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GENETIC DIVERSITY OF *Adansonia digitata* (L.) AND *Vitelleria paradoxa* (C.F. Gaertn) IN NIGERIA

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ABSTRACT

This study evaluated the genetic diversity of Adansonia digitata and Vitelleria paradoxa in three savanna ecological zones of Nigeria using ten microsatellite markers. Leaf samples were collected from three savanna ecological zones of Nigeria. Genomic deoxyribonucleic acid (DNA) was extracted from 0.020g powder of silica gel dried leaves using the modified Doyle and Doyle (1990) protocol. Polymerase Chain Reaction (PCR) was performed in a volume of 25µl in a DNA thermal cycler. PCR products were separated by electrophoresis using 2% agarose gel inTBE buffer. Fragment sizes were estimated using ladder – DNA sizing marker and analysed using CERVUS. Averagely high genetic diversity (He) was recorded for A. digitata and V. paradoxa genotypes for Sudan Savanna (0.6572, 0.5792) and Guinea Savanna (0.6050, 0.5302) but significantly higher in Derived Savanna ecological zone (0.8093, 0.7372). A good strategy to protect these endangered tree species is to protect more of their habitat and also to develop their plantation outside their natural habitat.

Key words: *Adansonia digitata*¸ *Vitelleria paradoxa*, Genetic diversity, conservation, Microsatellites.

INTRODUCTION

Adansonia digitata and *Vitelleria paradoxa* are multipurpose tree species, belongs to the Bombacaceae and Sapotaceae families respectively. They are native deciduous trees from the African savannas and generally known as the African Baobab and Shea butter (Allaye *et al.* 2008 and Amusa *et al.* 2017). Almost all parts of *A. digitata* and *V. paradoxa* trees are used as medicines and also possess high nutritional values. Leaves, bark and fruits of these species are traditionally employed as food stuffs and for medicinal purposes, and for that reason baobab is also named "the small pharmacy or chemist tree" (Gebauer *et al.*, 2002; Johri, 2008; Rabi'u and Murtala, 2013). More so, *A. digitata* has been reported to be a highly economic tree species and the leaves are used in the preparation of soup, flower is eaten raw and the seeds provide flour which is very rich in vitamin B and protein (Rabi'u *et al*., 2013; Rabi'u and Murtala, 2013). While the fat and oil obtained from the Shea kernel of *V. paradoxa* is referred to as Shea butter and it is the most valued product from the Shea tree (Warra and Komo, 2014). The Shea butter is widely utilized for domestic purposes such as cooking, lightning, soap manufacture, skin moisturizer to relieve rheumatic and joint pains, applied to open wounds to quicken healing times, burns, ulcers, dermatitis and prevent infection (Soro *et al.* 2011). Despite their potential, very little is known about their genetic diversity (Sugandha *et al.*, 2010). These trees have been facing crisis of survival and enlisted as endangered species in the Red data book with only 30 to 40 trees available in India (Johri, 2008).

Globally, about one billion people depend on wild foods mostly from plants (Aberoumand, 2009). Chao (2012) estimated that between 300 and 350 million people depend almost entirely on forests for their nutrition and livelihood support. Onyekwelu *et al*., (2015) revealed some indigenous tree species that produce important fruits and other non-timber forest products (NTFPs) of importance to the people. *A. digitata* and *V. paradoxa* species are still considered wild (ICRAF, 2000); because of this, fruits and nuts for consumption, sale or processing are still collected from the wild, raising questions of full and sustained exploitation (Ugese *et al.,* 2005). Rabiu and Murtala (2013) opined that fruit trees are currently being overexploited in their natural habitats owing to unsustainable farming systems, absence of livelihood option, intensive human pressure on the forests and poverty. The swiftly declining of fruit trees poses threats not only to food security, but also to wildlife, environment, traditional medicine and human beings. The growing realization of the importance of fruit trees and non-wood forest products (NWFP) has brought about a gradual change in forest management in many countries to conserve genetic diversity of species for mating and sustainable use (Okunomo, 2010).

