

Original Research Article

GENOTYPING OF PIGEON PEA (*Cajanus cajan* (L.) Millsp.) ACCESSIONS OBTAINED
FROM INTERNATIONAL INSTITUTE OF TROPICAL AGRICULTURE (IITA) GERMPLASM USING
RANDOM AMPLIFIED POLYMORPHIC DNA

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. RAPD primers were used for PCR DNA amplification and bands were visualized under UV light using ethidium bromide. Data analyses were computed using power 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

Introduction

According to Snook *et al.* (2011), 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 Bao *et al.* (2008) and He *et al.* (2008), 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 Lewis *et al.* (2005), 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, Varshney *et al.* (2009), 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 its centre of origin and diversity (Saxena *et al.*, 2010) is an excellent protein source and mineral elements (CNCP, 2002; Damadi *et al.*, 2004; Olawuyi and Fawole, 2005; Sousa *et al.*, 2011; Singh *et al.*, 2012), therapy for jaundice, inflammation, sores of the mouth (Parrot, 2001), malaria (Aiyeloja and Bello, 2006); fodder and feedstuff (Phillips, 2002; Rao *et al.*, 2002) and the improvement of soil fertility (Damadi *et al.*, 2004). 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 (Kui *et al.*, 2001; Naylor *et al.*, 2004; Varshney *et al.*, 2009; Odeny *et al.*, 2009; Saxena *et al.*, 2009).

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

(Saxena et al., 1990). Though the production of two Genetic Male Sterility (GMS) systems (Saxena et al., 1983) 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)(Tikka et al., 1997; Saxena and Kumar, 2003; Wanjari and Patel, 2003) producing GTH-1 and ICPH-2671 (Saxena et al., 2008). This however, seemed not to have unmasked the problems in pigeon pea breeding research. According to Varshney et al. (2009), 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 (Yadav et al., 2007; Malviya and Yadav, 2010; Walunjkar et al., 2013; Shende and Raut, 2013; Yadav et al., 2014) 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.

100 **Materials and methods**

101 **Seed collection and green house planting**

102 Twenty two pigeon pea accessions were obtained from the Genetic Resource Unit
103 (GRU) of International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. These
104 were; TCc 1; TCc 2; TCc 4; TCc 6; TCc CITA2; TCc 8; TCc CITA 1; TCc CITA 5; TCc ICPL
105 87; TCc CITA 3; TCc 8127; TCc 8126; TCc 151; TCc 8104; TCc 8111; TCc 8125; TCc
106 8129; TCc 87; TCc 8863; TCc Ao 78-99; TCc Ao/7B-9 and TCcp 5877-161. Three seeds
107 of each accession were sown per hole in plastic pots at 3-4cm deep in a completely
108 randomized design (CRD). Young leaves were harvested from each accession 14
109 days post seedling emergence, labelled in bags and stored at -80°C prior to DNA
110 extraction.

111 **Genomic DNA extraction and quantification**

112 DNA was isolated from young fresh leaves using the SDS (Sodium dodecyl
113 sulphate) method of extraction (Dellaporta *et al.*, 1983) as modified by IITA. The
114 leaves were freeze dried, cut into sizeable pieces and then transferred into
115 extraction tubes containing steel balls for grinding using the electric genomic grinder
116 (Model 2000). 700µl of extraction buffer (1M Tris-HCl at pH of 8.0, 5M NaCl, 0.5M EDTA
117 at pH 8.0, 20% SDS, 1% PVP, 2% β-mercaptoethanol, 70% ethanol, 25ml of CIA
118 (chloroform Isoamylalcohol), 5M potassium acetate) were added. The suspension
119 was mixed well and placed in a water bath for 25mins at 65°C. Sample was
120 removed from the water bath and placed on ice to cool for 5mins and 200µl of 5M

Potassium acetate was added to the mixture and carefully inverted and placed on ice for 20mins. 350µl of CIA (Chloroform-isoamylalcohol) was added in a ratio of 24:1, mixed gently and centrifuged at 5000g for 10 mins. Supernatant was decanted into eppendorf tubes containing iced cold isopropanol in a ratio of 2:3 (400µl of supernatant: 600µl of Isopropanol) and mix gently for 2-3 mins. The mixture was placed in -20°C freezer for 30mins to enhance precipitation. Precipitated DNA was collected by centrifugation at 4000g for 20mins and supernatant discarded.

