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Climate change and Fading Genetic Resources of *Parkia biglobosa* (Jacq.) in Nigeria based on SSR markers

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Abstract: African locust bean (*Parkia biglobosa* (Jacq.) is a multi-purpose economic tree with genetic potentials in sub-Saharan Africa. Its cultivation and production is declining with increased aging and genetically threatened throughout its natural ranges. Research efforts are needed to change the present scenario to sustainable cultivation and utilization, hence this present study. This study was aimed at evaluating genetic diversity and geographical spread relationships of twenty landraces collected from different ecological zones of Nigeria using simple sequence repeat (SSR) markers. Ten SSR markers were screened and five primers (PbL02, PbL03, PbL04, PbL05 and PbL09) were selected based on clear amplification products and reproducible scorable bands. The SSR primers detected a total of 55 alleles ranged from 10 to 14 alleles with a mean of 11. The percentage polymorphisms were high and ranged from 68.75 % in PbL04 to 84.21 % in PbL05 with a mean of 74.16 %. The polymorphic information content (PIC) was in the range of 0.31 in PbL02 to 0.37 in PbL09. The genetic diversity and heterozygosity values ranged from 0.39 to 0.50 and 0.00 to 0.68 while the average genetic distance for all pair wise comparisons was 0.31.The first five Principal Component (PC) accounted for 70.20 % of the total variation out of which PC1 (31.50%) and PC2 (19.20%) extracted 49.70% molecular similarity. The dendrogram resulted in separation of the 19 landraces into three major clusters based on unweighted pair group method with arithmetic average. Cluster I comprised of five landraces: ABNo130 and BENo023; OYNo11, KANo125 and NiNo262 while cluster II had only one (BANo116). Cluster III was diverse comprising 13 landraces: ZANo188, KNNo162, KENo220, GMNo076 and EbNo260, ADNo64, EdNo164, KANo137, KENo217, KwNo270, NiNo241, OsNo206 and PLNo120. The homogeneity of alleles among the studied landraces suggested suspicion of loss of genetic intra-specific variation among the landraces of *P. biglobosa* which calls for concerted efforts toward better cultivation, conservation, management, utilization and genetic improvement of the species in Nigeria.

Keywords: African locust bean; climate change; cluster analysis; genetic intra-specific diversity; Polymorphic information content; food and nutrition security.

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1. Introduction

Global food sufficiency, food security and agricultural production sustainability are currently confronted with negative effects of climatic change and greenhouse gases. The environment is facing serious threat while forest trees are reducing in cultivation, production and utilization. Legumes are generally believed to have capacity to mitigate the negative effects of climate change and greenhouse gases based on their inherent features [1]. They provide important sources of oil, fiber, and protein-rich food and feed while supplying nitrogen (N) to agro-ecosystems via their unique ability to fix atmospheric nitrogen in symbiotic relationship with the soil bacteria rhizobia [2-4]. Legumes increases soil nitrogen content, stimulates organic fertilizer and enhances crop productivity. However, many indigenous legume species have been neglected and underutilized which could contribute significantly to food and nutritional security in Africa [1]

African locust bean (*Parkia biglobosa* (Jacq.)) of the Fabaceae family is one of the important woody forest tree legumes in the semi-arid and sub-tropical farming systems of West Africa [5]. It is a perennial multi-purpose tree species widely used for food, medicine and ecological purposes [5,6]. The pods containing-seeds are highly nutritious and rich in protein [6-8]. Locally, the seeds are prepared as spicy food/condiments and consumed in soups in many African households [9,10]. Different parts of *P. biglobosa* such as seeds, bark, roots and flowers are reportedly used to treat myriads of diseases and ailments [11-13]. Scientific findings indicated that the condiments from fermented seeds of *P. biglobosa* control activities of certain enzymes relevant to cardiovascular diseases and endothelial function [14]. Generally, locust bean tree is used as source of firewood, charcoal and as timber for making pestles, mortars, bows, hoe handles, and seats while the husks and pods are good food for livestock [13,15-17].

Globally, the area of cultivation and production of the species is declining without reforestation strategies in sight. Recent studies have shown a reduced regeneration of the species [5]. Genetic resources are fading away, and improved tree management practices are lacking while genetic improvement are not sufficiently promoted. The species is threatened by over-exploitation, bush fires, and a progressive habitat degradation leading to fragmentation of tree populations [18]. In addition, overgrazing by domestic animals causes a lack of regeneration and an over-aging of tree individuals in savanna parklands; additional potential threats are also envisaged as a result of the absence or declining number of pollinators [5,6,17]. Our recent survey of the species in Nigeria also indicated poor conservation and poor management of its genetic resources throughout out its ecological range, yet there is increased demand for its use and derivable products [19]. Thus, there is need for concerted research efforts to improve its cultivation, conservation, management of its genetic resources and utilization toward sustainable utilization for food and protein security.

P. biglobosa is a diploid genome with different chromosome numbers (2n = 2x = 22, 24, and 26) [20] and thus genetic diversity is expected to be high. However, there is lack of adequate and consistent data on genetic diversity with no improved varieties or breeding lines in Africa. Few genetic diversity assessments have been carried out [5]. In addition, most of the landraces available are represented in few farmlands and open spaces with poor cultivation and management practices while majority are aging. In the light of climate change, safeguarding the genetic diversity of the species is crucial to foster adaptation and to support its long-term survival.

