



Biodiversity assessment of African locust bean (*Parkia biglobosa*) accessions from Savanna and Forest zones of Nigeria as revealed by seed storage proteins and RAPD markers

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Abstract: Understanding the level and distribution of genetic diversity in African locust bean (*Parkia biglobosa*) would strengthen breeding and conservation programmes towards domestication and sustainable use of this species. Sixteen accessions of *P. biglobosa* were assessed for variability based on seed morphology, seed protein and DNA profiling. Significant variation in seed characteristics were observed across locations. Seed protein profiling by SDS-PAGE revealed homogeneity as most bands were found common in all accessions, indicating that the protein profiles are highly conserved. Protein profiling separated the 16 accessions into four major clusters at 0.93 similarity coefficient. Most accessions grouping into Cluster 1 had a similarity coefficient of close to 100% and were from the Derived Savanna suggesting the presence of duplicates. Accessions NH/2016/P14, NH/2016/P03 and NH/2016/P04 grouped into clusters II, III and IV; respectively. Sixteen RAPD markers generated a total of 256 bands of which 63.67% were polymorphic. Gene diversity ranged from 0.41 to 0.93 and Polymorphic Information Content (PIC) from 0.39 to 0.93. The RAPD-based dendrogram separated accessions into six groups at 0.68 similarity coefficient. Based on a polymorphic seed storage protein marker a genetically distinct accession NH/2016/P04 could be exploited for breeding purposes. The homogeneity of alleles and narrow genetic base as revealed by RAPD and SDS-PAGE analyses suggests possible loss of intraspecific genetic diversity. Thus, intensification of germplasm collections across the different agroecological zones and characterization using specific markers will give a better understanding of diversity of *P. biglobosa* in order to enhance selection towards conservation, breeding and sustainable utilization.

Keywords: African locust bean, genetic diversity, agroecological zone, RAPD, SDS-PAGE, *Parkia biglobosa*

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Introduction

In Sub-Saharan Africa several indigenous agroforestry systems exist containing woody species known as multipurpose trees. Africa locust bean [*Parkia biglobosa* (Jacq.) R. Br. ex G. Don] is a well-known indigenous

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agroforestry fruit tree species belonging to the sub-family *Mimosoideae* and family *Fabaceae* (Amusa *et al*, 2014; Houndonougbo *et al*, 2020). The species grows in multiple climatic zones and is widely distributed from Senegal and Guinea in West Africa to Uganda in Central Africa (Lompo *et al*, 2018). *Parkia biglobosa* is maintained in the parklands of Africa for non-timber forest foods (Houndonougbo *et al*, 2020; Leakey, 2012; Okoye *et al*, 2014). *P. biglobosa* is a valuable resource for the livelihoods of local people in Sub-Saharan Africa because of its multipurpose function as a source of food, nutrition, medicine and income (Matig *et al*, 2002; Nikiema, 2005; Awodoyin *et al*, 2015; Sankhon *et al*, 2014; Shao, 2002; Sina and Traore, 2002; Teklehaimanot, 2004). The fruit pulp and seeds are both suitable for human consumption. The mealy pulp from the species' fruits is a major source of energy and nutrients including carbohydrates, proteins, lipids, carotenoids, vitamins A, B, C, and oligoelements (Nyadanu *et al*, 2017). The seeds are an important source of plant protein and essential amino acids among rural communities who often have limited access to animal proteins due to high cost (Akin-Idowu *et al*, 2018; Alabi *et al*, 2005) and are also rich in energy value, saccharose, vitamin C, lipids, carbohydrates (Orwa *et al*, 2010) and bioactive components such as phenolic compounds which may contribute in health promoting properties (Dedehou *et al*, 2016; Okoye *et al*, 2014). *P. biglobosa* seeds are fermented to make a food condiment called Dawadawa (Iru) which is rich in dietary protein and serves as an important substitute for animal protein (Lamien *et al*, 2011; Nyadanu *et al*, 2017). The leaves, bark, roots and flowers are also used in the treatment of many diseases such as hypertension, wound healing and malaria (Dedehou *et al*, 2016; Ouedraogo, 1995).

In spite of the importance of this species in traditional agriculture, regular cultivation of the fruit tree has rarely gone beyond conservation of natural stands *in situ* (Hopkins, 1983; Oni *et al*, 1998). Several studies on *P. biglobosa* population structure have shown low regeneration rates and ageing of the stands which may lead to extinction (Padakale *et al*, 2015; Ræbild *et al*, 2012a). Overexploitation, late fruitification for most progenies and climate change are some of the factors suggested to explain the phenomenon (Boffa, 1999; Kwon-Ndung *et al*, 2009; Teklehaimanot, 2004). Improved tree management practices for *P. biglobosa*, such as establishment of new trees or protection of natural regeneration are currently not sufficiently promoted (Ræbild *et al*, 2012b). Thus, African locust bean remains undomesticated despite increasing demand for its use.

The conservation and sustainable use of genetic resources are critical to maintain tree resource availability, especially in the face of significant environmental changes, erosion of cultural heritages and declining cultivation and production activities of neglected indigenous species leading to genetic resources being

threatened. The process of plant domestication and conservation requires characterization of genetic resources for identification of cultivars and effective utilization of germplasm.

Genome size variation has been identified as an associate of evolutionary divergence (Dobes *et al*, 2019). Variation in genome size and chromosome number becomes taxonomically significant if associated with some degrees of morphological and ecological differentiation (Murray, 2005). Sina (2006) studied genetic diversity among populations of *P. biglobosa* species in Burkina Faso using enzyme electrophoresis. Dobes *et al* (2019) tested for linkage of relative genome size variation with geography, leaf morphology and population genetic variation in 58 individuals from 15 populations covering most of the distribution of *P. biglobosa* species in Burkina Faso. Most of the variation was found within populations and there was no evidence from the karyological data for structured intraspecific taxonomic heterogeneity (Dobes *et al*, 2019).