Understanding patterns of genetic diversity within plant species is of fundamental importance to the development of conservation strategies, both for defining appropriate units for in-situ conservation and for developing effective sample collection strategies for ex-situ conservation (Hogbin and Peakall, 1999). This is done with the aim to protect the variability of taxa so as to preserve ecological processes, rate of establishment, survival and fecundity (Miller *et al.,* 2000). Koskela *et al.,* (2007) observed that genetic diversity ensures survival of tree species and thus the stability of forest ecosystems as its quantity and quality determines the potential of population that will adapt to the changing environmental condition. A large number of studies have been undertaken to assess the extent of genetic diversity in threatened plant species (Gitzendammer and Soltis, 2000). This reflects the importance accorded to the maintenance of genetic diversity in conservation programmes, as its loss may reduce the evolutionary viability of populations by decreasing their ability to adapt to changing environmental conditions (Ennos, 1996). Hence, this study examined the genetic variation within and between the populations of *Adansonia digitata* and *Vitellaria paradoxa* using chloroplast microsatellite markers.

MATERIALS AND METHODS

Study Area

The three agro-ecological zones used in this study is presented in Figure 1. The study was carried out in purposively selected three savanna ecological zones in Nigeria which are Oyo State (Derived Savanna), Kaduna State (Guinea Savanna) and Kano State (Sudan Savanna). The Derived Savanna (in Oyo State) is located between the latitude 70^{o} 3' and $9^{o}12'$ North of the equator and longitude 2^{o} 47' and 4^{o} 23' East of the meridian (Ikoku *et al.,* 2013). Northern Guinea Savanna (Kaduna State) lies between latitude 10^{o} 20' N and 10^{o} 40' N, and longitude 7^{o} 20' E and 7^{o} 28' E (Wapwera and Gajere, 2017) while the Sudan Savanna (Kano State) between latitude 11^{o} 30' N and 11^{o} 50' N and longitude 8^{o} 30' E and 8^{o} 50' E (Dogara *et al.,* 2012). The savanna generally refers to landscape characterized by pure grassland to dense woodland vegetation with a continuous grass layer beneath or between the trees. It lies between the equatorial rainforest and the deserts of the subtropics (Ker, 1995).

Collection of Plant Materials

The locations of *A. digitata* and *V. paradoxa* population sampled in this study (using GIS tool) is presented in Figure 2. Two juvenile leaves were collected from 95 and 83 stands of *A. digitata* and *V. paradoxa* species found in Oyo State (Derived Savanna), Kaduna

State (Guinea Savanna) and Kano State (Sudan Savanna). The leaves were cleaned and preserved with silica gel in sealed nylon and brought to the Biosafety Laboratory of the Federal University of Technology, Akure, Nigeria for DNA extraction. Ten (10) chloroplast microsatellite molecular markers were used in the analysis of their genetic similarity and diversity.

DNA Extraction Protocol

Genomic DNA was extracted from 0.020 g powder of silica gel dried leaves using the modified Doyle and Doyle (1990), protocol.

Quantification of DNA using UV Spectrophotometer

Tris-Ethylenediamine tetraacetic acid (Tris-EDTA/TE) buffer of 900µl was taken into 1 ml corvette. 10 µl of the extracted DNA sample was taken and added to the TE buffer. The UV-Spectrophotometer was then set to 260 wavelengths and reading was taken at that wavelength.

Concentration of DNA Sample

Concentration of DNA (µg/ml) = Absorbent @260 × Dilution factor × Constant ……1

Dilution Factor = Total Volume ÷ Initial Volume ……2

Total Volume = (Volume of TE (Tris EDTA) pH8 + Volume of DNA) ……....3

Initial volume = Volume of DNA used ……4

DNA Normalization for Polymerase Chain Reaction

To normalized DNA sample:

$C_IV_I = C_2V_2$ …….5

Where;

C_I = Concentration value of DNA sample

V_I = Volume taken from the DNA sample

C_2 = Value use to normalize all DNA samples (10ng μl^{-1})

V_2 = Amount to which DNA will be normalized to (?)

