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Genetic Diversity Assessment of Neem (*Azadirachta indica* A. Juss) in Northern Nigeria

B.P. Mshelmbula[1,2], G.O. Anoliefo[2], B. Ikhajiagbe[2,3*] and B.O. Edegbai[2]

[1]*Department of Plant Science and Biotechnology, Federal University of Lafia, PMB 146, Lafia, Nigeria.*
[2]*Environmental Biotechnology and Sustainability Research Group, Dept. of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria.*
[3]*Applied Environmental Biosciences and Public Health Research Group, Department of Microbiology, University of Benin, Benin City, Nigeria.*
**Corresponding author, Email address:* Beckley.ikhajiagbe@uniben.edu

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beckley.ikhajiagbe@uniben.edu
Phone: +2348038097770

Abstract
Neem is a tropical tree that can adapt to a wide range of places and particularly to semi-arid conditions. As at present, it is grown in many Asian countries and in the tropical regions of the western hemisphere. Genetic variability and diversity are a major requirement needed for both immediate results and the ones thereafter for the adaptation of plant types in their original domain. The evaluation of genetic diversity of any species is extremely crucial for their sustainability, continuity, survival and gene manipulation. Major breakthroughs in the field of molecular biology were able to develop several tools for the investigation of genetic diversity at the genome level to determine phylogenetic relationships among inter or intra-species. The advent of molecular markers for the detection and exploitation of DNA polymorphism is one of the major breakthroughs in the world of molecular genetics. The importance of genetic diversity in plant germplasm conservation, especially in economically important species such as *Azadirachta indica*, is enormous, particularly in Nigeria. The question is whether *A. indica* from different Agro-ecological zones have genetic variations or similarities. This was the bane of the current study, which used RAPD to look at genetic diversity of 27 randomly selected neem trees within the agro-ecological zones in Northern Nigeria. A total of 9 primers were employed out of which only 5 were responsive (OPA-02, OPA-03, OPA-15 and OPA-19). These primers showed dissimilarities in the visible DNA bands among the various tree samples. There was evidence of genetic dissimilarities among the trees sampled. Differences in percentage polymorphism was reported, where it was reportedly highest among the Borno State tree samples (97.44%), compared to those in Yobe State with no polymorphism.

1. Introduction

Azadirachta indica A. Juss (Meliaceae) is a well adaptable plant that lives in forests where agriculture is practiced of the semiarid and arid tropics and is native to the dry forest zones of Asia [1,2]. It is widely distributed, and this may be the result of its wide acceptability, particularly for its economic importance.Neem trees have been broadly developed within the tropics. Its seedlings are raised in the nursery from where they are transplanted unto the field. It is a tree in Northern Nigeria that is almost synonymous with the term "tree of life". This is so because there is virtually hardly any man-made settlement without the neem tree. It flourishes in nutrient-poor dry soils and is tolerant of temperatures when high but is vulnerable to over-the-top cold or ice. It has uses in ethno medicine, construction, energy and fuel, environmental protection, traditional significance, fencing and

agricultural intervention. Neem trees have been broadly developed within the tropics. Its seedlings are often grown in the nursery and later raised in the field.
Due to the cosmopolitan nature of the tree, it may be exposed to a wide range of environments and ecosystems, particularly since it has been connected to nearly every agro-ecological zone in Nigeria. It is unclear if this ability is inherited or provides genetic diversification. Genetic diversity is an essential factor in species stability in any ecosystem, as it provides important transformations to the dominant biotic and abiotic natural conditions, as well as allowing changes in genetic make-up to adapt to changes in the environment.
The assessment of hereditary difference of any species is extremely crucial for their preservation and gene improvement [3]. The objective of this study is to evaluate the genetic variability among individual neem species using Randomly Amplified Polymorphic DNA (RAPD). The study also hopes to determine the genetic similarities and differences among the individuals of the selected plant population within the agro ecological zones of northern Nigeria. Dhillon et al. [4] utilized RAPD markers to assess the genetic variety in *Azadirachta indica* population from various eco-geological locale of India.

2. Methodology

2.1 Plant Sample Collection

The study occurred between February and July of 2019. The research randomly targeted 27 individual Neem tree species scattered within the 4 agro-ecological zones of Northern Nigeria (Figure 1). The plant samples were not obtained from the wild; they were obtained from within cultivated or built environment. This is because the plant is very common either as wind break, aesthetic, horticultural or for phytomedicinal purposes. Formal identification of the samples was made possible with assistance from Plant Taxonomy and Herbarium Unit of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, and the Department of Botany, Federal University, Lafia, Nasarawa State, Nigeria. Care was taken to ensure that tree species of interest were not sampled within a single, but a wider location in so far as they represent designated ecological zones of interest.

2.2 Seeds storage

For RAPD analysis, seeds were used. Seeds from mature fruits were collected from each tree as described by (5). Selection of tree for sample collection was based on the following conditions: that the tree should have a visible bole of not less than 3m, a basal girth of not less than 50 cm and must show evidence of fruiting. Seed samples were immediately covered with aluminum foil paper and placed in ice packed coolers. The seeds were then labeled according to geographic locations of trees as provided on Table 1.

2.3 RAPD Analysis.

In order to obtain clear reproducible amplification products, different preliminary experiments were carried out in which a number of factors were optimized. These factors included PCR annealing temperatures and concentration of each of the template DNA [6]. A total of 9 random DNA oligonucleotides were independently used in the PCR reaction (Table 2). PCR was performed with 10 µl volumes using Thermo Fisher Scientific PCR mix. The final concentrations were as follows: 1 X Taq buffer, 2.5 mM, MgCl2, 0.32 mM, dNTP mixture, 0.25 µM for each primer, 0.5 U Taq polymerase and 10 ng genomic DNA. All reactions were performed using a Wagetech Projects Master Cycler (Eppendorf, Hamburg, Germany) with an initial denaturing cycle of 5min at 95 °C, 40 cycles of 30 s

at 93 °C, 1min at 43 °C, 1 min at 72 °C, and a final extension cycle of 10 min at 72 °C. The pure DNA was stored at – 20 °C.

