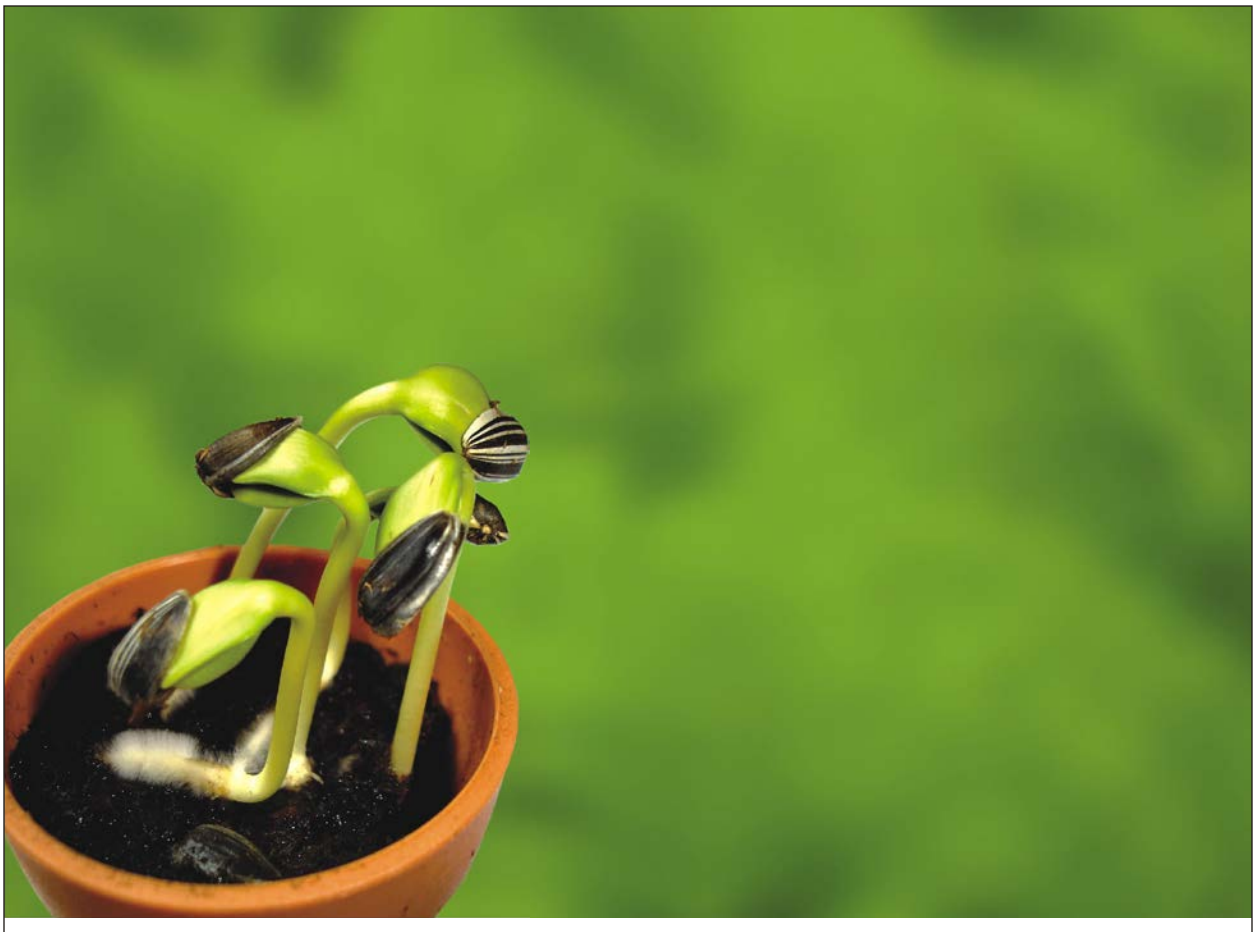

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Research Article

Assessing the Genetic Diversity in Cowpea (*Vigna unguiculata* L. Walp) Accessions Obtained from IITA, Nigeria Using Random Amplified Polymorphic DNA (RAPD)

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Abstract

The aim was to assess genetic diversity in cowpea using Random Amplified Polymorphic DNA (RAPD). Twenty cowpea accessions were obtained from International Institute of Tropical Agriculture (IITA), Nigeria and planted in the screening house of the same institute. The seeds were planted in pots filled with top rich soil, arranged in a Complete Randomized Design (CRD). DNA extraction and quantification and RAPD analysis of the DNA was carried out. A dendrogram was constructed using Unweighted Pair Group Method Arithmetic Mean (UPGMA). Analysis of molecular variance (AMOVA) was done. Result revealed 87.03% polymorphism. The major allele frequency ranged from 0.3875-1.0000 while, genetic diversity was from 0.000-0.7313. Polymorphic Information Content (PIC) ranged from 0.0000-0.6934. The result further revealed that the genetic distance across countries ranged from 0.6620-0.9423 showing that the widest distance was cowpea accessions obtained from Nigeria and Benin. The UPGMA based cluster analysis generated dendrogram with six clusters. The dendrogram revealed that accessions obtained from the same continent were clustered together, which was corroborated by the PCoA and AMOVA results. Generally, genetic diversity parameters were higher in the cowpea accessions obtained from the Asian continent when compared with accessions from African origin. It is therefore, reasonable to conclude that there is a wide genetic diversity of the cowpea accessions adopted from the Asian continent by International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria. However, from the UPGMA-based dendrogram, it could be suggested that selection be made on accession Tvu 9696 adopted from Egypt as stock for introgression.

