; geared towards its genetic improvement and conservation.

2. MATERIALS AND METHODS

2.1 Source of Plant Materials

Fifteen (15) accessions of African yam bean were collected from the International Institute for Tropical Agriculture (IITA), Ibadan and 25 local accessions were collected from different parts of Nigeria including Enugu. Plate1 shows picture ofAfrican yam bean seeds taken during sample collection.

Plate 1. African yam bean (*Sphenostylis stenocarpa*) seeds

2.2 Sodium Dodecyl Sulfate – polyacrylamide Gel Electrophoresis (SDS-PAGE) Analysis

SDS–PAGE was carried out on 40 accessions of African yam bean(*Sphenostylis stenocarpa*) in the Virology Unit, International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. The procedure for protein extraction and SDS – PAGE analysis are outlined sequentially below:

2.2.1 Protein extraction

Approximately 0.2 g of the seeds were ground into powder using mortar and pestle, then put into 1.5 ml eppendorf tube. Nine hundred and fifty micro liters (950 µl) of protein extraction buffer (0.05 M Tris HCl, pH 7.4 with 2.3% SDS, 5% 2-β-Mercaptoethanol, 10% glycerol, 0.1% bromophenol blue) was added to the powder in the eppendorf tube and vortexed for 5 minutes to mix properly. The mixture was kept at room temperature for 1 hour and thereafter centrifuged for 15 minutes at 10,000 rpm. After centrifugation, the supernatant was transferred into new 1.5 ml eppendorf tube and kept at -4^{O}C till electrophoretic separation.

2.2.2 Procedure for SDS-PAGE

The following procedures were undertaken using the protocol of Laemmli, 1970 [30].

a) Vertical slab type, model 45 – 2020 (PEGLAB Biotechnologies GmbH) was used.
b) A 15% resolving gel and a 5% stacking gel were prepared and the glass plate sandwich assembled.
c) Approximately 0.15 ml of 10% (w/v) ammonium persulphate (APS) and 0.02 ml tetramethylenediamine (TEMED) were added sequentially to the resolving gel and mixed carefully to avoid formation of bubbles.
d) Using a pipette, the resolving gel solution was poured between the glass plates with ¼ of the space left free for the stacking gel. The top of the resolving gel was carefully covered with 50% isopropanol plus 0.1% SDS solution and water until the resolving gel polymerized.
e) The water, isopropanol and SDS solution was discarded by gently washing with double-distilled water.
f) The stacking gel solution containing 0.1% SDS solution was then poured and APS

and TEMED were carefully added with a pipette to avoid formation of bubbles.

g) The combs were inserted and the gel allowed to polymerize for 60 minutes.
h) The combs were carefully removed and the gel put into the electrophoretic tank, (bottom and top reservoirs) with fresh 1X-Tris-glycine-SDS buffer, and the gel wells covered with the buffer.
i) Page Ruler Prestained Protein Ladder (10 to 170 KDa) was loaded onto the first well serving as a ladder or standard. Aliquots (70 µl) of the protein samples extracted from each of the 40 accessions *Sphenostylis stenocarpa* (African yam bean) were mixed with loading buffer in the ratio of 1:1 before loading into each of the gel wells. The set-up was connected to electricity and a constant current of 120 Volts was applied (for 30 minutes) and later changed to 200 volts as the bands migrated into the resolving gel, until the tracking dye (bromophenol blue) have travelled considerable toward the end of the gel plate.
j) At the end of the run, the gels were stained with Coomasie Brilliant Blue R250 in 180 propanol/water/acetic acid (4:5:1. v/v/v) overnight. They were de-stained by boiling in microwave at 5 minutes intervals until cleared for visual observation of bands using the automated gel documentation system [30].

2.3 Data Analysis

The electrophoregram of each sample (accession) was scored for the presence (1) or absence (0) of bands and were entered in a binary data matrix. Based on electrophoretic band spectra, Jaccard's similarity index (JSI) was calculated by the formula below as given by Sneath and Sokal [31]:

$$S = \frac{W}{(A+B-W)},$$

Where W is the number of bands of common mobility; A is the number of bands in type A and B is the number of bands in type B (representing rows and columns in relation to presence or absence of bands entered as (1) and (0) respectively, into a binary data matrix). All analyses were carried out using the statistical software MVSP, version 3.2. Genetic relationships were determined by cluster analysis using UPGMA method according to Nei and Li [32] and the MVSP, version 3.2 software was used to construct a dendrogram based on the similarity indices.