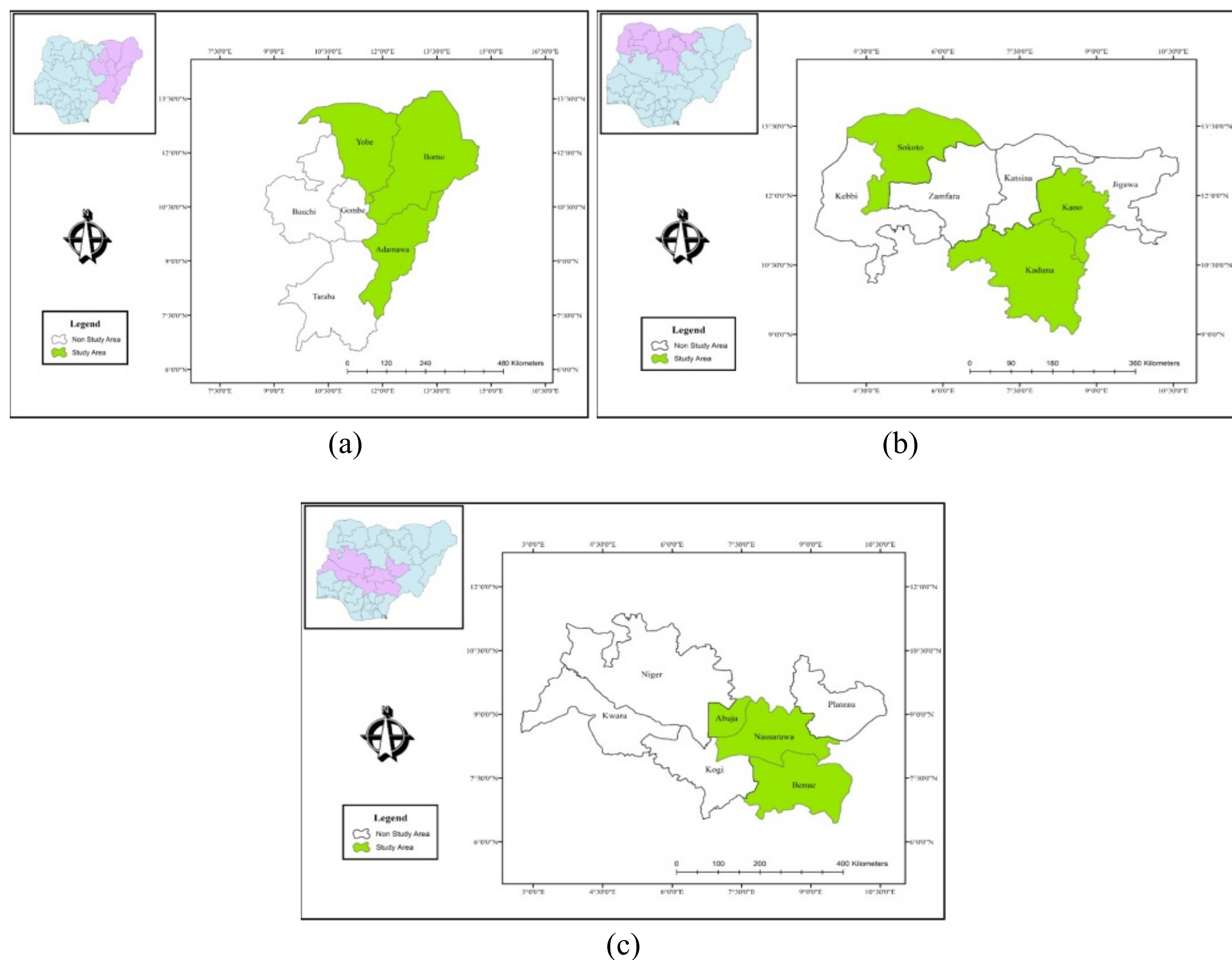


Figure 1: Map showing study area at the (a) North Eastern, (b) North West and the (c) North Central region of the country

2.4 Agarose Gel Electrophoresis.

The PCR products were visualized using ethidium bromide dye in 2 % agarose gels. The gel matrix was prepared following the protocol of [7]. The solution was then poured on a gel tray with a comb inserted in it. Using a micro pipette, 1 µl of loading dye was added to 9 µl of the final product and the mixture was loaded into the wells. The tank was connected to a power source at 90 V for 60 mins. The gel photograph was taken using a digital camera.

2.5 Data Analysis

The DNA bands were counted and fragment sizes compared with those of the DNA Ladder. The bands were scored and used for genotyping. The presence or absence of each DNA band was treated as a binary character in a data matrix (coded 1 and 0, respectively) to calculate genetic similarity and to predict variations among the fruit forms. The genetic distance between the three fruit forms was calculated using GeneAlEx 6.502 software which was used to construct a dendogram on MEGA 7.0.21 software [8].

Table 1: Sample codes for collection of plant samples during the study

Code	Plant physical location	State	Agro-ecological zone
YB-BK-1	Along Malari motor park, Malari ward, Damaturu.	Yobe	Sahel savanna
YB-BK-2	Potiskum cattle market	Yobe	Sahel savanna
YB-BK-3	Federal University, Gashua. Sabon gari, Nguru-Gashua Road, Damasak road, Gashua	Yobe	Sahel savanna
AD-BK-1	Federal college of education, Yola	Adamawa	Southern Guinea savanna
AD-BK-2	Emirs palace, Mubi	Adamawa	Southern Guinea savanna
AD-BK-3	College of Agriculture, Ganye	Adamawa	Southern Guinea savanna
BN-BK-1	E.Y.N Church, Wulari, Maiduguri	Borno	Sahel savanna
BN-BK-2	Army University, Biu	Borno	Sahel savanna
BN-BK-3	University of Maiduguri	Borno	Sahel savanna
KN-BK-1	Gidan Buhari Road, T/Wada Kano	Kano	Sudan savanna
KN-BK-2	BUK, Kano state	Kano	Sudan savanna
KN-BK-3	Dala Orthopedic Hospital, Kano	Kano	Sudan savanna
SK-BK-1	Usman Dan Fodio University, Sokoto	Sokoto	Sudan savanna
SK-BK-2	Sani Dingyadi Sec. Sch. Farfaru, Sokoto	Sokoto	Sudan savanna
SK-BK-3	Sokoto main market, Sokoto	Sokoto	Sudan savanna
KD-BK-1	Ahmadu Bello University, Zaria	Kaduna	Northern Guinea savanna
KD-BK-2	Sabo motor park, Kaduna	Kaduna	Northern Guinea savanna
KD-BK-3	Close to Stanbic IBTC	Kaduna	Northern Guinea savanna
NS-BK-1	OBKi-1 opposite Nasarawa Polytechnic, Lafia	Nasarawa	Southern Guinea savanna
NS-BK-2	Hope academy secondary school, Akwanga	Nasarawa	Southern Guinea savanna
NS-BK-3	Awe Motor Park, Awe LGA	Nasarawa	Southern Guinea savanna
AJ-BK-1	Lungi Barracks, Maitama Abuja.	Abuja	Derived savanna
AJ-BK-2	MaBKilla Barracks, Asokoro Abuja	Abuja	Derived savanna
AJ-BK-3	Opposite State House Medical Centre, Asokoro Abuja	Abuja	Derived savanna
BN-BK-1	University of Agriculture, Makurdi	Benue	Derived savanna
BN-BK-2	Benue State University, Makurdi	Benue	Derived savanna
BN-BK-3	Peace House, Gboko	Benue	Derived savanna