Over the years, molecular markers have proven to be highly discriminatory, easy and rapid in the assessment of genetic diversity among plant species. Markers such as simple sequence repeat (SSR), amplified fragment length polymorphism (AFLP), sequence-related amplified polymorphism (SRAP) and single nucleotide polymorphisms (SNPs), have several advantages including abundant, independent from the environment, suitability for early and rapid evaluation, and having non-tissue specific characteristics [21-23]. SSRs (microsatellites) or short tandem repeats (STR) are widely present in eukaryotic genomes and very useful for a number of reasons including co-dominant inheritance, high polymorphism, high variability and suitability for automated allele sizing and cross-species transferability[24,25]. Currently, SSR markers have not been applied to study and characterize the landraces of *Parkia biglobosa* from Nigeria. In this study, we present the first evaluation of genetic diversity of the threatened *P. biglobosa* landraces in Nigeria using SSR markers

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in the face of climate change and over-exploitation of the species. Hence, this study uses SSRs to analyze genetic diversity among 19 selected landraces of *Parkia biglobosa* collected in Nigeria; to generate allele frequency and heterozygosity values useful towards conservation, management, breeding and genetic improvements of the species.

2. Materials and Methods

Plant samples and areas of collection

A total of 20 landraces of *P. biglobosa* were selected from the field survey on sample collection of the genetic diversity assessment of under-exploited African plants for genetic improvement and food security [19] but 19 analyzed. The selected landraces, codes and areas of collection of the accessions used for this study are as listed in Table 1. One of the samples was identified by the first author with the voucher specimen number (*Pb*/CUBio/H812) and deposited in the depository of the Department of Biological Sciences, Covenant University, Ota, Nigeria.

Sample Preparation

Young fresh leaf samples of the collected accessions from the survey were silica gel dried in well labeled zip-lock bags. The crystals were removed and moisture-free samples kept at – 80 °C at the Molecular Biology Laboratory of the Department of Biological sciences, Covenant University, Ota, Ogun State, Nigeria. The samples were transferred and lyophilized for three days at Bioscience Laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria for molecular analysis.

Sources of Primers

Ten microsatellite primers developed for *Parkia biglobosa* [26] were adopted, tested and used for this study. The oligonucleotides (10 bases F/R) with code number (NG2018/049) were synthesized and supplied by Ahava Biotechnology and Forensic Services Ltd. The locus name, sequences, repeat motifs and the allele size range are as reported by Lassen *et al* [26].

DNA extraction and Quantification

Genomic DNA was extracted using modified SDS protocol as described by Dellarpotal et al. NanoDrop spectrophotometer (ND-1000) and 1% agarose gel were used to determine the quality and quantity of the extracted DNA prior to amplification.

PCR amplification

The PCR reactions of 10 µl contained 3.0µl of genomic DNA (100 ng / µl), 1.0µl of 10 X PCRbuffer, 0.4 µl of MgCl2 (50mM), 0.5 µl each of forward and reverse SSR primer mix in 5 µM. 0.8µl of 2.5mM DNTPs, 0.8 µl of DMSO, 0.1 µl of taq polymerase (5 u/ul) and 2.9 µl of sterile double distilled water on a GeneAmp PCR system 9700, USA with the following programmes; initial denaturation at 94.0°C for 5min, final denaturation at 94.0 °C for 15 sec, annealing at 55.0°C for 20 sec and extension at 72.0°C for 30 sec (9cycles). The reactions also followed another 30 cycles of 94.0°C for 15 sec, 45.0°C for 20 sec, 72.0°C for 30 sec and a final extension at 72.0°C for 7 min. The PCR products were loaded on 1.5 % agarose gel with a 1000 bp ladder plus generuler (Thermo Scientific).

SSR PAGE Analysis

The amplified products were resolved on 6 % (w/v) polyacrylamide gel electrophoresis (PAGE) for 2.5 hours in 1 X Tris/borate/EDTA buffer with 7.5 M urea at 70 W according to the manufacturer's protocol. The gels were stained with silver nitrate. The size of DNA bands in base pairs was estimated using the 1000-bp ladder. Gels output files were saved as TIFF format for scoring and analysis.

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142 Data analysis

143 Cytogenetic studies of *Parkia biglobosa* showed diploid genome with different chromosome
144 numbers (2n = 2x = 22, 24, and 26) [20] which hampers the identification of alleles from homologous
145 chromosomes on specific loci. Thus, SSR bands were scored as dominant. The bands were considered
146 polymorphic when absent in some samples. Percent polymorphism for each marker was generated
147 by the formula:

148 $$\frac{Number\ of\ polymorpic\ bands}{Total\ number\ of\ scord\ bands}\ X\ 100.$$

149 Alleles were scored in binary form ('1' for presence and '0' for absence) and the pair-wise genetic similarity
150 between genotypes generated through Jaccard's co-efficient. Gene diversity, heterozygosity and polymorphic
151 information content (PIC) for each of the markers were calculated using Power Marker v.3.25 software [27].
152 Dendrogram was also generated using Liu and Muse [27] following the unweighted pair group method
153 average (UPGMA) clustering. Multivariate principal coordinate analysis (PCoA) was generated with
154 GenAlex 6.5 [28] to analyse the genetic divergence between *Parkia biglobosa* landraces. Genetic distances
155 between the landraces were calculated using Nei [29].

