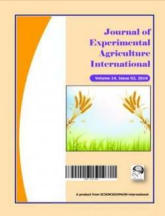

Journal of Experimental Agriculture International

17(3): 1-12, 2017; Article no.JEAI.26971
Previously known as American Journal of Experimental Agriculture
ISSN: 2231-0606

Genotyping of Pigeon Pea [*Cajanus cajan* (L.) Millsp.] Accessions Obtained from International Institute of Tropical Agriculture (IITA) Germplasm Using Random Amplified Polymorphic DNA

O. U. Udensi[1*], N. E. Edu[1], E. V. Ikpeme[2], O. O. Onung[1], L. I. Emeagi[1], B. I. Nwanze[1] and E. R. Ejiyere[1]

[1]Plant Genetic Resource Management and Genomics Unit, Department of Genetics and Biotechnology, University of Calabar, P.M.B. 1115, Calabar, Nigeria.
[2]Animal Genetics and Genomics Unit, Department of Genetics and Biotechnology, University of Calabar, P.M.B. 1115, Calabar, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author OUU designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors NEE and EVI reviewed the experimental design and all drafts of the manuscript. Authors OOO and LIE managed the analyses of the study. Authors BIN and ERE collected the accessions and did the bench work. Author OUU performed the statistical analysis. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JEAI/2017/26971
Editor(s):
(1) Lanzhuang Chen, Laboratory of Plant Biotechnology, Faculty of Environment and Horticulture, Minami Kyushu University, Miyazaki, Japan.
Reviewers:
(1) Abe Shegro Gerrano, University of the Free State – Department of Plant Sciences, South Africa.
(2) Karine Kettener, University of Sao Paulo State, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/20689

Original Research Article

Received 12th May 2016
Accepted 29th August 2016
Published 28th August 2017

ABSTRACT

Genotyping of crop plants, especially pigeon pea is very strategic in the global quest for crops that will be suitable for the precarious climate change as it provides genotypes for introgression. 22 pigeon pea accessions obtained from IITA germplasm were evaluated for genetic diversity. Genomic DNA was extracted using SDS protocol from young leaves of each accession and quantification was done. 12 RAPD primers were used for PCR DNA amplification and bands were visualized under UV light using ethidium bromide. Data analyses were computed using power

**Corresponding author: E-mail: princeuou4u@yahoo.com, princeudensi@unical.edu.ng;*

marker version 3.25 and GenAlex 6.41 software. Results obtained revealed that the polymorphic information content ranged from 0.6458-0.9436 while percentage polymorphism ranged from 71.4-100. Average percentage polymorphism was 88.6%. UPGMA- based cluster generated two major clusters with 12 and 10 accessions, respectively. Principal coordinates (PCoA) contributed 20.75% to the total diversity. Though showing two clusters, it was not population-dependent. AMOVA result gave 0.00% to variation among the population while variation within population was 100%. Additionally, genetic diversity parameters such as heterozygosity, mean Nei unbiased genetic distance, genetic identity were 0.015, 0.006 and 0.994, respectively. However, Shannon's information index, diversity and unbiased diversity were 1.858±0.02, 0.648±0.005 and 0.503±0.005. Pigeon pea accessions used in this present study revealed very narrow genetic diversity suggesting that they came from one population. The little variability observed may have been contributed by the accession TCc CITA 3. The implication of the results taking together is that further genotyping should be carried out using other DNA markers before explicit conclusion can be made.

Keywords: Pigeon pea; genotyping; RAPD; selection; introgression.

1. INTRODUCTION

According to [1], farming practice, especially in the developed countries of the world has evolved from subsistence agriculture to a market-orientated endeavour that is dominated by modern cultivars, which has resulted in the erosion of crop genetic resources, thus altering their diversity. For emphasis, landraces involve much of the original diversity as well as accumulated mutations and genetic recombination since domestication. As was decried by [2] and [3], these landraces are rapidly disappearing and are unfortunately being replaced by improved, higher yielding cultivars. The major challenge is the fact that concerns of climate change and its attendant problems such as drought, flooding, desertification, erosion, increased pests/diseases epidemics and their likes demand urgent search for landraces of crop plants that possess inherent capacity to withstand these biotic and abiotic stressors militating crop productivity. On the other hand, the existing diversity in crop species is not used to the extent for increased food productivity as well as improving the sustainability of production systems.

Interestingly, many global fora are now focusing energies in strategic planning and policy formulation towards mitigating the impact of climate change on agricultural productivity. Obviously, farmers and plant breeders are much interested in germplasm collection that possess or are likely to possess agronomic traits need for breeding programmes, which can be achieved through evaluation, characterization, identification as well as establishment of such crop plants. According to [4], leguminosae is the 3rd largest family of flowering plant with 800 genera and 20,000 species. Of the species, pigeon pea has been reported to be drought tolerant, which makes it best fit in the changing climatic conditions in the globe. Regrettably [5], observed that there is a narrow genetic diversity in the cultivated germplasm of pigeon pea, which retards efficient and effective utilization of the genetic resource. Broadening the genetic base of pigeon pea through injection into the gene pool pigeon pea genotypes with wide genetic base will obviously increase holistic performance. This could be done by introgressing useful agronomic traits identified through characterization/ evaluation into locally adapted or elite materials for use in breeding and base-broadening breeder's material through incorporation of genotypes with wide genetic base.