Key words: *Vigna unguiculata* (L.) walp, molecular analyses, selection, improvement

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cowpea (*Vigna unguiculata* L. walp) is a global legume and presently is the second most important legume in Africa (NRC., 2006; FAO., 2009). Informatively, more than 5.4 million tons of dried cowpeas are produced worldwide with Africa producing approximately 5.2 million tons. Nigeria being the largest producer as well as consumer accounts for 61% of production in Africa and 58% worldwide (Ronner and Giller, 2013). Regrettably, this increase in cowpea production in Sub-Saharan Africa notwithstanding, its yield has remained one of the lowest among all food legumes. The FAO (2001) estimated average cowpea yield in West Africa to be 483 kg ha^{-1}, which was still 50% below the estimated potential production yield.

Generally, plant breeding has undergone different era of development including, the utilization of commercial heterosis and biometrical techniques (Troyer, 1996), exploitation and induction of dwarfing genes (Jain and Yadav, 2009), discovery of various mutagens and genome-doubling agents and their utilization for crop improvement (Ahloowalia *et al*., 2004; Kloen and Speckmann, 1959; Ciftci *et al*., 2006; Boureima *et al*., 2009; Brisibe *et al*., 2011; Udensi *et al*., 2012a; Udensi and Ontui, 2013), micropropagation as well as several molecular techniques. As laudable these strides are, Rauf *et al*. (2010) observed that plant breeding has drastically reduced genetic diversity. According to Wilkes and Williams (1983), the future of plant breeding depends on the utilization and conservation of plant breeding resources for the improvement of mankind's conditions. Ghalmi *et al*. (2010) observed that *ex-situ* conservation of potentially useful populations requires a clear understanding of the genetic variation and distinctiveness of the populations. The implication therefore, is that the characterization of accessions of crop plants is a sine-quo-non to guiding selection and subsequent breeding/improvement.

This characterization of germplasm of crops is feasible by studying the genetic diversity between and within the collection. Genetic diversity is the sum of genetic characteristics within any species or genus (Rao and Hodgkin, 2002) while, genetic variability is the variation within the genetic characteristics. Thus analysis of genetic relationships in crop species is an important component of crop improvement (Udensi and Edu, 2015). Undoubtedly, various researchers have adopted different approaches in the bid to estimate genetic diversity in a given germplasm, which includes principal component analysis, divergence analysis, coefficient of parentage, utilization of morphological, agronomical as well as biochemical data (Matus and Hayes, 2002; Mohammadi and Prasanna, 2003; Jaradat *et al*., 2004; Ahmad *et al*., 2008). However, due to the labourious and time-consuming nature of these methods in drawing meaningful conclusions from generated data, molecular marker-based techniques were explored and exploited by plant breeders. Molecular markers are highly heritable and polymorphic enough to enable the discrimination of closely related genotypes. O'Neill *et al*. (2003) affirmed that molecular genetic techniques using DNA polymorphism have been increasingly used to characterize and identify a novel germplasm for use in the crop breeding process.

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). Specifically for cowpea, RAPD has been employed (Akundabweni, 1995; Menendez *et al*., 1997; Mignouna *et al*., 1998; Tosti and Negri, 2002; Ba *et al*., 2004; Ghalmi *et al*., 2010; Prasanthi *et al*., 2012; Patil *et al*., 2013). Genetic diversity using RAPD marker fingerprinting has potential applications including determination of cultivar purity, efficient use and management of genetic resources as it pertains to the identification of diverse genotype for hybridization, providing genetic barriers against different biotic and abiotic stressors (Hughes and Stachowicz, 2004) and necessary for genetic mapping as well as marker-assisted selection in crop breeding (Lapitan *et al*., 2007).

The thrust of this present research stems from the fact that though Nigeria is the largest producer of cowpea, it may not translate into optimal utilization of the potential of the inherent genetic resources in the germplasm collected, especially at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Aware that increase in cowpea production in Sub-Saharan African countries including Nigeria notwithstanding, its yield has remained the lowest when compared with other food legumes. What this might suggest is that the potential of these accessions in the germplasm is yet to be harnessed and optimized. Thus assessing genetic diversity in the germplasm becomes imperative, especially in Nigeria.

