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MORPHOLOGICAL CHARACTERIZATION OF *GARCINIA KOLA* HACKEL (BITTER KOLA) FROM SOUTHERN NIGERIA

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ABSTRACT

Garcinia kola is an important indigenous fruit tree that has a huge medicinal and economic value in Nigeria. It is often referred to as 'wonder plant' because all its parts have specific medicinal properties and have been employed traditionally for the treatment of diverse ailments. This study is aimed to determine the genetic variation that exists between and within G.kola species in selected states of Southern part of Nigeria using morphological and morphometric markers. Phenotypic traits were measured on site according to descriptors of tropical fruits by Bioversity International in the course of an exploration. The geographical positioning system (GPS) data of individual trees were also documented. Measurements were taken with the aid of a meter rule and direct observations. Data obtained were subjected to one-way analysis of variance and revealed F-value of 58.49 and a corresponding p-value >0.000. The result of the descriptive statistics also shows that diameter at breast height (DBH), crown diameters (CD-) ranged from 2-8m, while leaf related traits revealed that leaf length (LL), leaf width, average petiole length (APTL) and index of leaf length and leaf width ranged from 2.8-1, 1.66-6.66 ,0.6-1.48, 1-7.12 respectively. Also mean and standard deviation of DBH, CD, APTL, LL, LW, LL /LW are 4.63±1.44, 1.95±0.59, 1.04±0.33, 11.51±3.39, 1.85±1.77 and 2.78±0.36 respectively. The study revealed high variability between and within the different G.kola individuals sampled from Southern Nigeria.

KEY WORDS: *indigenous, morphological markers, medicinal, phenotypic, variation*

INTRODUCTION

Bitter kola (*Garcinia kola* Hackel) belongs to the genus *Garcinia* (Family: *Guttiferae*), is an indigenous medicinal tree that is often referred to as 'wonder plant' because all its parts have medicinal properties (Manourova *et al*., 2017). The genus consists of over 200 species distributed in the tropics of the world majorly in Africa, Asia and Polynesia (Parthasarathy & Nandakishore 2014). *Garcinia kola* is well branched, evergreen polygamous trees and are found in moist forests throughout West and Central Africa (Anegbeh *et al*. 2006). This fruit tree has a regular fruiting cycle and tree produces fruits every year making it one of the most important trees valued in Nigeria (Anegbeh *et al*. 2006), West and Central Africa. The seeds, leaves and bark of *G. kola* are highly medicinal with high pharmacological uses. The seeds have been reportedly used for the treatment of coughs, throat infections, bronchitis, hepatitis (inflammation of the liver), and liver disorders (Farombi *et al*., 2005; Anegbeh *et al*. 2006). *G.kola* seeds have also been found to exhibit inhibitory effects on lipid peroxidation in rat liver homogenate (Manourova *et al*., 2017). The seed's bitter stimulant is also used as snake repellent when they are placed around the compound (Antwi-Boasiako & Abubakari 2011). Other medicinal uses include: purgative, antiparasitic, antimicrobial. The seeds are used to prevent and relieve colic, cure head or chest colds.

The seeds constituents include biflavonoids, xanthones and benzophenones and other antioxidant and protective properties which could be widely exploited (Antwi-Boasiako & Abubakari 2011). The antimicrobial properties of this plant are attributed to the benzophenones, flavanones. According to Orie & Ekon, (1993) as cited by Anegbeh *et al*. (2006) this plant has shown bronchodilator effect, anti-inflammatory, antimicrobial, antibacterial and antiviral properties (Ebana *et al*., 1991; Akoachere *et al*., 2004). Anegbeh *et al* (2006) also reported that in the laboratory tests, *Garcinia kola* was found to halt the deadly disease caused by Ebola virus in its tracks. The virus causes Ebola haemorrhagic fever - an often-fatal condition. Compounds from the plant have also proved effective against some strains of flu, a contagious respiratory disease also commonly known as influenza (Iwu, 1993). Its by-products are also useful: the wood makes excellent fuel wood; its dense rounded crown makes it an ideal tree for shade around homestead; the branches are used as chewing stick because of its bitter taste and antibacterial activities of its extracts (Taiwo *et al*., 1999). The genus is the source for a natural diet ingredient hydroxycitric acid (HCA: 1,2 dihydroxypropane-1,2,3-tricaarboxylic acid) present in the fruits and leaves is an antiobesity compound which is also known as to inhibit lipid and fatty acid synthesis in living systems (Manourova *et al*., 2019). The HCA present in *Garcinia* spp is also a hypocholesterolemic agent and constitutes 20-30% in fruits on a dry weight basis (Adebayo & Oladele 2012).