Polymerase Chain Reaction (PCR) Amplification and Gel Electrophoresis

DNA was amplified by PCR using five universal microsatellite primers (Ccmp1, Ccmp3, Ccmp4, Ccmp6 and Ccmp10) described by Weising and Gardner, (1999) and five tobacco microsatellites (Ntcp7, Ntcp9, Ntcp19, Ntcp23, and Ntcp28) described by Bryan *et al.,* (1999). PCR reactions were performed in a volume of 25µl in a DNA thermal cycler (Eppendorf, mastercycler gradient). The reaction mixture contained 4µl of 40ng DNA, 12µl of Go Taq Green master Mix, 2x (Promega), 1µl of each primer and 6.5µl of Nuclease-free water. Optimal amplification conditions were one cycle of 4 min at 94 ^oC (initial denaturation), followed by 30 cycles of 30 s at 94^0C (denaturation), 1 min at 56 ^oC (annealing) and 1 min at 72 ^oC (extension). A final step of 5 min at 72 ^oC ensured full extension of all amplified products. The PCR products were store at 4 ^oC until analysis. Amplified PCR products were separated by electrophoresis using 2% agarose gel (TBE buffer), stained with 5 µl ethidium bromide and visualize under UV light. Fragment sizes were estimated using 50-1000 bp Bench Top PCR Markers (ladder – DNA sizing marker).

Data Analysis

Scored chloroplast marker data was analyzed as a haploid dominant marker for the analysis of total allelic diversity using the CERVUS program version 1.32 (Yeh *et al.,* 1999). The frequencies of the bands were used to calculate the number of alleles per locus, observed heterozygosity (Ho), expected heterozygosity (He), polymorphic information content (PIC) as described by Sorkheh *et al.,* (2007).

RESULTS

Genetic Diversity within the Population of *Adansonia digitata* in Savanna Ecological Zones of Nigeria

The genetic diversity within the population of *A. digitata* of Nigeria is presented in Table 1. In Derived Savanna (Oyo State), all loci were polymorphic. The ten chloroplast microsatellite primers assayed on 23 individuals gave 73 difference alleles. The number of alleles (*K*) ranged from 3 to 13 per locus, with a mean number of 7.3 per locus. Locus "ccmp10" had the highest number alleles, while the least number of alleles was found in "Ntcp19". The observed heterozygosity (H_o) ranged from 0.174 to 0.957 and expected heterozygosity (H_e) ranged from 0.410 to 0.999 respectively. It was discovered that expected heterozygosity was higher than the observed heterozygosity in about 90% of the loci investigated. The polymorphic information content (PIC, which is the primer's discriminating power) values ranged between 0.333 and 0.877. The highest polymorphic information content (PIC) was recorded for ccmp10 (0.877).

In Guinea Savanna (Kaduna State), ten chloroplast microsatellite primers assayed on 51 individuals generated a total of 51 polymorphic and 1 monomorphic loci (Ntcp19). This set of loci is expected to give a good sampling of the total genome and a good assessment of the genetic diversity. The number of alleles (*K)* per locus ranged from 1 to 12. ccmp4 and ccmp10 had the highest number of alleles per locus while Ntcp19 had the lowest number of allele. The observed heterozygosity (H_o) ranged from 0.000 to 0.412 and expected heterozygosity (H_e) ranged from 0.000 to 0.841 respectively. In all the loci investigated, expected heterozygosity was higher than the observed heterozygosity. Also, the highest polymorphic information content (PIC) was recorded for Ccmp10 (0.842).

For *A. digitata* in Sudan Savanna (Kano State), all loci were polymorphic. The ten chloroplast microsatellite primers assayed on 23 individuals gave 47 difference alleles. The number of alleles (*K*) per locus ranged from 2 to 9 per locus. Ccmp10 had the highest number of alleles while Ntcp28 had the lowest number of alleles per locus. The observed heterozygosity (H_o) ranged from 0.238 to o.952 and expected heterozygosity (H_e) ranged from 0.298 to 0.885 respectively. The highest polymorphic information content (PIC) was recorded for ccmp10 (0.814).