In Nigeria, genetic diversity of *P. biglobosa* accessions have been evaluated based on morphological characterization of trees (Gbadamosi *et al*, 2005; Okunlola *et al*, 2011) and seedlings (Adesoye *et al*, 2013). However, these characters are known to be more influenced by the environment. Uyoh *et al* (2011) reported variation in banding pattern of proteins obtained from leaf samples of three accessions of *P. biglobosa* collected from locations within Cross River State, Nigeria. Adesoye and Apo (2015) reported low levels of genetic diversity when 34 accessions of *P. biglobosa* were screened using seed protein electrophoresis. Owing to the limited information on genetic variation and relationship of *P. biglobosa* accessions in Nigeria, characterization using a combination of morphological, biochemical and molecular markers is essential for species identification and genetic variability establishment. This will facilitate the domestication, conservation and management of its genetic resources towards sustainable use.

Morphological characterization has been used to reveal phylogenetic relationships among crop populations, but this has its limitations as it is influenced by environmental factors (Ferreira, 2006). Selection, according to genetic variability using biochemical and molecular markers, has proved advantageous compared with the use of phenotypic markers (Alghamdi *et al*, 2019).

Biochemical analysis, particularly electrophoresis of seed proteins has been effective in studying genetic diversity at intraspecific levels in several legumes including soybean and chickpea (Durán *et al*, 2005; Signor *et al*, 2005; Malik *et al*, 2009; Sammour *et al*, 2007a,b) and for cultivar identification (Krochko and Bewley, 2000; Mustafa and El-Kholy, 2008).

The use of polyacrylamide gel electrophoresis (PAGE) in fractionating seed proteins and evaluating genetic diversity among accessions of several legume species including groundnut, African yam bean, grass pea, buckwheat, bambara groundnut and underground vetches

has also proven effective (la Rosa and González, 2010; Javaid et al, 2004; Machuka, 2001; Przybylska et al, 2000).

Molecular markers are known as one of the best approaches to study genetic material as well as to assess genetic variation in crop gene pools (Badr, 2008; Mondini et al, 2009; Wang et al, 2016). A number of PCR based molecular markers such as Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeats (ISSR) and Amplified Fragment Length Polymorphism (AFLP) have been used to assess genetic variability and cultivar identification in soybean (Barakat, 2004; Baránek et al, 2002; Chowdhury et al, 2001; El-Kholy, 2013) and other plant populations (Hoque and Hasan, 2012; Lin et al, 2009). RAPD is often used successfully to assess genetic diversity among species as it is fast, less technical and expensive and may reveal dominant molecular marker of good potential (Badr et al, 2012; Prasanthi et al, 2012).

This study aimed to estimate the genetic diversity of *P. biglobosa* accessions across different agro-ecological zones in Nigeria using SDS-PAGE and RAPD markers.

Materials and Methods

Plant material and morphological description

Seeds of *P. biglobosa* were obtained from 16 locations across seven states in Nigeria (Figure 1). Thirteen of the sixteen accessions studied were collected from locations in the Derived Savanna agroecological zone (AEZ); two were collected from the Guinea Savanna and one from Humid Forest (Figure 1, Table 1). The sampling of *P. biglobosa* accessions was not conducted systematically across all AEZs because the species is reported to be widely adapted and naturally distributed throughout the varied savannas of Sub-Saharan Africa (Lompo et al, 2016). The seed morphological characteristics of each collected accession, including seed shape, seed coat color and seed coat texture were observed. One hundred (100) seed weight in grams of each accession was also recorded.

Seed preparation and total protein extraction

Dried seeds were milled into flour and defatted with n-hexane at a flour:hexane ratio of 1:10 (w/v) prior to protein extraction (de la Rosa et al, 1992).

Seed total protein extraction was carried out by suspending defatted flour samples (100 mg) in 2.0 mL of pre-chilled Tris buffer 50mM Tris-HCl, pH 7.6, 1mM DDT, 150 mM NaCl and 1mM EDTA for 2h at room temperature according to the method of Zarkadas et al (2007). The resulting homogenates were centrifuged at 10,000 g for 20 min and supernatants were stored at -20°C until used.

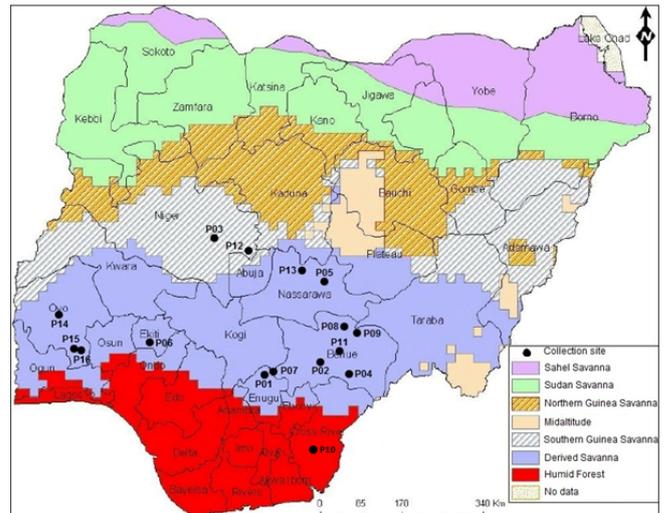


Figure 1. Map of Nigeria showing agroecological zones of the collection sites of sixteen *Parkia biglobosa* accessions

One dimensional SDS-PAGE

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the procedure of Laemmli (1970). Protein extracts were diluted in a sample buffer that contained 60 mM Tris-HCl (pH 6.8), 2% w/v SDS, 3.33% v/v β -mercaptoethanol, 10% glycerol and 0.05% of bromophenol blue. The samples were heated at 98°C for 5 min before loading onto a vertical slab gel (Mini-PROTEAN II Electrophoresis Cell) using a 4% (w/v) stacking and 12% gradient separating (acrylamide/bis acrylamide) gel containing 0.1% SDS. The separating polypeptide bands were calibrated using a protein molecular weight marker which ranged from 11 to 130 kDa. Electrophoresis was done at 125 V for about 2 h. At the end of the run, the gels were stained with Coomassie brilliant blue R-250 (SigmaAldrich) in methanol/water/acetic acid (40:50:10) and destained in a solution containing methanol/water/acetic acid (50:40:10).

