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Morphological Variation and Interrelationship among Traits of African Walnut (*Plukenetia Conophora* Mull Arg.) Accessions from Nigeria

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ABSTRACT

Plukenetia conophora is a woody climber with high demand for food, raw material, medicine and income generation but has not been improved upon. Therefore, this study was conducted with the aim of evaluating its variability and character association. Seven accessions sown directly into polythene bags filled with top soil were laid out in a completely randomized design in five replications. Data on height, collar diameter, number of leaves, leaf dry weight, stem dry weight, root dry weight, biomass and leaf area were subjected to Analysis of Variance and Principal component analysis. For most of the traits, there were significant differences among the accessions indicating variations among the accessions. The first three axes accounted for 79.5% of the overall variations. Clustering analysis grouped the seven accessions into three clusters. Members of cluster one which had the highest biomass and leaf area could serve as genes for parental lines that might be used in hybridization programs to improve this species. A positive significant correlation existed between leaf area and biomass as well as leaf dry weight. This therefore will facilitate the selection of promising phenotypes. This result will constitute a database for the improvement of Walnut.

Keywords: *Plukenetia conophora* - traits, variability – correlation – improvement - hybridization.

INTRODUCTION

Plukenetia conophora formally called *Tetracarpidium conophorum.*is a multipurpose fruit woody climber belonging to the family *Euphobiaceae*, popularly known as Walnut. It has enormous uses which ranges from food, raw material, medicine and income generation, which is in high demand. The high demanded seeds have been shown by several authors to be rich in protein, oil, vitamins and other nutrients (Agbo and Baiyeri 2019, Amadi *et. al.* 2019). The macerated leaves, roots and seeds are used for treatment of asthma and hypertension (Okafor 1991), it is also useful in area of cholesterol control, blood sugar and improvement of cardiovascular functions, (Okafor 1975).

Therefore, improvement of this multipurpose plant demands an urgent attention to meet the increase demand for its fruits and other products derived from the plants. It is equally an opportunity to improve farmers' incomes and even develop the rural economy. There are gaps in research areas of walnut improvement as most researchers over the years had focused on its nutritive aspect (Akpuaka and Nwankwor 2000, Odoemenam 2003, Oboh and Ekperigin 2004, Ajaiyeoba and Fadare 2006, Isong *et al.* 2013, Apeh *et al.* 2014 and Amadi *et. al.* 2019). Walnut has not been studied in detail to provide information on its improvement and conservation capabilities (Awodoyin *et al*. 2000). This neglect in this aspect of research according to Amadi (2014) is due to their morphological characteristic as climbers. She further added that this climbing ability makes it difficult to establish in orchards and plantation thereby discouraging traits improvement research efforts on them.

The main step into traits improvement is to determine the variability that exists within and between genotypes. Variation is an important aspect of improvement in any particular plant species. Hettasch *et al*. (2009) defined variation "as the observable differences between and within individuals of a species". Some of these observed differences could be attributed to differences in the genetic make-up of the individual tree, other variation could be as a result of the impact of the environment on the tree (Hettasch *et al.* 2009). Variation enables species to adapt to changing environments and to survive. It is a survival fitness mechanism whereby species which are unable to alter their genetic make-up to suit a changed environment become extinct and species that are able to pioneer new environments dominate the entire space. According to Hettasch *et al*. (2009), genetic variation is the foundation on which tree breeding is based. Therefore, exploitation of existing variability among germplasm accessions is the first step and short-term strategy for improving this species (Alaje *et al*. 2019).

The existence of variations could be assessed by morphological traits evaluation using quantitative data. Data generated from morphological traits in plants have been analyzed and used to study genetic diversity. This information on genetic relationships is used to determine genetic distance among the genotypes tested (Fatimah *et al.* 2018). This will be used to categorize the tested genotypes into subgroup based on their similarities or differences. This will be important in hybridization program in selecting genetically divergent parental lines derived from the different genetic groups (Mayes *et al.* 2015) which is expected to produce high heterosis in the progenies (Falcon 1981), thereby increasing the chance of obtaining superior segregants in subsequent generations.