3. RESULTS

3.1 Protein Banding Pattern

Protein bands were seen at specific sites and their relative sizes estimated based on the distance migrated and using the weights of the standard protein ladder. Electrophoregrams showing protein banding pattern of accessions of *Sphenostylis stenocarpa*are given in plates 2. A total of fifteen (15) reproducible bands were obtained, with only one band being monomorphic in all the accessions of *Sphenostylis stenocarpa*. The relative weights for separated proteins estimated using the protein ladder ranged from 14.1 to 160.2 kDa.

3.2 Cluster Analysis and Similarity Coefficients

Cluster analysis result is presented in Fig. 1. The dendrogram revealed four main clusters (cluster 1(C_1), cluster 2 (C_2,) cluster3 (C_3) and cluster4 (C_4)) at 0.52 coefficient. C_1 contained only one accession (Aliade Benue (E); C_2 contained two accessions (Nkerefi Congo (O) and Chaschanji Taraba 2M). Cluster 3 contained seventeen accessions (Aliade Benue (K), Gboko Benue (I), Oduma Imo 2 (Q), Abejukolo Kogi (AC), Umana Imo (Y), Okigwe 1 (V), NSK Enugu (T), Igala Benue (G) and other locally collected accessions except Sokoto (AF), KogiIgala (AA), Oduma podded Enugu, OfugoAnkpaKogi (AE) and IjaleKogi (AD) which were in cluster 4. Cluster 4 contained twenty accessions including all the improved lines from IITA germplasm and three accessions from Ghana (TSs-68, TSs-76 and TSs-77) as well as some locally collected accessions from Nigeria (Sokoto (AF), KogiIgala (AA), Oduma podded Enugu, Ofugo Ankpa Kogi (AE) and IjaleKogi (AD). At a coefficient of similarity of 0.11, all the accessions showed similarity with one another. The highest similarity coefficient was 1. Aliade Benue (K) and Gboko Benue (I) accessions were 100% similar. Abejukolo Kogi (AC), Umana Imo (Y), Okigwe 1 (V), NSK Enugu (T) and Igala Benue (G) were also 100% similar based on the protein landmarks from SDS-PAGE.

Also, Agbani Enugu (S), Ezeagu (R), Oduma Enugu 4 (P), Chanchanji, Taraba 3(N), Chanchanji Taraba (L), Otukpo Benue (F), Isiukwuoabo Imo (D), UzoakaliAbia (C) and

Iheonjilsialangwa (B) accessions were 100% similar. TSs-93 and TSs-91; TSs-94, TSs-90; TSs-89, TSs-86, TSs-84, TSs-83 and TSs-82 were also 100% similar. Furthermore, TSs-79 and TSs-77; Sokoto (AF) and Kogilgala (AA) as well as Oduma podded Enugu, Ofugo Ankpa Kogi (AE), Ijale Kogi (AD) and TSs-68 accessions also showed 100% similarity based on the protein landmarks from SDS-PAGE. The least similarity percentage was 6.7%. This was obtained when accessions, Iheonji Isialangwa (B), Uzoakali Abia (C), Isiukwoabo Imo (D), Otukpo Benue (F), Chanchanji Taraba (L), Chanchanji Taraba 3(N), Oduma Enugu 4 (P), Ezeagu (R) and Agbani Enugu (S) were compared with accessions TSs-82, TSs-83, TSs-84, TSs-86, TSs-89, TSs-90 and TSs-94, respectively. Interestingly, most of the TSs accessions were clustered together (cluster 3) except TSs–81 and TSs-68 which were found in cluster 4.