Genomic DNA extraction, RAPD amplification and electrophoresis

For the genetic analysis, ten viable seeds of each of the sixteen accessions of *P. biglobosa* collected from the different locations were grown in a greenhouse. Prior to sowing, the seeds were immersed in concentrated sulphuric acid (H_2SO_4) for 15 minutes to break seed dormancy, rinsed thoroughly in running water and air dried at room temperature. Total genomic DNA was extracted from young leaves of *P. biglobosa* accessions. About 0.2g of a composite of 5 samples per accession was ground in extraction buffer following the cetyltrimethyl ammonium bromide (CTAB) method of Dellaporta et al (1983).

The DNA quality was evaluated using 1% agarose gel electrophoresis with known concentrations of undigested lambda DNA (Sigma, St Louis, MO, USA). Quantification of DNA was done using a spectrophotometer

Table 1. *Parkia biglobosa* accessions used in this study and collection sites.

Accession code	Collection site	Agroecological Zone	Geographical Coordinates (DD)	
			Longitude	Latitude
NH/2016/P01	Obollo-Afor, Enugu State	Derived Savanna	6.9153°N	7.5139°E
NH/2016/P02	Oju, Benue State	Derived Savanna	7.0383°N	8.3451°E
NH/2016/P03	Minna, Niger State	Guinea Savanna	9.5836°N	6.5463°E
NH/2016/P04	Vandeikya, Benue State	Derived Savanna	6.7835°N	9.0695°E
NH/2016/P05	Agyaragu, Nasarawa State	Derived Savanna	8.3471°N	8.5679°E
NH/2016/P06	Ikere, Ekiti State	Derived Savanna	7.5000°N	5.2333°E
NH/2016/P07	Orokam, Benue State	Derived Savanna	6.9786°N	7.5572°E
NH/2016/P08	Gbajimba, Benue State	Derived Savanna	7.8197°N	8.8587°E
NH/2016/P09	Kwatan-Sule, Benue State	Derived Savanna	7.8112°N	9.2429°E
NH/2016/P10	Ogoja, Cross River State	Humid Forest	6.6548°N	8.7977°E
NH/2016/P11	Mbatiav, Gboko, Benue State	Derived Savanna	7.2788°N	8.7953°E
NH/2016/P12	Suleja, Niger State	Guinea Savanna	9.2003°N	7.1723°E
NH/2016/P13	Garaku, Nassarawa State	Derived Savanna	8.8444°N	8.1311°E
NH/2016/P14	Iseyin, Oyo State	Derived Savanna	7.9765°N	3.5914°E
NH/2016/P15	NIHORT, Ibadan, Oyo State	Derived Savanna	7.4052°N	3.8499°E
NH/2016/P16	Oje Market, Ibadan, Oyo State	Derived Savanna	7.3891°N	3.9092°E

(Beckman Coulter DU530) at 260 nm. Extracts were diluted to obtain DNA concentrations of 25ng/ μ l. To generate DNA profiles, 40 decamer oligonucleotide DNA primers were initially screened for polymorphisms and only 16 which were polymorphic were used in the PCR reactions (Table 2).

To ensure reproducibility of RAPD markers, RAPD-PCR amplification was performed in triplicate for each primer and accession. The PCR reaction contained the following reagents with the final concentrations: 1x PCR buffer, dNTPs (0.2 mM), MgCl₂ (2.0 mM), primer (25 pmol), template DNA (25ng/ μ l), Taq polymerase (1.25 U) and nuclease-free water to 50 μ l. Amplification was carried out in a 2400 Perkin Elmer Gene Amp PCR thermo-cycler as follows: pre-denaturation for 3 min at 94°C; then 40 cycles each consisting of a denaturation step for 1 min at 94°C; an annealing step for 1 min at 36°C; an extension step for 90 sec at 72°C. Amplification was terminated by a final extension of 7 min at 72°C. PCR products were electrophoresed on a 1.5% (w/v) agarose gel in 1X Tris/Borate/EDTA (TBE) buffer at 100 volts for 2h and visualized under UV light after staining with ethidium bromide.

Data analysis

Data on 100-seed weight of all accessions were subjected to ANOVA and means were separated using Duncan's multiple range tests ($p \leq 0.05$). Distribution frequencies of the qualitative traits were calculated using Microsoft Excel 2010. The protein bands and RAPD amplified DNA fragments were scored qualitatively, whereby (0) stands for the absence and (1) stands for the presence of bands in the profile of each accession. All values were pooled together to generate a binary data matrix, which was analyzed using the Numerical Taxonomy and Multivariate Analysis System (Rohlf, 2002). Genetic

relationship among accessions was evaluated based on Jaccard's similarity index (Jaccard, 1908). Dendrograms were generated by using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Analysis of genetic diversity (GD) was calculated according to the method of Nei (1987). Polymorphic information content (PIC) for each primer was determined from allele frequencies (Nei and Li, 1979).

Results and Discussion

Variation in seed morphology

Improvement of the quality of indigenous fruit trees across Sub-Saharan Africa involves assessment of fruit/seed traits; this has shown significant variation both among and within provenances and has enhanced selection in breeding programmes (Ræbild *et al*, 2012a). In this study, significant differences ($p \leq 0.05$) were observed in the 100-seed weight of *P. biglobosa* collected from 16 different locations (Table 3), which ranged from 17.93g (NH/2016/P09) to 26.11g (NH/2016/P05). The highest 100-seed weight was recorded for accession NH/2016/P05 collected from Agyaragu, Nassarawa State; this was significantly ($p \leq 0.05$) higher than that of all other accessions. The lowest 100-seed weight was recorded for NH/2016/P09 collected from Kwatan-Sule, Benue State. The mean 100-seed weight (21.46g) of all accessions of *P. biglobosa* collected from the Derived Savanna zone was higher than the mean 100-seed weight collected from the Guinea Savanna and Humid Forest (Figure 2a). This variation in seed weight can be attributed to a wide range of factors such as environment (soil, climate), anthropogenic activities and phenotypic plasticity, thus promoting the presence of ecotypes.

Table 2. List of primers used and RAPD polymorphisms among *Parkia biglobosa* accessions.