300µl of 70% alcohol was used to rinse the DNA pellets twice. Pellets were dried until no trace of alcohol was noticed. At this point 110µl of TE buffer and 1 µl of RNase A were added to remove RNA molecules that may be present, gently mixed and incubated for 30mins 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 -20°C until further analysis.

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 (Ba *et al.*, 2004). 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.

RAPD analysis was carried out in a total reaction volume of 25µl containing 10xbuffer (2.5µg), MgCl₂ (1.2µl), DNTPs (2.0µl), DMSO (1.0µl), primers (1.0µl), Taq polymerase (0.3µl), DNA sample (2.0µl) and H₂O (15µl), 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 94°C for 2 mins followed by 40 cycles of denaturation at 94 °C for 1min, 37 °C for 1 min and 72 °C for 2 mins. The amplification was completed within 7 min of final extension for 72 °C. The amplified products were subjected to gel electrophoresis of 1.5% agarose gels stained with 0.5µl/ml ethidium bromide solution. A 50bp 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. Details of the RAPD primers used are seen below.

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

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 (Liu *et al.*, 2005) 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 (Peakall and Smouse, 2006). Additionally, principal coordinate analysis was carried out using the GenAlex software.

Results and Discussion

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.

192 Table 2: Genetic parameters of the RAPD markers used as revealed by Power
193 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

194

195 Table 3: Percentage polymorphism of RAPD primers used

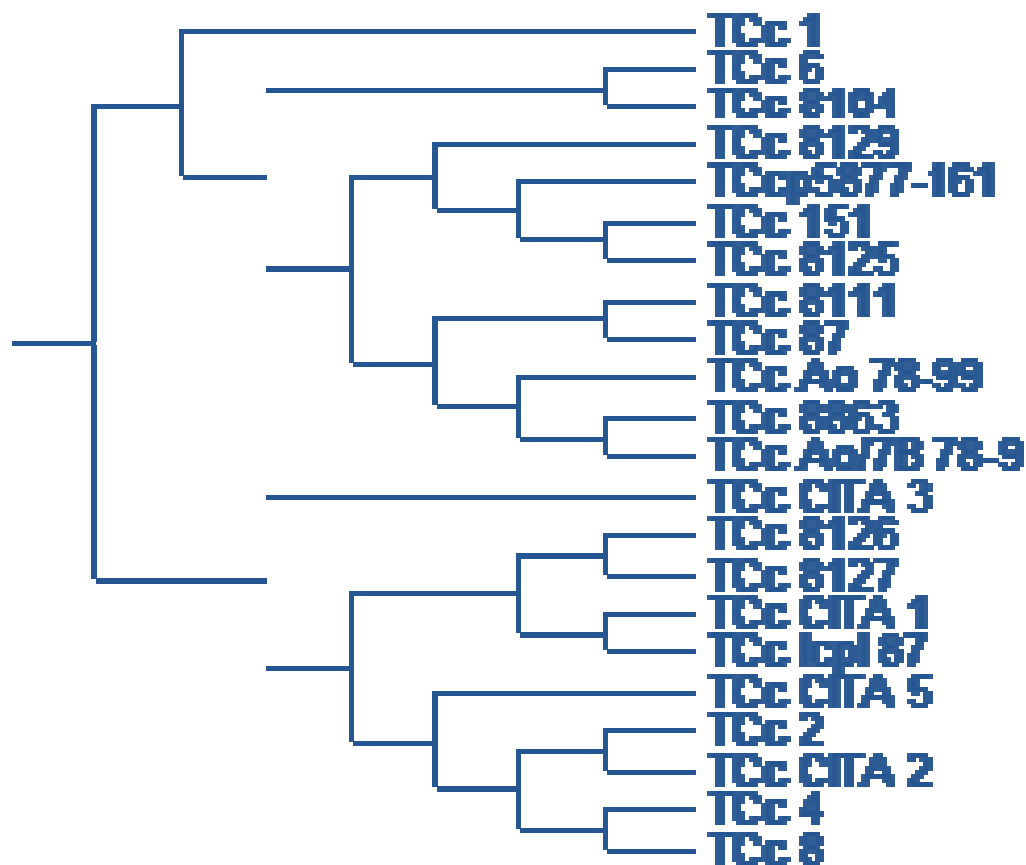
RAPD Markers	Number of bands	Number of polymorphic bands	Percentage polymorphism (%)
OPH05	7	7	100.0
OPB07	5	4	80.0
OPH08	8	8	100.0
OPB10	7	5	71.4
OPT14	10	9	90.0
OPT17	8	7	87.5
OPB02	9	9	100.0
OPH04	10	9	90.0
OPH06	5	5	100.0
OPT11	6	5	83.3
OPT15	7	6	85.7
OPT19	4	3	75.0
Mean	7.2±0.56	6.4± 0.6	88.6±2.9