MATERIALS AND METHODS

Seed collection and screen house experiment: Twenty cowpea accessions were obtained from the Genetic Resource Unit (GRU) of IITA, Ibadan, Nigeria (Table 1). Planting was

Table 1: Twenty cowpea (*Vigna unguiculata* L. walp) accessions obtained from IITA, Ibadan, Nigeria and their countries of collection

Cowpeas	Cultivar names	Biological status of accessions	Countries	Continents
Tvu-9081	IC 26042	Traditional cultivar/landrace	India	Asia
Tvu-9389	KHED NO.2	Traditional cultivar/landrace	India	Asia
Tvu-9390	KHED NO.1	Traditional cultivar/landrace	India	Asia
Tvu-9618	IBYAR RED EYE 3	Traditional cultivar/landrace	Egypt	Africa
Tvu- 9696	DISUQ RED EYE 5	Traditional cultivar/landrace	Egypt	Africa
Tvu-10235	P 1279	Traditional cultivar/landrace	India	Asia
Tvu-10253	P 1347	Traditional cultivar/landrace	India	Asia
Tvu-10299	IGBATTI NO.8	Traditional cultivar/landrace	Nigeria	Africa
Tvu-10332	IC 20665	Traditional cultivar/landrace	India	Asia
Tvu-10399	EX TVU 1297	Breeding/research material	USA	America
Tvu-10594	DAN KALABA–EARLY	Traditional cultivar/landrace	Nigeria	Africa
Tvu-10610	KOYA BABBA SATA	Traditional cultivar/landrace	Nigeria	Africa
Tvu-10931	SWUIEX NDALA	Traditional cultivar/landrace	Benin	Africa
Tvu-11490	UPLB-895	Traditional cultivar/landrace	Philippines	Asia
Tvu-11529	UPLB-969	Traditional cultivar/landrace	Philippines	Asia
Tvu-11578	EX TVU 7180	No information	India	Asia
Tvu-11789	MW80-144	Traditional cultivar/landrace	Malawi	Asia
Tvu-12337	TN80-145	Traditional cultivar/landrace	Niger	Africa
Tvu-12397	ZM 1245	Traditional cultivar/landrace	Zambia	Africa
Tvu-12951	APC 857	Traditional cultivar/landrace	India	Asia

carried out in the screen house of the institute. Cowpea seeds from the 20 accessions (Table 1) were planted in pots filled with top rich soil, which was arranged in a Complete Randomized Design (CRD). Eight seeds were planted in each pot, 2 per hole in a depth of 3-4 cm. Young and healthy leaves were harvested separately from each of the accessions two weeks after planting. They were put in sample collection bags in buckets filled with ice and then transferred to a freezer of -80°C for DNA extraction. This study lasted for 8 months (October, 2014 to May, 2015).

DNA extraction and quantification: DNA was isolated from young fresh leaves using the SDS (Sodium dodecyl sulphate) method of extraction (Dellaporta *et al*., 1983). 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). Seven hundred microliter of extraction buffer (1 M 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 chloroform Isoamylalcohol (CIA), 5 M potassium acetate were added. The suspension was mixed well and placed in a water bath for 25 min at 65°C. Sample was removed from the water bath and placed on ice to cool for 5 min and 200 µL of 5 M Potassium acetate was added to the mixture and carefully inverted and placed on ice for 20 min. 350 µL of Chloroform-isoamylalcohol (CIA) was added at a ratio of 24:1, mixed gently and centrifuged at 5000 rpm for 10 min Supernatant was decanted into eppendorf tubes containing ice cold isopropanol, which was added at the ratio (2:3) i.e., (400 µL of supernatant: 600 µL of Isopropanol) and mixed gently for 2-3 min. The mixture was placed in -20°C freezer for 30 min to enhance precipitation. Precipitated DNA was collected by centrifugation at 4000 rpm for 20 min and supernatant discarded.

Three hundred microliter 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 RnaseA were added to digest the RNA, gently mixed and incubated for 30 min 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: Fourteen primers (Eurofins MWG Operon LLC technologies, USA) were screened for PCR amplification. Primers used were carefully chosen from previous cowpea genetic diversity studies by Menendez *et al.* (1997) and Ba *et al.* (2004). After preliminary testing on a few sample, ten primers that gave clear polymorphic and reproducible bands patterns were selected to assess the genetic variability of the landraces selected. DNA amplification was carried out in 25 µL reaction mixture containing 10X buffer at 2.5 and 2.4 µL of $MgCl_2$, 2.0 µL of dNTPs, 1 µL of primer, 1.0 µL $DMSO_4$, 0.3 µL taq polymerase, 13.8 µL distilled autoclaved water and 2 µL of genomic DNA.

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 min followed by 40 cycles of denaturation at 94°C for 1min, 37°C for 1 min and 72°C for 2 min. The amplification was completed within 7 min final extension for 72°C. The amplified products were subjected to gel electrophoresis of 1.5%

agarose gels stained with 0.5 µL mL^{-1} 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.