Economically, pigeon pea [*Cajanus cajan* (L.) Millsp.] being important pulse crop predominating grown in the tropics and semi-tropics with India as it centre of origin and diversity [6] is an excellent protein source and mineral elements [7,8,9,10], therapy for jaundice, inflammation, sores of the mouth [11], malaria [12]; fodder and feedstuff [13,14] and the improvement of soil fertility. These economic benefits notwithstanding, little research attention have been directed towards its breeding as well as improvement, especially as it pertains to the application of genomics tools [5,15,16, 17,18].

The extent of research in pigeon pea may not be unconnected with the breeding challenges. For instance, the cross-pollinated nature with an insect assistance limits the use of different selection and mating designs, possible in self-

pollinating species [19]. Though the production of two Genetic Male Sterility (GMS) systems [20] and subsequent commercialization was hampered due to high cost of hybrid seed production, it paved way to a more efficient system known as Cytoplasmic Genetic Male Sterility (CGMS) [21,22,23] producing GTH-1 and ICPH-2671 [24]. This however, seemed not to have unmasked the problems in pigeon pea breeding research. According to [5], the narrow genetic diversity in cultivated germplasm of pigeon pea possesses as hindrance to the optimization and maximization of both conventional breeding and molecular-based breeding. However, to be able to inject into the existing gene pool new species with the bid to broadening the genetic base, a more robust genetic diversity analysis becomes imperative.

Although there are shortcomings observed in the use of Random Amplified Polymorphic DNA (RAPD) in genetic diversity analysis of crop species, which borders, especially on its dominant status, which affects the precision of data, many researchers have reported its use in pigeon pea genetic diversity studies [25,26, 27,28] with varying results on genetic diversity parameters. This present study seeks to evaluate the genetic diversity of some pigeon pea accessions obtained from IITA, Ibadan using RAPD fingerprinting with the view to preliminarily selecting accessions for breeding and subsequent improvement.

2. MATERIALS AND METHODS

2.1 Seed Collection and Green House Planting

Twenty two pigeon pea accessions were obtained from the Genetic Resource Unit (GRU) of International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. These were; TCc 1; TCc 2; TCc 4; TCc 6; TCc CITA2; TCc 8; TCc CITA 1; TCc CITA 5; TCc ICPL 87; TCc CITA 3; TCc 8127; TCc 8126; TCc 151; TCc 8104; TCc 8111; TCc 8125; TCc 8129; TCc 87; TCc 8863; TCc Ao 78-99; TCc Ao/7B-9 and TCcp 5877-161. Three seeds of each accession were sown per hole in plastic pots at 3-4 cm deep in a completely randomized design (CRD). Young leaves were harvested from each accession 14 days post seedling emergence, labelled in bags and stored at -80ºC prior to DNA extraction.

2.2 Genomic DNA Extraction and Quantification

DNA was isolated from young fresh leaves using the SDS (Sodium dodecyl sulphate) method of extraction [29] as modified by IITA. The leaves were freeze dried, cut into sizeable pieces and then transferred into extraction tubes containing steel balls for grinding using the electric genomic grinder (Model 2000). 700 µl of extraction buffer (1M Tris-HCl at pH of 8.0, 5M NaCl, 0.5M EDTA at pH 8.0, 20% SDS, 1% PVP, 2% β-mercaptoethanol, 70% ethanol, 25 ml of CIA (chloroform Isoamylalchohol), 5M potassium acetate) were added. The suspension was mixed well and placed in a water bath for 25 mins at 65ºC. Sample was removed from the water bath and placed on ice to cool for 5 mins and 200 µl of 5M Potassium acetate was added to the mixture and carefully inverted and placed on ice for 20 mins. 350 µl of CIA (Chloroform-isoamylalchohol) was added in a ratio of 24:1, mixed gently and centrifuged at 5000 g for 10 mins. Supernatant was decanted into eppendorf tubes containing iced cold isopropanol in a ratio of 2:3 (400 µl of supernatant: 600 µl of Isopropanol) and mix gently for 2-3 mins. The mixture was placed in -20ºC freezer for 30 mins to enhance precipitation. Precipitated DNA was collected by centrifugation at 4000 g for 20 mins and supernatant discarded.

300 µl of 70% alcohol was used to rinse the DNA pellets twice. Pellets were dried until no trace of alcohol was noticed. At this point 110 µl of TE buffer and 1 µl of RNase A were added to remove RNA molecules that may be present, gently mixed and incubated for 30 mins at room temperature. The purified DNA was quantified by using Nanodrop spectrophotometer (ND-1000). The quality of genomic DNA was checked by using 1.5% agarose in the presence of Ethidium bromide. DNA samples were stored at -20ºC until further analysis.

2.3 RAPD Analysis

Twenty RAPD primers (Eurofins MWG Operon LLC Technologies, USA) were screened for PCR amplification. Primers used were carefully chosen from previous genetic diversity studies on legumes [30]. After preliminary testing on a few sample, twelve primers that gave clear polymorphic and reproducible bands patterns were selected to assess the genetic variability of the landraces selected.