The dearth of information on the morphological variability and traits relationship towards the improvement of this multipurpose tree species facilitated this research. This research was therefore conducted to determine the morphological variability among the seedling traits of Walnut as well as to determine the extent of association among the seedling growth traits for its improvement and conservation.

MATERIALS AND METHOD

Experimental site

The experiment was conducted at Tree Physiology and Breeding nursery of Sustainable Forest Management Department, Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria. FRIN is located on the longitude 070 23'18"N to 070 23'43"N and latitude 03051'20"E to 03051'43"E. The climate of the study area is the West African monsoon with dry and wet seasons. The dry season is usually from November through March and is characterized by dry cold wind of harmattan. The wet season usually starts from April to October with occasional strong winds and thunderstorms. Mean annual rainfall is about 1548.9 mm, falling within approximately 90 days. The mean maximum temperature is 31.9oC, minimum 24.2oC while the mean daily relative humidity is about 71.9% (FRIN 2015).

Sources of Accessions

The matured fruits were collected from their natural stand in each of the under listed States from Nigeria (Table 1). The seeds were extracted from the fruit manually.

Experimental set up and data collection

The collected fruit were extracted manually with the use of knife to obtain the seeds. Fifty seeds from each of the accessions were used for the experiment. The selected seeds from each accession were propagated in 10"X8'' polythene pots containing forest soil sourced from FRIN arboretum at one seed per pot at a uniform depth of 5cm. A total of 350 seeds from the seven accessions were used. The pots were arranged in completely randomized deign in five replications and placed in a nursery shed. Water was done daily to maintain soil moisture and manual weeding was done as at the appearance of weed in the pots. After germination, the seedlings were assessed fortnightly for six months.

Table 1: Source of *Plukenetia conophora* accessions used

Genotypes	Accessions Number	Source of collection	State of collection	Longitude	Latitude
G1	Accs1	Igbajo	Osun State	4.82	7.91
G2	Acces2	Akure	Ondo State	5.21	7.25
G3	Acces3	Ijebu ode	Ogun State	3.92	6.83
G4	Acces4	Ibadan1	Oyo State	3.86	7.67
G5	Acces5	Benin	Edo Sate	5.62	6.32
G6	Acces6	Ore	Ondo Sate	4.88	6.75
G7	Acces7	Ibadan2	Oyo State	3.86	7.67

Data collection and analysis

The following quantitative data were collected:

1. Plant height: the length of the liana was taken from the base to the tip by the use of meter rule graduated in cm
2. Collar diameter: the diameter at collar height was taken with the aid of digital venner caliper
3. Number of leaves were counted physically
4. Leaf dry weight: The uprooted seedlings were washed off the soil and segmented into leaf, stem and root. These were dry in oven at constant temperature until a constant weight is achieved. Sensitive weighing balance set in gram was used to take the readings
5. Stem dry weight was also done as in leaf dry weight
6. Root dry weight was also done as in leaf dry weight
7. Biomass: this is the total dry weight component measured in gram
8. Leaf area: The leaf area was taken by the use of portable leaf area meter.

Accessions average were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS 2000) was employed for data analysis to establish the variability that existed among the accessions. Significant means were separated using Turkey at 5% level of probability ($P \leq 0.05$).

Principal component analysis (PCA) was also used to determine the variability between traits of the studied accessions. The eigenvector for each principal component's axis were evaluated from PCA, solutions were accepted when Eigen values were greater than one (*Hair et al*. 1995, Hajjar *et al*. 2008). The character loading was used to determine the contribution of the traits to the total variations observed. Factor loading equal to or greater than 0.3 were considered to be defining part of a principal component (Hajjar *et al*. 2008). The accessions were subjected to hierarchical clustering using Average linkage which classified them into homogenous groups.