Data analysis: The amplified products for RAPD analysis were scored visually based on the presence as (`1`) or absence (`0`) of band 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 were 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. Powermarker version 3.25 (Liu and Muse, 2005) was used for cluster analysis. Analysis of molecular variance (AMOVA) was done firstly to analyze the intra-and inter-variability in landraces and secondly among and within geographical regions and this was imputed in the GeneAlex 6.41 Software (Peakall and Smouse, 2006). Additionally, principal coordinate analysis was carried out using the GenAlex software.

RESULTS

RAPD primer parameters: Of the 14 primers used, 10 produced 54 bands of which 47 were polymorphic. This gave an average of 4.7 polymorphic bands per primer.Percentage polymorphism ranged from 75-100%. Figure 1 shows a representative of the RAPD banding profile. However, major allele frequency ranged from 0.15-0.50. Genetic diversity of the primer used ranged from 0.620-0.015, where OPB07, OPT15 as well as OPB10 revealed the highest genetic diversity. Additionally, Polymorphic Information Content (PIC) showed a range of 0.5711-0.9087 with OPB07 having the highest value of PIC (Table 2).

Major allele frequency, genetic diversity and PIC of location of selected cowpea accessions: In the 20 cowpea accessions obtained from the germplasm collection of IITA, Ibadan, Nigeria, the following countries were represented: Benin, Egypt, India, Niger, Nigeria, Philippines, USA and Zambia. Results obtained from this study showed that major allele

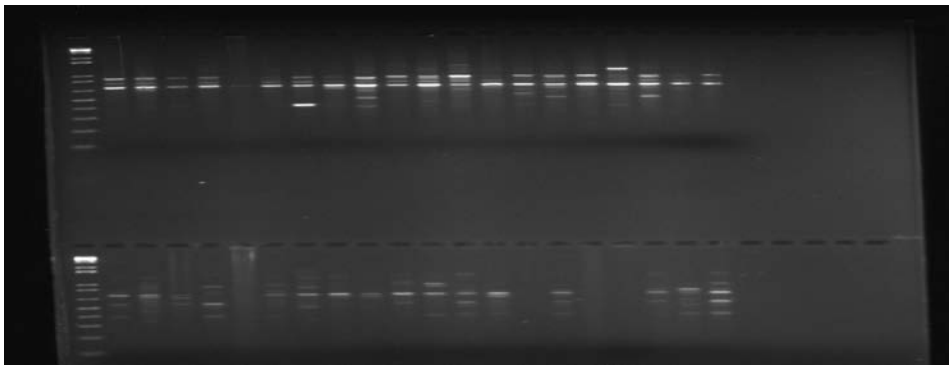

Fig. 1: Representative RAPD banding profile of 20 cowpea *Vigna unguiculata* (L.) accessions, M: 50 bp DNA ladder

Table 2: Primers used for RAPD studies among cowpea landraces showing its sequence, number of bands, number of polymorphic bands, percentage polymorphism, major allele frequency genetic diversity and the polymorphic information content

RAPD primers	Sequences (5`→3`)	No. of bands	No. of polymorphic bands	Polymorphism (%)	Major allele frequency	No. of bands (Allele No.)	Genetic diversity	PIC
OPB07	GGT GAC GCA	8	7	87.5	0.15	14	0.9150	0.9087
OPB10	CTG CTG GGA	5	4	80.0	0.20	14	0.9050	0.8979
OPH04	GGA AGT CGC	5	4	80.0	0.55	5	0.6200	0.5711
OPB20	GGA CCC TTA	6	6	100	0.25	13	0.8850	0.8760
OPT10	CCT TCG GAA	6	5	83.3	0.20	7	0.8400	0.8192
OPH05	AGT CGT CCC	4	3	75.0	0.50	6	0.6800	0.6422
OPT15	GGA TGC CAC	7	7	100	0.15	13	0.9100	0.9030
OPT14	AAT GCC GCA	5	4	80.0	0.25	9	0.8500	0.8334
OPT17	CCA ACG TCG	4	3	75.0	0.25	9	0.8350	0.8150
OPB02	TGA TCC CTG	4	4	100	0.35	8	0.7850	0.7579
Total		54	47					
Mean					0.285	9	0.8225	0.8025

RAPD: Random amplified polymorphic DNA, PIC: Polymorphic information content

frequency ranged from 0.3875-1.0000 while genetic diversity was from 0.000-0.7313. The PIC ranged from 0.0000-0.6934 (Table 3). However, from the same table it could be observed that some countries have major allele frequency of 1.00 and 0.000 gene diversity and PIC. This is due to the fact that they had one cowpea accession only.

