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Provenance Variation in African Wall Nut (*Plukenetia conophora* Mull Arg) from Southwestern Nigeria

[1]J.O. Amadi, [2]A.O. Adegeye, and [1]V.I. Alaje

[1]*Forestry Research Institute of Nigeria*

[2]*University of Ibadan, Ibadan, Nigeria*

Correspondence*: alaje.vi@frin.gov.ng*

ABSTRACT

Walnut is a multipurpose liana of high economic importance and vital potentials that are yet to be exploited due to lack of adequate germplasms. The study was aimed at documenting the variation in provenance in early growth in order to generate information necessary for its management and conservation. Seeds collected from four States: Ibadan in Oyo, Igbajo in Osun, Ijebu-Ode in Ogun and Akure in Ondo were sown directly into polythene bags filled with top soil. After germination, at the two-leaf stage, thirty uniformly growing seedlings from each provenance were selected and transferred into the open nursery, and laid out in a completely randomized design in six replications. Data on length, collar diameter, number of leaves, biomass and leaf area were subjected to analysis of variance and significant means were separated using Least Significant Differences p≤005. Net assimilation ratio (NAR), relative growth rate (RGR), and absolute growth rate (AGR) were also determined. Results showed that the effect of seed source on all the variables, except collar diameter, was not significant at p≤0.05. Ibadan source had the highest length, number of leaves, biomass, leaf area, NAR, RGR and AGR. The study concludes that Ibadan provenance could serve as superior gene pool.

Keywords: liana - seed source improvement – conservation - P*lukenetia conophora* - biomass.

INTRODUCTION

The Nigeria tropical forest is enriched with edible non-timber forest resources which are vital to the existence of man. These resources contribute to welfare and livelihood of the populace most especially the rural dwellers by generating substantial cash income (Ladipo 2000, Okafor 1993). They are also vital in providing and augmenting daily nutrients with relevant minerals and vitamins. They also provide herbs, medicines and raw materials for industries (Amadi *et al*. 2019).

However, a large percentage of the population could not make use of the enormous potentials of the forest due to overexploitation of the forest, being found in the wild. A few of these non-timber forest product species that enjoy little domestication for example, *Dacryodes edulis* are not established in commercial plantations (Degrande *et al.* 2013). They are found in isolated stands in farmlands, home gardens or existing agro forestry and cash crop plantation (Anegbe *et al.* 2003). For those growing in their natural habitat, little or no in-situ regeneration study has been carried out in the forest, nor ex-situ conservation for their potential propagation. However, sustainable management options for most of these products are necessary to curb over exploitation leading to extinction (Ladipo 2000), which could only be possible if research effort is focused on improvement strategies to manage potentials of these species on a sustained yield basis, with insight on the structure and functioning of tropical ecosystems including the silvicultural processes involved in regeneration, improvement and conservation of these species (Philips *et al.* 2002).

Most neglected are lianas (climbers), due to their physiological and morphological characteristic which makes them difficult to be established in orchards thereby preventing research from being carried out on them for improvement (Awodoyin *et al.* 2000). However, lianas are vital part of the tropical rain forest ecosystem. Most of them have vital potentials that are yet to be exploited. Among these is *plukenetia conophora* (walnut), a multipurpose liana of high economic importance.

Plukenetia conophora which belongs to *Euphobiaceae* is a wild woody perennial climber (liana) whose length ranges between 12 and 30 metres (Hutchinson and Dalziel 1963, Okafor 1975, 1991, Awodoyin *et al.* 2000), originated from Sierra Leone where it is known as 'Awusa' from where it spread to other parts of West Africa. Information from the herbarium (F.H.I) of the Forestry Research Institute of Nigeria (FRIN) revealed that the species is abundant in Oyo, Osun, Ogun and Ondo State but can also found in Benin province and Cross River State.

It is a non-timber forest resources protected on farmlands and sparingly cultivated for its oil rich fruits that serve as good source of income to the people living in rural areas (Awodoyin *et al.* 2000).

As demand for its fruits is increasing, the supply from forests is threatened by increasing deforestation and unsustainable farming practices (Anegbe *et al.* 2003). It is therefore pertinent to establish this in plantations and orchards to meet the demand for its fruit and for conservation purposes. This will require a detailed study on the variation that exists among and within provenance of *Plukenetia conophora*. This study was therefore carried out to identify the superior provenance of *P. conophora* from four sources in Southwestern Nigeria with a view to generating information necessary for its management and conservation.