According to Igwea *et al* (2007) as cited by Antwi-Boasiako & Abubakari (2011) reported that *G.kola* is ranked third among medicinal plants in Benin where it is incorporated in scores of recipes and has proved as one of the several non-timber forest products of high socio-economic importance. In addition to their anti-oxidant and protective properties, *G.kola* also inhibit tumour initiation, promotion and progression (Adebayo & Oladele 2012). It has been established that genetic diversity assessment has potential uses in breeding, conservation of genetic resources and evolution. The extent of genetic variability in accessions is pertinent to farmers, especially as it relates to selection of superior cultivars (Jamnadass *et al*., 2009; Enaberue *et al*., 2014). Genetic diversity serves as a fortitude to conservation of genetic resources, for present and future use. Enabberue *et al* (2014) asserted that genetic diversity provides plant breeders with options of developing productive crops that are resistant to virulent pests and diseases as well as crops that are well adapted to changing environments. However, the genetic resources of *G. kola* in Nigeria is under severe threat of erosion due to destruction of their habitat through wide scale deforestation and urbanization and climate change. Despite the immense benefits this species can offer, there are still very scant study of the genetic variability that exist within and between the species. Therefore, this study is aimed to determine the genetic variation that exists between and within *G. kola* species in selected states of Southern part of Nigeria.

MATERIALS AND METHODS

The Garcinia *kola* genotypes considered in this study were naturally established but selected and preserved by farmers in their cropping areas alongside other trees of economic importance. 22 bitter kola genotypes from four southern states in Nigeria were used. The geographical coordinates and area of each location were taken using a geographical positioning system (GPS) apps from a mobile phone, Nokia Lumia 1320 (Table1). The exploration was conducted in Anambara, Imo, Edo, and Osun states to have four locations during field exploration and sample collections. Fresh young leaf samples were collected and stored in silica gel for future DNA/Molecular analysis while mature leaves were collected for herbarium storage and leaf related morphometric analyses. Morphological and morphometrics data were collected on site with reference to descriptors of *Garcinia kola* by Bioversity International.

Data on quantitative and qualitative traits were collected according to descriptors of Bioversity International formerly known as IPGR. Quantitative traits were determined by measurements with a measuring tape rule, counting and weighing with a weighing balance. The traits were measured on traits related to tree, leaves and fruits. Diameter at breast height (m), trunk height (m), crown diameter (m). Qualitative traits considered were trunk color (TC), trunk texture (TT), branch pattern (BP), plant growth habit (PGH). Leaf related traits measured are leaf length (LL), leaf width (LW) petiole length (PTL) and index of leaf length and leaf width (LL/LW). Fruits related traits measured were fruit length (FL) fruit width (FW) and the index of fruit length and fruit width (FL/FW).

Data collected were subjected to analysis of variance for within and among the geopolitical region of Nigeria. Descriptive statistics (mean, ranges and standard deviation) was carried out to summarize the data. The data was also subjected to statistical software Paste version 2.0 for further analysis and generate a similarity matrix (dendrogram) or hierarchical cluster analysis using Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Principal component analysis (PCA) was also performed to determine the pattern of variation among the genotypes and relative importance of the observed variables in the description of the observed variance of trees sampled.