3. Results and Discussion

Nine (09) random primers were obtained and screened for responsiveness to the neem genome. This was done to select the most suitable primer for the RAPD study. Only primers OPA-02, OPA-03, OPA-15 and OPA-19 turned out to be responsive. Primers OPA-04, OPA-05, OPA 6, and OPA-11 were not responsive. They did not amplify any region of the neem genome tested. Information of the random primers used for this study are presented in Table 2. RAPD primers variations in the study of genetic diversity of Neem tree has been presented on Table 3. Among the primers used in the study, Primer OPA-15 was most effective. Mean number of effective alleles ranged from 1.095 – 1.625. OPA-15 had the highest diversity index compared to others used in the study. Expected Heterozygosity ranged from 0.088 – 0.405. heterozygocity was higher with Primer OPA-15.

Table 2a: Primer information on 9 primers for RAPD study

S/No	Primer name	Primer Sequence
1	OPA-02	5’ TGCCGAGCTG 3’
2	OPA-03	5’ AGTCAGCCAC 3’
3	OPA-04	5’ AATCGGGCTG 3’
4	OPA-05	5’ AGGGGTCTTG 3’
5	OPA-07	5’ GAAACGGGTG 3’
6	OPA-11	5’ CAATCGCCGT 3’
7	OPA-17	5’ GACCGCTTGT 3’
8	OPA-19	5’ CAAACGTCGG 3’
9	OPA-15	5’ TTCCGAACCC 3’

Table 2b: Random primer screening.

S/No	Primer name	Primer Sequence	Response
1	OPA-02	5’ TGCCGAGCTG 3’	+
2	OPA-03	5’ AGTCAGCCAC 3’	+
3	OPA-04	5’ AATCGGGCTG 3’	+
4	OPA-05	5’ AGGGGTCTTG 3’	No response
5	OPA-07	5’ GAAACGGGTG 3’	-
6	OPA-11	5’ CAATCGCCGT 3’	No response
7	OPA-17	5’ GACCGCTTGT 3’	-
8	OPA-19	5’ CAAACGTCGG 3’	+
9	OPA-15	5’ TTCCGAACCC 3’	+

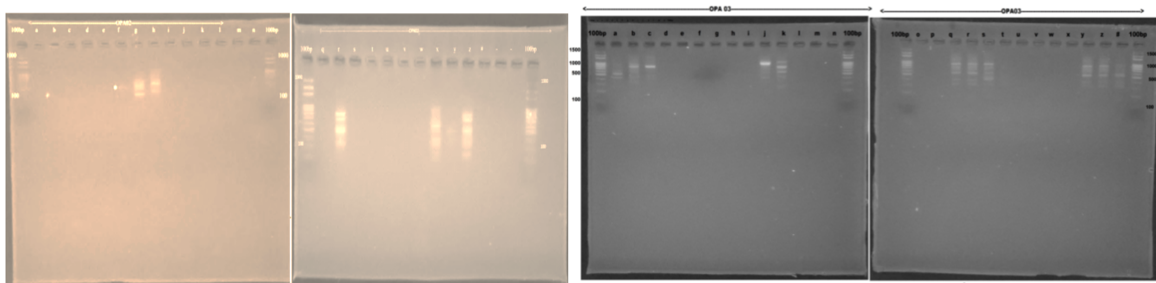
Table 3: RAPD primers variations in the study of genetic diversity of Neem tree

Primers	Na	Ne	I	He	uHe
OPA-04- 1	0.444	1.153	0.129	0.088	0.105
OPA-04- 2	0.556	1.095	0.106	0.067	0.080
OPA-04- 3	0.556	1.212	0.151	0.108	0.130
OPA-04- 4	1.000	1.307	0.257	0.175	0.210
OPA-04- 5	1.000	1.365	0.280	0.196	0.235
OPA-04- 6	1.000	1.307	0.257	0.175	0.210
OPA-04- 7	1.000	1.307	0.257	0.175	0.210
OPA-04- 8	0.667	1.201	0.182	0.121	0.145
OPA-04- 9	0.889	1.190	0.212	0.133	0.160
OPA-02-1	0.667	1.259	0.204	0.142	0.170
OPA-02-2	0.667	1.259	0.204	0.142	0.170
OPA-02-3	0.667	1.259	0.204	0.142	0.170
OPA-02-4	0.667	1.259	0.204	0.142	0.170
OPA-02-5	0.667	1.259	0.204	0.142	0.170
OPA-02-6	0.556	1.153	0.129	0.088	0.105
OPA-02-7	0.556	1.153	0.129	0.088	0.105
OPA-02-8	0.778	1.201	0.182	0.121	0.145
OPA-02-9	0.889	1.249	0.235	0.154	0.185
OPA-02-10	0.444	1.153	0.129	0.088	0.105
OPA-03-1	0.778	1.259	0.204	0.142	0.170
OPA-03-2	0.889	1.259	0.204	0.142	0.170
OPA-03-3	0.889	1.201	0.182	0.121	0.145
OPA-03-4	0.889	1.201	0.182	0.121	0.145
OPA-03-5	1.000	1.307	0.257	0.175	0.210
OPA-03-6	1.000	1.249	0.235	0.154	0.185
OPA-03-7	1.000	1.307	0.257	0.175	0.210
OPA-03-8	0.778	1.201	0.182	0.121	0.145
OPA-15-1	0.667	1.201	0.182	0.121	0.145
OPA-15-2	0.667	1.201	0.182	0.121	0.145
OPA-15-3	0.889	1.249	0.235	0.154	0.185
OPA-15-4	1.333	1.402	0.363	0.242	0.290
OPA-15-5	1.778	1.614	0.515	0.350	0.420
OPA-15-6	1.667	1.566	0.462	0.317	0.380
OPA-15-7	1.667	1.625	0.484	0.338	0.405
OPA-19-1	0.778	1.201	0.182	0.121	0.145
OPA-19-2	0.889	1.259	0.204	0.142	0.170
OPA-19-3	0.889	1.259	0.204	0.142	0.170
OPA-19-4	1.000	1.307	0.257	0.175	0.210
OPA-19-5	1.000	1.307	0.257	0.175	0.210
Grand mean	0.875	1.270	0.228	0.155	0.186