156 **3. Results**

157 **3.1. Selected landraces and areas of collection**

158 The selected landraces of *P. biglobosa* cut across five (5) Southern states (Oyo, Osun, Edo, Abia
159 and Bayelsa) and ten (10) Northern states (Kano, Kaduna, Niger, Kwara, Plateau, Gombe, Bauchi,
160 Kebbi, Zamfara and Sokoto) of Nigeria. The areas of sample collections with codes are shown in Table
161 1 and Figure 1.

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163 Table 1: Codes and areas of collection of *Parkia biglobosa* landraces used for this study

S/N	Code	Areas of collection	L/G	State	Latitudes(N)	Longitudes (E)
1	AdNo064	Bunyayi	Maibelwa	Adamawa	8.564	11.564
2	BeNo023	Katsina-ala	Katsina-ala	Benue	7.167	9.287
3	AbNo130	Dakwo/Abai	Shangai	Abia	8.961	7.542
4	BaNo116	Soro	Ganjuwa	Bauchi	10.766	10.288
5	EdNo164	Ehanle-Ewu	Esan central	Edo	6.82	6.25
6	EbNo260	Ezillo	Ishielu	Ebonyi	6.47	7.83
7	GMNo076	Ture-Balam	Katungo	Gombe	9.787	11.42
8	KaNo125	Aboro Village	Shangai	Kaduna	8.542	9.486
9	KeNo220	Besse	Koko-Besse	Kebbi	10.16	4.768
10	KnNo162	Gwarmai	Ikara	Kano	11.871	8.246
11	KaNo137	Igwa	Kajuru	Kaduna	9.366	7.301
12	KeNo217	Dada village	Koko-Besse	Kebbi	11.924	4.427
13	KWNo270	Onipako	Mokwa	Kwara	8.795	4.825
14	NINo241	Wawa	Kainji	Niger	9.483	4.419
15	NINo262	Tashabu	Zugurma	Niger	8.966	4.384
16	OSNo50	Ejigbo	Ejigbo	Osun	7.8972	4.3365
17	OYNo011	Iseyin Road	Ojongbodu	Oyo	7.8537	3.8932
18	SONo206	Shagari road	Shagari	Sokoto	11.924	4.99
19	PLNo120	Anglai Jos	Riyom	Plateau	8.771	9.644
20	ZaNo188	Kadauri	Maru	Zamfara	12.621	6.314

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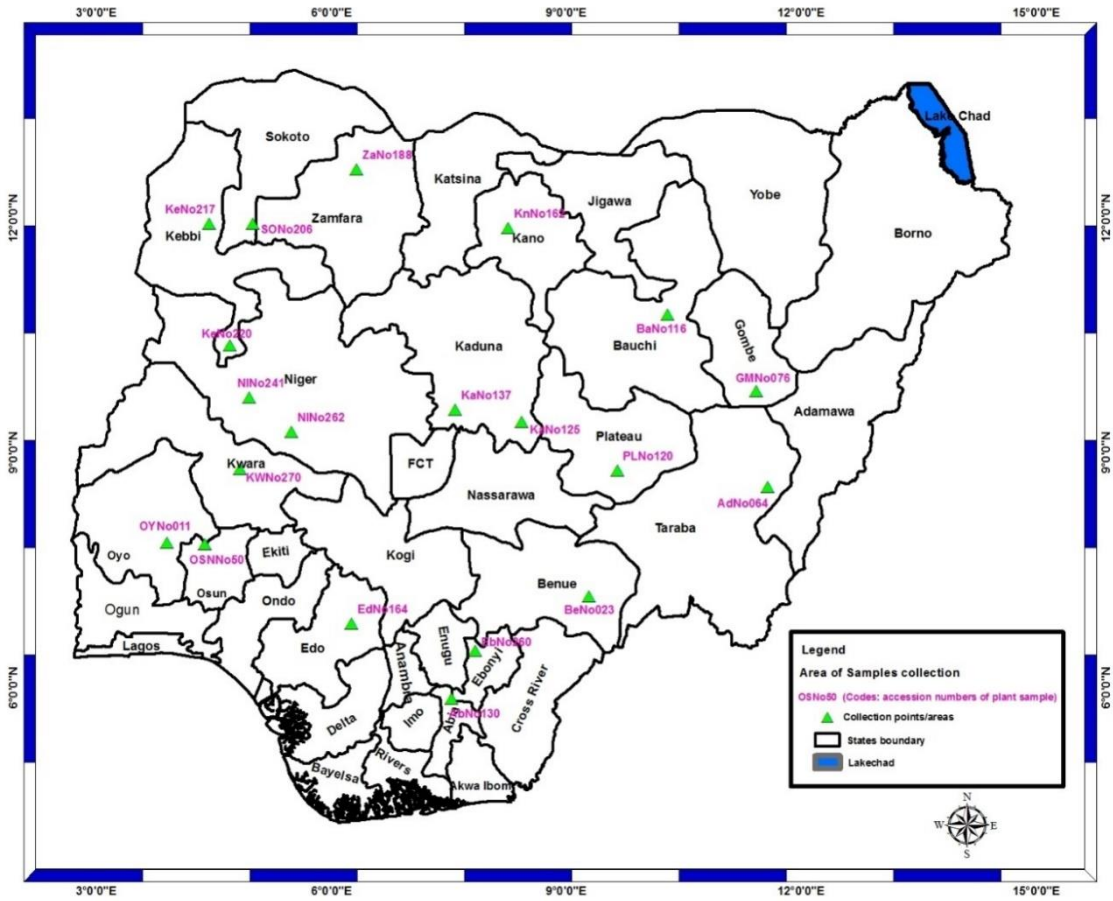