Average Linkage Cluster Analysis (ALCA) was used to summarize the level of relatedness among the accessions. Morphological dendrogram was drawn from the average linkage cluster analysis summary.

Correlation coefficient was computed to determine the extent of association among the traits of Walnut accessions.

RESULTS

The analysis of variance revealed that there were variations among the plant height, number of leaves, stem dry weight, root dry weight, biomass and leaf area evaluated in 7 accessions of African Walnut as shown by the significant differences (p≤0.05) observed (Table 2). However, collar diameter and leaf dry weight showed no significant different.

Table 2: ANOVA for morphological characters in 7 *Plukenetia conophora* accessions

Plant traits	Sources of variation	Sum of Squares	Df	Mean Square	F	Sig.
Plant height	Accessions	10757.5	6	1792.91	11.827	0.00
	Error	4244.73	28	151.597		
	Total	15002.2	34			
Collar diameter	Accessions	81.115	6	13.519	1.979	0.103
	Error	191.269	28	6.831		
	Total	272.384	34			
Number of leaves	Accessions	85.577	6	14.263	3.537	0.01
	Error	112.916	28	4.033		
	Total	198.493	34			
Leaf dry weight	Accessions	0.02	6	0.003	0.635	0.701
	Error	0.148	28	0.005		
	Total	0.168	34			
Stem dry weight	Accessions	0.158	6	0.026	4.653	0.002
	Error	0.159	28	0.006		
	Total	0.317	34			
Root dry weight	Accessions	4231.01	6	705.168	25014.8	0.00
	Error	0.789	28	0.028		
	Total	4231.8	34			
Biomass	Accessions	4286.76	6	714.46	17431.9	0.00
	Error	1.148	28	0.041		
	Total	4287.91	34			
Leaf area	Accessions	419.421	6	69.904	36.855	0.00
	Error	53.109	28	1.897		
	Total	472.53	34			

Results from Table 3 revealed that plant height showed a high level of variability of above 440% while characters such as collar diameter, leaf dry weight and stem dry weight exhibited low level of variability. The result also revealed that accessions collected from Akure and Ijebu ode had higher plant height which is significantly different from other accessions while accessions collected from Igbajo recorded the least plant height. Higher number of leaves was observed in Ijebu-ode and Ibadan 1 accessions which is significantly different from number of leaves from other accession. Ore had the highest stem dry weight when compared to the other accessions. Ibadan1 recorded the highest Root dry weight, biomass as well as leaf area (89.588).

Table 3: Mean performance of 7 accessions of *African Walnut evaluated* for Its morphological traits

Accessions	Plant height (cm)	Collar diameter (mm)	Number of leaves	Leaf dry weight (g)	Stem dry weight (g)	Root dry weight (g)	Biomass (g)	Leaf area (cm^2)
Igbajo	35.812c	4.838a	6.88b	0.700a	0.42b	7.088d	8.208d	83.948c
Akure	69.644ab	3.998a	9.636ab	0.642a	0.454b	6.878d	7.978de	80.264d
Ijebu-ode	87.806a	5.238a	11.298a	0.650a	0.432b	6.548e	7.63e	70.546d
Ibadan 1	83.838a	4.134a	11.71a	0.712a	0.520ab	31.618a	32.85a	89.588a
Benin	64.838ab	4.864a	10.352ab	0.792a	0.522ab	28.592b	29.816b	86.520bc
Ore	50.644bc	4.724a	8.278ab	0.692a	0.628a	27.884c	29.204c	86.210bc
Ibadan2	50.232bc	4.850a	10.064ab	0.684a	0.536ab	27.884c	28.800c	87.466ab
CV (%)	441.241	0.011	5.838	0.005	0.009	124.465	126.115	13.898

Table 4 shows that the total variation accounted for could be attributed to a total of seven principal axes. However meaningful contribution could only be attributed to the first three axes which had Eigen values equal to or greater than 1 (*Hair et al*. 1995). Principal component 1 axis accounted for the most variations observed in *Plukenetia conophora* accessions used accounting for 44.6%. Principal axes 2 and 3 accounted for 23.6% and 13.3% respectively while axes 4 and 5 accounted for 9.1% and 6.1% of the total variation seen.