196

197 Cluster analysis based on UPGMA dendrogram

198 Two major clusters A and B were revealed by the analysis. Cluster A has 12 pigeon
199 pea accessions while cluster B had 10 accessions (Fig. 1). TCc 1 and TCc CITA 3
200 though found in cluster A and B, respectively, are quite diverse from other
201 accessions from the same cluster. During the course of the analysis, we could not
202 retrieve from ILTA germplasm unit the location from where these accessions were

203 obtained. As such we assumed that cluster A and B represent two different pigeon
204 pea populations.



205

206 Figure 1: Dendrogram based on UPGMA clustering of 22 pigeon pea
207 accessions

208 **Principal coordinate analysis (PCoA)**

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

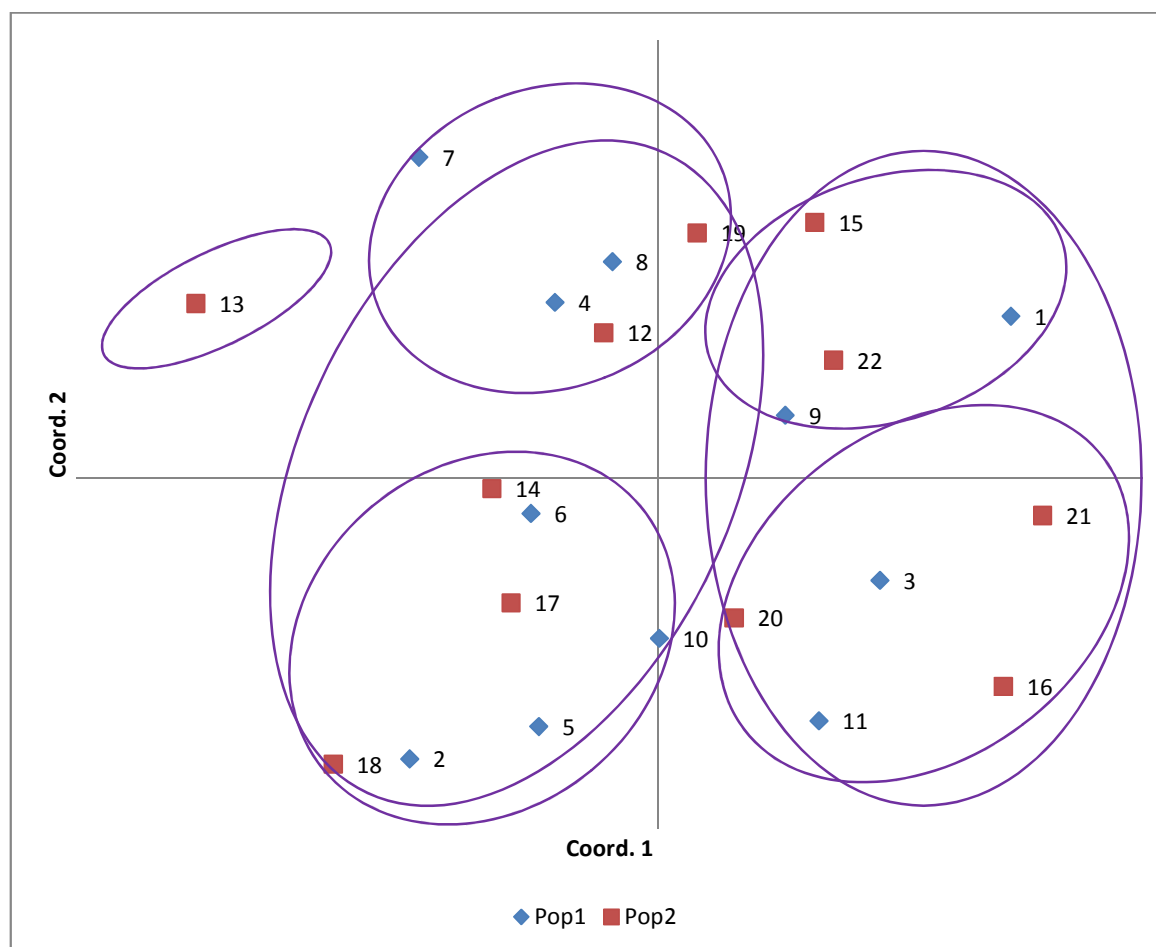


Figure 2: Principal coordinate analysis (PCoA) of pigeon pea accessions using GenAlex computer software.

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 lcpl 87;18 =TCc CITA 5;19=TCc 2;20=TCc CITA 2;21 =TCc 4;22=TCc 8

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 figure 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.

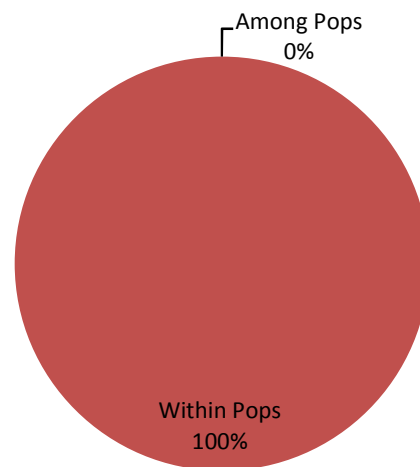


Figure 3: Percentage molecular variation in pigeon pea accessions obtained from IITA, Ibadan

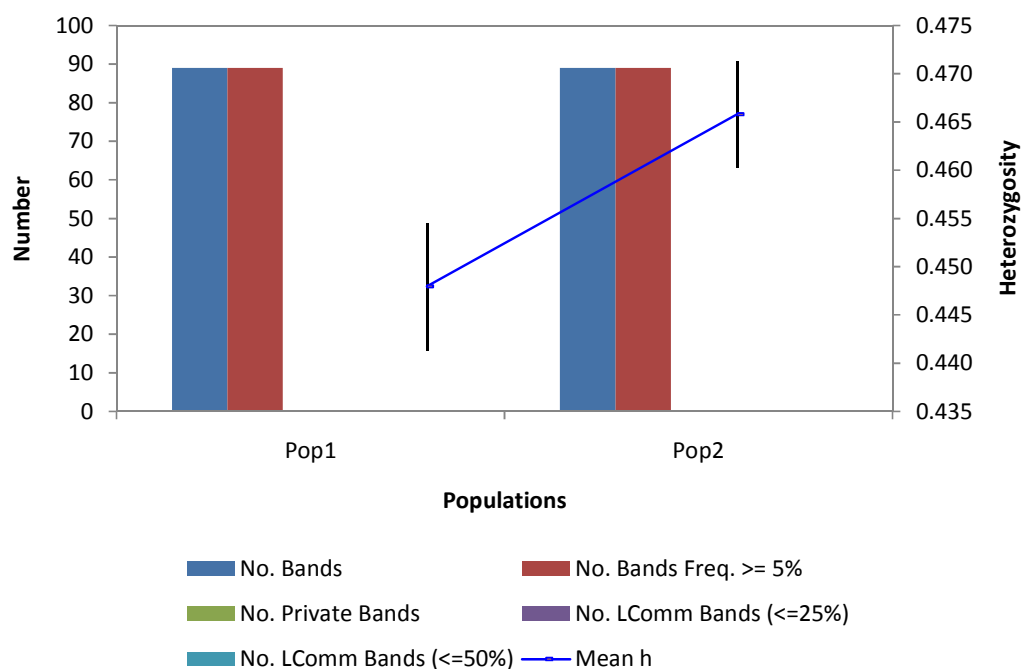


Figure 4: Band patterns across pigeon pea populations studied

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.