Figure 1: Areas of sample collection

3.2. *Genetic Summary of the SSR markers used for this study*

Ten SSR primers were tested out of which five (50%) selected based on clear amplification and scorable bands and used for this study. The primer sequences, repeat motif and allele size range are as reported by Lassen *et al* 2014. The amplification products allowed for analysis of the 20 landraces of *P. biglobosa*, however OSNo50 (from Osun) did not show appreciable amplification and not included in the analysis. The data in Table 2 showed the summary of the genetic parameters of the five SSR markers used for this study. The number of bands per locus ranged from 16 (PbL04) to 19 (PbL05 and PbL09) with an average of 17.8. The number of scored bands was higher in PbL05 and PbL09 with 16 and 14 bands, respectively, compared to 12 bands in PbL03 while PbL02 recorded 13 bands. The percentage polymorphisms was generally high and ranged from 68.75 % in PbL04 to 84.21 % in PbL05 with an average of 74.16 %. A total of 55 alleles were detected and the number of alleles per marker ranged from 10 to 14 with an average of 11. The polymorphic information content were in the range of 0.31 in PbL02 to 0.37 in PbL09 with an average of 0.35 (Table 2). Higher PIC value (0.37) and maximum number of alleles (14) were shown by the locus PbL09 while locus PbL02 generated lower PIC and minimum number of alleles per marker across the 19 landraces of *P. biglobosa* studied. Major allele frequency was higher in PbL02 (0.74) and lower in PbL09 (0.53) while PbL03, PbL04 and PbL05 recorded 0.61, 0.63 and 0.66, respectively with an average of 0.63. The genetic diversity values were high across the markers and ranged from 0.39 in PbL02 to 0.50 in PbL09 with an average of 0.46. Heterozygosity and fixation index values ranged from 0.00 and 0.0001 in PbL02 to 0.68 and 0.5 in PbL05 with average of 0.48 and 0.035, respectively.

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Table 2: Genetic Summary of the five SSR markers used for the *Parkia biglobosa* landraces studied

Marker	MAF	NA	GD	Het	NB	TSB	% Polymorphic	PIC	F
PbL02	0.74	10	0.39	0.00	18	13	72.22	0.31	0.0001
PbL03	0.61	10	0.48	0.58	17	12	70.58	0.36	-0.185
PbL04	0.63	11	0.47	0.63	16	11	68.75	0.36	-0.3333
PbL05	0.66	10	0.45	0.68	19	16	84.21	0.35	-0.5
PbL09	0.53	14	0.50	0.53	19	14	73.68	0.37	-0.0286
Mean	0.63	11	0.46	0.48	17.8	13.2	74.16	0.35	-0.035

MAF = Major Allele Frequency, NA = Number of alleles, GD = Genetic Diversity, Het = Heterozygosity, NB = Number of bands, TSB = Total number of scored bands, % Polymorphic = Percentage Polymorphic, PIC = Polymorphic Information Content, F = Fixation Index

3.3 Genetic distance and similarity among the landraces of *P. biglobosa* studied

Genetic distance among the 19 *P. biglobosa* landraces was calculated to identify the relatedness between the landraces. The average genetic distance for all pair wise comparisons was 0.31. Higher genetic distance of 0.90 was recorded between landraces ZANo188 and KANo125; ZANo188 and NiNo262 and OyNo11 while GMNo076 and ABNo130; GMNo76 and BENo023; KANo125 and KNNo162; KANo125 and EbNo260; KNNo162 and NiNo241 and OyNo11 showed genetic distance of 0.70. More than 10 landraces had genetic distance of 0.60: ABNo130 and ADNo064; ABNo130 and EdNo164; ABNo130 and KENo270; ABNo130 and KWNo270; ABNo130 and NiNo241; OsNo260; PLNo120; ADNo64and BENo023: NiNo262; OyNo11; BENo023 and EdNo164. Other genetic distance relatedness among the 19 landraces studied was presented in Table 3.

