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Genetic diversity for nutritional traits in the leaves of baobab, *Adansonia digitata*

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Genetic divergence of baobab (*Adansonia digitata*) in the North East and West of Nigeria was investigated. The seeds collected from 36 populations of *Adansonia digitata* were thoroughly washed from the pulp, dried and sown in polyethylene pots and arranged in completely randomized design (CRD) with three replications in the nursery of Savanna Forestry Research Institute of Nigeria, (FRIN), Zaria Station. The leaves of the seedlings were assessed at 16 weeks after sowing (WAS) for nutritional traits such as carbohydrate, crude protein, fat, moisture content, fiber and ash. Highly significant differences were observed in all the traits assessed showing that high variability exists among the populations. Mean separation and comparisons were also consistent with the observed variability in all the traits of the genotypes in the 36 populations. Mahalanobis D^2 statistic and wards minimum variance grouped the 36 populations into four clusters: I (25), II (3), III (7) and IV (1). Clusters IV and I had the highest inter-clusters value and intra-cluster distance. Clusters II and I had the least values of D^2 and $\sqrt{D^2}$ of the inter cluster and intra cluster distance. Furthermore, cluster I had the highest population (25) and cluster IV was observed to be an isolated cluster with only one population and 0 intra-cluster values. Crosses can be made among the genetically divergent populations of baobab to develop genotypes for higher nutritional quality in the leaves as well as withstand both biotic and abiotic stress arising from the environment.

Key words: *Adansonia digitata*, Genetic variability, diversity, populations, and clusters.

INTRODUCTION

Adansonia digitata (L.) commonly referred to as baobab belongs to the family of bombacaceae (Keay, 1989), consisting of about eight species (Baum and Oginuma, 1994), and that only one of the species exists in Africa. *A. digitata* originated in Madagascar and was introduced to the rest of African countries by long distance dispersal before the breaking of West Gondwanaland block through Continental drift at Cretaceous. (Assogbadjo et al., 2006; Baum et al., 1998). In Nigeria, *A. digitata* is found in all the ecological zones. Keay (1989) reported that the tree is evenly distributed in arid and semi-arid zones of Nigeria. It is reported to be widely distributed in savannah regions of Nigeria, particularly north west and north east regions, where it is called Kuka tree (Esenowo,

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1991; Baum 1995; Ibrahim and Otegbeye, 2004). It is also found in the southern region of Nigeria where the Yorubas call it *Ose* tree, Nupe (*Much*i), Edo *(Usi*) (Keay, 1989). The tree is used in parkland agro-forestry system in the arid and semi regions of Nigeria where it grows undistributed and survives until their natural death. The tree is also grown in courtyards of villages and cities of Northern Nigeria. Baobab seems to be the most protected tree in the arid and semi-arid zones of Nigeria. Esenowo (1991) noted that the tree is protected by those who know its value while ICRAF (2003) stated that in hot and dry part of the world, where the average per capita income may not reach 300 USD a year, the tree is vital for food security and that the tree is cherished and even worshipped.

The tree contributes significantly to the socio-economic activities of the people of Northern Nigeria. Recently, attention has been diverted on the use of indigenous trees which may be due to the problems obtained in the use of exotic trees agro-forestry system. The problems which, resulted into poor acceptability of the exotic trees by the farmers, could be, among many, unfavorable competition with the crops for nutrient, water, and sunlight, more importantly, is the problem of allelopathic influence (Momodu et al., 1997). In contrast, indigenous multipurpose tree species (MPTS) such as *A. digitata* have evolved and adapted over time to the local farming systems and the additional advantages of multipurpose uses in the production of fuel-wood, edible fruit, leaves, oils, tannin and medicaments (Ibrahim and Otegbeye, 2004). Many publications and calls have been made in recent times on the need for the domestication of indigenous multipurpose tree species (Momodu et al., 1997; ICRAF, 1997; ICRAF, 2003; Ibrahim and Otegbeye, 2004), but there is no any practical action taken in this direction, as there is little or no research study on the genetic and nutritional improvement of this tree in Nigeria (Ibrahim and Otegbeye, 2004). This is probably the first genetic and variability study on an indigenous tree species in Nigeria. Most of the edible indigenous MPTS are becoming endangered due to large-scale deforestation of the natural ecosystem. This problem may affect the reproductive potential, species extinction and genetic resources, which would be used for future, tree improvement (ICRAF, 2003). According to Stephenson et al. (2000), malnutrition is the most important cause of mortality in the global human population. More than a quarter of children less than five years old suffer from protein-energy malnutrition, as determined by rates of stunting and underweight. Of these, 70% are in Asia, 26% in Africa, and 4% in Latin America. Stunting in poor-resource population is usually associated with reduced mental development