Figure 1 represents the scree plot of the 7 walnut genotypes showing the Eigen value and the character component. All variations observed could be partitioned into eight components, however only three components are significant.

Table 4: Eigen value and the Proportion of total variation accounted by Principal Component Axes

Principal Component axes	Eigen value	Proportion of variability (%)	Cumulative proportion (%)
1	3.408	42.6	42.46
2	1.888	23.6	66.2
3	1.068	13.3	79.5
4	0.7313	9.1	88.6
5	0.484	6.1	94.7
6	0.2667	3.3	98.0
7	0.153	2.0	100
8	-0.00	-0.00	100

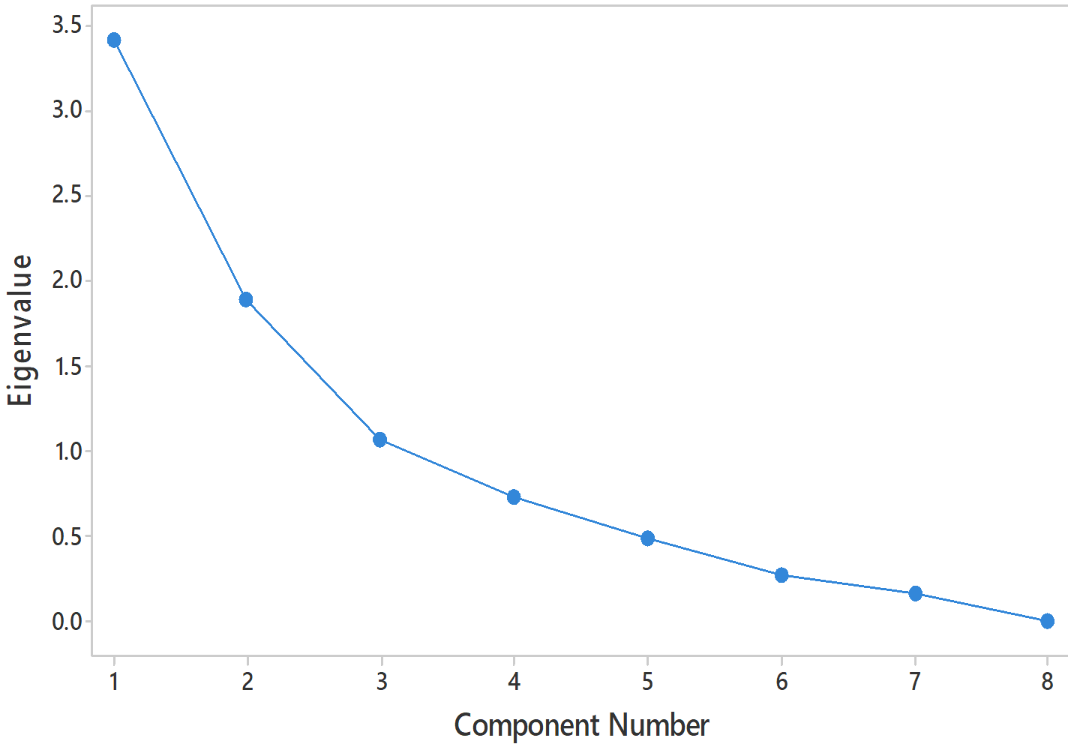

Figure 1: The scree plot showing the Eigen value and the character component of 7 *Walnut* genotypes

Table 5: The five axes and their character loading as well as number of clusters evaluated in *P. conophora* traits