Genetic distance across countries where cowpea accessions was obtained as well as among accessions: Our results also revealed that the genetic distance across countries ranged from 0.6620-0.9423. It showed that the widest distance was cowpea accessions obtained from Nigeria and Benin, while the least distance was between accessions from India and Benin. It was also noticed that the accessions from USA was distant apart from those obtained from Philippines (Table 4). Generally, however, the cowpea accessions showed wide genetic distances from each other. Using the computer software GeneAlex, the genetic distance of the cowpea accessions was generated. However, the programme excludes the only accession from USA, because it could not form a single population as the accessions classified into African and Asian populations. The result showed that Tvu 10594 and Tvu 1295 was 26.804 distance apart from each other with Tvu 10253 and Tvu 1033 showing the least genetic distance of 10.00.

Table 3: Major allele frequency, genetic diversity and polymorphic index content in respect to the countries where the cowpea accessions were collected

Country/regions	Major allele frequency	No. of bands (Allele No.)	Gene diversity	PIC
Benin	1.0000	1.0	0.0000	0.0000
Egypt	0.5500	1.9	0.4500	0.3375
India	0.3875	5.2	0.7313	0.6934
Malawi	1.0000	1.0	0.0000	0.0000
Niger	1.0000	1.0	0.0000	0.0000
Nigeria	0.5333	2.4	0.5111	0.4346
Philippines	0.5000	2.0	0.5000	0.3750
USA	1.0000	1.0	0.0000	0.0000
Zambia	1.0000	1.0	0.0000	0.0000

(N/B) values with 0.000 are so because sample locations have just one individual, countries where the cowpea accessions were collected, RAPD: Random amplified polymorphic DNA, PIC: Polymorphic information content

UPGMA based cluster analysis: Using the Powermarker version 6.25, UPGMA based cluster analysis generated dendrogram with six clusters. Clusters 1-3 contained two cowpea accessions each while cluster-4 contained eight accessions while cluster-6 had only one accession (Tvu 9696) (Fig. 1). The dendrogram revealed generally that accessions obtained from the same continent were clustered together except in cluster-1 where Tvu 10235 came from Asia while, Tvu 1093 was obtained from Africa, cluster-4 where Tvu 1149 came from Asia and cluster-5 with Tvu 10399 from USA (Fig. 2).

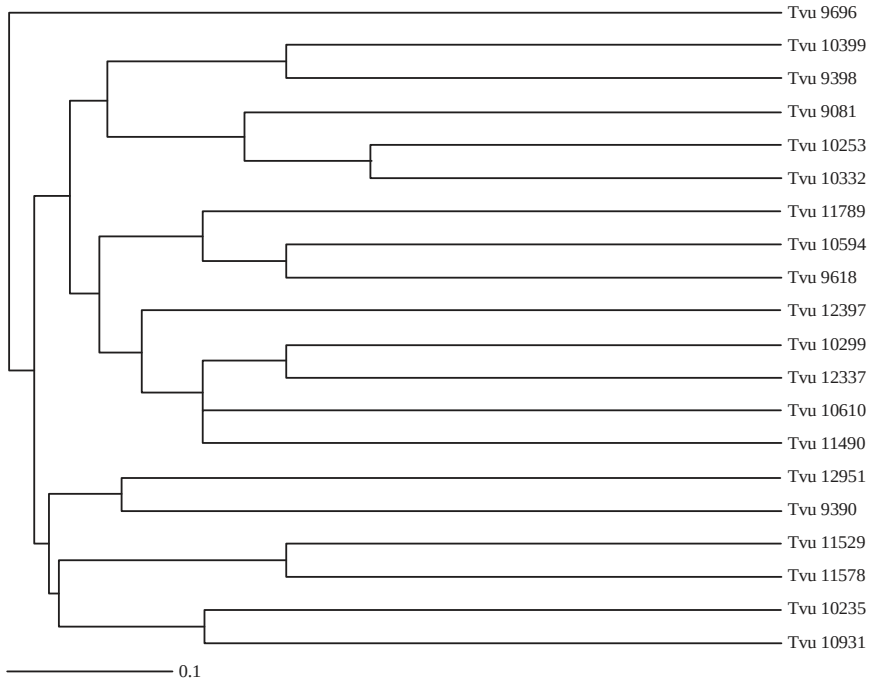

Fig. 2: UPGMA-based dendrogram from RAPD data illustrating the genetic relationships among 20 accessions of *Vigna unguiculata* (L) walp, UPGMA: Unweighted pair group method arithmetic mean, RAPD: Random amplified polymorphic DNA

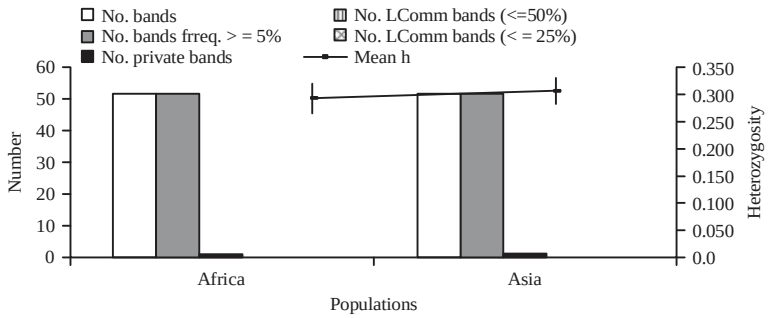

Fig. 3: Band patterns across populations of cowpea accessions obtained from African and Asian continents in germplasm of IITA, Ibadan, Nigeria