MATERIALS AND METHOD

Reconnaissance survey

A reconnaissance survey of the natural distribution of *Plukenetia conophora* within the South Western part of Nigeria was carried out prior to flowering and fruiting. Stands with desirable traits were noted, selected and labeled. The plants were mainly on farms owned by the villagers. Necessary personal contacts were made with the farmers to ascertain appropriate period for fruit procurement. Based on information obtained from Forest Herbarium Ibadan (FHI) and preliminary survey of the endemic areas of this species, four sources were selected in South Western Nigeria (Figure 1). Ibadan in Oyo State, Igbajo in Osun State, Ijebu-Ode in Ogun State and Akure in Ondo State).

Ibadan is located on Lat 7°24´ 45"N and Long 3°55´ 45"E, Ijebu Ode – Lat 6°47´N and Long 3°58´E, Igbajo – Lat 7°56´ and Long 4°41´E, Akure – Lat 5°8´N and Long 4°6´E.

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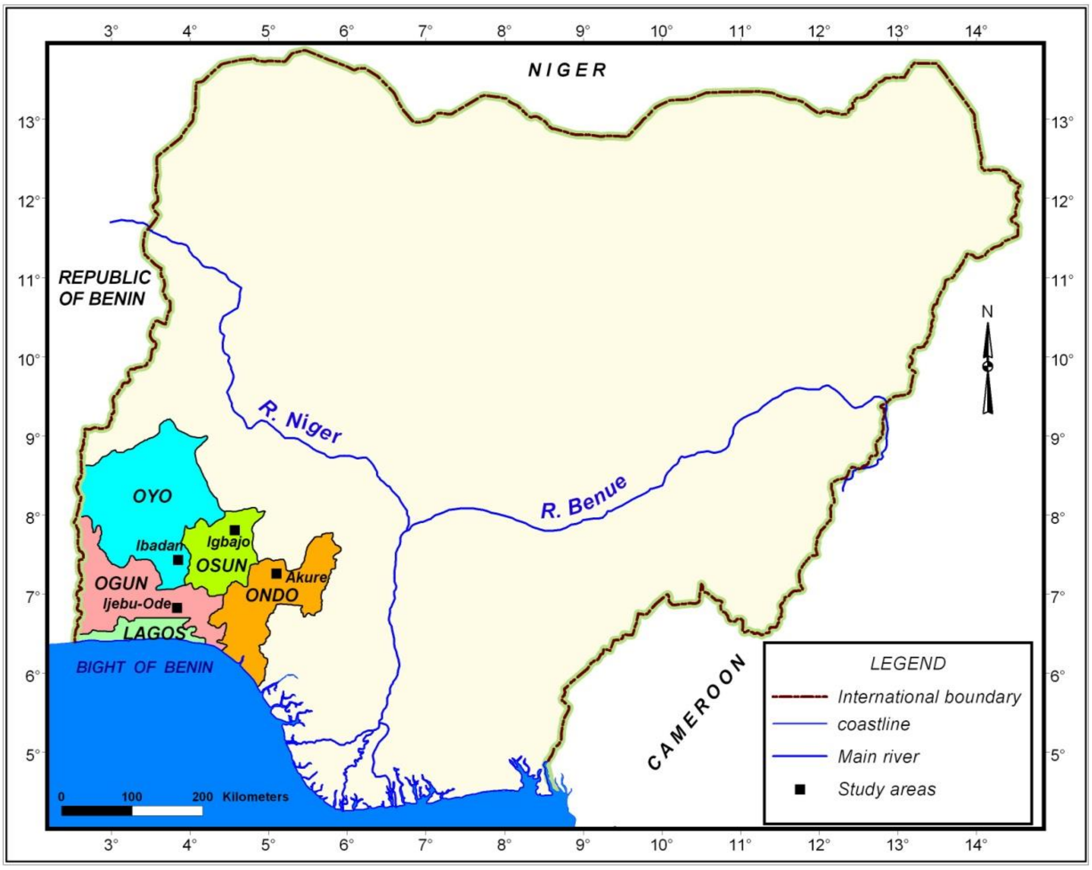

Fig. 1: Map of Nigeria Showing Seed Sources

The Experiment site

The experiment was carried out at Physiology and Tree breeding nursery of Forestry Research Institute of Nigeria, Ibadan which is located at Jericho Hills of Ibadan North West Local Government Area – Lat 7°25°N and 3°51°E and altitude of 277m above sea level.

The climate is of the West Africa monsoon type with dry and wet seasons. The dry season is normally from November through March and is characterized by dry cold wind of harmattan. The rainy season normally starts from the month of April to October. The annual rainfall is approximately 1,300mm; annual average relative humidity is about 90% at 7.00 a.m. and 67% at 4.00pm. Day temperature fluctuates between 22°C and 34°C with little variations throughout the year (FRIN, 2016).

