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GENETIC VARIABILITY AND INTERRELATIONSHIP AMONG MORPHOLOGICAL TRAITS IN AFRICAN PEAR FRUIT (*DACRYODES EDULIS* (G.DON) H.J. LAM) ACCESSIONS

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ABSTRACT

African pear fruit (*Dacryodes edulis*) is a highly sought for multipurpose agroforestry tree species that has the potential for both local and international trades, Nigeria is among the leading producer of the fruit, but its pattern and extent of its genetic variability is currently unknown. Hence, a study was carried out to determine the pattern and extent of genetic variability and interrelationships among 13 morphological traits of African pear fruit accessions. The experiment was laid in a Completely Randomized Design in 5 replications. Data collected on number of leaf production, leaf length, leaf breadth, leaf length/breadth ratio, internode distances, plant height, collar diameter, fresh leaf weight, fresh stem weight, fresh root weight, dry leaf weight, dry stem weight, dry root weight and biomass accumulation were subjected to Analysis of Variance. Treatment means were separated using Duncan's Multiple Range Test at 5% probability level. Results showed significant ($p<0.05$) differences among the *D. edulis* genotypes for most of the traits evaluated indicated variation in the performance of the genotypes and pattern of clustering indicated that there was no association between eco-geographical distribution of genotypes and genetic diversity. PCA revealed that fourteen axes accounted for 100% of the variations observed while the first five axes contributed 73.23% of the total variation observed. The first axis accounted for 25.61% of the total variation while the second and third accounted for 15.43 and 15.01%, respectively. The fourth axis contributed 9.82% of the total variation while the fifth axis contributed 7.36% of the total variation. Significant phenotypic and genotypic correlations were observed between most of the plant characters evaluated and biomass accumulation which is an indication that the phenotypic association will be a good index for the genotypic association.

Keyword: *Accessions, Genotypic correlation, genetic variation, Principal Component Analysis, Phenotypic correlation, Dacryodes eduli,s*

INTRODUCTION

Pear fruit (*Dacryodes edulis* (G. Don) H.J. Lam), is an agroforestry plant species belonging to the Burseraceae family. It is one of the most important edible fruit trees indigenous to the Gulf of Guinea and Central African regions. The species of edulis are perennial plants growing to 40m tall. Phenotypic variations among the species are enormous (Waruhiu *et al.*, 2004). The flowers are yellow and are arranged in large inflorescence. The fruit is an ellipsoidal drupe

which varies in length from 4 to 12 cm. The skin colour of the mature fruit is dark blue or violet while the immature colour is pink (Anegbe *et al.*, 2005, Ajibesin, 2011).

The species is not only endowed with enormous morphological variations but also the plants are adaptated to a wide range of climate and soil types that are widely distributed in Africa (Kengue *et. al.* 2002, Onana, 2008; Omonhinmin, 2012). However, due to human activities, it current geographical distribution extends beyond its area of origin to as far as tropical Asia (Kengue, 2002).

In the last few years, agroforestry plant species have been fully recognized to contribute to both biodiversity conservation and livelihood development (Ndoye *et al*., 1998; Leakey, 2010) and this has led to greater interest in understanding the genetic variability of trees within the ecosystem (Munjuga *et al*., 2008). The success of any breeding programme depends largely on the available genetic variability in the base population. An understanding of genetic variability of different characters will help in identifying useful character (s) and rapid gain in genetic advance.

Sustainable production in tree species will depend on the extent of genetic diversity since great loss of characteristics in any population may limit its chances of survival and require greater human efforts for successful production (Trethowan and Kazi, 2008).

Inspite of the immense importance of the plant, not much work has been done on its genetic improvement in Nigeria leading to lack of information on many aspects. Reports on variability and association among different traits in *Dacryodes edulis* are few, detailed experiment on morphological and qualitative variability, and are absent. Breeding a crop for new and targeted environments requires the use of a range of cultivars/genotypes since it allows us to quantify intraspecific variability for different traits and their interactions.

This study was conducted with the following objectives:

i. To determine genetic variability among the seedling growth characteristic of *Dacryodes edulis* accession.

ii. To determine the extent of association among the seedling growth characteristics.

iii. To determine the level of relatedness among the 30 accessions using cluster analysis

MATERIALS AND METHODS

Source of planting materials, experimental set-up and plant management

Seeds of thirty accessions of *Dacryodes edulis* were collected from Edo, Delta, Ondo and Oyo States of Nigeria (Table 1). In each state, samples were collected from trees at a distance exceeding 20m from each other to avoid collecting multiple seeds from the same parents. From each parent, seeds were collected, placed in labeled plastic bags and kept for use.