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206 Table 3: Genetic distance and similarity among the landraces of *P. biglobosa*

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OTU	ABNo130	ADNo64	BANo116	BENo023	EbNo260	EdNo164	GMNo076	KANo125	KANo137	KENo217	KENo220	KNNo162	KwNo270	NiNo241	NINo262	OSNo206	OYNo11	PLNo120	ZANo188
ABNo130	0	0.6	0.5	0	0.5	**0.6**	**0.7**	0.2	0.6	0.6	0.5	**0.7**	**0.6**	**0.6**	0.2	0.6	0.2	**0.6**	**0.7**
ADNo64	0.6	0	0.3	0.6	0.1	0	0.1	0.6	0	0	0.1	0.1	0	0	0.6	0	0.6	0	0.3
BANo116	0.5	0.3	0	0.5	0.4	0.3	0.2	0.3	0.3	0.3	0.4	0.4	0.3	0.3	0.3	0.3	0.3	0.3	**0.6**
BENo023	0	0.6	0.5	0	0.5	**0.6**	**0.7**	0.2	0.6	0.6	0.5	0.7	0.6	0.6	0.2	0.6	0.2	0.6	**0.7**
EbNo260	0.5	0.1	0.4	0.5	0	0.1	0.2	**0.7**	0.1	0.1	0.2	0.2	0.1	0.1	0.7	0.1	0.7	0.1	0.2
EdNo164	**0.6**	0	0.3	0.6	0.1	0	0.1	0.6	0	0	0.1	0.1	0	0	0.6	0	0.6	0	0.3
GMNo076	**0.7**	0.1	0.2	0.7	0.2	0.1	0	0.5	0.1	0.1	0.2	0.2	0.1	0.1	0.5	0.1	0.5	0.1	0.4
KANo125	0.2	0.6	0.3	0.2	0.7	0.6	0.5	0	0.6	0.6	0.5	**0.7**	**0.6**	**0.6**	0	**0.6**	0	0.6	**0.9**
KANo137	0.6	0	0.3	0.6	0.1	0	0.1	0.6	0	0	0.1	0.1	0	0	0.6	0	0.6	0	0.3
KENo217	0.6	0	0.3	0.6	0.1	0	0.1	0.6	0	0	0.1	0.1	0	0	0.6	0	0.6	0	0.3
KENo220	0.5	0.1	0.4	0.5	0.2	0.1	0.2	0.5	0.1	0.1	0	0.2	0.1	0.1	0.5	0.1	0.5	0.1	0.4
KNNo162	**0.7**	0.1	0.4	0.7	0.2	0.1	0.2	**0.7**	0.1	0.1	0.2	0	0.1	0.1	0.7	0.1	**0.7**	0.1	0.2
KwNo270	**0.6**	0	0.3	0.6	0.1	0	0.1	0.6	0	0	0.1	0.1	0	0	0.6	0	0.6	0	0.3
NiNo241	**0.6**	0	0.3	0.6	0.1	0	0.1	0.6	0	0	0.1	0.1	0	0	0.6	0	0.6	0	0.3
NINo262	0.2	0.6	0.3	0.2	**0.7**	0.6	0.5	0	0.6	0.6	0.5	**0.7**	**0.6**	**0.6**	0	**0.6**	0	0.6	**0.9**
OSNo206	0.6	0	0.3	0.6	0.1	0	0.1	0.6	0	0	0.1	0.1	0	0	0.6	0	0.6	0	0.3
OYNo11	0.2	0.6	0.3	0.2	**0.7**	**0.6**	0.5	0	0.6	0.6	0.5	0.7	0.6	0.6	0	0.6	0	0.6	**0.9**
PLNo120	0.6	0	0.3	0.6	0.1	0	0.1	0.6	0	0	0.1	0.1	0	0	0.6	0	0.6	0	0.3
ZANo188	**0.7**	0.3	0.6	**0.7**	0.2	0.3	0.4	**0.9**	0.3	0.3	0.4	0.2	0.3	0.3	**0.9**	0.3	**0.9**	0.3	0

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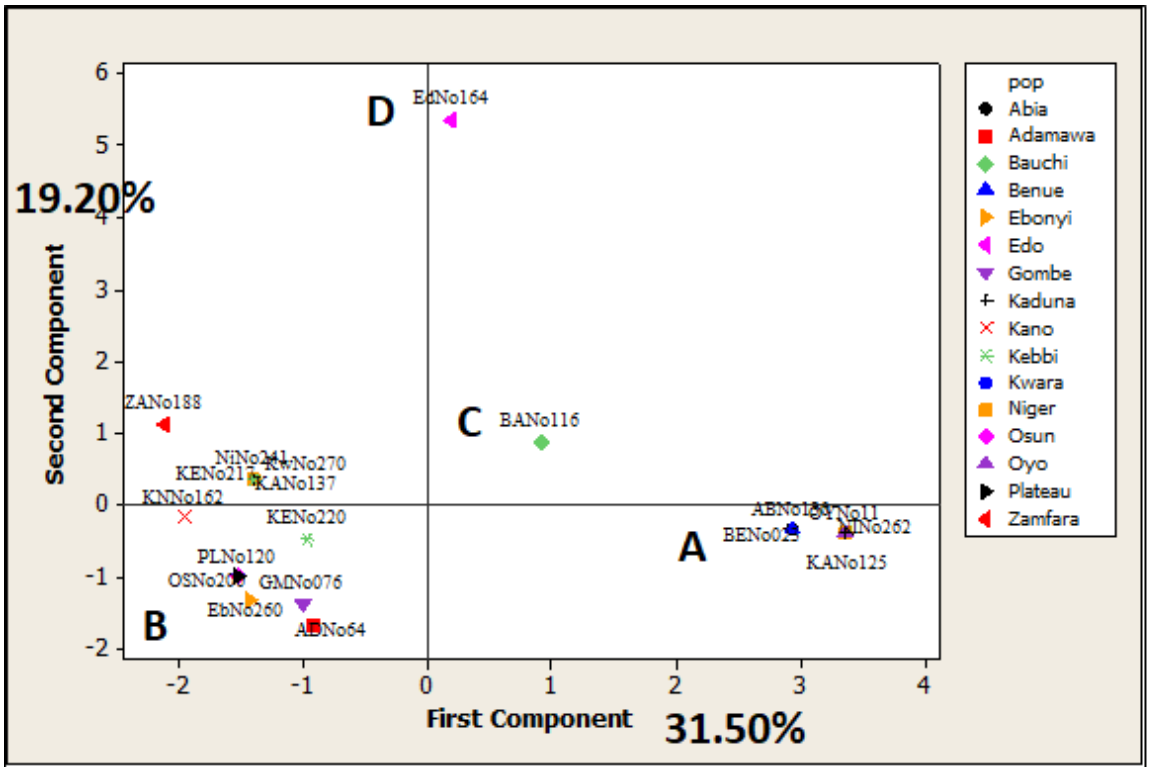
210 3. 4 Principal Component Analysis (PCA) of the landraces of *P. biglobosa* studied