Traits	PC1	PC2	PC3
Plant height	0.046	**-0.655**	-0.001
Collar diameter	-0.208	**-0.369**	-0.044
Number of leaves	0.081	**-0.643**	-0.12
Leaf dry weight	0.162	0.091	**-0.855**
Stem dry weight	**0.358**	0.028	**0.495**
Root dry weight	**0.526**	0.073	0.003
Biomass	**0.526**	-0.072	0.002
Leaf area	**0.491**	0.035	0.084
Number of clusters	4	2	1
Accessions	6,7,5,4	2,3	1

The bolded denote the characters that contribute significantly to the total variation and which scored above 0.3 (Hajjar *et al.* 2008) as presented in Table 5. The scores above 0.3 are considered meaningful to the total variation seen. The first axes were loaded with stem dry weight, root dry weight, biomass and leaf area. The principal axis 2 were loaded with plant height, collar diameter and number of leaves while the third axis were loaded with leaf dry weight and stem dry weight. Ore, Ibadan2, Benin and Ibadan 1 (6, 7, 5, 4) clustered together on principal axis one. Principal axis 2 has Akure and Ijebu-ode (2, 3) while principal component axis 3 had only one accession (Igbajo).

The dendrogram from the Average Linkage Cluster Analysis is presented in Figure 2. This illustrates the relationship between the genotypes. At 100% level of similarity, all the genotypes had formed a single cluster, meaning that the genotypes were distinct at 100% level of similarity. At 81% similarity and a distance of 1.106, the walnut accessions had formed 6 clusters and a similarity of 0% and a distance of 5.844, all the 7 genotypes had formed a single individual cluster (Table 6).