Data collection and experiment procedures

Mature fruit were sourced from high yielding mother tree between the ages of 4-5 years from the four sources identified. In each of the sources, fruit were collected from the four corners of the trees and five trees are selected per source. Seeds were manually extracted using hands from the collected fruits. The extracted seeds from each of the sources were sown directly into polythene bags of dimension 6” by 8” filled with forest (top) soil. After germination, at the two-leaf stage, thirty (30) uniformly growing seedlings from each provenance were selected making a total of 120 plants. Fifty seedlings from each source for biomass estimation were also selected, making a total of 320 seedlings for the entire experiments. The seedlings were transferred into the open nursery and laid out in a completely randomized design with six replicates of five plants per replicate. Watering was done to field capacity once daily for about 6 months.

The following growth variables were assessed fortnightly:

- Length of liana (cm): this was measured using meter rule taken from the base to the tip of the apical bud.
- Collar diameter of liana: the seedling stem diameter at the collar was measured using digital vernier caliper.
- The number of leaves: the number of leaves on each seedling were counted and recorded.

Biomass assessment

Biomass assessment of seedlings was carried out bi-monthly for 5 months after germination. Five seedlings were randomly selected from each provenance for each of the assessment making a total of 200 seedlings for the entire biomass assessment. The seedlings were uprooted by placing them in a bowl containing water; the soil around the root was carefully washed off. The uprooted seedlings were then separated into root and shoot components. Each component of the seedlings were put into separate envelopes and labeled on treatment basis for ease of identification. The envelopes containing the components were weighted in the laboratory using a sensitive weighing balance to determine the fresh weight of the samples. After which they were oven dried for 24 hours at 70°C until constant weight was obtained. The result and leaf area were used to calculate the relative growth rate (RGR), net assimilation rate (NAR) and Absolute Growth Rate (AGR) according to the following formulae:

Relative Growth Rate (RGR) this

$$RGR = \frac{InW2 - InW1}{T1 - T2} \quad (gm/t)$$

Net Assimilation Rate (NAR)

$$NAR = \frac{W2 - W1}{A2 - A1} X \frac{InA2 - InA1}{T2 - T1} \quad (gm/t)$$

Absolute Growth Rate (AGR)

$$AGR = \frac{W2 - W1}{T2 - T1} \quad (gm/t)$$

Where:

A1 = Initial Leaf Area
A2 = Final leaf Area
W2 = Final Dry Weight
W1 = Initial Dry Weight
T2 = Final Harvest Time
T1 = Initial Harvest Time
In = Natural Logarithm (log l)

Leaf area

Rapid estimation method was used to calculate the leaf area as follows.

- Leaves were collected bi-monthly
- Their margins were traced on graph to determine the areas
- Leaf length excluding the petiole was taken for each leaf.

Data analysis

All data collected were subjected to analysis of variance (ANOVA) and where the means are significant, least significant difference LSD at $p<0.05$ were used as post hoc.

Results

The result of the analysis of variance for the effect of seed source on early growth of wall nut revealed that the effect of seed source was not significant ($p \leq 0.05$) on length of liana as well as number of leaves (Table 1). However, the effect of seed source was significant ($p \leq 0.05$) on collar diameter of *P. conophora*. The seeds sourced from Igbajo produce the highest collar diameter of 1.146cm which was significantly different ($p \leq 0.05$) from other sources (Table2). The seed sourced from Igbajo also produce the highest liana length of 142.4cm which was not different from the other provenance according to LSD at $p \leq 0.05$. Akure produced the highest number of leaves though not significantly different from others.

Table 1: Analysis of variance for the effect of seed sources on early growth of *Plukenetia conophora.*

Variables	Source of Variation	Degree of Freedom	Sum of square	Mean Square	F - value	P-value
Collar diameter	Between group	3	0.44982	0.150	3.125	0.048
	Within group	20	0.9706	0.048		
	Total	23				
Length of liana	Between group	3	2025	675ns	2.755	0.069
	Within group	20	4900	245		
	Total	23				
Number of leaves	Between group	3	33.09	11.03ns	0.994	0.416
	Within group	20	222	11.1		
	Total	23				

*Significant at P< 0.05, Ns = not significant

Table 2: Effect of fruit sources on early growth of *P. conophora*

Source	Collar diameter	Liana length	Number of leaves
Ibadan	0.934b	129.2a	11.02a
Ijebu-Ode	0.957b	125.1a	11.58a
Akure	0.894c	129.5a	12.15a
Igbajo	1.146a	142.4a	9.9a

Means followed by the same alphabet in a column are not significantly different from each other according to LSD at 5% level of probability.