Table 4: Genetic distance across the countries where cowpea landraces were obtained

Countries	Benin	Egypt	India	Malawi	Niger	Nigeria	Philippines	USA	Zambia
Benin	0.0000								
Egypt	0.8586	0.0000							
India	0.6620	0.8030	0.0000						
Malawi	0.8000	0.7586	0.7244	0.0000					
Niger	0.9000	0.9293	0.8327	0.9000	0.0000				
Nigeria	0.9423	0.7043	0.7399	0.6635	0.6212	0.0000			
Philippines	0.8586	0.9500	0.6301	0.7879	0.7879	0.7153	0.0000		
USA	0.9000	0.8586	0.7681	0.9000	0.8000	0.8029	0.9293	0.0000	
Zambia	0.9000	0.8586	0.7266	0.9000	0.8000	0.7367	0.7172	0.9000	0.0000

Table 5: Genetic diversity parameters over loci per population

Parameter/continents	Africa	Asia
Genetic diversity (H)	0.293±0.026	0.3070.024
Unbiased diversity (UH)	0.332±0.030	0.344±0.027
Number of alleles (Na)	1.722±0.072	1.796±0.067
Number of effective alleles (Ne)	1.516±0.052	1.529±0.048
Shannon's information index (I)	0.431±0.037	0.456±0.033

Table 6: Pooled genetic diversity parameters over loci per population

Over loci and populations	(Africa and Asia)
Genetic diversity (H)	0.300±0.049
Unbiased diversity (UH)	0.338±0.020
Number of alleles (Na)	1.759±0.049
Number of effective alleles (Ne)	1.523±0.035
Shannon's information index (I)	0.444±0.025

Genetic diversity parameters over loci per population and over loci and populations: The result showed that genetic diversity among accessions obtained from Africa was 0.29 while for Asian cowpea population; it was 0.3070. Number of alleles for Asian population was 1.722 while 1.796 was the number of alleles in Asian population. Shannon's information index was 0.431 for Asian population. The result revealed that cowpea accessions from Asian origin had higher genetic diversity parameters. However, when the two populations were pooled together, genetic diversity, number of alleles and Shannon's information index were 0.300, 1.759 and 0.444, respectively (Table 5 and 6). Heterozygosity estimates were 0.3000 while number of private bands was very low. Heterozygosity estimated between both populations was the same (0.350) while, the number of private bands was estimated to be 0.01 (Fig. 3).

Principal coordinates analysis and percentage molecular variation (AMOVA): Result obtained from the Principal Coordinate Analysis (PCoA) showed that the percentage variation in the 20 cowpea accessions was explained by the three axes with the first axis accounting for 14.0% variations while the second axis accounted for 26.3% variation. However, the first three axes accounted for 36.47% genetic variation in 20 accessions studied. From Fig. 3, three major clusters were generated based on genetic relationship. Cluster-1 is made up of 13 accessions while cluster-2 had only Tvu11578. Cluster-3 contains five (Tvu 10235, Tvu 11529, Tvu 10931,Tvu 9390 and Tvu 12951) accessions (Fig. 4). Cluster -1 was sub-clustered with two; where sub-cluster A contains 9 accessions and sub-cluster B 4 accessions. This result revealed that the clustering was generally population-based. Additionally, molecular variation within populations was 96% while among populations was 4% (Fig. 5).

DISCUSSION

According to Udensi *et al.* (2012b), the sustainability in food security in the sub-Saharan African countries especially