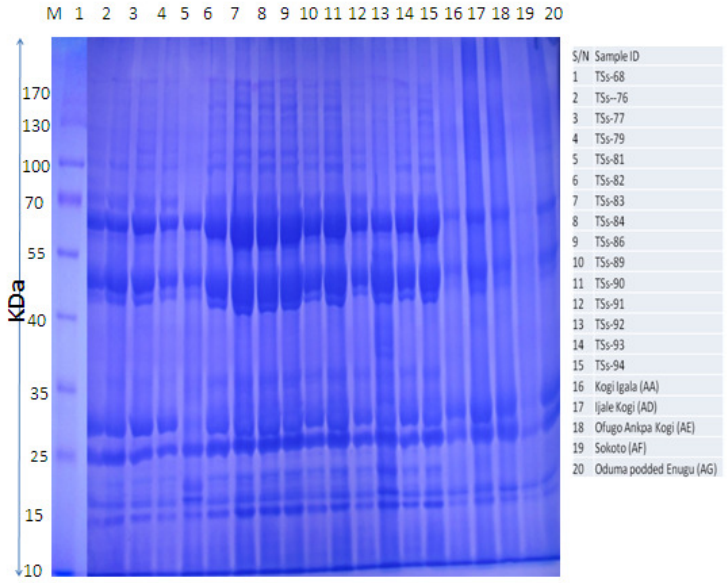

Plate 2. Electrophoregram of African yam bean samples using - Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

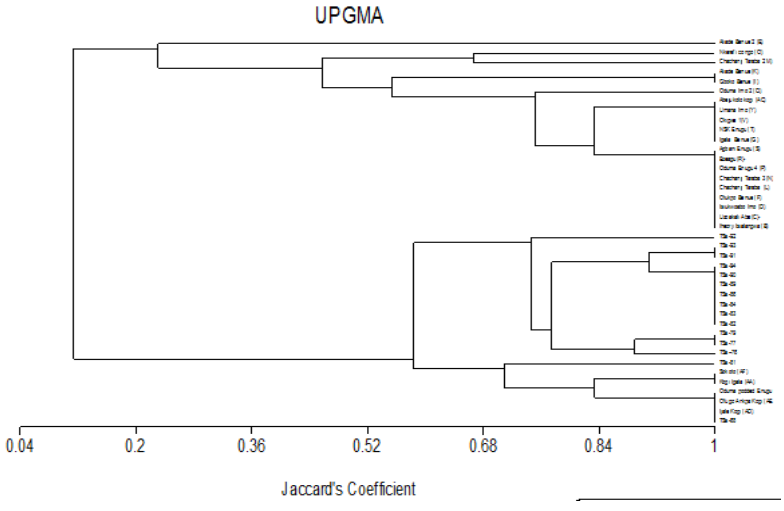

Fig. 1. UPGMA cluster analysis showing the diversity and relationship among 40 accessions of African yam bean based on SDS-PAGE

4. DISCUSSION

This study evaluated the genetic diversity on forty (40) accessions of African yam bean (*Sphenostylis stenocarpa*) obtained from IITA germplasm bank and other parts of Nigeria. In this study, the genetic differences among the accessions were detected by the presence or absence of bands, which could be used as a basis for variation among accessions. The variability in the SDS-PAGE profiling in this research revealed the extent and organization of genetic diversity within twelve (12) improved accessions of African yam bean obtained from IITA germplasm, three Ghanaian accessions (TSs-68, TSs-76 and TSs-77) and twenty-five (25) locally collected accessions of African yam bean from Nigeria. The cluster analysis tends to group the accessions based on their geographic origin and this indicated possible genetic divergence between the accessions, reflecting different growing environments. The Ghanaian lines (TSs-68, TSs-76 and TSs-77) were clustered together. Similarly, the improved lines from IITA germplasm and the locally collected lines from different geographical areas in Nigeria were clustered together respectively, suggesting close genetic resemblance. This result is in harmony with the findings of Soetan and Fafunso [33]. The information obtained will be helpful in further molecular studies. Interestingly, one of the Ghanaian accessions (TSs-68) was 100% similar to Nigerian accessions (Oduma podded Enugu, IjaleKogi (AD), and Ofugo, AnkpaKogi (AE) based on the protein profiles, indicating close genetic relatedness which could be useful in selection and breeding. This is in harmony with some studies [34-37] showing genetic diversity in different plants investigated; distributed among the populations due to natural selections and environmental conditions. Protein content may be influenced by age of plant at harvest and environmental conditions [37]. In a similar vein, the protein bands obtained from this study may be attributed to the age of plant at harvest and the prevailing environmental conditions of the different areas where these plant accessions were cultivated. Also, the protein bands could be associated with desirable traits in breeding, pointing that this study could help in improving the efficiency of African yam bean breeding programmes and germplasm conservation.