Genetic Diversity among *Adansonia digitata* Populations in Savanna Ecological Zones of Nigeria

The result of genetic diversity among populations of *A. digitata* in the three savanna ecological zones of Nigeria is presented in Table 2. The number of individual sampled in each population varied from 21 in Sudan Savanna (Kano State) to 51 in Guinea Savanna (Kaduna State). The few number of *A. digitata* found in Derived and Sudan Savanna respectively was as a result of the anthropogenic destruction. Allelic richness was significantly higher in Derived Savanna, followed by the alleleic richness in Guinea and Sudan Savanna respectively. The observed heterozygosity was significantly the same for Derived and Sudan Savanna respectively but significantly low in Guinea Savanna. The mean expected heterozygosity obtained in Derived Savanna is significantly different from the mean expected heterozygosity of Guinea and Sudan Savanna respectively.

Genetic Diversity within the Population of *V. paradoxa* in Savanna Ecological Zones of Nigeria

Genetic diversity within the population of *V. paradoxa* in three savanna zones of Nigeria is presented in Table 3. The ten chloroplast microsatellite primers assayed on 24 individuals in Derived Savanna generated a total of 45 polymorphic and 1 monomorphic loci (Ntcp19). The number of alleles (*K*) ranged from 3 to 10 per locus, with a mean number of 4.6 per locus. The observed heterozygosity (H_o) ranged from 0.000 to 0.720 and expected heterozygosity (H_e) ranged from 0.000 to 0.972 respectively. Locus "ccmp10" revealed the highest

diversity in terms of K and He (K = 10, H_e = 0.972) and the highest polymorphic information content (0.817). It was discovered that the expected heterozygosity was greater than the observed heterozygosity in all the loci investigated.

In Guinea Savanna, all loci were polymorphic. The ten chloroplast microsatellite primers assayed on 37 individuals generated a total of 62 difference alleles. The number of alleles (K) ranged from 3 to 13 per locus, with a mean of 6.2 per locus. The observed heterozygosity (H_o) ranged from 0.216 to 0.811 and expected heterozygosity (H_e) ranged from 0.204 to 0.871. Expected heterozygosity was higher than the observed heterozygosity in about 90% loci investigated.

All loci were polymorphic in Sudan Savanna. The ten chloroplast microsatellite primers assayed on 37 individuals generated a total of 46 difference alleles. The number of alleles (K) ranged from 2 to 8 per locus, with a mean number of 4.6 per locus. The observed heterozygosity (H_o) ranged from 0.225 to 0.820 and expected heterozygosity (H_e) ranged from 0.333 to 0.846 respectively. The polymorphic information content (PIC) ranged from 0.300 to 0.804.

Table 1: Genetic Diversity of *Adansonia digitata* in Derived, Guinea and Sudan Savanna Respectively using Ten (10) Microsatellites markers

Location	Locus	Ccmp1	Ccmp3	Ccmp4	Ccmp6	Ccmp10	Ntcp7	Ntcp9	Ntcp19	Ntcp23	Ntcp28
Derived Savanna in Oyo State	K	6	8	12	9	13	5	9	3	4	4
	N	23	23	23	23	23	23	23	23	23	23
	HObs	0.696	0.826	0.913	0.783	0.913	0.565	0.957	0.174	0.522	0.391
	HExp	0.862	0.905	0.99	0.885	0.99	0.802	0.87	0.41	0.72	0.65
	PIC	0.680	0.799	0.854	0.774	0.877	0.63	0.784	0.333	0.575	0.487
Guinea Savanna in Kaduna State	K	3	3	12	3	12	3	8	1	4	3
	N	51	51	51	51	51	51	51	51	51	51
	HObs	0.412	0.431	0.588	0.431	0.549	0.431	0.549	0.000	0.412	0.412
	HExp	0.670	0.662	0.698	0.55	0.841	0.62	0.837	0.000	0.671	0.501
	PIC	0.573	0.582	0.776	0.468	0.842	0.569	0.803	0.000	0.617	0.386
Sudan Savanna in Kano State	K	4	5	7	5	9	3	5	3	4	2
	N	21	21	21	21	21	21	21	21	21	21
	HObs	0.714	0.81	0.952	0.619	0.952	0.524	0.952	0.238	0.476	0.524
	HExp	0.773	0.695	0.877	0.712	0.885	0.53	0.792	0.298	0.480	0.530
	PIC	0.579	0.608	0.782	0.639	0.814	0.554	0.724	0.272	0.497	0.375