Primer	Sequence 5' to 3'	Major allele frequency	Allele number	Gene Diversity	Polymorphic Information content (PIC)
OPB10	CTGCTGGGAC	0.13	15	0.93	0.93
OPT07	GGCAGGCTGT	0.13	14	0.92	0.92
OPB04	GGACTGGAGT	0.13	14	0.92	0.92
OPT12	GGGTGTGTAG	0.13	14	0.92	0.92
OPT08	AACGGCGACA	0.13	13	0.91	0.91
OPT06	CAAGGGCAGA	0.19	13	0.91	0.90
OPH07	CTGCATCGTG	0.19	12	0.90	0.89
OPB08	GTCCACACGG	0.19	9	0.87	0.85
OPT20	GACCAATGCC	0.25	9	0.84	0.82
OPH06	ACGCATCGCA	0.38	9	0.80	0.78
OPT09	CACCCCTGAG	0.38	9	0.80	0.78
OPT10	CCTTCGGAAG	0.44	10	0.77	0.76
OPT01	GGGCCACTCA	0.44	7	0.74	0.71
OPT16	GGTGAACGCT	0.56	6	0.64	0.61
OPB05	TGCGCCCTTC	0.56	5	0.63	0.59
OPT05	GGGTTTGGCA	0.75	4	0.41	0.39
Mean		0.31	10	0.81	0.79
Total			163		

Leakey (2017) reported that fruit and/or kernel size was greater in more humid sites for *Adansonia digitata* and *Vitellaria paradoxa*; but greater in drier sites for *Balanite aegyptiaca*. Also, results from tests of *B. aegyptiaca* and *Prosopis Africana* indicated that provenances from drier sites had significantly better aboveground growth than provenances from more humid sites (Leakey, 2017). It is recommended that fruit trees germplasm should be collected in drier sites for future plantings in parklands, especially as this germplasm appears to be better adapted to dry conditions (Leakey, 2017).

The seeds of *P. biglobosa* accessions collected from the different locations in this study had distinct morphology and exhibited variation for seed shape, seed coat colour and seed coat texture (Table 3). Seeds of the sixteen *P. biglobosa* accessions were predominantly round oval in shape, black in colour with smooth seed coat and were mostly obtained from the Derived Savanna zone (Figure 2B, C, D). Seeds collected from the two sites in Guinea Savanna differed in shape and texture as seeds from one site were flat oval, black and smooth (NH/2016/P03) (Figure 3B); while seeds from the other site were round oval, black and rough (NH/2016/P12) (Figure 3C). Seeds collected from the same agroecological zone (Derived Savanna) exhibited distinct variation in colour and texture, NH/2016/P15 seeds were flat oval, brown and smooth (Figure 3E); while seeds of NH/2016/P04 were flat oval, black and wrinkled.

The morphological variation may be due to gene flow across regions because of seed dispersal since *P. biglobosa* is an open pollinated tree crop having

main pollinators such as bat, honeybees, rodents and humans (Lassen et al, 2012; Lassen, 2016; Lompo et al, 2017). This variation offers great potential for future selection and domestication.

Lack of a clear association between genome size and morphological, geographical, or ecological patterns of differentiation has been reported for two species of *Artemisia* (Asteraceae) (Dobes et al, 2019; de Xaxars et al, 2016).

Seed storage protein analysis

Seed storage protein profiling has been used as an effective tool for varietal identification and determination of phylogenetic relationship in several plant species populations (Emre, 2009; Hameed et al, 2012; Machuka, 2001).

In this study, the SDS-PAGE profile of sixteen accessions of *P. biglobosa* showed similar electrophoretic patterns with respect to the number of protein bands and their band intensities (Figure 4). The uniformity in the protein band profiles of all sixteen accessions suggests a low level of genetic diversity, which also corroborates earlier studies on *P. biglobosa* using seed protein electrophoresis (Adesoye and Apo, 2015).

Occurrence of a narrow genetic base from the accessions of *P. biglobosa* would suggest that they resulted not only from a common gene pool, but also from the outcrossing nature of the species over a long time, leading to a low level of inter-population diversity. Low variation was observed in thirteen mungbean varieties using SDS-PAGE (Hameed et al, 2012) and also in genetic diversity assessment of groundnut using SDS-PAGE (Javaid et al, 2004).

Table 3. Seed characteristics of *Parkia biglobosa* accessions used in this study. Values are mean \pm Standard Error. Means within the same column with different letters are significantly ($p \leq 0.05$) different.

Accession code	Weight of 100 seeds (g) Mean \pm SE	Shape	Colour	Seed coat texture
NH/2016/P01	23.11 ^d \pm 0.18	Round Oval	Black	Smooth
NH/2016/P02	18.59 ^k \pm 0.16	Round Oval	Brownish black	Smooth
NH/2016/P03	21.75 ^f \pm 0.15	Flat Oval	Black	Smooth
NH/2016/P04	18.30 ^k \pm 0.32	Flat Oval	Black	Wrinkle
NH/2016/P05	26.11 ^a \pm 0.21	Round Oval	Black	Smooth
NH/2016/P06	23.38 ^d \pm 0.10	Flat Oval	Brownish black	Smooth
NH/2016/P07	19.44 ⁱ \pm 0.14	Round Oval	Black	Smooth
NH/2016/P08	20.87 ^g \pm 0.19	Round Oval	Black	Rough
NH/2016/P09	17.93 ^l \pm 0.23	Round Oval	Brownish black	Smooth
NH/2016/P10	18.54 ^k \pm 0.13	Round Oval	Black	Smooth
NH/2016/P11	21.46 ^f \pm 0.06	Round Oval	Black	Smooth
NH/2016/P12	20.32 ^h \pm 0.26	Round Oval	Black	Rough
NH/2016/P13	23.74 ^c \pm 0.23	Round Oval	Black	Rough
NH/2016/P14	19.00 ^j \pm 0.12	Flat Oval	Brownish black	Rough
NH/2016/P15	24.59 ^b \pm 0.11	Flat Oval	Brown	Smooth
NH/2016/P16	22.48 ^e \pm 0.13	Flat Oval	Brownish black	Smooth

The number of clearly visible protein bands ranged from eight to nine among the sixteen accessions of *P. biglobosa* with molecular weights ranging from 11 to 130 kDa. Two major clusters of bands were observed between 11–17 kDa and 34–53 kDa and one minor band was observed above 130 kDa (Figure 4). This is similar to results of Adesoye and Apo (2015) on albumin and globulin fractions of *P. biglobosa* in which two major bands were observed at the 16 and 50 kDa regions.