The researchers further stated that genetic relatedness are necessary in genetic studies, selection and breeding programmes.

The results from this present study revealed high polymorphism (≈93%), indicating high intra-specific genetic variation with potential linkage to traits desired by breeders. Torkpo et al. [38] documented that polymorphism can be used to examine possible associations of important traits like drought and disease resistance, crucial for selection and breeding depending on the interest of the plant breeder. The variation in the seed protein of the different accessions of African yam bean investigated in the study may be attributed to presence or absence of genes encoding the proteins. This result is in line with the findings of Shah et al. [26], stating that the differences in seed proteins profile were attributed to the presence or absence of genes encoding the proteins. Selective pressures in different environments may have influenced the seed storage protein fractions in the studied samples; giving rise to polymorphism. The result is concomitant to other findings [26,34].

The clustered accessions revealed that accessions Aliade Benue 2 (E) in cluster 1 and Otukpo Benue (F) and Aliade Benue (K) in cluster 2; are genetically different from each other; although they originated from the same locality (Benue State). Also, accession Oduma podded Enugu in cluster 4 and Agbani Enugu (S) in cluster 2 are genetically different; both originated from Enugu State. This result is similar with the documented research of Moyib et al. [39] that reported that high genetic diversity among African yam bean accessions collected from different locations in Nigeria. The clustered accessions also indicated close genetic relatedness or proximity. For instance, accessions Aliade Benue (K) and Gboko Benue (I) were in the same cluster and had similar banding pattern. This may be due to duplication of plant materials, which does not agree with the findings of Moyib et al. [39].

The average similarity index in the 40 accessions based on Jaccard's coefficient ranged from 0.07% (Otukpo Benue (F) and TSs-82 accessions) and 1.00% (TSs-93 and TSs-91), indicating high intra-specific genetic variation among the accessions characterized. Moyib et al. [39] also reported considerable genetic diversity among 24 African yam bean accessions of Nigerian origin using Random Amplified Polymorphic DNA (RAPD) markers with a similarity index ranging from 0.42 to 0.96%, which is similar to the result of this present study. Furthermore, Asif et al. [40] reported on high genetic variability among 151 accessions of

groundnut from five continents, using seed protein marker. Similarly, the present study showed that diversity existed based on protein profiles of seed storage proteins. In contrast, Adewale [41] reported a low polymorphism (26%) among 80 accessions of African yam bean using Amplified Fragment Length Polymorphism (AFLP) markers. The study revealed high similarities across some of the genomic loci of the characterized population, which is not in harmony with the result of this present study.

Independent and distant related accessions have the highest diversity, reflecting their genetic uniqueness [22]. This was evident in this study, where the least similarity of 6.7% was obtained when the local Nigerian accessions (Iheonji Isialangwa (B), Uzoakali Abia (C), Isiukwabo Imo (D), Otukpo Benue (F) Chachanji Taraba (L), Ezeagu (R) and Agbani Enugu (S) were compared with accessions (TSs-82, TSs-83, TSs-84, TSs-86, TSs-89, TSs-90 and TSs-94) respectively; which is in tandem with the documented report of Leyla et al. [22]. Germplasm variation could help plant breeders in developing new cultivars and design their hybridization programmes with greater success [42]. Maity et al. [43] reported that contrasting parents may be identified and used in the crossing programme for generating index variability, useful in selection and crop improvement based on the distance between species in different clusters. Generally, the genetic diversity obtained among the locally collected African yam bean accessions in Nigeria was considerably high as shown in the clustering pattern and similarity indices. This result is similar to the report of Nnamani et al. [44] where high level of genetic variability was observed in different accessions of African yam beans; a reflection of adequate genetic diversity, suitable for enhance breeding program for genetic improvement and germplasm conservation. These accessions with high genetic variation or diversity should be conserve, in order to avoid the erosion of some important indigenous species of African yam bean. It is in harmony with the finding of Nnamani et al. [45], documenting that there is urgent need to scale up conservation through robust linkages between contemporary scientific domains and indigenous peoples in order to harness, incorporate the rich indigenous knowledge in local communities for enhanced scientific knowledge, biodiversity conservation and its sustainable utilization for food security. Therefore, large numbers of new varieties, which incorporate multiple traits, could be obtained from these local accessions through hybridization and breeding.