Na = No. of different alleles; Ne = No. of effective alleles; I = Shannon's Information Index; He = Expected Heterozygosity; uHe = Unbiased Expected Heterozygosity

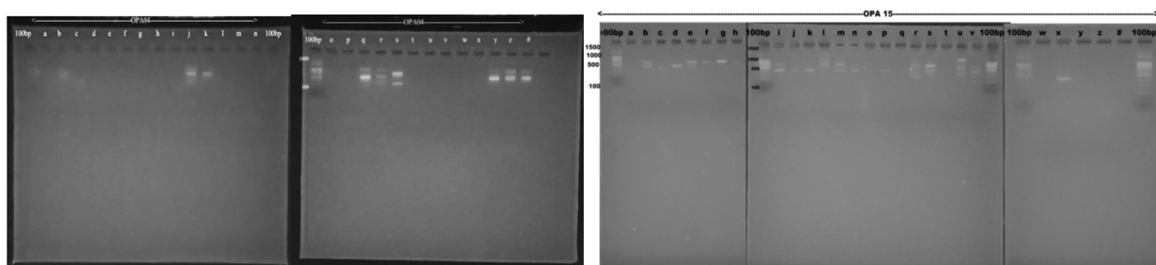
Using Primer OPA-02, visible bands were reported in samples collected from Abuja-2, Adamawa-3, Benue-1, Benue-2, Borno-3, Sokoto-3 and Yobe-2 (Figure 2). The implication is that among the samples collected from Abuja, one was genetically distinct from the other. This was similar to those collected in Adamawa, Borno, Benue, Sokoto and Yobe States respectively. Using Primer OPA-03 (Figure 3), amplifications were recorded for samples collected from Abuja-1, Abuja-2, Abuja-3, Kaduna-1, Kaduna-2, Borno-3, Nasarawa-1, Nasarawa2, Yobe-1, Yobe-2, Yobe-3. Using Primer OPA-02, samples collected from Yobe State were of similar genetic identity as their banding patterns were similar. They also had similar amplifications with samples collected from Borno State.

Gels of responsive Primer OPA-04 showed amplifications for all Abuja samples, Kaduna-1 and Kaduna-2, Borno-2 and Borno-3, Nasarawa-1, as well as all samples from Yobe State (Figure 4). These identities had distinct banding patterns indicating genetic dissimilarities. More than 80% of the samples collected showed amplified bands using Primer OPA-15, with majorly dissimilar characteristics (Figure 5). Samples from Borno and Yobe States had similar banding patterns, indicating possible genetic identities (Figure 6).



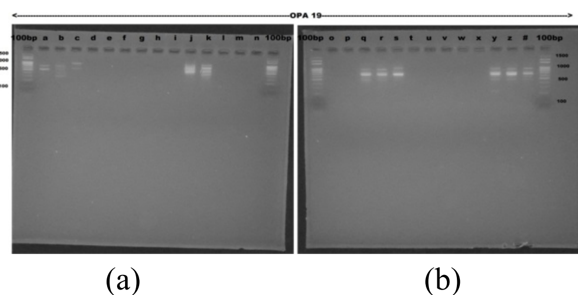
(a)	(b)	(a)	(b)

Figure 2: Gels of responsive Primer OPA-02 **Figure 3:** Gels of responsive Primer OPA-03



(a)	(b)	(a)	(b)

Figure 4: Gels of responsive Primer OPA-04 **Figure 5:** Gels of responsive Primer OPA-15



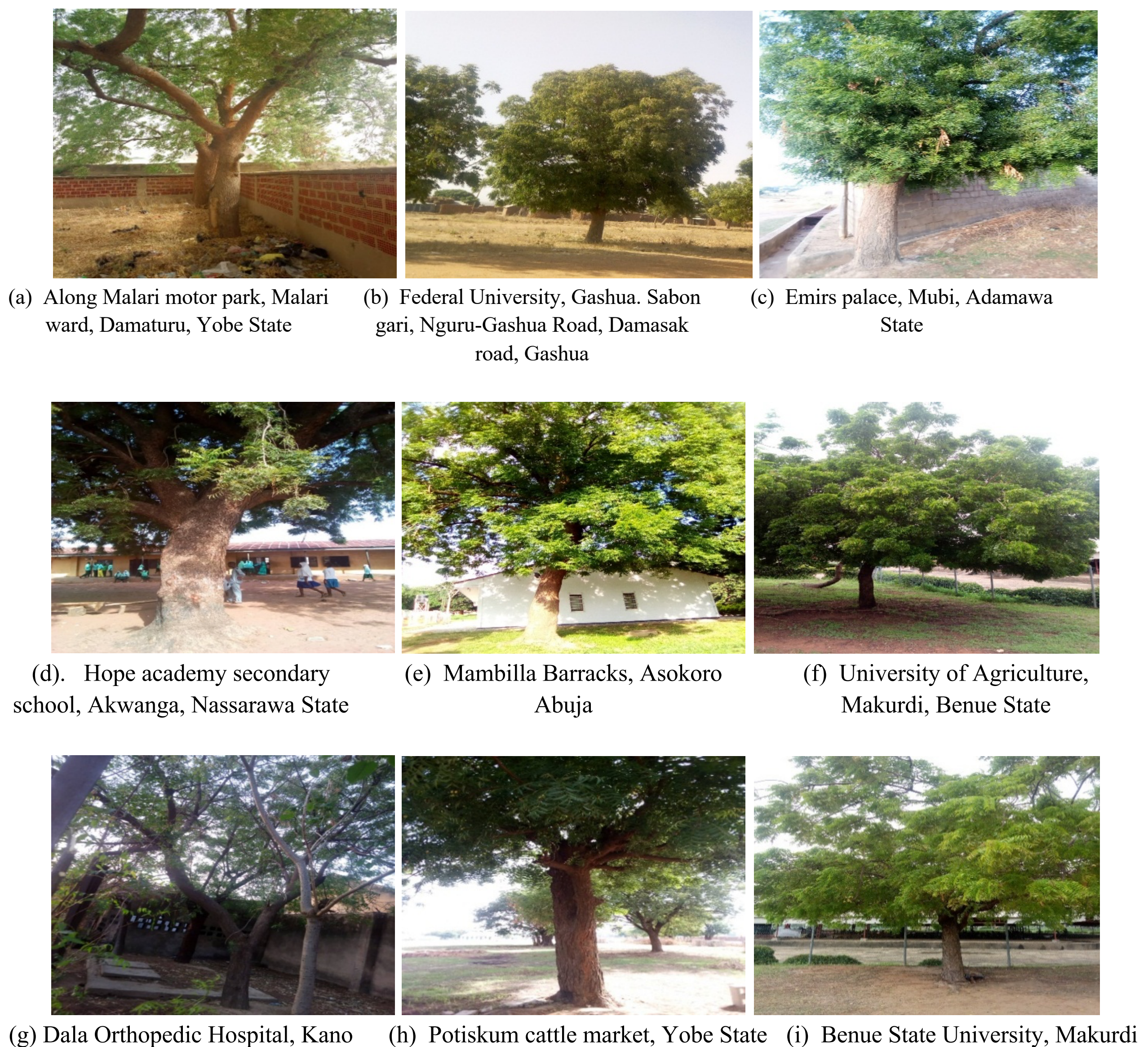
(a) (b)