Table 1. List of RAPD primers used for PCR analysis

S/N	Primer	Sequence (5' ⟶ 3')	% G+C content	Number of base pairs
1	OPHO5	AGTCGTCCCC	70	10
2	OPBO7	GGTGACGCAG	70	10
3	OPHO8	GAAACACCCC	60	10
4	OPB10	CTGCTGGGAC	70	10
5	OPT14	AATGCCCTGG	60	10
6	OPT17	CCAAGGTCGT	60	10
7	OPBO2	TGATCCCTGG	70	10
8	OPHO4	GGAAGTCGCC	70	10
9	OPHO6	ACGCATCGCA	60	10
10	OPT11	TTCCCCGCGA	70	10
11	OPT15	GGATGCCACT	60	10
12	OPT19	GTCCGTATGG	60	10

RAPD analysis was carried out in a total reaction volume of 25 µl containing 10x buffer (2.5 µg), $MgCl_2$ (1.2 µl), DNTPs (2.0 µl), DMSO (1.0 µl), primers (1.0 µl), Taq polymerase (0.3 µl), DNA sample (2.0 µl) and H_2O (15 µl), making the cocktail. Amplification was performed in thin–walled PCR tubes using a thermo–cycler (Eppendorf Germany, GeneAmp PCR system 9700) programmed for initial denaturation at 94ºC for 2 mins followed by 40 cycles of denaturation at 94ºC for 1 min, 37ºC for 1 min and 72ºC for 2 mins. The amplification was completed within 7 min of final extension for 72ºC. The amplified products were subjected to gel electrophoresis of 1.5% agarose gels stained with 0.5 µl/ml ethidium bromide solution. A 50 bp DNA ladder was used as molecular weight markers for comparison of the amplified products. The DNA bands were visualized under UV light and gel images taken using Gel Documentation System (Enduro TM GDS Imager). The reaction was repeated twice to test for reproducibility of RAPD markers. Table 1 above is details of the RAPD primers used.

2.4 Data Analysis

The amplified products from RAPD analysis were scored visually based on the presence as (`1`) or absence (`0`) of bands for each primer. Each RAPD fragment was treated as a unit character and only clear and unambiguous bands were scored. For each primer, the number of different bands and the frequency of polymorphic bands were calculated. The data obtained was used to generate a distance matrix for expressed RAPD and to construct a dendrogram using Unweighted Pair Group Method Arithmetic Mean (UPGMA) as imputed in the computer package Power marker version 3.25 [31] as well as for cluster analysis. Analysis of molecular variance (AMOVA) was done to analyze the intra– and inter – variability of landraces and this was imputed in the GenAlex 6.41 software [32]. Additionally, principal coordinate analysis was carried out using the GenAlex software.

3. RESULTS AND DISCUSSION

3.1 Genetic Parameters and Percentage Polymorphism of RAPD Markers Used

Generally, PICs for all the primers used were comparatively high. Major allele frequency ranged from 0.0909-0.5000 with mean of 0.2386±0.034 while genetic diversity ranged from 0.6818-0.9463 with 0.8650±0.022 as mean. The mean polymorphic information content was 0.8521±0.024. Specifically, OPH08 gave the highest major allele frequency of 0.5000 with OPT14 having the least. However, OPH04 had the highest number of allele, followed by OPT14. OPH04 had the highest gene diversity, which was followed by OPT14. Polymorphic Information Content (PIC) for OPH04 was the highest (0.9436), which was followed by OPT14 (0.9301) (Table 2). From Table 3, it was observed that primer OPH05, OPH08, OPB02 and OPH06 revealed 100 percent polymorphism. The 12 RAPD primers used gave a mean percentage polymorphism of 89%. The implication is that the primers used were good enough for diversity study in pigeon pea accessions.

Table 2. Genetic parameters of the RAPD markers used as revealed by Power marker Computer Software

RAPD markers	Major allele frequency	Allele number	Gene diversity	PIC
OPH05	0.3636	10.0000	0.8140	0.7975
OPB07	0.1818	13.0000	0.9008	0.8927
OPH08	0.5000	7.0000	0.6818	0.6458
OPB10	0.2273	10.0000	0.8636	0.8494
OPT14	0.1364	18.0000	0.9339	0.9301
OPT17	0.1818	11.0000	0.8843	0.8732
OPB02	0.1364	16.0000	0.9215	0.9162
OPH04	0.0909	20.0000	0.9463	0.9436
OPH06	0.3636	8.0000	0.7810	0.7536
OPT11	0.2727	12.0000	0.8678	0.8564
OPT15	0.2273	15.0000	0.9008	0.8939
OPT19	0.1818	11.0000	0.8843	0.8732
Mean	0.2386±0.034	12.5833±1.145	0.8650±0.022	0.8521±0.024

Table 3. Percentage polymorphism of RAPD primers used

RAPD markers	Number of bands	Number of polymorphic bands	Percentage polymorphism (%)
OPH05	7	7	100.0
OPB07	5	4	80.0
OPH08	8	8	100.0
OPB10	7	5	71.4
OPT14	10	9	90.0
OPT17	8	7	87.5
OPB02	9	9	100.0
OPH04	10	9	90.0
OPH06	5	5	100.0
OPT11	6	5	83.3
OPT15	7	6	85.7
OPT19	4	3	75.0
Mean	7.2±0.56	6.4± 0.6	88.6±2.9

3.2 Cluster Analysis Based on UPGMA Dendrogram

Two major clusters A and B were revealed by the analysis. Cluster A has 12 pigeon pea accessions while cluster B had 10 accessions (Fig. 1). TCc 1 and TCc CITA 3 though found in cluster A and B, respectively, are quite diverse from other accessions from the same cluster. During the course of the analysis, we could not retrieve from IITA germplasm unit the location from where these accessions were obtained. As such we assumed that cluster A and B represent two different pigeon pea populations.