TABLE 1: Location and geographical coordinates of *Garcinia kola* in Southern Nigeria

Collection site/location	Latitude	Longitude	Altitude (m)	Number of trees sampled	Zone
Anambara state	6º0.435´E	6º56.536´E	121	22	Southeast
Cross river state	5º48.260´E 5º48.417´E	8º4.703´E 8º4.883´E	87.0 99.0	2	South-south
Edo state	7º56.514´E	7º56.514´E		1	South-south
Osun state	7º56.514´E	4º16.259´E	409.0	2	Southwest

RESULTS AND DISCUSSIONS

Based on the quantitative morphological data obtained from the study, tree related traits revealed that diameter at breast height (DBH), crown diameters (CD-) ranged from 2-8m, while leaf related traits revealed that leaf length (LL), leaf width, average petiole length (APTL) and index of leaf length and leaf width ranged from 2.8-1,1.66-6.66,0.6-1.48, 1-7.12 respectively. Also mean and standard deviation of DBH, CD, APTL, LL, LW, LL /LW are 4.63±1.44, 1.95±0.59, 1.04±0.33, 11.51±3.39, 1.85±1.77 and 2.78±0.36 respectively (Table 2). The results obtained from one way analysis of variance revealed that there is high variation between and within the *G. Kola* trees tested (p>0.000). With respect to tree related traits (p>0.000) there is also significant variation in the population sampled. As indicated in table 3, F-value of 58.49 was obtained and the corresponding P-value is given as 4.366E-55.

Principal Component Analyses of Morphological Characteristics of *Garcinia kola* individuals sampled

Principal component analysis (PCA) was performed using 10 quantitative characters. Eleven components were selected for as meaningful factors with eigenvalues >1. These components explained 64% of the variation. The principal component (PC1) explained 68.2% of the total variation, (Table 3) second component (PC2) explained 17%, and the third principal component (PC3) explained 7% of variation. Also (PCs 4-11) the other principal components explained 3.7%, 2.3% 0.53%, 0.29% and 0.28% of the variation. Fig.2 shows the principal component analysis and revealed that component 2 comprises of orANA3, orANA4 and orANA5 are represented in quadrant I, while also quadrant II contained orANA14, orCRO1, orEDO1, orANA1 and orANA9 as represented in Fig3 in the cluster analysis.

TABLE 2: One-way analysis of variance for Orogbo (*Garcinia kola*) from Southern Nigeria

	Sum of sqrs	df	Mean square	F	p (same)
Between groups:	2291.24	10	229.124	58.49	4.366E-55
Within groups:	818.67	209	3.91708		
Total:		3109.91	219		

omega^2: 0.7232

TABLE 3: Pairwise comparison of the ten traits

	DBH	TG	TSE-W	TSN-S	APTL	LL	LB	LL/LB	DBH/TG
DBH		7.01E-05	0.001342	0.000558	1.19E-05	1.03E-05	0.000718	1.29E-05	0.6775
TG	6.756		0.9991	0.9999	0.9992	1.03E-05	0.9999	1.03E-05	0.07316
TSE-W	5.808	0.9475		1	0.9221	1.03E-05	1	1.03E-05	0.342
TSN-S	6.105	0.6505	0.2971		0.9712	1.03E-05	1	1.03E-05	0.227
APTL	7.691	0.9353	1.883	1.586		1.03E-05	0.9606	1.03E-05	0.008627
LL	13.9	20.66	19.71	20.01	21.6		1.03E-05	0.000292	1.03E-05
LB	6.02	0.7355	0.212	0.08502	1.671	19.93		1.03E-05	0.2571
LL/LB	7.579	14.34	13.39	13.68	15.27	6.326	13.6		1.03E-05
DBH/TG	2.556	4.2	3.252	3.549	5.135	16.46	3.464	10.14	