Seeds of the 30 accessions were sown in a 17cm deep by 20 cm diameter depth propagating pots that were arranged in a completely randomized design (CRD) with five replications. The soil was well drained, with an average P^H value close to 7.0. The cultural operations carried out were manual weeding and well necessary adequate watering to maintain soil moisture.

Experimental site

The experiment was conducted at Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria. Nigeria lies between 4-14^{0}N and 3-15^{0}E in the Southern edge of West Africa. The country is characterized by two main seasons: dry and wet seasons. The wet season commences from April till November, whereby, precipitation is usually distributed in a distinct bimodal pattern and with high proportion in July and September with a short dry spell in August. The dry season is from December to April. The dry spell is accompanied by a cold wind from the Sahara desert, known as harmattan. The climate is classified into: Tropical rainforest eco-climate, tropical savannah eco-climate and highland climate. Oyo state is suitated on 7^o 16'N; 3^o 47'E; 255m above sea level.

Data collection

Data were collected on five plants per replicate making a total of 25 plants per accessions and the following parameters were assesses.

Data analyses

Data were analyzed using the procedure Statistical Analysis System (SAS 2000). Means were compared using Duncan Multiple Range Test at 5% level of probability ($P \leq 0.05$).

Phenotypic correlation and genotypic correlation coefficient were computed to determine the extent of association among the seedling growth characteristics.

Principal component analysis (PCA) was also performed to determine the pattern of variability in seedling characteristics among the thirty *Dacryodes edulis* accessions. It was performed using correlation matrix to identify pattern of multi trait variations in the accessions. This was used to evaluate the eigenvector for each principal components axis, solutions were accepted when Eigen values were greater than one (Hajjar *et al*., 2008). The character loading was used to determine the contribution of the characters to the total variations observed in the *D. edulis* genotypes and to determine the cultivar components scores which were used to construct the PCA bi-plots. Factor loading equal to or greater than 0.3 were considered to be defining part of a principal component (Hajjar *et al*., 2008). Biplot display of PCA was used to visualize the variation pattern. Lastly, the accessions were subjected to hierarchical clustering using squared Euclidean method of Ward (1963), which classified them into homogenous groups.

Table 1: List of 30 Pear fruit accessions used in the study and sources of collection

Genotypes	Accessions Codes	Source	Latitude	Longitude
G1	FOR1	Jericho, Oyo State	3.86	7.67
G2	FOR2	Agbofieti, Oyo State	3.86	7.67
G3	FOR3	Ofosu, Ondo State	5.14	6.75
G4	FOR4	Ore, Ondo State	4.88	6.75
G5	FOR5	Akoko, Ondo State	5.42	7.36
G6	FOR6	Okpanam,Delta state	6.24	6.65
G7	FOR7	Ibusa, Delta State	5.78	6.55
G8	FOR8	Asaba, Delta State	6.20	6.73
G9	FOR9	Umunede, Delta state	6.27	6.31
G10	FOR10	Iseleukwu, Delta State	6.22	6.48
G11	FOR11	Okogbo, Edo State	5.88	6.20
G12	FOR12	Iguere, Edo State	6.24	6.66
G13	FOR13	Iduonmiwina, Edo State	5.90	6.18
G14	FOR14	Igbekhue, Edo State	5.90	6.15
G15	FOR15	ObozogbeniroEdo State	5.30	6.15
G16	FOR16	Ugbokoniro, Edostate	5.96	6.14
G17	FOR17	Ubokonumagba, Edo State	6.56	6.58
G18	FOR18	Ugbougo, Edo State	6.00	6.13
G19	FOR19	Evbousa, Edo State	5.62	6.15
G20	FOR20	Aideyanoba Edo State	5.92	6.11
G21	FOR21	Idu, Edo state	6.22	6.81
G22	FOR22	Iguemokhia, Edo State	5.83	6.14
G23	FOR23	Ugo Edo State	6.00	6.09
G24	FOR24	Evbousa, Edo State	5.62	6.15
G25	FOR25	Avbugo, Edo State	5.82	6.20
G26	FOR26	Evbowe, Edo State	6.06	6.14
G27	FOR27	Ona, Edo State	5.64	6.20
G28	FOR28	Ekobi, Edo State	6.46	6.63
G29	FOR29	Urhonigbe, Edo State	6.51	6.58
G30	FOR30	Sakpoba,Edo State	5.64	6.20

Number of leaves per plant
Plant height (cm)
Collar diameter (mm)
Leaf length (cm)
Leaf breadth (cm)
Leaf length/ breadth ratio
Fresh weights (g)
Dry weights (g)
Biomass accumulation (g)

RESULTS

The analysis of variance revealed significant effect for all the traits evaluated except plant height (Table 2). Significant differences observed indicated variation in the performance of the genotypes.