K, number of alleles per locus; N, number of individual typed; HObs, observed heterozygosity; HExp, expected heterozygosity; PIC, polymorphic information content

Genetic Diversity among *Vitelleria paradoxa* Populations in Savanna Ecological zones of Nigeria

Genetic diversity among the populations of *V. paradoxa* in the savanna ecological zones of Nigeria is presented in Table 4. The number of individual sampled in each population varied from 21 in Sudan Savanna to 37 in Guinea Savanna. Allelic richness was significantly higher in Guinea Savanna (6.2), followed by the alleleic richness in Derived (4.6) and Sudan Savanna (4.4) respectively. The observed heterozygosity was statistically the same for Derived (0.440) and Guinea Savanna (0.4917) but significantly high in Sudan Savanna (0.6143). The expected heterozygosity obtained in Derived Savanna (0.7372) is significantly higher than the expected heterozygosity in Guinea (0.5302) and Sudan Savanna (0.5792).

Table : 2 Savanna Zones of Nigeria

Population	Genetic Variables				
	N	No of loci	K	Ho	He
Derived Savanna	23	10	7.3^{a}	0.674^{a}	0.8093^{a}
Guinea Savanna	51	10	5.2^{b}	0.4215^{b}	0.6050^{b}
Sudan Savanna	21	10	4.7^{bc}	0.6761^{a}	0.6572^{bc}

K, number of alleles per locus; N, number of individual typed; Ho, observed heterozygosity; He, expected heterozygosity; a, b and c represent "significant difference between the savanna ecological zones"

Table 3: Genetic Diversity of *V. paradoxa* in Derived, Guinea and Sudan Savanna respectively using Ten (10) Microsatellites markers

Location	Locus	Ccmp1	Ccmp3	Ccmp4	Ccmp6	Ccmp10	Ntcp7	Ntcp9	Ntcp19	Ntcp23	Ntcp28
Derived Savanna in Oyo State	K	3	3	8	4	10	3	8	1	4	2
	N	25	25	25	25	25	25	25	25	25	25
	Hobs	0.440	0.600	0.440	0.440	0.720	0.360	0.600	0.000	0.480	0.320
	HExp	0.695	0.679	0.955	0.601	0.972	0.640	0.920	0.000	0.735	0.490
	PIC	0.591	0.592	0.753	0.530	0.817	0.554	0.778	0.000	0.670	0.365
Guinea Savanna in Kaduna State	K	3	3	10	3	13	4	13	5	5	3
	N	37	37	37	37	37	37	37	37	37	37
	Hobs	0.459	0.432	0.622	0.432	0.811	0.378	0.757	0.216	0.486	0.324
	HExp	0.51	0.666	0.721	0.45	0.871	0.623	0.855	0.204	0.705	0.505
	PIC	0.534	0.583	0.79	0.369	0.861	0.571	0.829	0.195	0.674	0.389
Sudan Savanna in Kano State	K	3	3	7	4	8	3	7	3	4	2
	N	21	21	21	21	21	21	21	21	21	21
	Hobs	0.524	0.524	0.81	0.619	0.952	0.429	0.857	0.238	0.714	0.476
	HExp	0.701	0.650	0.815	0.623	0.820	0.640	0.743	0.225	0.705	0.475
	PIC	0.563	0.584	0.780	0.607	0.804	0.555	0.798	0.300	0.636	0.374

K, number of alleles per locus; N, number of individual typed; Hobs, observed heterozygosity; HExp, expected heterozygosity; PIC, polymorphic information content

Table 4: Comparison of Genetic Diversity among *V. paradoxa* Populations in Savanna Ecological Zones of Nigeria

Location	Genetic Variables				
	N	No of loci	K	Ho	He
Derived Savanna in Oyo State	25	10	4.6^{b}	0.440^{b}	0.7372^{a}
Guinea Savanna in Kaduna State	37	10	6.2^{a}	0.4917^{b}	0.5302^{b}
Sudan Savanna in Kano State	21	10	4.4^{a}	0.6143^{a}	0.5792^{b}