A. digitata leaves have been reported to be a low to moderate source of pro-vitamin A (Delisle et al., 1997; Nordeide et al., 1996) and very rich in calcium (Glew et al., 1997; Becker, 1983). Leaf crude protein values for *A. digitata* have been reported in the range of 10.6% (Yazzie et al., 1994) to 12.5% (Kerharo, 1980). Leung et al. (1968) also reported that the nutritional composition of fresh baobab leaves per 100 g edible portion is: water 77 g, energy 289 kJ (69 kcal), protein 3.8 g, fat 0.3 g, carbohydrate 16.1 g, fibre 2.8 g, Ca 402 mg, P 65 mg, ascorbic acid 52 mg. Vos et al., (1995) used amplified fragment length polymorphism (AFLP) analysis to find the intra-specific genetic diversity of those locally recognized morphotypes and on the whole populations in order to assess the genetic diversity and differentiation within and between baobabs in Benin. Assogbadjo et al. (2010) used this AFLP technique to investigate the traditional classifications of *A. digitata* confirmed by genome-level genetic differentiation and also if there is within the species some genetic variations which should be conserved for the benefit of local people.

A lot of researches have been conducted on the nutritional value of baobab leaf compared to other fruit trees. This study evaluates the nutritional variability and genetic diversity among different populations of *A. digitata* trees at seedling stage with the aim of evaluating if the seedlings could be used as garden crop.

MATERIALS AND METHODS

Locations for the collection of population samples were selected based on the survey conducted by Momodu et al*.* (1997). These sites were surveyed to have prevalence of high population of *A. digitata* where high genetic variability is obtained in the arid and semi-arid zones of Nigeria.

Fruits samples were collected from 36 populations in North West and North East Nigeria (Table 1). Eight mother trees were randomly selected from each of the populations and four fruits were collected from round the crown of each of the sampled mother trees (four fruits per tree) in each of the populations. This is to ensure that fruits from different pollen sources were collected. The collected fruits from each population were then pooled together and stored in a well-ventilated seed room at optimum temperature of 25°C after which the seeds were then removed, washed properly and subjected to pre-germination treatment; soaking the seeds in a boiled water containing one teaspoon of potash for 1 h. Seeds from each population were sown in polyethylene pots containing mixtures of cow dung, river sand and top soil in ratio of 2:2:1, respectively, in the nursery of Savanna Forestry Research Station, Zaria. (11° 11'N, 07° 38'E, attitude 688 m above sea level). The experiment was replicated three times in complete randomized design (CRD).

The pots were watered daily morning and evening and number of germinated seeds were counted daily till 30th days after sowing. Agrocivicultural treatment was applied to promote seedling growth such as weeding, watering and application of Basagram to check termite activities. Proximate analysis of the leaves was conducted at the end of 16 WAS. The lower leaves of the seedlings per population were harvested and bulk and then taken to the Food Science Technology of the Institute for Agricultural Research Ahmadu Bello University, Zaria for the proximate analysis following the method described by AOAC (1980). Each treatment was repeated three times. The data were statistically subjected to analysis of variance and mean ranking using Domcan multiple range test (DMRT) to determine the variability that exists among the populations. The genetic diversity and genetic distance of 36 genotypes (populations) were estimated using Mahalanobis D^2

Table 1. List of the locations of population samples collected, agroecology coordinates and their serial number.