3.3 Principal Coordinate Analysis (PCoA)

The result showed that the first axis contributed 7.50% while the second contributed 6.82% to the genetic diversity in pigeon pea accessions studied. The third axis gave 6.43% giving a cumulative percentage of 20.75%. The result revealed that the distribution on the different coordinates was not dependent on the assumed population of pigeon pea. From the Fig. 2, it can be observed that there are two major clusters. The first cluster is composed of 9 accessions while second cluster has 12 accessions. However, TCc CITA 3 was independent of the major clusters.

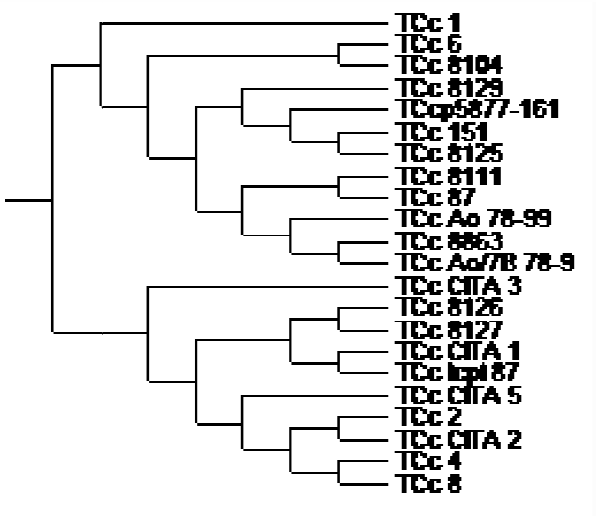

Fig. 1. Dendrogram based on UPGMA clustering of 22 pigeon pea accessions

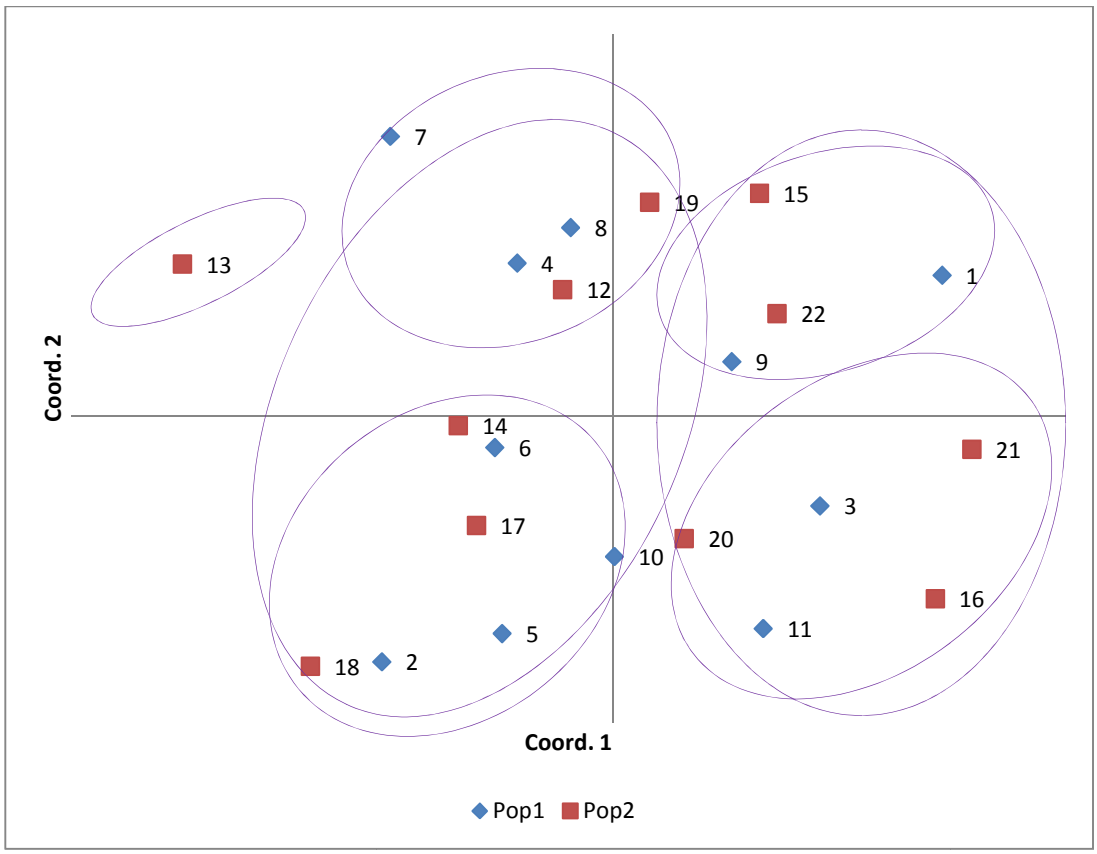

Fig. 2. Principal coordinate analysis (PCoA) of pigeon pea accessions using GenAlex computer software

1=TCc1; 2=TCc 6; 3=TCc 8104; 4=TCc 8129; 5=TCcp 5677-161; 6=TCc 151; 7=TCc 8125; 8=TCc 8111; 9=TCc 87; 10=TCcAo 78-99; 11=TCc 8863; 12=TCcAo7B-78-9; 13=TCc CITA 3; 14=TCc 8126; 15=TCc 8127; 16=TCc CITA 1; 17=TCc Icpl 87; 18=TCc CITA 5; 19=TCc 2; 20=TCc CITA 2; 21=TCc 4; 22=TCc 8

3.4 Analysis of Molecular Variation and Band Patterns across Populations

Analysis of molecular variation (AMOVA) is aimed of evaluating variability on the molecular level. The result showed that variation among the populations was 0% while molecular variation within the populations was 100%. The band pattern across the populations is as shown on Fig. 3. It revealed that heterozygosity was 0.015 with no private bands. Genetic diversity for population 1 was 0.448±0.07 while population was 0.466±0.006. Unbiased genetic diversity estimates was 0.493±0.009 and 0.512±0.006 for the different population. Nei genetic distance comparing the population was 0.086 while Nei genetic identity was 0.918. However, Nei unbiased genetic distance was 0.006 while Nei genetic identity was 0.994.