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 (Figure3). 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.

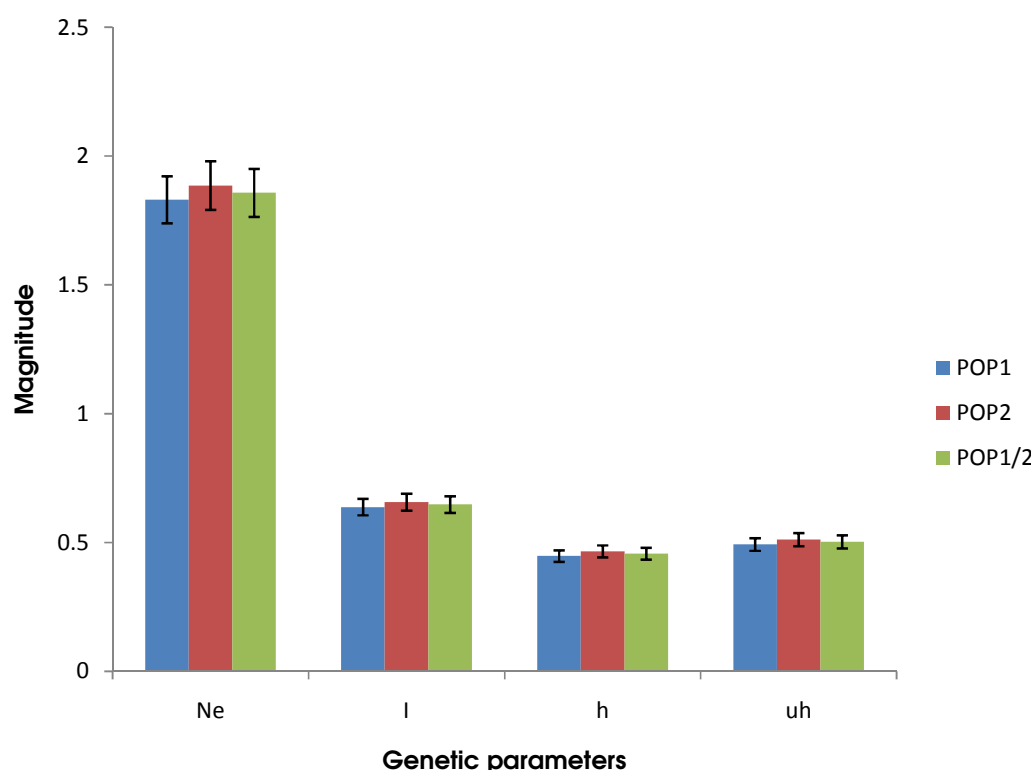


Figure 5: Genetic parameters of pigeon pea accessions as recorded by GenAlex Software (Note: Ne=Number of effective alleles; I=Shannon's information index; h=Genetic diversity; uh=Unbiased diversity)

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 (Khan *et al.*, 2013), identify breeding stock for crop improvement (O'Neil *et al.*, 2003; Welsh and Williams, 2010).

According to O'Neill *et al.* (2003), 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 (Skroch and Nienhuis, 1995; Mignouna et al., 1998; Beebe et al., 2000; Brown-Guedira et al., 2000; Amadou et al., 2001; Tosti and Negri, 2002). 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 (Hughes and Stachowicz, 2004), genetic mapping as well as markers-assisted selection in crop breeding (Lapitan et al., 2007).