It has been suggested that high molecular weight proteins facilitate the development of seed hardness (Coelho *et al.*, 2007); therefore, the presence of a high molecular weight band observed above the 130 kDa in this study may account for the seed hardness of *P. biglobosa*. The seeds of *P. biglobosa* are very hard and require treatment with sulphuric acid to induce germination.

A polymorphic polypeptide with molecular weight of approximately 24 kDa was absent in NH/2016/P04 but present in all other accessions (Figure 4A) and can be used for its identification. Differences in the presence or absence of bands among accessions are indicative of differences in the genes controlling the different polypeptide subunits (Osanyinpeju and Odeigah, 1998). Also, the presence or absence of polypeptide bands has been found to be linked to the expression of certain characteristics such as wrinkled seeds and insect resistance (Rao and Pernolet, 1981; Odeigah and Osanyinpeju, 1996). The seed shape of NH/2016/P04 was observed to be wrinkled and the absence of the 24 kDa band in this accession may be responsible for this seed texture as it was the only accession expressing this characteristic. Seed storage proteins are non-enzymatic and have the sole purpose of providing proteins (nitrogen and sulphur source) required during germination and establishment of a new plant (Hameed

et al., 2012). Legume seed storage proteins are mostly 7S and 11S globulins, which tend to be deficient in sulphur-containing amino acids (Tang and Sun, 2010). In mung bean, SDS-PAGE revealed that 11S globulin was composed of two bands: 40 kDa and 24 kDa, 8S vicilin was composed of 60 kDa, 48 kDa, 32 kDa and 26 kDa bands; and basic 7S globulin was composed of 28 kDa, 17 kDa and 16 kDa bands (Hameed *et al.*, 2012; Mendoza *et al.*, 2001).

In this study, peptides with molecular weights of 40 kDa and 24 kDa were detected that may correspond to 11S globulin, peptides with molecular weights of 48 kDa and 32 kDa were detected that may correspond to the 8S vicilin subunit, while 28 kDa and 16 kDa peptides were also detected that may correspond to the 7S globulin subunit. Barakat (2004) reported low levels of protein polymorphism among six cultivars of soybean in which five major bands were identified at the 72, 36, 32, 20 and 16 kDa regions.

In African yam bean, SDS-PAGE separated identical globulin, albumin and vicilin patterns for all 26 accessions studied (Machuka, 2001). Strelec *et al.* (2012) reported that barley varieties could be partially discriminated by albumin/globulin banding patterns using SDS-PAGE, whereas this was not possible with native PAGE which showed more or less identical protein patterns for all varieties.

Through the use of SDS-PAGE on total proteins it is possible to detect a useful band polymorphism to explore the diversity of *P. biglobosa* as was detected in this study, albeit at a low level.

Cluster analysis

A dendrogram was constructed based on protein banding patterns using Jaccard's similarity coefficients

(Table 4). The dendrogram grouped the sixteen accessions into four clusters at 0.93 similarity coefficient (Figure 5). The first cluster grouped together the largest number (thirteen) of accessions which consisted of NH/2016/P01, NH/2016/P02, NH/2016/P05, NH/2016/P16, NH/2016/P15, NH/2016/P06, NH/2016/P13, NH/2016/P12, NH/2016/P11, NH/2016/P10, NH/2016/P09, NH/2016/P08 and NH/2016/P07. The majority of the members of this cluster had similarity coefficient of 1.00 suggesting them to be genetically identical. Cluster II consisted of NH/2016/P14. Accession NH/2016/P03, which was collected in Minna, Niger state and belonging to the Guinea

Savanna agroecological zone, was separated alone into Cluster III, displaying pattern of geographic origin. Cluster IV consisted of NH/2016/P04 and was the most divergent of the sixteen tested accessions. The genetic similarity coefficient was 0.85 among NH/2016/P03, NH/2016/P04 and NH/2016/P14. Clustering showed pattern of geographic origin as eleven of the thirteen accessions grouped in Cluster I were collected from the Derived Savanna agro-ecological zone.

Genetic distance values give some idea of the level of genetic variability among selected species or accessions. The variation in genetic diversity among the sixteen *P. biglobosa* accessions ranged from 0.85