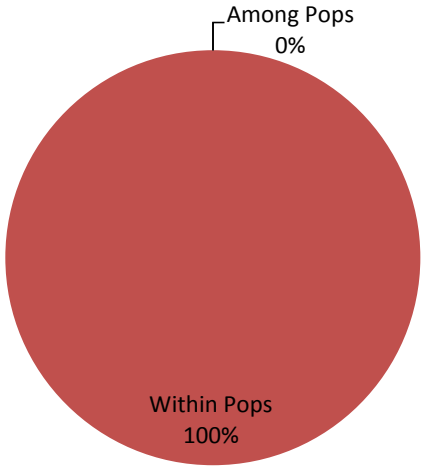

Fig. 3. Percentage molecular variation in pigeon pea accessions obtained from IITA, Ibadan

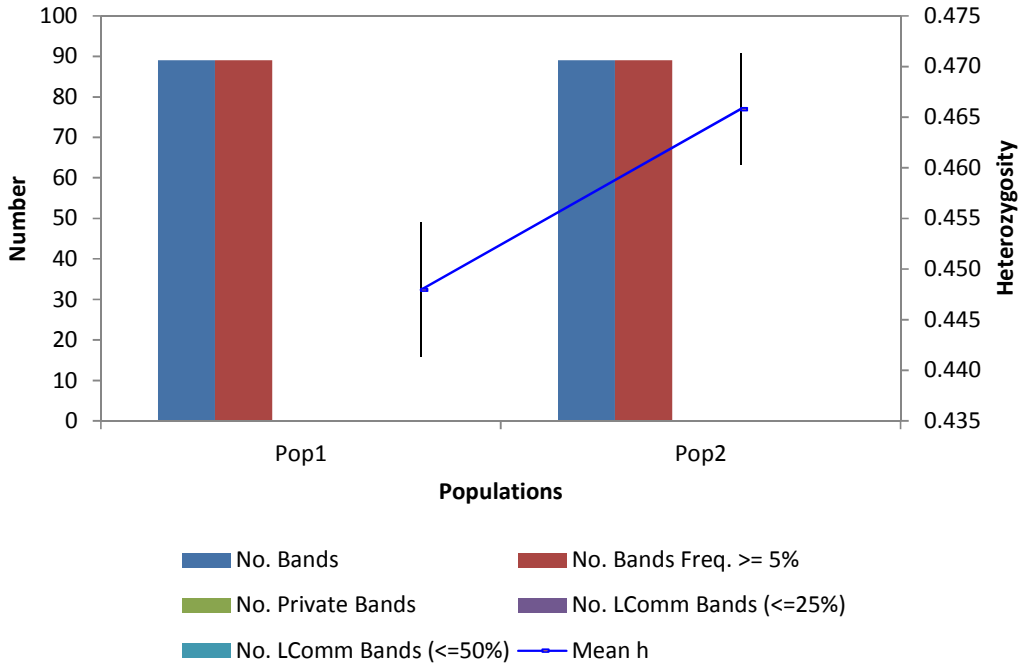

Fig. 4. Band patterns across pigeon pea populations studied

3.5 Linear Genetic Distance among Pigeon Pea Accessions

The linear genetic distance result revealed that most of the pigeon pea accessions have narrow genetic distance, which ranged from 5.657 to 7.810. However, the linear genetic distance between TCc 8127 and TCcCITA 5 was the widest (7.810) while TCc 5877-161 and TCcAo 78-99 as well as TCc 8 and TCcCITA 3 showed the narrowest linear genetic distance of 5.657 (Table 2). TCc 1 had a linear genetic distance of 7.550 with TCcCITA 5 while TCc 6 was 7.348 distant from TCc 8.

3.6 Genetic Diversity Parameter over Loci for Each Population and over Loci and Population

Comparing the population (clusters), all the genetic parameters were higher in population 2 than population 1 (Fig. 3). For example, Shannon's information index is 0.638±0.007 in population 1 while 0.657±0.006 in population 2. Unbiased diversity in population 1 is 0.493±0.007 while in population 2 it is 0.512 ±0.006. However, when these genetic parameters were computed over loci and population number of effective alleles, Shannon's information index, diversity and unbiased diversity were 1.858±0.02, 0.648±0.005, 0.457±0.004 and 0.503±0.005, respectively.

3.7 Discussion

Plant breeders and famers, especially pigeon pea should pay greater attention on plant genetic resources if food security and sustainability is worth combating. This is premised on the fact that their diversity evaluation generates baseline data to guide selection of parental lines used in designing breeding scheme [33], identify breeding stock for crop improvement [34].