Figure 6: Gels of responsive Primer OPA-19

Abuja 2- b, Abuja 3- c; Adamawa 1- d, Adamawa 2- e, Adamawa 3- f; Benue 1- g, Benue 2- h, Benue 3- I; Kaduna 1- j, Kaduna 2- k, Kaduna 3- L; Kano 1- m, Kano 2- n, Kano 3- O; Borno 1- p, Borno 2- q, Borno 3- r; Nasarawa 1- s, Nassarawa 2- t, Nassarawa 3- u; Sokoto 1- v, Sokoto-2; w, Sokoto 3 x; Yobe 1- y, Yobe 2- z, Yobe 3- #

In the Abuja samples, 20 bands were observed; all these Different Bands had a Frequency ≥ 5% (Table 4). However, the Borno samples had the highest number of bands (38). Of the 38 bands in the Borno samples, 20 were locally common bands found in 50% or fewer populations. Mean number of available alleles ranged from 0.154 (Kano samples) to 1.949 (Borno samples) (Table 5). In spite of this difference, number of effective alleles between these two locations were comparable (1.046 – 1.686). Table 6 shows the percentage polymorphism of RAPD markers across the nine locations in the study. Percentage polymorphism was highest among the Borno samples (97.44%), compared to samples in Yobe State with no polymorphism. Mean percentage polymorphism was 39.60%.

Nei's pairwise genetic distance and genetic identity among nine populations have been presented (Table 7). The Yobe samples were more genetically distant from Benue samples than they were when compared with other locations. In terms of genetic identity, the Sokoto tree were most likely more identical to samples located in Borno State. From the Analysis of molecular variance using RAPD markers among locations, it was recorded that estimated variance was more within the populations than among the populations (Table 8). Figure 7a-i shows the test tree at various sampling locations.



(a) Along Malari motor park, Malari ward, Damaturu, Yobe State
(b) Federal University, Gashua. Sabon gari, Nguru-Gashua Road, Damasak road, Gashua
(c) Emirs palace, Mubi, Adamawa State
(d). Hope academy secondary school, Akwanga, Nassarawa State
(e) Mambilla Barracks, Asokoro Abuja
(f) University of Agriculture, Makurdi, Benue State
(g) Dala Orthopedic Hospital, Kano
(h) Potiskum cattle market, Yobe State
(i) Benue State University, Makurdi

Figure 7(a – i): Test tree at various sampling locations

The findings of this study corroborate previous reports of the neem tree's economic importance to Nigerians' socioeconomic lives, especially in the north (Table 1) [9,10]. The tree is also adaptable to a wide range of climatic and topographic conditions, thriving in sandy, stony shallow soil sand as well as soils with hard calcareous or clay pan, according to the report. The neem tree just requires a little water and a lot of sunlight to thrive [11]. The pH differences between soil depths were minor. Organic carbon levels were high (1.61 – 1.97 percent) and magnesium levels were moderate (2.05 – 2.82 Cmole/kg), according to [13], regardless of location in the Northeast or soil depth. Similarly, nitrite levels (0.01–0.04 ppm) were similar regardless of position and depth (Table 5). Iron content, on the other hand, varied, with the lowest concentration in Borno (BN-BK-2) (2.29 – 5.78 ppm). Though iron is classified as a micronutrient since only small amounts are needed for normal plant development, Borno (BN-BK-2) had the lowest iron concentration, ranging from 2.29 to 5.78 ppm. The evaluation of genetic diversity of any species is very important for their conservation and gene manipulation [12]. Neem is thought to have a high cross-pollination rate. There have been records of inter-provenance differences in morphological and physiological characteristics in neem. To determine the degree and/or form of genetic (DNA) variation in neem, a powerful molecular technique must be used. The genetic variation of neem ecotypes has recently been determined using molecular techniques such as AFLP, RAPD, ISSR, and RFLP banding patterns [14]. To extract high-quality genomic DNA, 27 neem samples from Northern Nigeria were used. Nine [9] random primers were obtained and screened for responsiveness to the neem genome, with 5 of them proving to be responsive (Table 2). Because of its ability to search across regions of the genome, RAPD analysis is well suited for phylogenetic studies at the species level. Primers used in RAPD studies are usually random [9], necessitating the acquisition of a large number of primers for screening. This increases the likelihood of finding primers that answer. [13] investigated the degree and distribution of genetic diversity in *A. indica* from different eco-geographical regions of the world using RAPD markers and found that they were reliable.

The genetic similarities and variations among the neem samples examined varied significantly from one primer to the next. Variations in RAPD primers were discovered in the study of Neem tree genetic diversity. Primer OPA-15 was the most powerful of the primers used in the analysis. The average number of successful alleles was 1.095–1.625. When compared to the other samples included in the analysis, OPA-15 had the highest diversity index. Heterozygosity was expected to be between 0.088 and 0.405. Primer OPA-15 increased heterozygozity.