K, number of alleles per locus; N, number of individual typed; Ho, observed heterozygosity; He, expected heterozygosity; a, b and c represent "significant difference between the savanna ecological zones"

DISCUSSION

Expected heterozygosity (H_e), also known as gene diversity of a locus is a valuable parameter used to estimate the degree of genetic variation within a population (Toro *et al.*, 2009) and describes the proportion of heterozygous genotypes used by researchers to present genetic summary of populations (Vonholdt *et al.*, 2008; Andras *et al.*, 2011). It also means that pair of randomly sampled allele copies from a population are different (Harris and DeGiorgio, 2017). The expected heterozygosity recorded for Derived Savanna was significantly higher than those of Guinea and Sudan Savanna for both species. However, there is no significant difference between expected herozygosity for Guinea and Sudan Savanna respectively. This implies that the genetic diversity in *A. digitata* and *V. paradoxa* was higher in Derived savanna than the other two agro-ecological zones. Less genetic diversity in Guinea and Sudan savanna could be attributed to less number of trees per unit area and more anthropogenic activities.

The expected heterozygosity values for *A. digitata* across the three savanna ecological zones ranged from 0.6 to 0.8 while for *V. paradoxa* across the three agro-ecological zones ranged from 0.5 to 0.7. According to de Lafontaine *et al.*, (2013) and Mattioni *et al.*, (2013) species that records 0.6 to 0.8 expected heterozygosity are classified to have a high genetic diversity. As such, *A. digitata* could be said to have a high genetic diversity in the study area. The expected heterozygosity for *A. digitata* in Derived Savanna is higher than the H_e reported by Temunovic *et al.*, (2013) for *Fraxinus angustifolia* (0.72) in a forest of Portugal using microsatellite markers. Ellstrand and Elam, (1993), estimated 0.50 and 0.59 expected heterozyosity (H_e) for *Zelkova carpinifolia* and *Pterocarya fraxinifolia* in protected forest of South Caucasus. A low value of H_e (0.29) was recorded for *Cordia africana* in Ethiopia (Derero, 2007). Likewise Stefanon *et al.*, (2007), reported 0.27 expected heterozygosity (H_e) for *Araucaria angustifolia* in Brazil. Comparatively, genetic diversity of *A. digitata* in Derived Savanna of this study was higher than those recorded for other species in other studies that were threatened by extinction. The high levels of genetic variation present within populations of *A. digitata* and *V. paradoxa* suggested that large numbers of samples from a few populations would capture a sufficient amount of the species' genetic variability.

The highest diversity of *A. digitata* and *V. paradoxa* across the three agro-ecological zones was observed in Derived Savanna (H_e = 0.8093, K = 7.3; H_e = 0.7372, K = 4.6), followed by in Sudan Savana (H_e = 0.6572, K = 4.7; H_e = 0.5792, K = 4.4), with the lowest genetic diversity in Guinea Savanna (H_e = 0.6050, K= 5.2; H_e = 0.5302, K = 6.2) respectively. The slight decrease in genetic diversity of *A. digitata* and *V. paradoxa* in Guinea and Derived Savanna respectively suggests that the impact of logging on the

genetic diversity of the species was small. On the other hand, the slightly higher genetic diversity in Derived and Sudan Savanna respectively of *A. digitata* and *V. paradoxa* may be as a result of their higher ability to regenerate in open forest (FRIN, 1977). Several studies showed that, logging has adverse effects on genetic diversity (Prober and Brown, 1994; Buchert, *et al.*, 1997; Wickneswari, *et al.*, 1997), while in others, no distinct impact was observed (Lee, *et al.*, 2002; Zheng *et al.*, 2005).

CONCLUSION

High genetic diversity in *A. digitata* and *V. paradoxa* genotypes was recorded for Sudan and Guinea Savanna but significantly higher in Derived Savanna ecological zone. Most of the markers used in the study gave high polymorphic information content. This suggested that chloroplast microsatellite markers could be successfully applied for detecting genetic variability in natural population of *A. digitata* and *V. paradoxa.* A good strategy to protect these endangered tree species is to protect more of their habitat and also to develop plantation for these important trees outside their natural habitat.

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