According to [34], molecular genetic techniques using DNA polymorphism have been increasingly used to characterize and identify novel germplasm for use in the crop breeding scheme. Among these molecular markers, Random Amplified Polymorphic DNA (RAPD) has been adopted widely for the identification of genetic relationships among grain legumes [35,36,37,38, 39,40]. Fingerprinting populations of crops using RAPD markers has been reportedly used in the determination of cultivar purity, identification of diverse genotypes for hybridization, providing genetic barriers against different biotic and abiotic stressors [41], genetic mapping as well as markers-assisted selection in crop breeding [42].

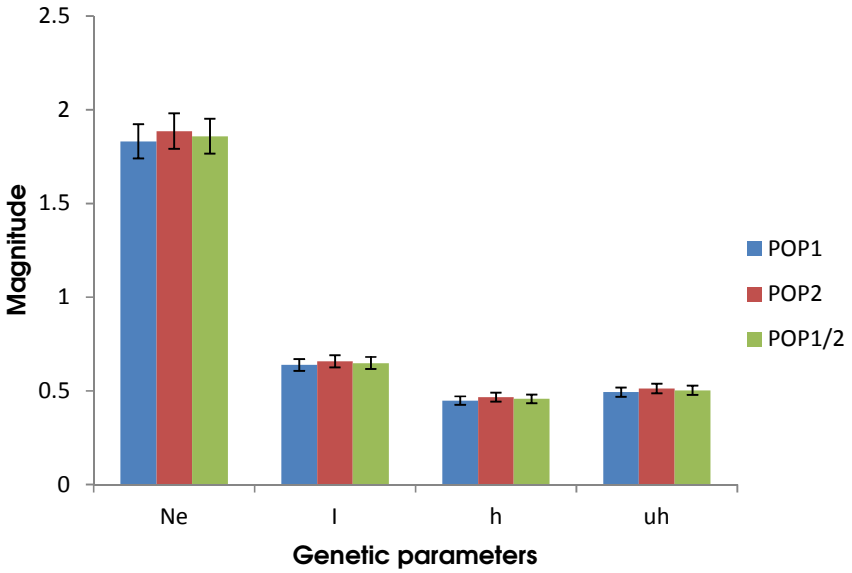

Fig. 5. Genetic parameters of pigeon pea accessions as recorded by GenAlex Software
(Note: Ne=Number of effective alleles; I=Shannon's information index; h=Genetic diversity; uh=Unbiased diversity)

Percentage polymorphism revealed by the 12 RAPD markers used in the current work was 88.6%. This suggests high percentage polymorphism. [27] reported 56.52% polymorphism using 15 pigeon pea genotypes with 5 RAPD primers while however, reported 71.2% polymorphic bands in their experiment using 17 pigeon pea genotypes with 17 RAPD markers. The percentage polymorphism reported in the current study showed that the primers used were suitable for assessing genetic diversity in the pigeon pea accessions investigated. [43] observed that high polymorphism revealed by molecular markers is due to the presence of repeated sequences AC, CA, AG and GA. This was corroborated by [44]. It can be observed that the primers that gave 100% polymorphism (OPH08 and OPH06) had the repeated sequences as reported by [43]. OPH05 and OPB02 that also gave 100% polymorphism had no repeated sequence. It might probably suggest that the ability to resolve genetic variation in any crop species germplasm is more directly correlated to the number of polymorphism detected by the marker technology.

Additionally, the results showed that the Polymorphic Information Content (PIC) for the primers used ranged 0.6458- 0.9436 with OPH04 having the highest value of PIC (0.9436). PIC results as reported by [27] reported PIC of 0.076-0.25 while [9] gave PIC of 0.11-0.91. What it does suggest is that the PIC as reported by [9] was closer to our present PIC result. However, it will be wise to mention that any conclusion made using PIC results might be misleading as the primer used, the number of primer used as well as the number of species/accessions differ.

Cluster analysis using UPGMA-based dendrogram revealed two major clusters A and B where cluster A has 12 accessions and cluster B had 10 pigeon pea accessions. During the course of the research, we were unable to determine the different locations from which the accessions were adopted from. This notwithstanding, we assumed that the two clusters were different pigeon pea populations. This was to aid in inputting the genetic data into GenAlex software. The result obtained from the principal coordinate analysis (PCoA) revealed that the three coordinates gave 20.75% contribution to the variability. The accessions were clustered or grouped in the different coordinates, the population notwithstanding. According to [45] who studied genetic diversity in cowpea accessions reported 36.4% of their PCoA axes with 20 accessions. This was higher than the present report though with pigeon pea. This suggests that there was narrow genetic diversity in pigeon pea accessions studied.

Also the results for analysis of molecular variance (AMOVA) showed that variation among the populations was 0% while molecular variation within the populations was 100%. However, [45] reported 4% and 96%, respectively in cowpea. The implication of the result is that the variability or diversity accounted by the principal coordinate analysis was concentrated within the accessions, suggesting that the different pigeon pea accessions obtained from IITA, Ibadan would have come from the same population. Taking the results obtained from the cluster analysis, PCoA and AMOVA together, the bifurcation of the dendrogram into two clusters A and B may have been as a result of the inherent genetic differences among the accessions as revealed by the PCoA and AMOVA.