Percentage polymorphism revealed by the 12 RAPD markers used in the current work was 88.6%. This suggests high percentage polymorphism. Shende and Raut (2013) reported 56.52% polymorphism using 15 pigeon pea genotypes with 5 RAPD primers while Yadav et al. (2013) 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. Ghalmi et al. (2010) 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 Ajibade et al. (2000). It can be observed that the primers that gave 100% polymorphism (OPH08 and OPH06) had the repeated sequences as reported by Ghalmi et al. (2010). 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 Walunjkar et al. (2013) reported PIC of 0.076-0.25 while Barbosa de Souse et al. (2011) gave PIC of 0.11-0.91. What it does suggest is that the PIC as reported by Barbosa de Souse et al. (2011) 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 Udensi et al. (2016) 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, Udensi *et al.* (2016) 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 (Udensi *et al.*, 2016). 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.

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 will be not advisable.

References

- Aiyeloga, A.A. and Bello, O.A. (2006). Ethnobotanical potentials of common herbs in Nigeria: A case study of Enugu State. *Educational Research and Review*, 1: 116 – 22.
- Ajibade, S.R., Weeden, N. F. and Chite, S. M. (2000). Inter simple sequence repeat analysis of genetic relationships in the genus *Vigna*. *Euphytica*, 111: 47-55.
- Amadou, H.I., Bebeli, P.J. and Kaltsikes, P.J. (2001). Genetic diversity in bambara groundnuts (*Vigna subterranea* L.) germplasm revealed by RAPD markers. *Genome*, 44; 995 -999.
- Ba, F.S., Pasquet, R. S. and Gepts, P. (2004). Genetic diversity in cowpea (*Vigna unguiculata* (L.) Walp.) as revealed by RAPD markers. *Genet. Resour. Crop Evol.*, 51: 539-550.

375 Bao, S., Yuhua, H., Zong, X., Wang, L., Lichi, L., Enneking, D., Rose, I. A., Leonforte, T.,
 376 Redden, R. J., and Paull, J. (2008). Collection of pea (*Pisum sativum*) and faba bean
 377 (*Vicia faba*) germplasm in Yunnan. *Plant Genet. Res News*. 156: 11–22
 378
 379 Barbosa de souse, A. C., Godoy, R., Sforca, D. A., Compos T., Zucchi, M. I., Jank, L.
 380 and Pereira de souse, A. (2011). Genetic diversity among pigeon pea genotypes
 381 adapted to South American regions based on microsatellite markers. *Sci. Agric.*
 382 (Piracicaba, Braz.), 68(4):431-439
 383
 384 Beebe, S., Skroch, P.W., Tohme, J., Duque, M.C., Pedraza, F. and Nienhius, J.
 385 (2000). Structure of genetic diversity among common bean landraces of
 386 middle. American Origin based on correspondence analysis of RAPD. *Crop*
 387 *Science Society of America*, 40: 264 – 273.
 388
 389 Brown-Guedira, G.L., Thompson, J.A., Nelson, R.L. and Warburton, M.L. (2000).
 390 Evaluation of genetic diversity of soybean introductions and North American
 391 ancestors using RAPD and SSR markers. *Crop Science Society of America*, 40:
 392 115 – 223.
 393
 394 Center for New Crops and Plants Products. *Cajanus cajan* (L.) Millsp. Purdue
 395 University. http://www.hort.purdue.edu/newcrop/duke_energy/Cajanus_cajun.html
 396 2002.
 397
 398 Dellaporta, S.L., Wood, J. and Hicks, J. B. (1983). A plant DNA miniprep: preparation:
 399 Version II. *Plant Mol. Biol. Rep.*, 1: 19-21.
 400
 401 Ghalmi, N., M. Malice, M., Jacquemin, J.M., Ounane, S.M., Mekliche, L. and
 402 Baudoin, J. P. (2010). Morphological and molecular diversity within Algerian cowpea
 403 (*Vigna unguiculata* (L.) Walp.) landraces. *Genet. Resour. Crop Evol.*, 57: 371-386
 404
 405 He, C., Liu, Y., Wu, K., Yuan, M., Feng, Q., Liu, Y., Yan, Q., Guan, J., Rose, I.
 406 A., Redden, R.J., and Enneking, D. (2008). Collecting and surveying landraces of pea
 407 (*Pisum sativum*) and faba bean (*Vicia faba*) in Qinghai province of China. *Pl. Genet.*
 408 *Res. News*. 156: 1–10.
 409
 410 Hughes, A.R. and Stachowicz, J. J. (2004). Genetic diversity enhances the resistance
 411 of a seagrass ecosystem to disturbance. *Proc. Natl. Acad. Sci. USA.*, 101: 8998-9002.
 412
 413 Khan, S.M., Ahmad, H., Nisar, M., Aslam, S., Inamullah, A., Ali, N. (2013).
 414 Genetic diversity in pea germplasm using randomly amplified polymorphic
 415 markers. *Pakistan Journal of Botany*, 45(4): 1259 – 1264.
 416
 417 Lapitan, V.C., Brar, D. S., Abe, T. and Redona, E. D. (2007). Assessment of genetic
 418 diversity of Philippine rice cultivars carrying good quality traits using SSR markers.
 419 *Breed. Sci.*, 57: 263-270.
 420
 421 Lewis, G., Schrire, B., Mackinder, B. and Lock, M. (2005). *Legumes of the World*. Royal
 422 Botanic Gardens, Kew, UK.
 423
 424 Liu, K. and Muse, S. V. (2005). Power Marker: An integrated analysis environment for
 425 genetic marker analysis. *Bioinformatics*, 21: 2128-2129.