Location	Coordinate	Sample	State	Groecology (savanna)
Yola	9.14N, 12.28E	Fruits	Adamawa	Sudan
Wurocheke	8.21N, 12.06E	Fruits	Adamawa	Sudan
Gire	9.12N, 12.33E	Fruits	Adamawa	Sudan
Song	9.49N, 12.37E	Fruits	Adamawa	Sudan
Minchika	10.36N, 13.23E	Fruits	Adamawa	Sudan
Bama	11.31N, 13.41E	Fruits	Borno	Sahel
Biu	10.37N, 12.13E	Fruits	Borno	Sudan
Kukawa/Ganjarma	12.55N, 13.31E	Fruits	Jigawa	Sudan
Damagum	11.41N, 11.21E	Fruits	Yobe	Sahel
Potiskum	11.43N, 10.58E	Fruits	Yobe	Sudan
Jam'aare	11.39N, 9.55E	Fruits	Bauchi	Sudan
Azare	11.40N, 10.12E	Fruits	Bauchi	Sudan
Dutse	11.48N, 9.19E	Fruits	Jigawa	Sudan
Samamiya	11.20N, 9.40E	Fruits	Jigawa	Sudan
Kwana Babaldu	12.22N, 9.31E	Fruits	Jigawa	Sudan
Gwaram	11.25N, 9.17E	Fruits	Jigawa	Sudan
Garki	12.25N, 9.11E	Fruits	Kano	Sudan
Jarkasa	11.19N, 9.52E	Fruits	Jigawa	Sudan
Garko	11.39N, 8,52E	Fruits	Kano	Sudan
Wudil	11.47N, 8.50E	Fruits	Kano	Sudan
Kwana Dangora	11.31N, 8.09E	Fruits	Kano	Guinea
Shagari	12.25N 5.11E	Fruits	Sokoto	Sudan
Dange Shuni	12.50N, 5.21E	Fruits	Sokoto	Sudan
Gwadabawa	13.24E, 5.03E	Fruits	Sokoto	Sudan
Tambuwal	12.23N, 4.40E	Fruits	Sokoto	Sudan
Talata Mafara	12.34N, 6.04E	Fruits	Zamfara	Sudan
Yandoto	12.22N, 6.12E	Fruits	Zamfara	Sudan
Kaura Namoda	12.35N, 6.36E	Fruits	Zamfara	Sudan
Zurmi	12.46N 6.47E	Fruits	Zamfara	Sudan
Funtua	11.30N, 7.19E	Fruits	Katsina	Guinea
Jibiya	13.01N, 7.01E	Fruits	Katsina	Sahel
Dutsin Ma	12.25N, 7.30E	Fruits	Katsina	Sudan
Yangoje	11.55N, 7.25E	Fruits	Katsina	Sudan
Yantabki	11.34N, 7.15E	Fruits	Katsina	Sudan
Gozaki	11.44N, 7.32E	Fruits	Katsina	Sudan
Wasai	12.01N, 8.12E	Fruits	Kano	Sudan

statistics (Mahalanobis, 1928). The D^2 values for the 'k' traits between i^{th} and j^{th} genotypes or (location) were computed as:

$$D^2 = (Y_i^{th} - Y_j^{th})$$

Where, D^2, Genetic distance; Y_i^{th}, mean character in the i^{th}population; Y_j^{th}, mean character in the j^{th} population.

RESULTS AND DISCUSSION

Highly significant differences ($P \leq 0.01$) was found in all the traits across the populations (Table 2) indicating that variability exists in the nutritional traits of the leaves and as such selection of genotypes with superior nutritional traits in the leaves could be made. This result is in conformity with the works of Sidibe et al. (1996) who noted that variability of vitamin C content exist in the pulp of *A. digitata* fruits among the trees assessed in Mali. Similarly, Sidibe et al. (1998) recorded difference in vitamin A content and other minerals in the leaves of different trees of baobab. Proximate analysis conducted by Odetokun (1996) shows similar result in the pulp.