211 The first five PC accounted for 70.20 % of the total variation out of which PC1 (31.50 %) and PC2
212 (19.20%) extracted 49.70 % molecular similarity. The scatter plot of the PCoA clustered the 19
213 landraces of *P. biglobosa* into four major groups (A – D). Group A comprised five (5) landraces
214 (ABNo130, OyNo11, BENo023, NiNo262 and KANo125), group B consisted of eleven (11) landraces
215 (ADNo64, EbNo260, GMNo076, OsNo206, PLNo120, KENo220, KNNo162, NiNo241, KwNo270 and
216 KANo137) while group C and D had one representative each EdNo164 and BANo116 (Fig. 2).



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218 Figure 2: Comparison of PC 1 and PC 2 of the landraces of *P. biglobosa* studied

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3.5 Cluster analysis

The cluster analysis resulted in separation of the landraces of *P. biglobosa* into three (3) major clusters (Fig. 3). Cluster I was comprised of five landraces subdivided into two sub clusters: ABNo130 and BENo023; OYNo11, KANo125 and NiNo262 while cluster II had only one landrace BANo116. Cluster III was larger comprising 13 landraces with 6 subclusters of which 5 were single cluster each: ZANo188, KNNo162, KENo220, GMNo076 and EbNo260 while the 6th sub-cluster comprised eight (8) landraces: ADNo64, EdNo164, KANo137, KENo217, KwNo270, NiNo241, OsNo206 and PLNo120 at similarity coefficient of 0.050 (Fig.3).

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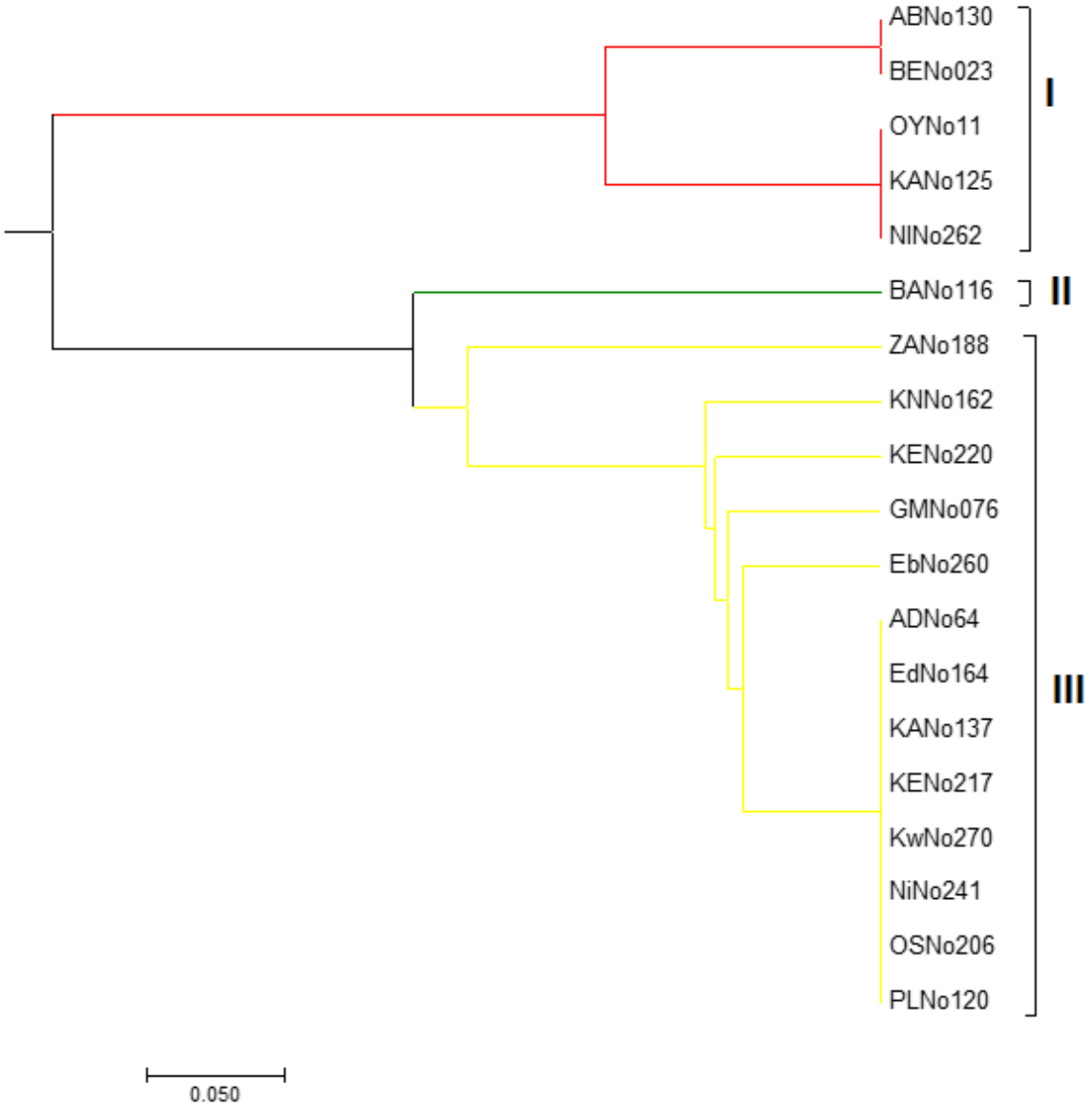