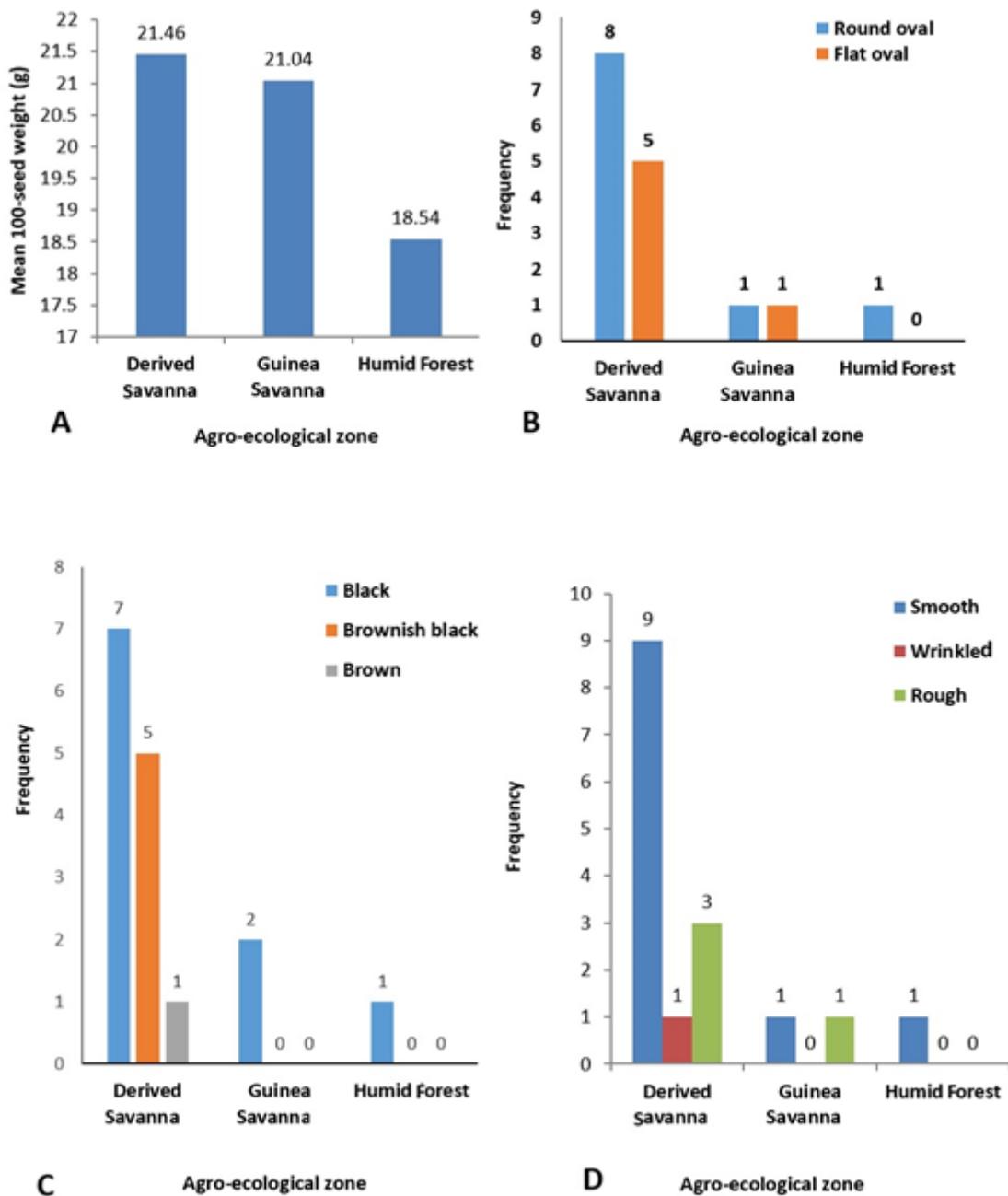


Figure 2. Distribution frequencies of seed characteristics of *Parkia biglobosa* across agro-ecological zones. A) 100-seed weight (g); B) Seed shape; C) Seed colour; D) Seed coat texture.



Figure 3. Shapes and colour of seeds with seed coat of *Parkia biglobosa*: A) Round oval shape (NH/2016/P02), B) Flat oval shape (NH/2016/P03), C) Black colour (NH/2016/P12), D) Brownish black colour (NH/2016/P14), E) Brown colour (NH/2016/P15).

to 0.92 depicting a low genetic diversity (Table 4). All accessions in Cluster I had a similarity coefficient of 0.92 in any pairwise comparisons with NH/2016/P03, NH/2016/P04 and NH/2016/P14. The low levels of variability observed in *P. biglobosa* is congruent with Sina (2006) who reported that the genetic distances among *P. biglobosa* trees in Burkina Faso were low (between 0 and 0.240), indicating that the populations were similar enough to belong to the same genetic group. Occurrence of a narrow genetic base from the accessions of *P. biglobosa* suggests that they may have resulted from a common gene pool and that fixation of variants through genetic drift has probably occurred.

Amusa *et al* (2014) had earlier reported that close natural populations like *P. biglobosa* exhibited high genetic similarity and low genetic distance attributed to a high rate of exchange of gene flow between

populations. Low genetic diversity was also observed in mung bean germplasm based on electrophoresis of seed storage proteins where a dendrogram analysis grouped the tested genotypes into three clusters at 93% homology (Hameed *et al*, 2012). Results of this study is similar to that obtained in cowpea in which seven landraces were separated into different clusters based on their geographical origin (Alghamdi *et al*, 2019). The high level of similarity in the accessions of *P. biglobosa* suggests that the seed storage proteins are highly conserved.

RAPD polymorphism

Genetic polymorphism is an indication of evolutionary adaptation, which has a main role in the survival of species in a changing environment (Stevens *et al*, 2007). In this study, Random Amplified Polymorphic DNA (RAPD) amplification profiles revealed a total of

Table 4. Jaccard's similarity coefficients of seed total protein profiles of *Parkia biglobosa* accessions.

	P01	P02	P03	P04	P05	P06	P07	P08	P09	P10	P11	P12	P13	P14	P15	P16
P01	1.000															
P02	1.000	1.000														
P03	0.923	0.923	1.000													
P04	0.923	0.923	0.846	1.000												
P05	1.000	1.000	0.923	0.923	1.000											
P06	1.000	1.000	0.923	0.923	1.000	1.000										
P07	1.000	1.000	0.923	0.923	1.000	1.000	1.000									
P08	1.000	1.000	0.923	0.923	1.000	1.000	1.000	1.000								
P09	1.000	1.000	0.923	0.923	1.000	1.000	1.000	1.000	1.000							
P10	1.000	1.000	0.923	0.923	1.000	1.000	1.000	1.000	1.000	1.000						
P11	1.000	1.000	0.923	0.923	1.000	1.000	1.000	1.000	1.000	1.000	1.000					
P12	1.000	1.000	0.923	0.923	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000				
P13	1.000	1.000	0.923	0.923	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000			
P14	0.923	0.923	0.846	0.846	0.923	0.923	0.923	0.923	0.923	0.923	0.923	0.923	0.923	1.000		
P15	1.000	1.000	0.923	0.923	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.923	1.000
P16	1.000	1.000	0.923	0.923	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.923	1.000	1.000

256 alleles out of which 163 were polymorphic showing 63.67% polymorphism among the studied accessions. This relatively high polymorphism suggests a significant amount of genetic diversity among the accessions. A similar result was obtained among 11 soybean cultivars

in which RAPD amplification profiles revealed 61.7% polymorphism using five primers (El-Kholy, 2013).