The mean separation and ranking among the populations is consistent with the variability observed (Table 3). Jarkasa population had the highest ash content, while the population from Azare had high protein content. Kukawa/Ganjarma and Dutsin Ma populations had high

Table 2. Analysis of Variance for Nutritional Traits of Leaves of *A. digitata* Seedlings at 16 WAS.

Sources of variance	df	Moisture	Fat	Ash	Protein	Fibre	Carbohydrate
Populations	35	1.5805**	0.0827**	0.0384**	0.3837**	0.0734**	2.5012**
Error	70	0.0141	0.0001	0.0002	0.0001	0.0001	0.0124

** = Significant at 1% level of probability.

Table 3. Result of proximate analysis for leaves of *A. digitata* for all the 36 locations at 16 WAS.

Population	% Moisture	% Fat	% Ash	% Protein	% Fibre	% Carbohydrate
1	78.14^{h-k}	0.97^{hi}	0.89^{l}	4.11^{s}	2.18^{c}	15.89^{hi}
2	78.31^{g-j}	0.85^{nop}	0.66^{o}	3.99^{w}	2.04^{g}	16.20^{efg}
3	76.38^{m}	1.00^{fg}	1.01^{ni}	4.87^{g}	1.86^{k}	16.74
4	78.07^{jk}	1.09^{a}	0.94^{k}	4.36^{m}	1.86^{k}	15.54^{jkl}
5	77.94^{k}	0.95^{j}	1.01^{hi}	4.47^{l}	2.08^{f}	15.64^{jk}
6	78.86^{bcd}	0.98^{ghi}	1.02^{gh}	4.28^{n}	1.64^{m}	14.86^{o}p
7	78.84^{bcd}	0.68^{r}	0.87^{lm}	4.21^{p}	1.88^{k}	15.41^{klm}
8	76.99^{l}	1.06^{bc}	1.03^{gh}	4.26^{o}	2.60^{a}	16.68^{c}
9	0.00^{n}	0.00^{v}	0.00^{p}	0.00^{g}	0.00^{o}	0.00^{u}
10	76.97^{l}	0.57^{s}	1.07^{cde}	4.17^{q}	2.20^{c}	17.20^{b}
11	80.00^{a}	1.03^{de}	0.96^{jk}	5.20^{b}	2.10^{e}f	12.82^{t}
12	78.69^{cde}	1.06^{b}	0.96^{jk}	5.64^{a}	2.10^{ef}	13.66^{r}
13	79.86^{a}	0.83^{p}	1.07^{cde}	5.05^{e}	2.04^{g}	13.19^{s}
14	78.15^{h-k}	0.98^{gh}	0.70^{n}	4.46^{l}	2.40^{b}	15.71^{ij}
15	78.93^{bc}	1.04^{cd}	0.94^{k}	4.11^{s}	1.82^{l}	14.99^{nop}
16	78.66^{def}	1.01^{ef}	0.99^{ij}	3.99^{w}	2.18^{c}	15.36^{lm}
17	78.09^{jk}	0.85^{op}	1.05^{c-g}	5.16^{c}	2.14^{d}	14.88^{op}
18	78.64^{def}	1.04^{bcd}	1.44^{a}	4.53^{k}	1.96^{j}	14.36^{q}
19	78.16^{h-k}	0.87^{lmn}	0.94^{k}	4.07^{t}	2.14^{d}	16.14^{fg}
20	78.13^{ijk}	0.92^{k}	1.08^{cd}	3.89^{x}	1.87^{k}	16.00^{gh}
21	78.16^{h-k}	1.02^{def}	0.69^{n}	4.02^{uv}	1.88^{k}	16.11^{fgh}
22	78.98^{ab}	1.04^{bcd}	0.99^{ij}	4.60^{j}	2.01^{h}	14.40^{q}
23	78.46^{efg}	1.06^{b}	0.87^{lm}	4.21^{p}	1.60^{n}	15.40^{klm}
24	76.14^{m}	0.56^{s}	0.95^{k}	5.01^{f}	2.12^{de}	17.34^{b}
25	78.27^{g-j}	0.79^{q}	1.05^{c-g}	4.66^{i}	2.10^{ef}	15.23^{mn}
26	78.64^{def}	0.98^{ghi}	1.08^{c}	4.24^{o}	1.96^{j}	15.06^{no}
27	78.66^{def}	0.69^{r}	1.22^{b}	5.11^{d}	2.04^{g}	14.33^{q}
28	78.38^{f-i}	0.83^{p}	0.85^{m}	4.21^{p}	2.08^{f}	15.73^{ij}
29	78.80^{l}	0.85^{nop}	1.06^{c-}f	4.66^{i}	1.86^{k}	16.63^{cd}
30	78.26^{g-j}	0.24^{u}	1.02^{gh}	4.14^{r}	1.96^{j}	16.34
31	78.17^{h-k}	0.89^{l}	1.04^{e-h}	5.00^{f}	2.04^{g}	14.90^{op}
32	76.18^{m}	0.86^{mno}	1.01^{hi}	4.03^{v}	2.04^{g}	17.92^{a}
33	0.00^{n}	0.00^{v}	0.00^{p}	0.00^{y}	0.00^{o}	0.00^{u}
34	76.94^{l}	1.02^{def}	1.06^{c-f}	4.12^{s}	2.00^{hi}	16.86^{c}
35	78.44^{efg}	0.88^{lm}	1.03^{fgh}	4.20^{p}	2.02^{gh}	15.44^{klm}
36	78.65^{def}	1.03^{de}	1.06^{c-f}	4.21^{p}	2.40^{b}	15.01^{nop}
X	73.86	0.85	0.93	4.20	1.92	14.67
Range	76.14-80	0.24-1.10	0.65-1.44	3.88-5.64	1.60-2.60	12.82-17.92
SE	0.02	0.002	0.002	0.002	0.002	0.02