- 426 Malviya, N. and Yadav, D. (2010). RAPD analysis among pigeon pea (*Cajanus cajan*
427 (L.) Millsp.) cultivars for their genetic diversity. Genetic Engineering and
428 Biotechnology Journal, 1:1-9
- 429
- 430 Mignouna, H.D., Ng, N.Q., Ikea, J. and Thottappilly, G. (1998). Genetic
431 diversity in cowpea as revealed by random amplified polymorphic DNA.
432 *Journal of Genetic Breed*, 52: 151 – 159.
- 433 Odeny, D.A., Jayashree, B., Gebhardt, C. and Crouch, J. (2009) New microsatellite
434 markers for pigeon pea (*Cajanus cajan* L. Millsp.). BMC Res. Notes. 2:35.
- 435 Olawuyi, O. J. and Fawole, I. (2005). Studies on genetic variability of some
436 quantitative and qualitative characters in pigeon pea (*Cajanus cajan* (L.) Millsp.)
437 (Fabaceae). Acta SATECH, 2(1):30-36.
- 438
- 439 O’Neil, R., Snowdon, R.J. & Kohler, W. (2003). Population genetic aspects of
440 biodiversity. *Progress in Botany*, 64: 115-137.
- 441
- 442 Parrotta, J. A. (2001). Healing Plants of Peninsular India. New York: CABI Publishing.
- 443
- 444 Peakall, R. and Smouse, P. E. (2006). GenAlex 6: Genetic analysis in Excel. Population
445 genetic software for teaching and research. Mol. Ecol. Notes, 6: 288-295.
- 446
- 447 Rao, S.C., Coleman, S.W., Mayeux, H. S. (2002). Forage production and
448 nutritive value of selected pigeon ecotypes in the Southern great plains.
449 *Crop Science*, 42: 1259-1263.
- 450
- 451 Saxena, K. B. (2008). Genetic improvement of pigeonpea—a review. Trop. Plant Biol.
452 1: 159–178.
- 453
- 454 Saxena, K., Kumar, R. and Sultana, R. (2010). Quality nutrition through pigeon
455 pea. *A Review; Health*, 2: 1335 – 1334.
- 456
- 457 Saxena, K.B., Singh, L. and Gupta, M. D. (1990). Variation for natural out-crossing in
458 pigeonpea. Euphytica 46: 143–148.
- 459
- 460 Saxena, K.B. and Kumar, R. V. (2003). Development of a cytoplasmic nuclear male-
461 sterility system in pigeonpea using *C. scarabaeoides* (L.) Thouars. Indian J. Genet.
462 63(3): 225–229
- 463 Saxena, R. K., Prathima, C., Saxena, K. B., Hoisington, D. A., Singh, N.K and Varshney,
464 R. K. (2009). Novel SSR markers for polymorphism detection in pigeon (*Cajanus* spp.).
465 Plant Breed, DOI: 10.1111/J.1439-0523.2009.01680.x
- 466 Saxena, K. B., Wallis, E. S. and Byth, D.E. (1983). A new gene for male-sterility in
467 pigeon pea (*Cajanus cajan* (L.) Millsp.). Heredity, 51:419-421
- 468 Shende, S and Raut, A. (2013). Analysis of genetic diversity in pigeon pea (*Cajanus*
469 *cajan* L.) by using PCR-based molecular markers. Recent Research in Science and
470 Technology, 5(2):20-23
- 471
- 472 Singh, N.K., Gupta, D.K., Jayarswai, P.K., Mahoto, A.K., Dutta, S., Singh, S.,