The analysis of variance for the effect of seed source on biomass and leaf area showed that source has no significant difference p≤0.05 on leaf dry weight, stem dry weight, root dry weight, biomass as well as leaf area (Table 3). Also, the four seed provenances are the same in terms of leaf area.

Table 3: Analysis of variance for the effect of seed source on biomass and leaf area of *Plukenetia conophora*

Source of variation		Degree of freedom	Sum of square	Mean square	F-value	P-value
Leaf Dry Weight	Between group	3	0.579	0.193ns	0.143	0.933
	Within group	20	26.98	1.349		
	Total	23				
Stem Dry Weight	Between group	3	1.068	0.356ns	0.061	0.9797
	Within group	20	109.7	5.845		
	Total	23				
Root Dry Weight	Between group	3		20.0ns	0.159	0.922
	Within group	20		125.8		
	Total	23				0.922
Biomass	Between group	3	8.07	24.21ns	0.160	
	Within group	20	3025.6	151.28		
	Total	23				
Leaf Area	Between group	3	87.69	29.23ns	2.091	0.1335
	Within group	20	279.6	13.98		
	Total	23				

Ns = not significant @ p≤0.05

The Mean Relative Growth Rate (RGR) g/t of seedling as affected by seed sources over the experimental period is presented in Table 4. The relative growth rate increased from RGR1 till RGR2 across the seed sources. However, the relative growth rate (RGR) showed a diminishing trend from the RGR3 week till the end of the experiment (RGR5). At RGR1, Ibadan had a mean relative growth rate of 0.088g/t, Ijebu Ode had 0.081g/t while Akure and Igbajo had 0.080g/t and 0.084g/t respectively. At 8 weeks of germination (RDR2), Ibadan source recorded the best relative growth rate with 0.095g/t. This was closely followed by Igbajo with 0.092g/t, Ijebu Ode and Akure had 0.089g/t and 0.084g/t respectively.

The least relative growth rate of 0.00325g/t was recorded in Akure source at 20 weeks of germination (RGR5) (Table 4.

Table 4: Mean Relative Growth Rate (RGR) g/t of seedling as affected by seed sources

Source	RGR1 (4weeks)	RGR2 (8 weeks)	RGR3 (12 weeks)	RGR4 (16 weeks)	RGR5 (20 weeks)
Ibadan	0.088	0.095	0.075	0.049	0.034
Ijebu-Ode	0.081	0.089	0.061	0.032	0.00675
Akure	0.08	0.084	0.053	0.02	-0.00325
Igbajo	0.084	0.092	0.06	0.031	0.00925
Mean	0.0833	0.09	0.0623	0.033	0.0112
S.E	0.0018	0.0024	0.0046	0.006	0.0079
CoefVar	4.32	5.21	14.8	36.28	135.4

Table 5: Mean Net Assimilation Rate (NAR) g/t of seedling as affected by seed sources

Source	NAR1 (4 weeks)	NAR2 (8 weeks)	NAR3 (12 weeks)	NAR4 (16 weeks)	NAR5 (20 weeks)
Ibadan	0.021	0.026	0.026	0.025	0.015
Ijebu-Ode	0.017	0.022	0.019	0.012	0.003
Akure	0.016	0.022	0.015	0.007	0.001
Igbajo	0.015	0.022	0.02	0.012	0.004
Mean	0.0172	0.023	0.02	0.014	0.0053
S.E.	0.0013	0.001	0.0023	0.0039	0.0034
Coervar (%)	15.25	8.7	22.73	55.02	130.47

The Net Assimilation Rate (NAR) (Table 5) increased from week 4 (NAR1) till the end of week 11 (NAR2) after germination (Table 5) and then a steady decline from week 12 (NAR3) till the end of the experiment (NAR5) across all the four seed sources. The highest value for all the four sources were observed in the second month (NAR 2) while the least values across the four sources were observed in NAR5. The highest NAR of 0.026g/t was recorded in Ibadan source at the 8th (NAR2) and 12th week of germination (NAR3) closely followed by the same source at 16th week of germination (RGR4). The least NAR was observed in seed sourced from Akure at the end of the experiment at week 20-24 (NAR5). The other three sources (Ijedu-ode, Akure and Igbajo) recorded their highest NAR of 0.022g/t at week 8-11 (NAR2). The least value of NAR for Ijebu ode and Igbajo is 0.003g/t and 004g/t respectively recorded at week 20-24 after germination (NAR5).