Values with same letter are not significantly different while those with different letters are significantly different using DMRT.

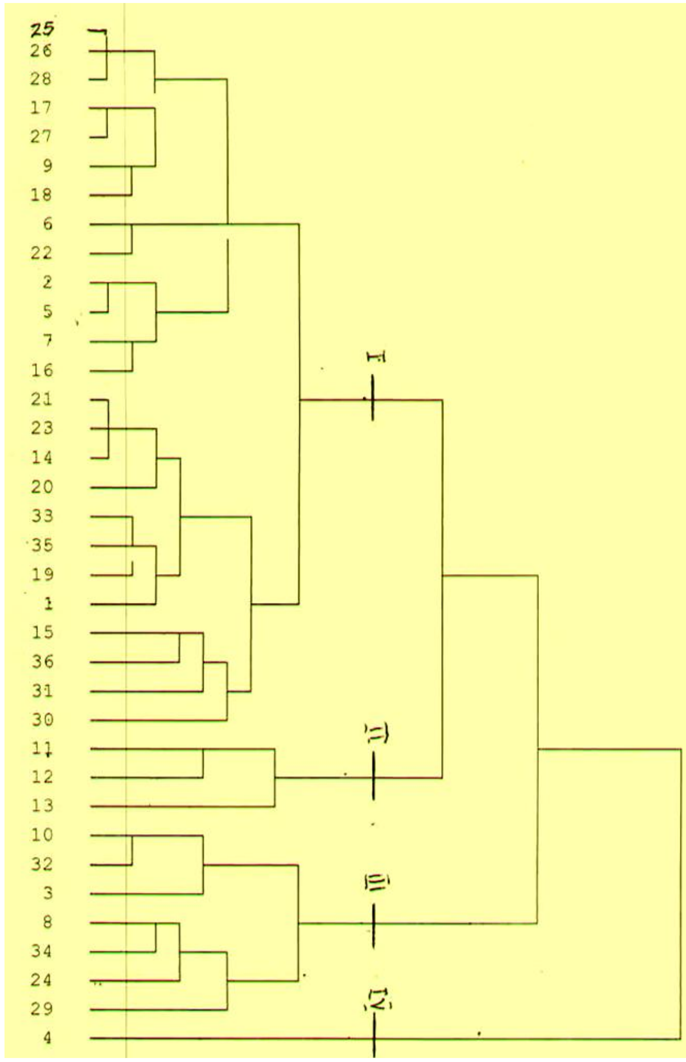

Figure 1. Dendogram showing populations of *Adansonia digitata* grouped into four clusters based on nutritional traits.

fiber and carbohydrate, respectively. Multiple crosses can be made from these populations to obtain desirable hybrid. In order to develop genotype with superior nutritional quality for crude protein, carbohydrate and ash content, multiple crosses can be made from the populations such as Jarkasa, Azare, Kukawa and Dutsin Ma.

Mean inter and intra cluster values (D^2) and distance ($\sqrt{D^2}$) using Mahalanobis D^2 statistics are presented in Tables 4 and 5. The study grouped the 36 populations into four clusters I-IV with 25, 3, 7 and 1 population(s) respectively, as displayed in dendrogram (Figure 1). The highest cluster value, 34.806, was found to be between Clusters IV and I and with intra-cluster distance of 5.900. Clusters II and I had the least values of D^2 and $\sqrt{D^2}$ as 11.602 and 3.406, respectively. Furthermore, cluster I had the highest intra-cluster D^2 value and intra-cluster distance $\sqrt{D^2}$, 66.534 and 8.157, respectively, and consequently had the highest population (25) followed by cluster III with intra-cluster D^2 and $\sqrt{D^2}$ as 16.88 and 4.109, respectively, with seven populations. However, cluster IV was observed to be an isolated cluster with only 1 population and 0 intra-cluster values.

The inter-cluster proximity of nutritional traits dendrogam (Figure 1) that was found to be minimum in clusters I, II, III and isolated in cluster IV is due to genetic influence as the genotypes in population 4 (Song) might not be affected by migration of the agents of dispersal. This result is in agreement with the result of Zehdi et al. (2005) who reported