- 473 Bhutani, S., Dogra, V., Prashat, G.R., Khare, A., Yadav, R., Raje, R.S., Singh,
474 M.N., Datta, S., Fakrudin, B., Wanjari, K.B., Kansal, R., Dash, P.K., Jain, P.K.,
475 Bhattacharya, R., Gaikward, K., Mohapatra, T., Srinivasan, R. and Sharma, T.R.
476 (2012). The first draft of the pigeon pea genome sequence. *Journal of Plant*
477 *Biochemistry and Biotechnology*, 21: 98 – 112.
- 478
479 Skroch, P.W. and Nienhuis, J. (1995). Quantitative and qualitative
480 characterization of RAPD variation among snap bean (*Phaseolus vulgaris*)
481 genotypes. *Theoretical and Applied Genetics*, 9: 1078 – 1085.
- 482
483 Snook, L. K., Dulloo, M. E., Jarvis, A., Scheldeman, X., and Kneller, M. (2011). Crop
484 germplasm diversity: The Role of gene bank collections in facilitating adaptation to
485 climate change. In: *Crop Adaptation to Climate Change*. Ch 25, pp. 495–506.
486 Yadav, S. S., Redden, R. J., Hatfield, J. L., Lotze-Campen, H., and Hall, A. E., Eds. John
487 Wiley and Sons, Blackwell Publishing Ltd.
- 488
489 Tikka, S.B.S., Parmar, L. D and Chauhan, R. M. (1997). First record of cytoplasmic-
490 genic male-sterility system in pigeonpea (*Cajanus cajan* (L.) Millsp.) through wide
491 hybridization. *GAU Res. J.* 22(2): 160–162.
- 492
493 Tosti, N. and Negri, V. (2002). Efficiency of three PCR-based markers in
494 assessing genetic variation among cowpea (*Vigna unguiculata* Sub. Sp.
495 *Unguiculata*) Landraces. *Genome*, 45: 268 – 275.
- 496
497 Udensi, O. U., Okon, E. A., Ikpeme, E. V., Onung, O.O. and Ogban, F. U. (2016).
498 Assessing the genetic diversity in cowpea (*Vigna unguiculata* L. Walp) accessions
499 obtained from IITA, Nigeria using Random Amplified Polymorphic DNA (RAPD). *Int. J.*
500 *Plant Breed. Genet.*, 10: 12-22.
- 501
502 Varshney, R. K., Close, T. J., Singh, N. K., Hoisington, D. A., and Cook, D. R. (2009).
503 Orphan legume crops enter the genomics era! *Curr. Opin. Plant Biol.* 12: 202–210.
- 504
505 Walunjkar, B., Parihar, A., Chaurasia, P., Pachchigar, K. and Chauhan, R. M.
506 (2013). Genetic analysis of wild and cultivated germplasm of pigeon pea
507 using random amplified polymorphic DNA (RAPD) and simple sequence
508 repeats (SSR) markers. *African Journal of Biotechnology*, 12(40): 5823-5832
- 509
510 Yadav, S. K., Yadav, A and Padey, V. P (2014). Comparative analysis of genetic
511 diversity among cultivated pigeon pea (*Cajanus cajan* (L.)Millsp.) and its wild
512 relatives (*C. albicans* and *C. lineatus*)using random amplified polymorphic DNA
513 (RAPD) and inter simple sequence repeats (ISSR) fingerprinting. *American Journal of*
514 *Plant Sciences*, 5:1665-1678.