Dhillon *et al.* [14] used RAPD molecular markers to assess genetic diversity in *Azadirachta indica* populations from various eco-geographical regions of India. A total of 40 decamer primers were used, and 24 of them resulted in polymorphic banding patterns. A total of 152 distinct DNA bands could be reliably collected, with 104 (68.4%) of them being polymorphic. To classify genetic relationships, polymorphisms were graded and used in band-sharing research. All 36 populations were grouped into two major groups using cluster analysis based on Jaccard's similarity coefficient and UPGMA.

Using Primer OPA-02, visible bands were reported in samples collected from Abuja-2, Adamawa-3, Benue-1, Benue-2, Borno-3, Sokoto-3 and Yobe-2 (Figure 2). The implication is that among the samples collected from Abuja, one was genetically distinct from the other. This was similar to those collected in Adamawa, Borno, Benue, Sokoto and Yobe States respectively. Findings indicated that samples collected from Abuja were genetically distinct from one another even though they are collected from the same state. This however doesn't agree with [15] who analyzed the RAPD to access genetic divergence among 29 neem accessions collected from two agro-ecological regions of India (11 agro-climatic sub-zones) and found out that 14 were polymorphic, generating a total of 3 amplifications products with an average of 5.21 products per polymorphic primer and estimated gene diversity of

0.49. Contrary observation was seen using Primer OPA-03, samples collected from Yobe State were of similar genetic identity as their banding patterns were similar (Figure 4). Figure 5 also showed majorly dissimilar characteristics where Primer OPA-15 had more than 80% of the samples collected showed amplified bands, with majorly dissimilar characteristics.

Table 4: Amplified bands detected with thirty-nine primers in Neem tree species across nine states in Nigeria (Primer OPA-15)

Population	Abuja	Adamawa	Benue	Kaduna	Kano	Borno	Nasarawa	Sokoto	Yobe
No. Bands	20	6	13	29	3	38	25	15	19
No. Bands Freq. >= 5%	20	6	13	29	3	38	25	15	19
No. Private Bands	0	0	0	0	0	0	0	0	0
No. LComm Bands (<=25%)	0	0	0	1	0	2	0	1	0
No. LComm Bands (<=50%)	3	2	9	11	0	20	7	11	5
Mean He	0.144	0.061	0.143	0.261	0.028	0.389	0.221	0.145	0.000
SE of Mean He	0.031	0.024	0.034	0.031	0.016	0.018	0.029	0.036	0.000
Mean uHe	0.173	0.073	0.172	0.313	0.033	0.466	0.265	0.174	0.000
SE of Mean uHe	0.037	0.029	0.041	0.037	0.019	0.022	0.035	0.043	0.000

Key
No. Bands = No. of Different Bands
No. Bands Freq. >= 5% = No. of Different Bands with a Frequency >= 5%
No. Private Bands = No. of Bands Unique to a Single Population
No. LComm Bands (<=25%) = No. of Locally Common Bands (Freq. >= 5%) Found in 25% or Fewer Populations
No. LComm Bands (<=50%) = No. of Locally Common Bands (Freq. >= 5%) Found in 50% or Fewer Populations
He = Expected Heterozygosity = 2 * p * q
uHe = Unbiased Expected Heterozygosity = (2N / (2N-1)) * He
Where for Diploid Binary data and assuming Hardy-Weinberg Equilibrium, q = (1 - Band Freq.)^0.5 and p = 1 - q.

Furthermore, a striking result is that they also had similar amplifications with samples collected from Borno State using both OPA-03 and OPA-15 (plates 3 and 5) indicating possible genetic identities. Therefore, the high percentage of similar banding profile generated in this study is not surprising but expected due to the close proximity between the two states. Also, samples from Born and Yobe had broader leaf size and had delayed seed emergence (see Table 8). Maletsema *et al.* [15] reported RAPD profiles of 17 accessions of neem from India were generated using 49 random DNA primers. The dendogram of similarities amongst the RAPD profiles suggested that there was less variation than expected within neem from India. In addition, the pattern of RAPD similarities obtained did not correspond to the pattern of geographical variation in neem. This result is not unusual when assessing provenance variation using molecular methods, and the use of additional genetic analyses would have assisted the interpretation of the results.

In the Abuja samples, 20 bands were observed; all these different bands had a Frequency ≥ 5% (Table 5). However, the Borno samples had the highest number of bands (38). Of the 38 bands in the Borno samples, 20 were locally common bands found in 50% or fewer populations.

Indication of significant polymorphism was reported in the study. Percentage polymorphism was highest among the Borno samples (97.44%), compared to samples in Yobe State with no polymorphism. Mean percentage polymorphism was 39.60%. Mean number of available alleles ranged from 0.154 (Kano samples) to 1.949 (Borno samples). In spite of this difference, number of effective alleles between these two locations were comparable (1.046 – 1.686). This is an indication that there is an allelic similarity between Borno and Kano samples. It is most likely that the parent plants were derived from the same mother plant, several years ago. From the Analysis of molecular variance using RAPD markers among locations, it was recorded that estimated variance was more within the populations than among the populations (Table 8). This however does not agree with [3] who reported RAPD profiles of 17 accessions of neem from India were generated using 49 random DNA primers.

The dendogram of similarities amongst the RAPD profiles suggested that there was less variation than expected within neem from India.

Table 5: Mean Biodiversity Statistics within Populations (Mean±SEM)

Population	Na	Ne	I	He	uHe
Abuja	0.897±0.151	1.245±0.057	0.215±0.045	0.144±0.031	0.173±0.037
Adamawa	0.308±0.117	1.106±0.044	0.089±0.035	0.061±0.024	0.073±0.029
Benue	0.667±0.153	1.264±0.065	0.206±0.048	0.143±0.034	0.172±0.041
Kaduna	1.436±0.141	1.444±0.059	0.388±0.044	0.261±0.031	0.313±0.037
Kano	0.154±0.086	1.046±0.028	0.042±0.024	0.028±0.016	0.033±0.019
Borno	1.949±0.051	1.686±0.046	0.569±0.022	0.389±0.018	0.466±0.022
Nasarawa	1.282±0.156	1.355±0.052	0.337±0.042	0.221±0.029	0.265±0.035
Sokoto	0.692±0.148	1.28±0.069	0.204±0.05	0.145±0.036	0.174±0.043
Yobe	0.487±0.081	1±0	0	0	0