5. CONCLUSIONS

The study demonstrated that seed storage protein using SDS-PAGE is an important tool for genetic diversity study in African yam bean, providing baseline information. Based on the results from this research, distantly related accessions such as TSs-68 and Aliade Benue 2 (E) could be exploited in breeding programs, while accessions like Aliade Benue (K) and Gboko Benue (I) may not be useful in breeding programs because their protein profiles are 100% similar. The range of variations observed among these accessions showed diverse genetic variability. This variations can provide sufficient scope for genotype selection by breeders, ecologist and biologist for germplasm conservation to avoid genetic erosion. Also these information will be useful in further molecular studies geared toward efficient breeding and conservation strategies for this crop.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Udensi OU, Okon EA, Ikpeme EV, Onung OO, Ogban FU. Assessing the genetic diversity in cowpea (*Vigna unguiculata L. Walp*) Accessions Obtained from IITA, Nigeria using random amplified polymorphic DNA (RAPD). International Journal of Plant Breeding and Genetics**.** 2016; 10(1):12-22.
2. Bhad PG, Mondal S, Badigannavar AM. Genetic diversity in groundnut (*Arachis hypogaea* L.) genotypes and detection of marker trait associations for plant habit and seed size using genomic and genic SSRs. Journal of Crop Science and Biotechnology. 2016;19(3):203–221.
3. Fofana B, Vekemans X, du Jardin P, Baudoin JP. Genetic diversity in Lima bean (*Phaseolus lunatus* L.) as revealed by RAPD markers. Euphytica. 1997;95: 157–165.
4. Dong Y, Zhao L, Liu B, Wang Z, Jin Z, Sun, H. The genetic diversity of cultivated soybean grown in China. Theoretical and Applied Genetics. 2004;108:931– 936.