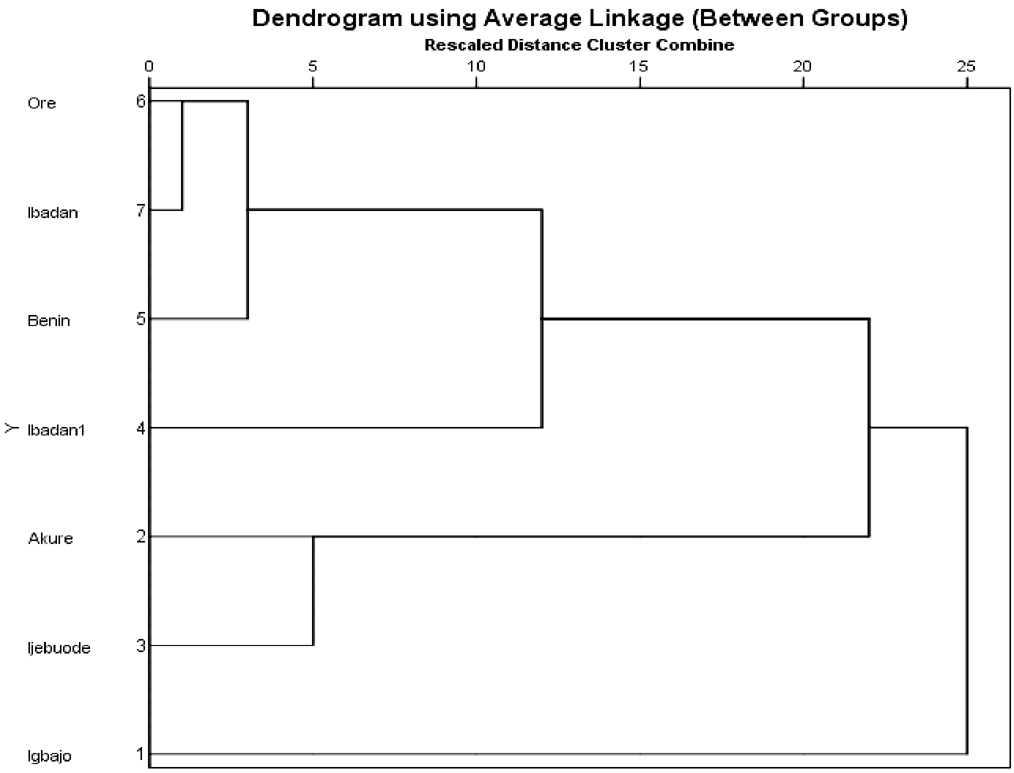

Figure 2: Dendrogram of 7 Walnut genotypes using Average linkage

Table 6: Cluster Partitioning in 7 Walnut Accessions

Number of clusters	Similarity level	Distance level	Cluster joined
6	81.082	1.1062	5 7
5	64.24	2.09	5 6
4	49.551	2.948	2 3
3	43.047	3.328	4 5
2	11.603	5.166	2 1
1	0	5.844	4 1

Morphological Traits correlation matrices as shown in Table 7 revealed some significant correlative responses. Collar diameter had a positive significant (r=34.719) relationship with number of leaf and a negative significant relationship with leaf area. Number of leaves also correlated negatively with traits such as root dry weight (r=7.988), biomass (r=-8.0533) and Leaf area (r=-3.183). The relationship between leaf dry weight and biomass revealed positive significant. Biomass and leaf area showed a positive significant relationship of coefficient correlation of 34.696.

Table 7: Intra-correlation coefficients among Morphological traits of African Walnut.

Traits	Plant height	Collar diameter	Number of leaves	Leaf dry weight	Stem dry weight	Root dry weight	Biomass
Collar diameter	-0.163						
Number of leaves	0.402	34.719*					
Leaf dry weight	0.018	-0.1794	-0.019				
Stem dry weight	0.0031	-0.078	-0.046	0.0131			
Root dry weight	0.0034	-0.692	-7.988*	5.1478*	0.184		
Biomass	0.0246	-0.949	-8.053*	5.165*	0.1885	0.619	
Leaf area	0.5349	-10.127*	-3.183*	0.792	0.0688	0.154	34.696*

DISCUSSION

Knowledge of morphological variability of African Walnut is vital towards its improvement, conservation and utilization. This will aid in designing breeding programs for this choice species. Genetic variability is an inherent character that largely influences the survival fitness of plant. Dart information is found on variations in Walnut accessions from Nigeria. Providing such information is prerequisites for its improvement. The morphological traits evaluated in this study except collar diameter and root dry weight varied significantly among the accessions. However, some of the traits have low coefficient of variation while some exhibited high coefficient of variation. These variations imply that the seven accessions used in this study are divergent and is as a result of combination of genetic and environmental factors the plants are exposed to. Many authors had worked on variations of tropical plants and had gotten similar results, *Chrysophyllum albidum* (Dadegnon *et al*. 2015), *Adansonia digitata* (Assogbajo *et al*. 2010), *Pentadesma butyracea* (Ewedje *et al*. 2015), *Dacryodes edulis* (Kengue *et al*. 2002, Alaje *et al*. 2019) and *Irvingia* gabonensis (Leakey 2002, Atangana *et. al*. 2002).

The high coefficient of variation recorded in plant height, root dry weight as well as biomass indicated that these traits are important in Walnut. Four out of the seven accessions performed better than the remaining three in at least one trait. Ibadan

1 accession recorded the highest number of leaves (11.71), root dry weight (31.618), biomass (32.85g) and leaf area (89.588), Ijebu ode accession had the best of Plant height (87.806cm), Collar diameter (5.238mm), while Benin accession and Ore had the best of leaf dry weight (0.792g) and stem dry weight (0.628g) respectively. This is an indication that morphological traits of *P. conophora* is not only affected by genetic variations but also environmentally controlled. This line of result is in agreement with Assogbajo *et al.* (2010). Therefore, there is potential for selection among the accessions.

Principal component analysis was employed to explain the variations observed. The result indicates that the overall variation observed could be explained by the first three axes which accounted for 79.5% of the total variation. At least one of the traits was important to the variations in one out of the three principal components. Since all the accessions are raised in the same environment, the differences in the contributions of different characters to the total variation might have been caused by the growing conditions the mother trees (original environment) from which the seeds are collected from are exposed to. The differences also could be due to the genetic make-up of the different accessions due to the inherent characters inherited from the mother trees or the influence of environment on the adaptation of the mother plant as well as selection of specific traits by farmers.