Figure 3: Cluster analysis of the 19 landraces of P. biglobosa segregated into three major clusters.

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4. Discussion

In the last decade, there had been an enormous increase in the use of molecular marker techniques to measure genetic variation in forest trees and legumes. The degree of genetic variation available in germplasm collections of species is directly linked to the success of any breeding/genetic conservation programmes [30,31]. So far, there are very few marker-based studies at hand assessing genetic diversity of *Parkia biglobosa* though phylogeography divergence among populations in West and Central Africa was recently reported using SSR markers [5,32].These SSR markers were specifically developed for *Parkia biglobosa* to study population structure and reproduction biology of the species [26].To our knowledge, this study is probably the first to assess genetic diversity of *P. biglobosa* in Nigeria using SSR markers. SSR marker techniques will continued to be relevant in the assessment of genetic variation in forest legume trees to estimate genetic diversity, population structure and reproduction biology.

SSR Markers and Genetic diversity of *P. biglobosa*

The five SSR primers were highly polymorphic and appeared valuable in detecting genetic diversity among the *P. biglobosa* landraces studied. The range of alleles (9 – 14 alleles) among the markers are comparable to the reports of Lassen *et al*. [26] and higher than previous values on some tree species [33,34]. The mean PIC which represent allele diversity was moderate (0.35) and similar to the report of Kaur *et al*. [35] for *Tribulus terrestris* but lower to the values reported by Popoola *et al*. [36]. Similarly, the mean percent polymorphism (74.16%) was high and comparable to previous reports on forest trees including *P. Biglobosa* [35-37]. The SSR markers amplified at least two or more than three fragments and recorded a PIC value of 0.35 which is an indicator of high polymorphism. The average values of heterozygosity found in this study also compares with the range of values reported for other forest trees; *Milicia excels* (He = 0.46 – 0.61) and *Vitellaria paradoxa* (He = 0.27 – 0.65) but lower than that of Lompo et al. [32] on *P. biglobosa* (He = 0.83). The relatively high values for genetic parameters such as major allele frequency, genetic diversity, percent polymorphism and heterozygosity indicated high genetic diversity among the landraces of *P. biglobosa* studied. This observation can be attributed to adaptation of the species to the different eco- geographical setting, continuous spreading and possibly the amphipolyploidy nature of *P. biglobosa* genome with different chromosome number (2n = 2x = 22, 24, and 26) [20]. Genomes of such nature have been reported to express relatively high genetic diversity among individuals and within populations of such species [38]. Africa indigenous forest trees such as *Milicia excels*, *Vitellaria paradoxa* and *Parkia biglobosa* have received attention from scientists during the last decade to draw attention of various policy makers and other stakeholders to negative effects of environmental degradation and deforestation [33,37,39].

Genetic similarity and Cluster Analysis of the *P. biglobosa* landraces

The dendrogram, PCA and genetic distance analyses from this study clearly showed high degree of genetic relatedness among the landraces of *P. biglobosa* studied. The higher genetic distance of 0.90 was recorded between landraces ZANo188 and KANo125; ZANo188 and NiNo262 and OyNo11 clustered in group I and III. GMNo076 and ABNo130; GMNo76 and BENo023; KANo125 and KNNo162; KANo125 and EbNo260; KNNo162 and NiNo241 and OyNo11 showed genetic distance of 0.70. Over 13 landraces had genetic distance of greater than 0.60 which indicated higher relatedness. The PCoA analysis supports the grouping as observed by UPGMA based dendrogram and revealed the segregation of the landraces into cluster groups based on alleles distribution among the landraces except EdNo164 which was further isolated from cluster group III of dendrogram.