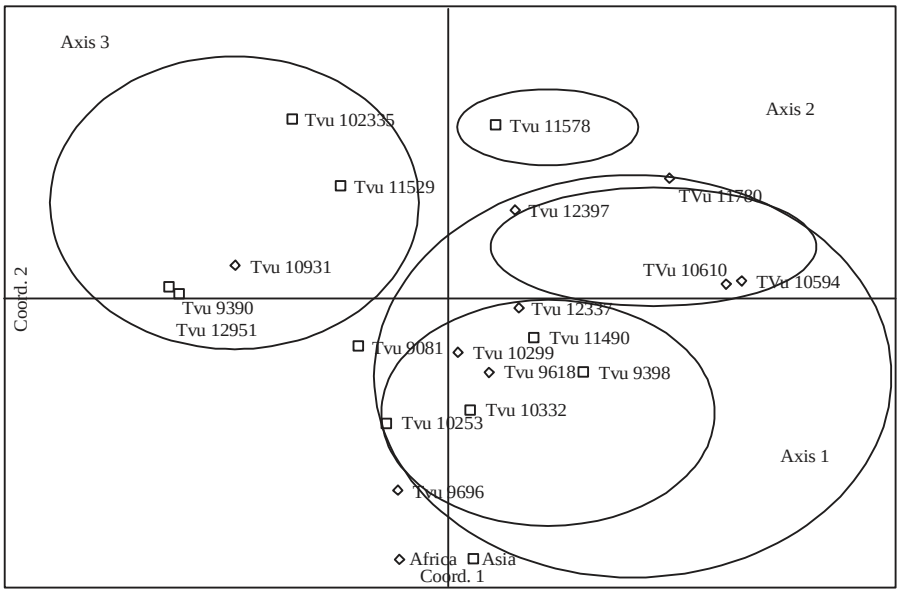

Fig. 4: Principal Coordinates Analysis (PCoA) based on the RAPD data illustrating the genetic relationship based on binary genetic distance among 19 accessions of cowpea and populations (Africa and Asia), RAPD: Random amplified polymorphic DNA

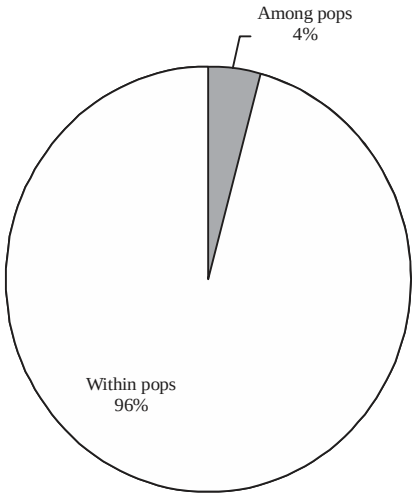

Fig. 5: Percentages molecular variance in two cowpea populations (Africa and Asia) obtained from IITA germplasm, Ibadan, Nigeria after RAPD-based fingerprinting

Nigeria will be hinged on the development, improvement as well as preservation of crop plants. Worthy to mention is the fact that the global threat to life in terms of food security orchestrated by the precarious and worsening climatic/weather conditions (Udensi *et al*., 2012a) is an issue of concern that demands pragmatic and urgent attention. Undoubtedly, cowpea has been reported to possess high variability, adaptability and drought tolerant amidst high and balanced proximate composition (Tharanathan and Mahadevamma, 2003; Costa *et al*., 2006; Udensi *et al*., 2011). With these in mind, analyses of genetic diversity and structure are critical in the management, research and utilization of plant germplasm. According to Rashid *et al*. (2012), the degree of genetic variation in a population specifies clearly (a) The kind of changes it might have experienced in the past (b) What the current situation is and (c) What the probability of sustenance is in the future.

According to Peleg *et al*. (2008) and Nevo *et al*. (2012), genetic diversity study is very critical for the study of crop evolution and genetic improvement of crop plants. This interestingly helps in the identification and correct interpretation of the associations between functional variation as well as molecular genetic diversity. The result of the current work on RAPD showed that of the 54 bands generated 47 were polymorphic with percentage polymorphism that ranged from 75-100%, with average polymorphism of 86.1%. Other researchers have reported varying results: 71.2% (Patil *et al*., 2013), 90.0% (Prasanthi *et al*., 2012), 55.0% (Khan *et al*., 2015), 46.5% (Pandey *et al*., 2004; Zannou *et al*., 2008), 64.5% (Sharawy and El-Fiky, 2003) and 58.44% (Ghalmi *et al*., 2010) polymorphisms. Going by these varying reports, the percentage polymorphism obtained from this present study is quite high, which indicates the primers used were good, revealing genetic diversity in the cowpea accessions studied, especially OPBO7, OPT15 and OPB10. According to Ghalmi *et al*. (2010), high polymorphism

revealed by molecular markers/primers is hinged on 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 had the repeated sequences as reported by Ghalmi *et al.* (2010) and corroborated by Ajibade *et al.* (2000). It might probably be that the ability to resolve genetic variation in any crop species germplasm is more directly related to the number of polymorphism detected by the marker techniques as well as the percentage of polymorphic RAPDs.

Additionally, our results showed that the Polymorphic Information Content (PIC) for the primers used revealed a range of 0.5711- 0.9087 with OPB07 having the highest value of PIC (0.9087). There seems to be a positive relationship between percentage polymorphism as revealed by the primers and the polymorphic information content of the same primers, such that the higher the polymorphism, the higher the PIC. Patil *et al.* (2013) reported a genetic distance that ranged between 0.321- 0.800 while working with 30 cowpea genotypes and 20 RAPD primers. However, our result revealed a higher genetic distance that ranged between 0.6620-0.9423. Though working with RAPD and ISSR, Chattopadhyay *et al.* (2005) reported high genetic distances among mungbean genotypes. The more distant apart an accession is to each other depicts the extent of genetic diversity between them. The wider the genetic distance, the wider will be the genetic diversity. The implication of the above is that there is high genetic diversity among the cowpea accessions obtained from IITA, Ibadan.