The seven accessions were group into three clusters. Cluster one and two had the highest of almost all the traits examined. Members of cluster one which had the highest biomass and leaf area might be used for selection of adapted genotypes for yield and other traits since biomass has been used as indicator to assess plant efficient use of available resources for growth and development and Leaf area growth determines light interception and is an important parameter in determining plant productivity (Gifford *et al.* 1984, Koester *et al.* 2014). These genotypes could serve as parental lines that might be used by hybridization programs to improve this species. The dendogram constructed using average linkage showed that Walnut from different region clustered together for example, accession from Ore, Benin and Ibadan clustered together while Akure and Ijebu ode clustered together. This type of relationship might indicate the degree of relatedness between accessions from different regions. This could be further explained and attributed to the transfer and or exchange of seeds between the different state of collection through human activities such as transfer of gene through seed and seedling exchange.

Correlations relationship among plant traits is important useful in designing an effective breeding program (Shabanimofrad *et. A.l* 2013). Also, the differences in magnitude and direction as well as number of significant relationships is important in the choice of breeding methods for selection on multiple traits (Freitas *et al.* 2011). For perennial plant such as Walnut, morphological descriptors will aid in selecting genotypes with high productive potential in the short term. Yield according to (Shabanimofrad *et. al.* 2013), is a complex quantitative plant characters inherited and influenced by genetic effects, as well as by genotype and environment interaction (GxE) and making selection to improve yield directly in the face of GXE is difficult and unpredictable and time consuming (Ojo 2000). Therefore, identification and use of associations between plant characters are appropriate. In this study, it was observed that there was positive statistically significant correlation between biomass and leaf area as well as leaf dry weight. This relationship is important as improvement program towards the selection of elite accessions to reproduce high biomass should be geared towards those with the best leaf area and leaf dry weight. Also, a high positive correlation relationship was noted between collar diameter and number of leaves. The strong positive correlation between these characters indicates that the genes which govern these characters are probably linked or have a pleiotropic effect. This therefore will facilitate the selection of promising genotypes.

It was further observed the leaf area had /negative significant relationship with collar diameter and number of leaves. This is expected as the walnut having high number of leaves produces smaller leaves but those with lesser number of leaves produces larger sizes of leaves. In the same vein, larger leaf area produces smaller girth.

CONCLUSION

This study carried out to determine the variation and inter relationship among the morphological traits of African walnut accessions from Nigeria had highlighted important morphological traits variability within the accessions. This offers potential for selection towards the improvement of this plant because the presence of morphological variability is essential for its future improvement by providing options to develop new varieties and hybrids.

Three axes contributed 79.5% of the total variations observed and cluster analysis classified the genotypes into three distinct clusters. Members of cluster one which comprised of Ore, Ibadan, Benin and Ibadan 1 had the highest biomass and leaf area. These accessions could serve as parental lines in hybridization programs to improve this species.

Strong positive correlation existed between some morphological traits which are indications that the genes which govern these characters are probably linked or have a pleiotropic effect. This therefore will facilitate the selection of promising genotypes,

The research recommended that the variability observed within the accessions studied and the associations between the characters obtained will constitute a database for the improvement of Walnut particular the development of high-yielding Walnut. The result will also contribute substantially to the management, conservation and character improvement of Walnut in Nigeria.

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all the authors.

Alaje V.I. and Amadi J.O. conceived the research.

Adegoke F.F. and Adebusuyi G.A. collected all the data used.

Alaje V.I. and Oyedeji O.F. interpreted the data and draft the first copy of the manuscript

Amadi, J.O. proof read the final manuscript.

All the authors read the final manuscript, and agreed with all contents.

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