There was no correlation whatsoever between cluster groups and areas of collection of the landraces. Higher genetic similarities were observed between landrace ABNo130 (collected from Abia state) and BENo023 (from Benue), OyNo011 (Oyo state), KANo125 (Kano) and NiNo262 (Niger) in cluster group I. We obtained similar trend in cluster group II with high genetic similarity among the landraces; ADNo64 (Adamawa), EdNo164 (Edo), KANo137 (Kaduna), KENo217 (Kebbi),

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KwNo270 (Kwara), NiNo241 (Niger) and OsNo206 (Osun). Cluster group II with single landrace BANo116 (Bauchi) showed clear genetic difference from other clusters. The clustering system of dendrogram and PCA indicated that cluster groups shared a large number of alleles resulting into random distribution of landraces in cluster group I and III except cluster group II (BANo116; Bauchi) and group D of PCA (EdNo164; Edo) both of which were isolated from the others. These observations clearly suggest that the landraces are becoming genetically homozygous as there was no clear differentiation according to areas of collection other than exchange of alleles from one location to another through trade routes, agents of pollination, gene flow and exchange of planting materials. The analyses further showed that landraces collected from northern region of Nigeria with endemic populations of the species were genetically grouped with representatives from southern Nigeria as illustrated in Fig. 2 and 3, respectively. Thus, it is more likely that *P. biglobosa* genetically spread from northern Nigeria particularly from Adamawa, Gombe, Plateau and Kaduna to southern Nigeria with North Central of Niger and Kwara states as point of entry. The summary of cluster analysis corroborated the observations as cluster group I; that was postulated to have genetically spread from Kaduna, cluster group III from Niger or Kano while the isolated cluster group II served as a bridging gap linked to Bauchi, North-east of Nigeria. *P. biglobosa* is highly adapted to Savanna ecological zones from the northern hemisphere of Nigeria to the derived guinea Savanna of the southern Nigeria. Though this study did not observed genetic barriers which could have created wide genetic diversity among the landraces, the genetic similarities and differences obtained are very important towards effective management and conservation of the species in Nigeria. The homogeneity of alleles observed in cluster group I and III reflects weak genetic diversity as reported by Amusa *et al*. [17] using Random Amplified Polymorphic DNA (RAPD) markers in 23 open-pollinated accessions of *P. biglobosa* in Nigeria. Although the genetic diversity parameters from the SSR markers used in this study demonstrated high genetic diversity, weak genetic diversity as observed from the multivariate analyses (PCA and dendrogram) might be linked to fewer number of landraces selected for this study. Consequently, the recent report of Lompo *et al*. [32] on phylogeography of *P. biglobosa* revealed a high degree of genetic differentiation and spatial structured populations in West and Central Africa using 1610 individuals and 84 populations. In addition, previous study on 24 populations of West and Central Africa indicated high genetic diversity using chloroplast markers [40]. The limitation of our study arose from less number of genotypes.

Genetic Loss and Conservation Status of *P. biglobosa* in Nigeria

In the course of field survey and sample collections on genetic diversity assessment of under-exploited African plants for genetic improvement and food security [19], we observed possible loss of genetic resources among the neglected but extensively versatile indigenous species in Nigeria. These genetic resources are vanishing at an alarming rate linked to massive unregulated developmental activities, political instability, poor or nonexistent conservatory programmes and erosion of cultural heritages. There was poor management and conservation of genetic resources of *P. biglobosa* throughout its ecological zones. Individual landraces affected by land clearing, bush fire, deforestation, old age and climatic change are not replaced through conscious effort of cultivation, afforestation and research. To our knowledge, there are no records of active germplasm banks on *P. biglobosa* to represent core collections of the species, available landraces were old, exposed to bush fire burning from time to time and over-exploitation by users. From this study, the homogeneity of alleles among the studied landraces further suggested the suspicion of loss of genetic intraspecific variation among the landraces. This loss is a threat to our environment and consequently to the well-being of the present and future generations. *P. biglobosa* is threatened in Nigeria and concerted scientific efforts are required toward systematic conservation and management of the genetic resources of the taxa using the combination of *ex situ* and *in situ* techniques. Plant genetic resources are the raw materials that farmers, breeders and researchers rely upon to improve the quality and the quantity of food produced, and to respond to new conditions, including changes in climate [41,42].

The conservation and sustainable use of genetic resources also provide important options for adapting agricultural production to the impacts of climatic change.

Conclusion

The present study significantly contributes fundamental genetic evidence towards the implementation of appropriate conservation and utilization plans as well as potential breeding trial programs for *Parkia biglobosa* genetic resources in Nigeria. Systematic cultivation, management and conservation of the species are recommended in view of changing climate and towards sustainable utilization in Nigeria. In addition, the weakening gene pool and diversity reported from this study can be mitigated through germplasm collections particularly from the endemic Northern regions for further systematic characterizations. Genetic insight is also relevant to guide sustainable harvest. Based on our observations and utilization of *P. biglobosa,* it has been limited to local uses, and in view of its strategic importance to boost food security and enhance other important utilization of the species, it is imperative to step up genetic diversity studies via germplasm collection and characterization. On the whole, this study is a timely contribution considering the multi-purpose economic importance of the species, its wide distribution, adaptation and ease of integration into commercial agricultural production.

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