Alleles, ranging in size from 250 to 1500 basepairs (bp) and above, were scored for estimation of genetic relationship among the sixteen *P. biglobosa* accessions (Figure 6). An average of 10 alleles per primer was obtained in this study ranging from a minimum of four alleles using primer OPT05 to a maximum of 15 alleles using primer OPB10 (Table 2).

Genetic diversity is one of the important indices for the evaluation of genetic diversity among crop species (Narzary et al, 2009; Tamboli et al, 2016). The genetic diversity in this study ranged from 0.41 to 0.93, suggesting a significant amount of variation among the 16 accessions evaluated (Table 2). An earlier report on genetic diversity assessment of 23 accessions of *P. biglobosa* from different agro-ecological zones using RAPD indicated weak genetic diversity (expected heterozygosity, $H_E = 0.05-0.18$ and observed number of alleles, $ON_A = 1.11-1.65$) (Amusa et al, 2014). The significance of the polymorphic information content

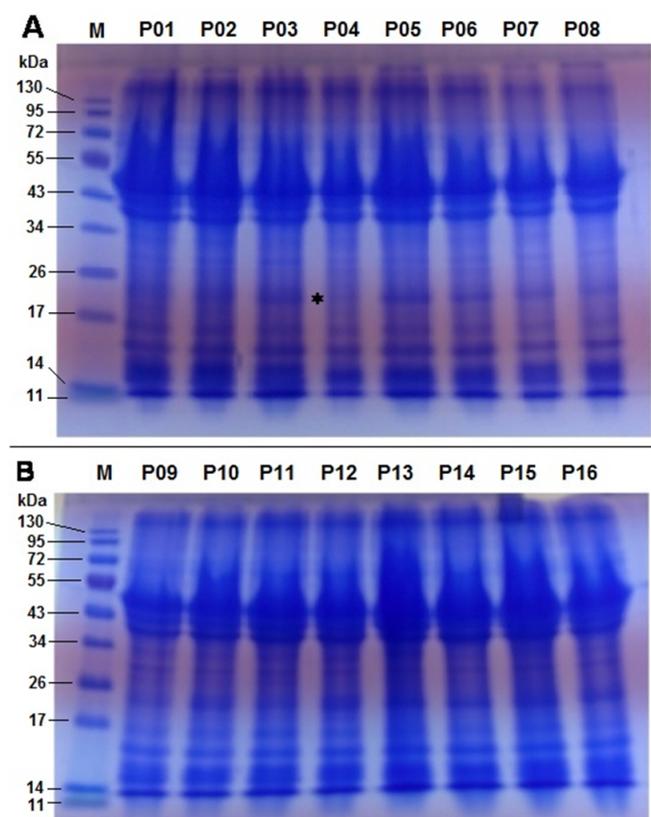


Figure 4. SDS-PAGE of *P. biglobosa* seed total proteins. A) Accessions NH/2016/P01 to NH/2016/08; B) Accessions NH/2016/P09 to NH/2016/P16. M stands for protein size marker. * indicates the missing 24 kDa band in NH/2016/P04.

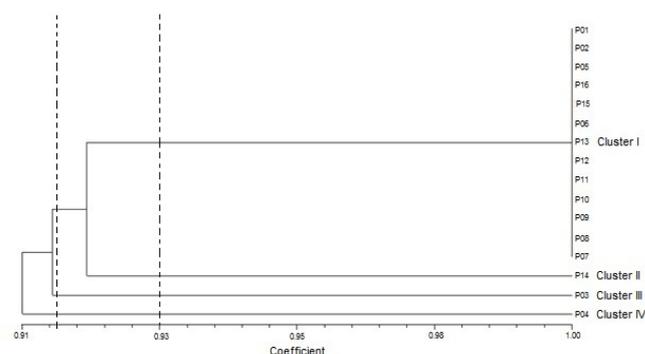


Figure 5. UPGMA dendrogram based on Jaccard's similarity of seed total protein profiles showing close relationship among *Parkia biglobosa* accessions.

(PIC) value is such that they are used to evaluate the amount of genetic diversity and are categorized as high ($PIC > 0.05$), medium ($PIC < 0.05$) and low ($PIC < 0.25$) (Abedian *et al*, 2012). Polymorphic information content ranged from 0.39 for primer OPT05 to 0.93 for primer OPB10 (Table 2).

Fifteen of the sixteen polymorphic primers had PIC values greater than 0.5 indicating that RAPD can develop high-locus polymorphism, which is useful to assess genetic variability of the accessions. All primers showed an average PIC value of 0.79 and nine out of the sixteen primers exceeded the average. This relatively high polymorphism has been observed in studies of genetic diversity on soybean cultivars by Chowdhury *et al* (2001) and Barakat (2004).

Cluster analysis based on UPGMA revealed six distinct clusters at a genetic similarity coefficient of 0.68 on the UPGMA dendrogram (Figure 7). Cluster I consisted of accessions NH/2016/P01, NH/2016/P05, NH/2016/P12, NH/2016/P13, NH/2016/P04, NH/2016/P02, NH/2016/P09, NH/2016/P14 and NH/2016/P15. All the accessions grouped together in Cluster 1 were collected from Derived Savanna except accession NH/2016/P12 which was collected from Guinea Savanna. This suggests that gene flow between the different locations may have occurred as a result of movement of germplasm and exchange of genetic material among farmers and markets. Cluster II consisted of NH/2016/P06; Cluster III consisted of NH/2016/P07 while Cluster IV consisted of NH/2016/P03. Accessions NH/2016/P08, NH/2016/P16 and NH/2016/P11 grouping together in Cluster V were collected from Derived Savanna. Accession NH/2016/P10 collected from Ogoja, Cross River State in the Humid Forest zone grouped alone in Cluster VI. Thus, in the UPGMA analysis of the RAPD profiles, accessions from same agroecological zone grouped together, while in other cases they were placed in different clusters. For most accessions in this study there was similar clustering pattern of geographically closer accessions, indicating significant association between genetic similarity and geographical distance. This does not agree with a report of Adesoye *et al* (2013) who reported that distribution of *P. biglobosa* genotypes among clusters did not correspond with their geographical patterns. The lowest similarity was recorded between NH/2016/P10 and NH/2016/P06, NH/2016/P07, NH/2016/P03. The highest similarity was recorded between accessions NH/2016/P12 and NH/2016/P13, followed by accessions NH/2016/P05 and both NH/2016/P12, NH/2016/P13. Accessions NH/2016/P05 and NH/2016/P13 with narrow genetic distance were collected from the same agro-ecological zone (Derived Savanna). This result corroborates findings of Hamrick and Godt (1989) in which species with small geographic ranges maintained less genetic diversity than geographically widespread species. RAPD clearly distinguished some accessions by accession specific fragments (Figure 6). Therefore, RAPD markers can successfully be used to produce variety specific finger-

prints in *P. biglobosa* accessions and are a valuable tool for assessing genetic diversity.