Na = No. of different alleles; Ne = No. of effective alleles; I = Shannon's Information Index; He = Expected Heterozygosity; uHe = Unbiased Expected Heterozygosity

Table 6. Percentage polymorphism of RAPD markers across nine locations in Nigeria

Population	Polymorphism (%)
Abuja	38.46
Adamawa	15.38
Benue	33.33
Kaduna	69.23
Kano	7.69
Borno	97.44
Nasarawa	64.10
Sokoto	30.77
Yobe	0.00
Mean	39.60

Table 7. Nei's pairwise genetic distance (below the diagonal) and genetic identity (above the diagonal) among nine populations

Location	Abuja	Adamawa	Benue	Kaduna	Kano	Borno	Nasarawa	Sokoto	Yobe
Abuja		0.825	0.771	0.872	0.832	0.896	0.860	0.685	0.732
Adamawa	0.192		0.960	0.908	0.994	0.890	0.965	0.890	0.481
Benue	0.260	0.040		0.832	0.957	0.867	0.916	0.957	0.428
Kaduna	0.137	0.097	0.184		0.904	0.927	0.928	0.763	0.675
Kano	0.184	0.006	0.044	0.100		0.896	0.962	0.873	0.500
Borno	0.109	0.117	0.143	0.076	0.110		0.924	0.978	0.754
Nasarawa	0.151	0.036	0.088	0.075	0.039	0.080		0.834	0.587
Sokoto	0.378	0.117	0.044	0.271	0.135	0.226	0.182		0.337
Yobe	0.312	0.732	0.848	0.392	0.694	0.283	0.532	1.086	

Table 8. Analysis of molecular variance using RAPD markers among locations

Source of variation	Df	SS	MS	Estimated Var.	Variance (%)
Among populations	8	106.370	13.296	2.716	35
Within populations	18	92.667	5.148	5.148	65
Total	26	199.037		7.864	100

Df = degree of freedom; SS = sum of square; MS = mean square; Var. = variance

Application of PCA tool and multivariate statistical analysis provide useful means to estimate morphological diversity within and between germplasm collections. The distinctive nature of Abuja samples among other samples (see Fig. 6 and 7) collaborates with [15]. The genetic relatedness is

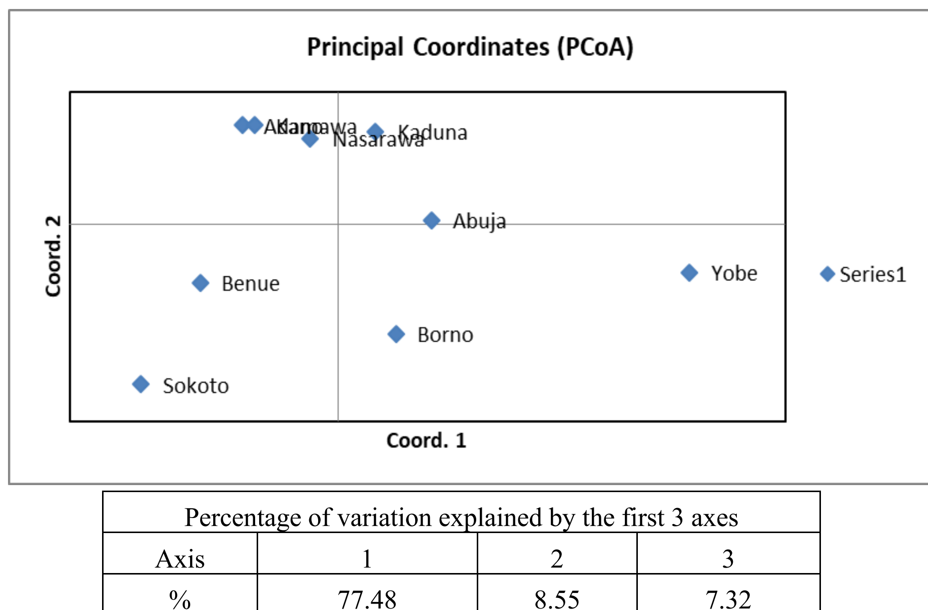
useful for selection of parental lines for hybridization in crop improvement. Among the locations, Adamawa, Nasarawa and Kaduna populations were clustered. This may indicate likely genetic identities (Fig. 8). The genetic relatedness is useful for selection of parental lines for hybridization in crop improvement [16]. Abuja tree samples were separated from the population (Fig. 9) in terms of phylogeny. Maletsema et al. [15] working on sorghum accessions reported that Accession 10 collected was distantly related with the other accessions which disagrees with the present findings. The diversity observed among the neem samples could be useful in improvement of neem for various traits [17].

Percentage of variation explained by the first 3 axes			
Axis	1	2	3
%	51.88	14.27	11.05

Figure 8: Principal Component Analysis showing Sample Clustering

Figure 9: Unweighted pair group method with arithmetic mean phylogeny of samples

Figure 8 shows Principal Component Analysis of samples. Results showed that Abuja 1 and Abuja 3 samples were distinct from the other groups which formed clusters. The UPGMA Phylogeny of Samples (Figure 9) also showed the district nature of the 2 Abuja tree samples. Among the locations, Adamawa, Nasarawa and Kaduna populations were clustered. This may indicate likely genetic identities (Figure 10). Again, as observed in Figures 4 and 5, the Abuja tree samples were separated from the population (Figure 11) in terms of phylogeny.



Percentage of variation explained by the first 3 axes			
Axis	1	2	3
%	77.48	8.55	7.32

Figure 10: Principal component analyses showing population clustering

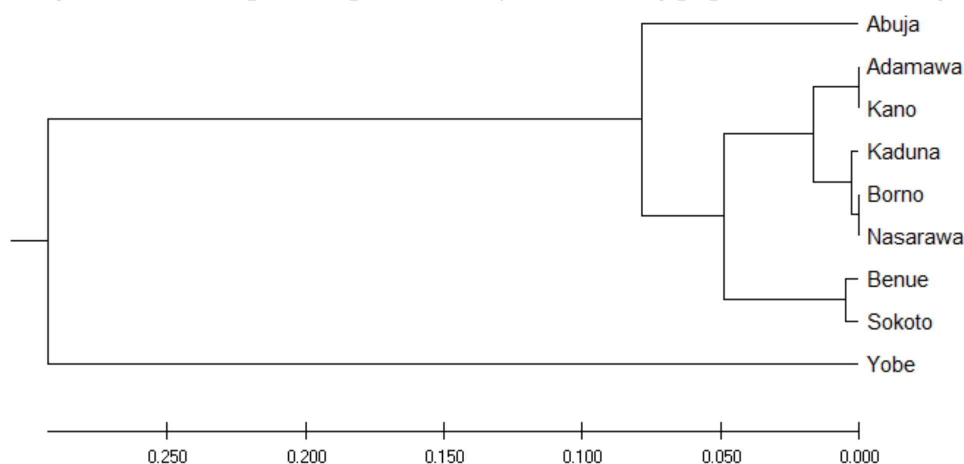


Figure 11: Unweighted pair group method with arithmetic mean phylogeny of populations