5. Eltayeb ARSM, Ali AO, Abou-Arab AA, Abu-Salem MF. Chemical composition and functional properties of flour and protein isolate extracted from Bambara groundnut. African Journal of Food Science. 2011; 5(2):82- 890.
6. Oboh H, Muzquiz M, Burbano C, Cuadrado C, Pedrosa MMM, Osasgie AU. Anti-nutritional constituents of six underutilized legumes grown in Nigeria. Journal of Chromatography. 1998;823:307-312.
7. Saka JO, Ajibade SR, Adeniyan ON, Olowoyo RB, Ogunbodede BA. Survey of underutilized grain legume production systems in the south west agricultural zone of Nigeria. Journal of Agric. Food Inform. 2004;61:93-108.
8. Klu GYP, Amoatey HM, Bansa D, Kunmaga FA. Cultivation and use of African yam bean (*Sphenostylis stenocarpa)* in the Volta Region of Ghana. The Journal of Food Technology in Africa. 2001;6(3):74-77.
9. Potter D, Doyle JJ. Origins of the African yam bean (*Sphenostylis stenocarpa*) (leguminosae) – evidence from morphology, isozymes, chloroplast DNA and linguistics. Economy Botany. 1992;46: 276-292.
Available:http://dx.doi.org/10.1007/BF0286 6626
10. Omeire GC. Amino acid profile of raw and extruded blends of African yam bean (*Sphenostylis stenocarpa*) and cassava flour. Am. J. Food Nutr. 2012;2:65–68. Available:http://dx.doi.org/10.2521/ajfn.201 2.2.3.65.68
11. Ene-Obong EE, Okoye FI. Inter-relationships between yield and yield composition African yam bean. Tropical Land Wirstschaft Vet. Med. 1992;3:283-290.
12. Agricultural research service, United State Department of Agriculture. Germplasm Resources Information Network (GRIN). Ag. Data Commons. 2015.
Available:http://dx.doi.org/10.15482/USDA. ADC/1212393
13. Adewale BD, Kehinde OB, Aremu CO, Popoola JO, Dumet DJ. Seed metrics for genetic and shape determination in African yam bean. African Journal of Plant Science. 2010;4(4):107-115.
14. Wu GYP, Amoatey HM, Bansa D, Kumaga FK. Cultivation and use of African yam bean (*Sphenostylis stenocarpa*) in the Volta Region Ghana. The Journal of Food Technology in Africa. 2001;6:74-77.
15. Potter D. Economic botany of *Sphenostylis stenocarpa (Leguminoase*). Economic Botany. 1992;46(3):262-275.
16. Aremu CO, Ibirinde DB. Bio-diversity studies on accessions of African yam bean (*Sphenostylis stenocarpa*). Int. J. Agric. Res. 2012;7:78–85.
Available:http://dx.doi.org/10.3923/ijar.201 2.78.85
17. Rameeh V. Heterosis and combining ability assessment for phenological traits, plant height and grain yield in winter spring combinations of rapeseed varieties. International Journal Plant Breed Genetics. 2011;5:349-357.
18. Goldstein DB, Linares AR, Cavalli-sforza, LL, Feldman MW. An evaluation of genetic distances for use with microsatellite. Genetics. 1995;139(1):463-471.
19. Papa R, Gepts P. Asymmetry of gene flow and differential geographical structure of molecular diversity in wild and domesticated common bean (*Phaseolus vulgaris)* from Mesoamerica. Theory Applied Genetics. 2003;106:239-250.
20. Oppong-Konadu EYR, Akromah HK, Adu-Dapaah, Okai E. Genetic diversity within Ghananian cowpea germplasm based on SDS-PAGE of seed proteins. African Crop Science Journal. 2005;13(2):117-123.
21. Malik MFA, Qureshi AS, Ashraf M, Khan MR, Javed A. Evaluation of genetic diversity in soya bean (*Glycine max)* lines using seed protein electrophoresis. Australian Journal of Crop. 2009;3(2):107-112.
22. Leyla A, Fatima O, Mecit V, Osman T, Sefa G. Analysis of genetic variation among accessions of critically endangered *Rhamponyicoidesiconiensis* and *Rhaponticoidesmykalea* based on RAPD and SDS-PAGE markers. African Journal of Biotechnology. 2009;8:2076-2082.
23. Khurshid H, Rabbani MA. Comparison of electrophoretic protein profiles from seed of different oil seed Brassica cultivars. Journal of Public Health and Biological Sciences. 2012;1:36-42.
24. Omonhinmin CA, Ogunbodede OO. Genetic diversity, taxonomy and legumins implications of seed storage protein profiling in *Fabaceae*. African Journal of Biotechnology. 2013;1:36-42.
25. Amouri AA, Fyad L, Zohra F, Noureddine Y. Genetic diversity in seed storage