DBH: diameter at breast height, TG: Tree Girth, TSE-W/TSE-N: crown diameter, APTL: average petiole length LL: leaf length, LB: leaf blade, LL/LB: index of leaf length and leaf width, DBH/TG: index of diameter at breast height and tree girth

Hierarchical cluster analysis of *Garcinia kola*

From the cluster analysis (fig3) it was observed that at the similarity coefficient status of 11.5 two major groups (I and II) were formed. Cluster I contained two sub clusters which comprises of orCR02 forming a single cluster at 6.4 coefficient status (Euclidean distance). Further analysis of this cluster indicated that orANA14 and orCR02 were grouped together while orANA15 formed a single cluster. Cluster II formed two major sub clusters which grouped orANA3 and orANA4 together as a sub cluster. At a similarity distance of 6.8, sub clusters were also formed grouping orANA5as a lone cluster while orANA2, orANA7, orANA8, orANA6, orANA12, orANA13 where all grouped. The cluster analysis revealed great diversity exist within and among the *G. kola* trees sampled with respect to the quantitative traits considered.

This study reveals the level of morphological diversity of *G. kola* in Southern Nigeria. Obtaining this information using morphological parameters is one of the first step in evaluation of genetic diversity which is an important step in conservation and management of genetic resources for breeding and selection of superior cultivars. According to Jamnadass *et al* (2009), phenotypic traits are pertinent to farmers as it assists the farmers in their selection processes for superior cultivars. *Garcinia kola* still remains largely undomesticated in Southern Nigeria, sampling and collection of leaves and fruits were stifled by the massive size of the trees, coupled with some sociocultural practices that prevent access to these trees. These results indicate the expression of high genetic variability in the *G. kola* tress tested. This study is in congruence with the study conducted by Manourova *et al* (2017) with respect to tree and fruit related traits. However, studies of this sort remained scant especially in Nigeria and Africa. The within-population variation observed in this study could be attributed to cross pollination exhibited by most tropical fruit trees species. Furthermore, the variability observed both within and between populations of *G. kola* tress tested for most characters suggests that there is unique genetic diversity inherent in each of the populations. The amount and pattern of genetic variation established is to determine its adaptability. Consequently the variability observed in this study is essential parameters of a long term stability of the ecosystem for which the trees are part. Majority of *G. kola* in the southwestern part still retain their gigantic form with little domestication, therefore preventing sampling and collections. Some social-cultural practices and believes of the people of the region still plays a major role in the domestication of the species. In the southeastern part also, there is a widespread belief that the grower of the tree crop will not enjoy the yield from it due to its very long duration of fruiting and maturity. For this reason interest in its domestication and propagation is reduced. Breeding programs that is directed to dwarf, early maturing trees and high yield should be encouraged, using appropriate molecular markers.