Using GenAlex software, the two populations revealed heterozygosity 0.015 with no private bands. Genetic diversity for population 1 was 0.448 ± 0.07 while population 2 was 0.466 ± 0.006. Unbiased genetic diversity estimates was 0.493 ± 0.009 and 0.512 ± 0.006 for the different population. Nei genetic distance comparing the populations was 0.086 while Nei genetic identity was 0.918. However, Nei unbiased genetic distance was 0.006 while Nei genetic identity was 0.994. It could be observed from the results that the genetic identity was very high corroborating the AMOVA result. This importantly goes further to validate the narrow genetic diversity in the pigeon pea accessions investigated.

The linear genetic distance between TCc 8127 and TCcCITA 5 was the widest (7.810) while TCc 5877-161 and TCcAo78-99 as well as TCC 8 and TCcCITA 3 showed the narrowest linear genetic distance of 5.657. TCc1 had a linear genetic distance of 7.550 with TCc CITA 5 while TCc 6 was 7.348 distant from TCc 8. Obviously, the wider the genetic distance, the more diverse will be the accessions concerned [45]. From the present result, TCc8127 and TCcCITA 5 pigeon pea accessions showed the widest genetic distance of 7.810. From the PCoA, the two accessions occupy different clusters, which might be the integral source of the variability recorded.

4. CONCLUSION

The present results are explicit indication that the 22 pigeon pea accessions obtained from IITA, Ibadan have narrow genetic diversity as revealed by the RAPD fingerprinting. This might imply that selection of accessions from this population as a breeding stock may not be promising.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Snook LK, Dulloo ME, Jarvis A, Scheldeman X, Kneller M. Crop germplasm diversity: The Role of gene bank collections in facilitating adaptation to climate change. In: Crop Adaptation to Climate Change. Ch. 25. 2011;495–506.
2. Bao S, Yuhua H, Zong X, Wang L, Lichi L, Enneking D, Rose IA, Leonforte T, Redden RJ, Paull J. Collection of pea (*Pisum sativum*) and faba bean (*Vicia faba*) germplasm in Yunnan. Plant Genet. Res News. 2008;156:11–22.
3. He C, Liu Y, Wu K, Yuan M, Feng Q, Liu Y, Yan Q, Guan J, Rose IA, Redden RJ, Enneking D. Collecting and surveying landraces of pea (*Pisum sativum*) and faba bean (*Vicia faba*) in Qinghai province of China. Pl. Genet. Res. News. 2008;156:1–10.
4. Lewis G, Schrire B, Mackinder B, Lock M. Legumes of the World. Royal Botanic Gardens, Kew, UK; 2005.
5. Varshney RK, Close TJ, Singh NK, Hoisington DA, Cook DR. Orphan legume crops enter the genomics era! Curr. Opin. Plant Biol. 2009;12:202–210.
6. Saxena K, Kumar R, Sultana R. Quality nutrition through pigeon pea. A Review; Health. 2010;2:1335–1334.
7. Center for New Crops and Plants Products. *Cajanus cajan* (L.) Millsp. Purdue University; 2002. Available:http://www.hort.purdue.edu/newcrop/duke_energy/Cajanus_cajun.html
8. Olawuyi OJ, Fawole I. Studies on genetic variability of some quantitative and qualitative characters in pigeon pea (*Cajanus cajan* (L.) Millsp.) (Fabaceae). Acta SATECH. 2005;2(1):30-36.
9. Barbosa de Souse AC, Godoy R, Sforca DA, Compos T, Zucchi MI, Jank L, Pereira de Souse A. Genetic diversity among pigeon pea genotypes adapted to South American regions based on microsatellite markers. Sci. Agric. (Piracicaba, Braz.). 2011;68(4):431-439.
10. Singh NK, Gupta DK, Jayarswai PK, Mahoto AK, Dutta S, Singh S, et al. The first draft of the pigeon pea genome sequence. Journal of Plant Biochemistry and Biotechnology. 2012;21:98–112.
11. Parrotta JA. Healing plants of peninsular India. New York: CABI Publishing; 2001.
12. Aiyeloga AA, Bello OA. Ethnabotanical potentials of common herbs in Nigeria: A case study of Enugu State. Educational Research and Review. 2006;1:116–22.
13. Philip TA. An agricultural notebook. McGraw Hill Publisher, London; 2002.
14. Rao SC, Coleman SW, Mayeux HS. Forage production and nutritive value of selected pigeon ecotypes in the Southern great plains. Crop Science. 2002;42:1259-1263.
15. Kui GYP, Amoatey HM, Bansa D, Kumaga FK. Cultivation and use of Africa yam beans (*Sphenostylis stenocarpa*) in the Volta region of Ghana. J. Food and Technol. Africa. 2001;6:74-77.
16. Naylor RB, Falcon WP, Goodman RM, Jahn MM, Sengooba T, Tefera H, Nelson RJ. Biotechnology in the developing world: A case for increased investments in orphan crops. Food Policy. 2004;29:15-44.
17. Odeny DA, Jayashree B, Gebhardt C, Crouch J. New microsatellite markers for pigeon pea (*Cajanus cajan* L. Millsp.). BMC Res. Notes. 2009;2:35.
18. Saxena RK, Prathima C, Saxena KB, Hoisington DA, Singh NK, Varshney RK. Novel SSR markers for polymorphism detection in pigeon (*Cajanus* spp.). Plant Breed; 2009. DOI: 10.1111/J.1439-0523.2009.01680.x
19. Saxena KB, Singh L, Gupta MD. Variation for natural out-crossing in pigeon pea. Euphytica. 1990;46:143–148.
20. Saxena KB, Wallis ES, Byth DE. A new gene for male-sterility in pigeon pea (*Cajanus cajan* (L.) Millsp.). Heredity. 1983;51:419-421.
21. Tikka SBS, Parmar LD, Chauhan RM. First record of cytoplasmic-genic male-sterility system in pigeon pea (*Cajanus cajan* (L.) Millsp.) through wide hybridization. GAU Res. J. 1997;22(2):160–162.
22. Saxena KB, Kumar RV. Development of a cytoplasmic nuclear male-sterility system

in pigeon pea using *C. scarabaeoides* (L.) Thouars. Indian J. Genet. 2003;63(3):225–229.