Conclusion

The study thus showed genetic dissimilarities among the trees investigated. These genetic disparities as observed through RAPD analysesexisted across the ecological zones. It is indeed unclear if this was caused by plant distribution, soil conditions, weather, or atmosphere. These plants, on the other hand, clearly differed in their ecotypes. The recorded potential for genetic diversity is a significant attribute for ecosystem management and germplasm conservation, given the economic value of this tree. Despite the molecular diversity found among neem trees in Northern Nigeria, it had no effect on their economic importance or significance in one area compared to another.

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Authors' contributions : The studywasdesigned by GOA and BI, and supervised by GOA, BI, and BOE. The fieldworkwascarried out by BPM. Data collection and interpretationwas by GOA, BI, BOE, and BPM. Statisticalanalysis of data was by BI. All authorsread, revised and approved the final manuscript.

Abbreviations:
AFLP: Amplified Fragment Length Polymorphism
RAPD:Random Amplified Polymorphic DNA
ISSR :Inter Simple Sequence Repeat
RFLP**:** Restriction Fragment Length Polymorphism
UPGMA: Unweighted Pair Group Method with Arithmetic Mean

Conflicts of Interest : The authorsdeclare no conflicts of interest

References

1. O. Koul, M.B. Isman, C.M. Ketkar, Properties and uses of neem (*Azadirachta indica*). *Canadian Journal of Botany*.68 (1990) 1-1; https://doi.org/10.1139/b90-001
2. C. Ketkar, M. Ketkar, Neem seed crush and deoild cake as manure and as nitrification inhibitors. The Neem Tree *Azadirachta indica* A. Juss. and other Meliaceous plants, ed. H. schmutterer. 68 (1990) 531-540; https://doi.org/10.1139/b90-001
3. S. Khan, L. Guo, Y. Maimaiti, M. Mijit, D. Qui, Entomopathogenic fungi as microbial biocontrol agent. *Molecular Plant Breeding*. 3(7) (2012) 3-6.
4. RS. Dhillon, T. Mohapatra, S. Singh, KS. Boora, K. Singh. Assessment of genetic diversity in *Azadirachta indica*based on DNA fingerprinting. *Indian Journal of Biotechnology* 6 (2007) 519-524
5. RP. Singh, R. Singh, K. Malik, GJ. Randhawa.Assessment of genetic diversity and genetic relationships among 29 populations of *Azadirachta indica* A. Juss. using RAPD markers, *Genetic Resources and Crop Evolution*. 52 (2005) 285-293
6. N. Kumari, SK. Thakur. Randomly Amplified Polymorphic DNA - A brief review. *American Journal of Animal and Veterinary Sciences*. 9(1) (2014) 6 – 13; doi:10.3844
7. LO. Ihase,R. Horn, GO. Anoliefo, CR. Eke, OC. kwuagwu and O. Asemota. Assessment of an oil palm population from Nigeria Institute for Oil palm Research (NIFOR) for simple sequence repeat (SSR) marker application. *African Journal of Biotechnology*. 13(14) (2014) 1529–1540; DOI:10.5897/AJB2013.13428
8. S. Kumar, G, Stecher, and K. Tamura. MEGA7*,* Molecular Evolutionary Genetics Analysis, version7. Molecular Biology and Evolution 33 (2016) 1870-1874; DOI:10:1093/moldey/msw054
9. O. Uko, T. Kamalu. The neem tree-uses and potentials. *Nigerian Journal of Experimental Applied Biolology*. 2 (2001) 223-229; DOI:10:3923/rjmp

10. N. Lale, A. Mustapha, Efficacy and acceptability of neem (*Azadirachta indica* A. Juss.) seed oil and pirimiphos-methyl applied in three storage devices for the control of *Callosobruchus maculates* (F.) (Caleoptera, Bruchidae). *Emir Journal of Food and Agriculture.* (2000) 310-315; DOI:10.1016/S0022-474X(98)00039-3
11. I. Ogbuewu, I. Okoli, M. Iloeje. Assessment of blood chemistry, weight gain and linear body measurements of pre-puberal buck rabbits fed different levels of Neem (*Azadirachta indica* A. Juss.) leaf meals. *Chilean Journal of Agricultural Research.* 70(3) (2010) 515-520; DOI: 10.4067/S0718-58392010000300020
12. AJ. Metson. Methods of Chemical Analysis of Soil Survey Samples. Govt. Printers, Wellington, New Zealand. 1961; 207; DOI: 10.12691/aees-4-3-1
13. S. Kota, N. Rao, P. Chary. In vitro response of select regions of *Azadirachta indica*. *Curricular Science.* 91(6) (2006) 770-776
14. N. Farooqui, S. Ranade, P. Sane. RAPD profile variation amongst provenances of neem. *Biochemical and Molecular Biology International*. 45 (1998) 931-939
15. AM. Maletsema, SLM. Hussein. S. Nemera. Genetic variability, heritability and genetic gain for quantitative traits in South African sorghum genotypes. *Australian Journal of Crop Science* 13(1) (2019) 1-10; doi: 10.21475/ajcs.19.13.01.p718
16. I. Dossou-Aminon, L. Yêyinou Loko, A. Adjatin, B. Ewédjè, A. Dansi, S. Rakshit, K. Akpagana. Genetic Divergence in Northern Benin sorghum (*Sorghum bicolor* L. Moench) landraces as revealed by agro-morphological traits and selection of candidates genotypes. *Science World Journal* (2015) 1 – 10; http://dx.doi.org/10.1155/2015/916476
17. R.K. Upadhyay, P. Dwivedi, S. Ahmad. Screening of antibacterial activity of six plant essential oils against pathogenic bacterial strains. *Asian Journal of Medical Science.* 2(3) (2009) 152-158

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