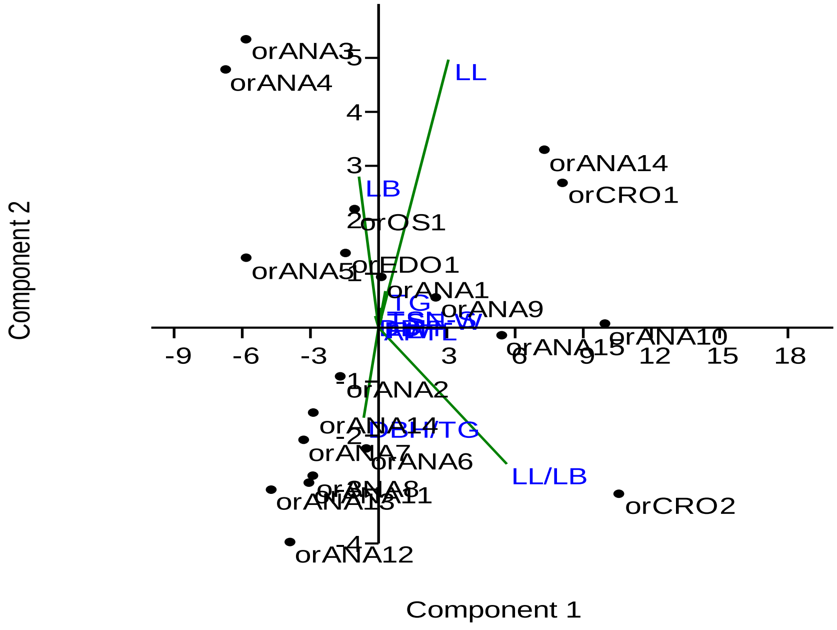

FIG 2: Bi plot graph of 20 *Garcinia kola* genotypes from Southern Nigeria based on10 quantitative characters

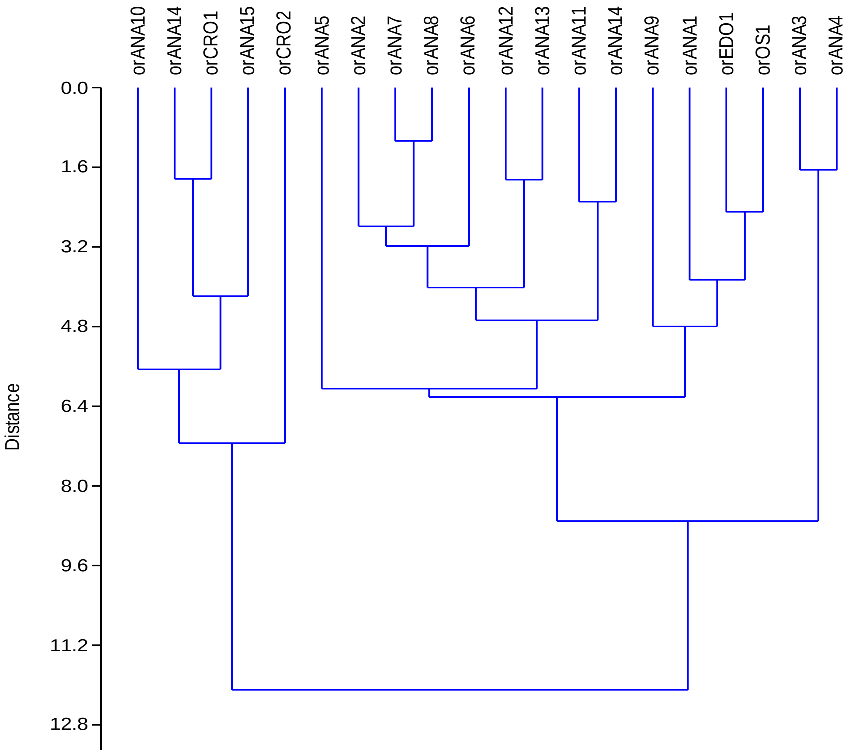

FIG 3: Hierarchical cluster analysis of quantitative traits of *G.kola* from Southern Nigeria

TABLE 4: Eigenvalue and percentage variance of Orogbo (*Garcinia kola*) from Southern Nigeria

PC	Eigenvalue%	variance
1	29.4187	68.276
2	7.46757	17.331
3	3.04729	7.0723
4	1.59894	3.7109
5	1.01582	2.3575
6	0.232236	0.53898
7	0.126103	0.29266
8	0.120667	0.28005
9	0.0291982	0.067764
10	0.0222177	0.051564
11	0.00919003	0.021329

CONCLUSIONS

This diversity observed should be targeted for conservation and further breeding programs for improvement. The grouping of the dendogram as well as the (biplot) principal component analysis demonstrated the depth of genetic diversity inherent in the *G. kola* genotypes tested. There is high genetic variability within and among the *G. kola* genotypes, an implication of a pool of resources for subsequent breeding and conservation programme. Further, the study reveals the potential genetic base that can be used for improvement of production of *G. kola* in Nigeria. However, further molecular approaches are needed to be employed to ascertain the diversity observed in this study.

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