protein in *Medicago truncatula* genotype in relation with salt stress tolerance. International Journal of Agriculture and Crop Sciences. 2014;7:55-59.
26. Shah SH, Khan MF, Khaliq A. Genetic characterization of some maize (*Zea mays L.*) varieties using SDS-PAGE. Asian Journal of Plant Science. 2003;2:1188-1191.
27. Sharma SC, Maloo SR. Genetic diversity and phylogenetic relationship among soya bean (*Glycine max* (L) Merrill) varieties based on protein, evolutionary and morphological markers. Indian Journal of Plant Genetics Resources. 2009;22:33-36.
28. Shitta NS, Abberton MT, Adesoye AI, Adewale DB, Oyatomi O. Analysis of genetic diversity of African yam bean using SSR markers derived from cowpea. Plant Genetic Resources. 2016;14(1):50-56.
29. Hou WW, Zhang XJ, Shi JB, Liu YJ. Genetic diversity analysis of Faba bean (*Viciafaba L.)* germplasms using sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Genetic Molecular Research. 2015;14(4):13945-13953.
30. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970;227(5259):680-685.
31. Sneath PH, Sokal RR. The estimation of taxonomic resemblance. In: Kennedy D, Park RB. (Eds.). Numerical taxonomy: The principles and practice of numerical classification. San Francisco: Freeman. 1973:129-132.
32. Nei M, Li WH (1979). Mathematic model for studying genetic variation in terms of restriction endonucleases. Proceeding of the National Academy of Sciences of the United States of America (PNAS). 1979;76:5269-5273.
33. Soetan KO, Fafunso MA. Biochemical characterization of three varieties of *Lablab purpureus* (lablab beans) seed proteins. International Journal of Biotechnology and Biochemistry. 2009;5:71-78.
34. Abraham SO. Evaluation of genetic diversity in fonio (*Digitaria exilis* and *D. iburua*) using morphological and protein markers. Unpublished M.Sc Thesis, Department of Genetics and Biotechnology, University of Calabar, Nigeria; 2016.
35. Alege GO, Abu NE, Sunday CE. Seed protein electrophoresis of some members of the family *Fabaceae*. African Journal of Biotechnology. 2014;13(36):3730-3735.
36. Adewale BD, Dumet DJ, Vroh-Bi I, Kehinde OB, Ojo DK, Adegbite AE, Franco J. Morphological diversity analysis of African yam bean and prospects for utilization in germplasm conservation and breeding. Genet. Res. Crop Evolution. 2012;59:927–936.
Available:http://dx.doi.org/10.1007/s10722-011-9734-1
37. Tesfaye D, Gulelat DH, Nigussie R, Ashagrie W, Mulatu G. Mineral and anti-nutritional contents of Niger seed (*Guizotia abyssinica* (L.f.) Cass., Linseed (*Linum usitatissimum L.*) and Sesame (*Sesamum indicum L.*) varieties grown in Ethiopia. Foods. 2017;6:27-27.
DOI: 10.3390/foods6040027
38. Torkpo SK, Danquah EY, Offei SK, Blag, ET. Esterase total protein and seed storage proteins diversity in okra (*Abelmoschus esculentus*). West African Journal of Applied Ecology. 2006;9:7-18.
39. Moyib OK, Gbadegesin MA, Aina OO, Odunola OA. Genetic variation within a collection of Nigerian accessions of African yam bean (*Sphenostylis stenocarpa*) revealed by RAPD primers. African Journal of Biotechnology. 2008;7:1839-1846.
40. Asif J, Abdul G, Rashid A. Seed storage protein electrophoresis in groundnut for evaluating genetic diversity. Pakistan Journal of Botany. 2004;36:25-29.
41. Adewale DB. Genetic diversity, stability and reproductive biology of African yam bean, *Sphenostylis stenocarpa.* Ph.D Thesis*,* Federal University of Agriculture, Abeokuta, Nigeria; 2011.
42. Volk GN, Richard CM, Henk AD, Reilley AX, Bassil NV, Postman JD. Diversity of wild *Pyruscommunis,* based on microsatellite analysis. Journal of American Society of Horticulture Science. 2006;131:408-417.
43. Maity MFA, Querch AS, Ashraf M, Ichan MR, Asif J. Seed polymorphism in nine species of jute (*Corchorus,* family: *Tihaceac*). Indian Journal of Science and Technology. 2009;2(1):34-36.
44. Nnamani CV, Ajayi SA, Oselebe HO, Atkinson CJ, Adewle DB, Igwe DO, Akinwale RO. Updates on nutritional diversity in *Sphenostylis stenocarpa* (Hoechst ex. A. Rich.) Harms. for food security and conservation. American J. of Agric. and Biological Sciences. 2018;13:

38-49.
Available:http://dx.doi.org/10.3844/ajabssp.2018.38.49

45. Nnamani CV, Ajayi SA, Oselebe HO, Atkinson CJ, Igboabuchi AN, Ezigbo EC. *Sphenostylis stenocarpa* (ex. A. Rich.) Harms., a fading genetic resource in a changing climate: prerequisite for conservation and sustainability. Plants. 2017;6(30):1-16.
Available:http://dx.doi.org/10.3390/plants6030030

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle3.com/review-history/49178