23. Wanjari KB, Patel MC. Fertility restorers isolated from germplasm for cytoplasmic male sterility in pigeon pea. PKV Res. J. 2003;27:111-113.
24. Saxena KB. Genetic improvement of pigeonpea—a review. Trop. Plant Biol. 2008;1:159–178.
25. Malviya N, Yadav D. RAPD analysis among pigeon pea (*Cajanus cajan* (L.) Millsp.) cultivars for their genetic diversity. Genetic Engineering and Biotechnology Journal. 2010;1:1-9.
26. Walunjkar B, Parihar A, Chaurasia P, Pachchigar K, Chauhan RM. Genetic analysis of wild and cultivated germplasm of pigeon pea using random amplified polymorphic DNA (RAPD) and simple sequence repeats (SSR) markers. African Journal of Biotechnology. 2013;12(40): 5823-5832.
27. Shende S, Raut A. Analysis of genetic diversity in pigeon pea (*Cajanus cajan* L.) by using PCR-based molecular markers. Recent Research in Science and Technology. 2013;5(2):20-23.
28. Yadav SK, Yadav A, Padey VP. Comparative analysis of genetic diversity among cultivated pigeon pea (*Cajanus cajan* (L.) Millsp.) and its wild relatives (*C. albicans* and *C. lineatus*) using random amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR) fingerprinting. American Journal of Plant Sciences. 2014;5:1665-1678.
29. Dellaporta SL, Wood J, Hicks JB. A plant DNA minipreparation: Version II. Plant Mol. Biol. Rep. 1983;1:19-21.
30. Ba FS, Pasquet RS, Gepts P. Genetic diversity in cowpea [*Vigna unguiculata* (L.) Walp.] as revealed by RAPD markers. Genet. Resour. Crop Evol. 2004;51:539-550.
31. Liu K, Muse SV. Power Marker: An integrated analysis environment for genetic marker analysis. Bioinformatics. 2005;21: 2128-2129.
32. Peakall R, Smouse PE. GenAlex 6: Genetic analysis in excel. Population genetic software for teaching and research. Mol. Ecol. Notes. 2006;6:288-295.
33. Khan SM, Ahmad H, Nisar M, Aslam S, Inamullah A, Ali N. Genetic diversity in pea germplasm using randomly amplified polymorphic markers. Pakistan Journal of Botany. 2013;45(4):1259–1264.
34. O'Neil R, Snowdon RJ, Kohler W. Population genetic aspects of biodiversity. Progress in Botany. 2003;64:115-137.
35. Skroch PW, Nienhuis J. Quantitative and qualitative characterization of RAPD variation among snap bean (*Phaseocus vulgaris*) genotypes. Theoretical and Applied Genetics. 1995;9:1078–1085.
36. Mignouna HD, Ng NQ, Ikea J, Thottappilly G. Genetic diversity in cowpea as revealed by random amplified polymorphic DNA. Journal of Genetic Breed. 1998;52:151–159.
37. Beebe S, Skroch PW, Tohme J, Duque MC, Pedraza F, Nienhius J. Structure of genetic diversity among common bean landraces of middle. American Origin based on correspondence analysis of RAPD. Crop Science Society of America. 2000;40:264–273.
38. Brown-Guedira GL, Thompson JA, Nelson RL, Warburton ML. Evaluation of genetic diversity of soybean introductions and North American ancestors using RAPD and SSR markers. Crop Science Society of America. 2000; 40:115–223.
39. Amadou HI, Bebeli PJ, Kaltsikes PJ. Genetic diversity in bambara groundnuts (*Vigna suberranea* L.) germplasm revealed by RAPD markers. Genome. 2001;44:995-999.
40. Tosti N, Negri V. Efficiency of three PCR-based markers in assessing genetic variation among cowpea (*Vigna unguiculata* Sub. Sp. Unguiculata) Landraces. Genome. 2002;45:268–275.
41. Hughes AR, Stachowicz JJ. Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. Proc. Natl. Acad. Sci. USA. 2004;101:8998-9002.
42. Lapitan VC, Brar DS, Abe T, Redona ED. Assessment of genetic diversity of Philippine rice cultivars carrying good quality traits using SSR markers. Breed. Sci. 2007;57:263-270.
43. Ghalmi N, Malice M, Jacquemin JM, Ounane SM, Mekliche L, Baudoin JP. Morphological and molecular diversity within Algerian cowpea (*Vigna unguiculata* (L.) Walp.) landraces. Genet. Resour. Crop Evol. 2010;57:371-386.

44. Ajibade SR, Weeden NF, Chite SM. Inter simple sequence repeat analysis of genetic relationships in the genus Vigna. Euphytica. 2000;111:47-55.
45. 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). Int. J. Plant Breed. Genet. 2016;10:12-22.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/20689