The mean Absolute Growth Rate (AGR) g/t (Table 6) of seedling as affected by seed sources across the experimental period is presented in Table 6. The AGR values ranged from 0.105g/t (AGR5) to 2.266g/t (AGR3).

Table 6: Mean Absolute Growth Rate (AGR) g/t of seedling as affected by seed sources

Source	AGR1	AGR2	AGR3	AGR4	AGR5
Ibadan	1.898	2.237	2.266	1.854	1.45
Ijebu-Ode	1.511	1.788	1.636	1.047	0.245
Akure	1.242	1.244	1.308	0.625	0.108
Igbajo	1.567	1.856	1.651	1.06	0.343
Mean	1.555	1.781	1.715	1.147	0.482
S.E.	0.135	0.205	0.2	0.257	0.337
CoefVar (%)	17.33	22.96	23.31	44.76	139.58

All the seed sources recorded their least AGR at 20-24 after germination (AGR5) whereas the highest value for each of the seed sources were observed at week 8-11 after germination (AGR2) except at Ibadan which recorded its highest vale at AGR3. Overall, the highest value of AGR was observed in seedlings from seed sourced from Ibadan (2.266g/t) at 12-15 week after germination (AGR3) and the least AGR were observed in seedlings produced from seed sourced from Akure (-0.108g/t) at the end of the experimental period (AGR5).

DISCUSSION

Tree improvement and breeding programmes depend to a larger extent on genetic diversity, which are the variation that exists within and among species as well as environmental variation. Plans for conservation are therefore often based on assessment of the eco-geographic variation of the distribution area of the population. The selection of any conservation method depends upon factors such as the variability of the species, its biology, specified objectives of conservation, local conditions and genetic makeup of the species (Hettasch *et al*. 2009). Improvement effort on plant species with high economic importance and demand like *Plukenetia conophora* need urgent attention to meet both the demand for conservation, production and consumption. This could be done firstly by knowing the exact location for germplasm collection and utilization. Improvement involves the utilization of germplasm of plant varieties with desirable traits, which is possible when the variation in the natural populations of the species have been documented.

The study revealed that the effect of provenance was pronounced on collar diameter as shown by the significant difference of the effect of seed sources on collar diameter. The seed sourced from Igbajo had the best collar diameter. Seeds and fruits often contain energy food reserves, which are used up during germination to produce shoot and root components of the seedlings thus, a good seed source is an indication that will manifest in strong and vigorous seedlings. Many authors have equally observed variations among and between populations of different tree species which include, Alaje *et al.* (2020) who investigated provenance variations in *Irvingia gabonensis*, Akinyele (2007) who investigated *Buchholzia coriacea*. Gbadamosi (2002) studied variation in *Enantia chlorantha* from different sources and Oyum (2003) who worked extensively on *Parkia biglobosa* sourced from different seed sources.

According to Mkonda *et al*. (2003) and Loha *et al.* (2006), these variations could be attributed to the seeds which vary in their degree of performance between and within populations and between and within individuals, causes of such variability is generally attributed either to genetic characters of source population/plant (Shu *et al*. 2012), or impact of mother plant environment (Singh *et al.* 2010).

The study also revealed that the effect of provenance was not pronounced on length of liana and number of leaves as shown by the non-significant different on these variables. This is in line with Abebe *et al*. (2018) who observed that the effect of seed provenance had no significant differences on the growth of Olea due to absence of drastic differences in the physical characteristics of the localities where the seeds were collected.

It was also observed from this study that Ibadan had the highest net assimilation ration, relative growth rate as well as absolute growth rate which are indication of higher photosynthetic ability and growth rate. The relative growth rate refers to the increase in dry matter per unit area. When plants do not produce enough leaves over a long period of time, the photosynthetic ability of the plant as well as the growth rate may be negative. Growth will only occur when photosynthesis is in excess of respiration (Nwoboshi 1992). The negative

RGR obtained in Akure is an indication of its poor growth rate which may not make such provenances suitable for selection. Similarly, the differences observed in the values of the NAR within and between sources is an indication of differences in photosynthetic efficiency of the seedlings of the species. This also is in agreement with the work of Atangana *et al.* (2002) and Leakey (2002) who studied the variation on morphological trait and size of different components of *Dacryodes edulis* and *Irvingia gabonensis* respectively. Thus, this may suggest that before embarking on any tree improvement programme for plantation or orchard establishment, source of seed is vital.

CONCLUSION

The effect of seed source on *P. conophora* was well-pronounced on collar diameter. Ibadan and Igbajo provenances had proven to be superior among the various provenances used; hence seed should be sourced from these provenances for the improvement programme in the management and conservation of *Plukenetia conophora.*

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