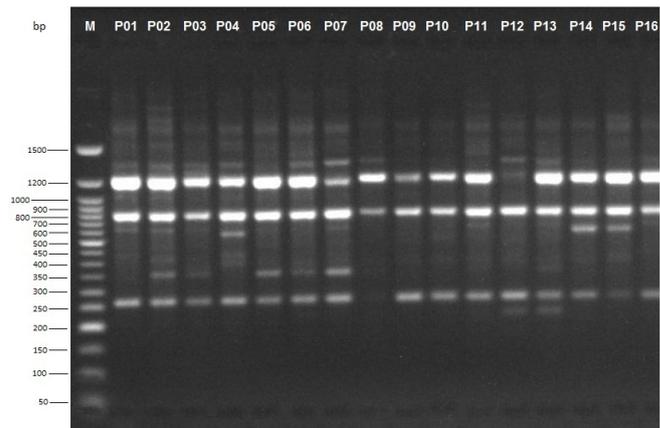


Figure 6. RAPD-PCR analysis of *Parkia biglobosa* accessions as revealed by primer OPB10. Accessions are numbered as in Table 1. M is the molecular size marker.

Conclusion

The low level of variation observed among the accessions as revealed by SDS-PAGE limits the method's applicability to distinguish accessions. Nevertheless, SDS-PAGE was able to detect a polymorphic polypeptide with molecular weight of approximately 24 kDa which was present in all accessions but absent in NH/2016/P04. Thus, SDS-PAGE may not be used to identify accessions based on intraspecific variation;

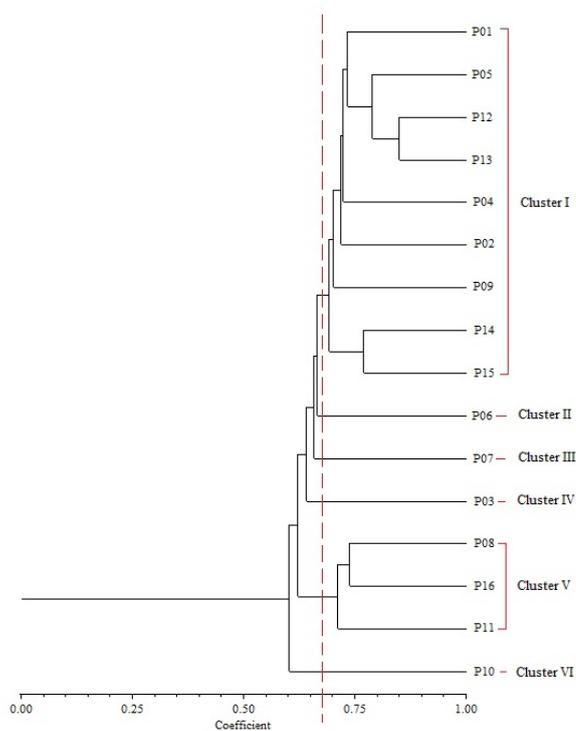


Figure 7. UPGMA dendrogram based on RAPD analysis of *Parkia biglobosa* accessions.

rather it might be more suitable to identify interspecific variation.

RAPD-PCR markers were more informative and could be used to discriminate between *P. biglobosa* accessions. Four RAPD primers (OPB10, OPT07, OPB04, OPT12) revealed high degrees of polymorphism among the accessions. The identification of accessions with maximum genetic divergence should be optimized in breeding programmes. The RAPD marker is therefore a more efficient tool to discriminate between accessions of *P. biglobosa*.

Implications of findings to the conservation and sustainable use of *Parkia biglobosa* genetic resources

- The variation observed in qualitative traits and 100-seed weight among the sixteen accessions of *P. biglobosa* across the three agro-ecological zones of Nigeria indicates their potential for domestication through selection towards conservation and breeding.
- Cluster analysis of the seed protein profile identified NH/2016/P04 as genetically distinct as it was clustered alone and was the most divergent of the sixteen tested accessions. The absence of the 24kDa band in accession NH/2016/P04 distinguishes it from all the other accessions. It can be adopted as parental line for heterosis crossing and should be exploited for conservation, breeding and sustainable utilization.
- Four selected RAPD primers (OPB10, OPT07, OPB04 and OPT12) can be used to discriminate the accessions of *P. biglobosa*.
- The highly diversified accessions of *P. biglobosa* indicate potential for domestication through selection in breeding programmes.
- Most accessions from the same agroecological zone clustered together suggesting that there is a strong correlation between genetic similarity and geographic proximity.
- The homogeneity of alleles among the studied *P. biglobosa* accessions suggests possible loss of intraspecific genetic diversity. The weakening gene pool and diversity observed can be enhanced through more germplasm collections, particularly from the diverse agroecological zones of Nigeria for better genetic characterization using more specific markers towards conservation, breeding and sustainable utilization.

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Author contributions

P. E. Akin-Idowu designed the experiment. P. E. Akin-Idowu, A. O. Aduloju and O. I. Akinyoola executed the experiment. O. I. Akinyoola, P. E. Akin-Idowu and D. O. Ibitoye performed the analysis. P. E. Akin-Idowu, O. I. Akinyoola, U. G. Adebo, U. Orkpeh, Y. O. Olagunju and D. O. Ibitoye wrote the manuscript. All the authors read and approved its submission.

Conflict of interest statement

The authors declare no conflict of interest.

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