narrow genetic diversity in date palm and that the clusters were grouped independent of their geographical origin. The isolation of the cluster 4 (Song) could be as a result non palatability of the fruits to agents of dispersal and pollination of the trees. Similarly, Sie et al. (2005) obtained three clusters of 79 genotypes of *Cola nitida,* which did not merge with the geographical origin,

Table 4. Mean intra and inter-cluster D^2 values for nutritional traits of the leaves of *Adansonia digitata* for the 36 populations.

Parameter	I	II	III	IV
I	66.534	11.602	12.677	34.806
II		5.637	26.171	24.698
III			16.88	15.084
IV				0

Table 5. Mean intra and inter-cluster ($\sqrt{D^2}$) values for nutritional traits of the leaves of *Adansonia digitat* for the 36 populations**.**

Parameter	I	II	III	IV
I	8.157	3.406	3.561	5.900
II		2.374	5.116	4.970
III			4.109	3.884
IV				0

showing that the study sites were not dependent on centers of dispersion. Although, contrary to this result, Montagnon et al. (1991) on coffee, McGranahan et al. (1997) on *Acacia aulacocarpa*, Wickneswari and Norwati (1993) on *Acacia auriculiformis* and House and Bell (1996) on *Eucalyptus pellita* had their centre of dispersion merged with their centre of origin, showing that the clusters were grouped alongside their centre of origin. Hybrids of *A. digitata* could be between clusters that are highly divergent or with higher genetic distance as this will reduce the danger posed by the harsh environment, pest, and diseases and drought. The nutritional problems reported by Obilana (2011) energy and protein deficiencies for Nigeria are 42% stunting, 11% wasting and underweight accounted for 24%. This could be alleviated in these zones by selection and hybridization among the populations with superior traits to improve the nutritional status of the trees.

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