According to Patil *et al.* (2013), the higher the genetic diversity detected in the cowpea accessions analyzed, might indicate that theses accessions were originally generated by different ancestors of cowpea in the past. This was corroborated by the findings of Ba *et al.* (2004) about wild cowpea relatives being more genetically diverse than the domesticated types. One would wonder the reason underlying the genetic distances reported between cowpea accessions obtained from Nigeria and Benin though both are in the West Africa. Undoubtedly, it should be understood that plant breeders introduce different plant species from diverse geographical areas in the bid to widen the genetic variability among the existing plant breeding stock (Vetelainen *et al.*, 1996; Singh, 2004). This might have contributed to the wide divergence observed .This was also confirmed in the genetic distances (26.804; 10.00) reported between cowpea accessions Tvu 10594 (Nigeria) and Tvu 12951(India) as well as Tvu 10253 (India) and Tvu 10332(India). Ghalmi *et al.* (2010), noted that there seems to exist a coherent relationship between genetic diversity within landraces and geographical distribution of these landraces. The UPGMA based cluster analysis showed that generally cowpea accessions obtained from the same geographical locations were found on the same cluster. This geographically based clustering of the accessions was affirmed by the genetic distances results. This might suggest that cowpea accessions on the same cluster were more genetically similar while those found on different clusters might be more genetically diverse. The more an accession is distant apart from each other the wider the genetic diversity, which also show their positions on the clusters. According to 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), RAPD markers have been widely applied for the identification of genetic relationships among grain legume crops. It might be therefore important to make selections of accessions for genetic improvement in clusters that are very genetically diverse.

Generally, genetic diversity parameters such as genetic diversity, unbiased diversity, number of alleles, numbers of effective alleles as well as Shannon's information index were higher in the cowpea accessions obtained from the Asian continent when compared with accessions from African origin. Importantly, all these parameters measure extent of genetic diversity, which is referred as the expected heterozygosity. It is the probability that two randomly chosen alleles from the population are different .This could suggest that cowpea accessions adopted from the Asian continent are probably more genetically distinct. The other interesting possibility is that Asian accessions may have shown lower intra-population similarity and higher proportion of polymorphic loci resulting to increased heterozygosity as compared with the African population (Khan *et al.*, 2015). According to Hughes and Stachowicz (2004), Hajjar *et al.* (2008), the higher genetic diversity reported for Asian cowpea accessions in the present study might provide genetic barrier against different biotic and abiotic stresses. One could reason that urbanization as well as the replacement of traditional agriculture systems by modern industrial methods may have affected/reduced diversity, especially in the African accessions or populations.

Result obtained from the principal coordinate analysis showed that the total variation among the cowpea accessions was 36.47%, while clustering was generally based on population. This was in agreement with the UPGMA-based dendrogram clustering. Our result also revealed that the molecular variation (AMOVA) within populations was 96.0% while among population variation was 4.0%. In a genetic diversity studies in cowpea using alloenzymes, RAPD as well as microsatellites, there was little variation within and

between cowpea accessions (Pasquet, 2000; Li *et al*., 2001; Tosti and Negri, 2002). This was a negation to our present report where variation within cowpea population was very high. Though according to Pasquet (2000), cowpea probably has been domesticated only once as compared with common bean (*Phaseolus vulgaris* L.) that has undergone two domestication events (Singh *et al*., 1991), within and between genetic diversity is relatively low in landraces of cultivated cowpea, which might have been as a result of an initial single bottleneck (Pasquet, 2000). Diouf and Hilu (2005) had observed that RAPD markers were efficacious in accessing intra-specific or inter-specific genetic variability in many crop species. This was in confirmation with our present results. It should be emphasized that plant breeder's aim is to select crop species better suited and adapted to human needs. According to Trethowan and Mujeeb-Kazi (2008), genetic diversity was necessary for the rapid genetic improvement of crop species. Interestingly, Li *et al*. (2001) opined that the utilization of introduced germplasm in breeding programmes is much more important than the direct release of introduced material for cultivation, implying that the future of plant breeding depends on the utilization and conservation of plant genetic resources. Results from this current report implicitly show that there is a wide genetic diversity in the cowpea accessions adopted from the Asian continent from International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria. Also, is the fact that molecular genetic variation within cowpea population was very high (96.0%) when compared with among cowpea population (4.0%). From the UPGMA-based dendrogram, it could be suggested that selection be made on accession Tvu 9696 adopted from Egypt as stock for introgression.

CONCLUSION

Genetic diversity using RAPD marker fingerprinting has potential applications including determination of cultivar purity, efficient use and management of genetic resources as it pertains to the identification of diverse genotype for hybridization, providing genetic barriers against different biotic and abiotic stressors and necessary for genetic mapping as well as marker-assisted selection in crop breeding. Thus, from the results obtained in this study, it could be reasoned that there is a wide genetic diversity of the cowpea accessions adopted from the Asian continent by International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria. However, from the UPGMA-based dendrogram, it could be suggested that selection be made on accession Tvu 9696 adopted from Egypt as stock for introgression.

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