Extent of association in 30 accessions of Dacryodes edulis

A significant positive genotypic correlation were observed between number of leaves and fresh leaf weight (0.89),dry leaf weight (0.99) and biomass(0.53) (Table 3). Leaf length had a significant but positive significant with leaf breadth (0.95), Leaf Breadth Ratio (0.78), internode distance (0.68), Plant height (0.80), Dry stem weight (0.36) and collar diameter (1.00). A negative significant exist between Number of Leaves and Leaf Breadth (-1.00), Number of Leaves and Collar diameter (-1.00), Leaf breadth and Fresh Leaf Weight (-0.36), Leaf Breadth and Dry root weight (0.42), internode distance and Fresh root weight (-0.49) and between Fresh root weight and Plant height (-1.00).

A positive significant phenotypic correlation exists between number of leaves and fresh leaf weight (0.86), DLW (0.89), biomass (0.47) (Table 4). Leaf length had a positive significant correlation with plant height(0.63) and CD(0.76).

The first five component axes and the scores of the major characters was presented in Table 5. The first five axes contributed 73.23% of the total variation observed. The first axis accounted for 25.61% of the total variation while the second and third accounted for 15.43 and 15.01%, respectively. The fourth axis contributed 9.82% of the total variation while the fifth axis contributed 7.36% of the total variation. *Hair et al*., (1995) suggested that Eigen values greater than one are considered significant and component loading greater than 0.30 were considered to be meaningful. Among the components, number of leaves, fresh leaf weight, fresh root weight dry leaf weight, dry stem weight, dry root weight and biomass yield contributed to the variation in first principal component axis. The second principal component was dominated by number of leaves, fresh leaf weight, fresh root weight and dry root weight. The third component axis was loaded by leaf length, leaf breadth, internode distance and plant height. Leaf length and leaf length /breadth ratio contributed to the fourth axis while the fifth axis had fresh stem weight.

The scree plot showing the Eigen value and the character component of thirty *Dacryodes edulis* genotypes is presented in Figure 1. Fourteen axes accounted for all the variations (100%) observed in the traits of *Dacryodes edulis* accessions used in this study.

The Means and Standard Deviation (SD) of Morphological characters of four clusters of *Dacryodes edulis* group using Ward Linkage clustering procedure is presented in Table7 . Cluster 1 comprises of 10 genotypes. They had the highest leaf length to breadth ratio and fresh stem weight. Eleven genotypes were assigned to cluster II, this group of genotypes had the highest leaf length, leaf breadth, internode distance, plant height and collar diameter. Cluster IV consist of five genotypes. They produced the highest number of leaves, fresh leaf weight, fresh root weight, dry leaf weight, dry stem weight, dry root weight and biomass production.

Table 2: Mean square in the analysis of variance for morphological characters evaluated in 30 *Dacryodes edulis* accessions

g	DF	NOL	LL	LB	LLBR	ID	FL	FS	FR	DL	DS	DR	PH	CD	BA
Rep	4	23.79	5.02	22.39	0.14	0.81	2.21	1.29	0.67	0.56	0.44	0.09	18.18	2.45	0.34
Genotypes(G)	29	62.26**	15.49**	39.75*	0.21**	4.33**	11.47**	32.83**	5.90**	4.17**	11.91**	3.15**	62.97ns	2.59*	22.11**
Error	116	30.81	8.86	2.2	0.08	1.26	2.19	717.28	0.28	0.58	0.1	0.07	26.78	1.02	0.78

*, ** Significant differences at 0.05 and 0.01 levels of probability respectively; ns not significant
NOL= leaf production; LL= leaf length; LB= leaf breadth; LLBR= leaf length/breadth ratio; ID= internode distances; FL= fresh leaf weight; FS= fresh stem weight; FR= fresh root weight; DL= dry leaf weight; DS= dry stem weight; DR= dry root weight; PH= plant height; LXG= location by genotype; CD= collar diameter; BA= biomass accumulation,

Table 3: Genotypic correlation coefficients between plants characters evaluated among thirty accessions of *Dacryodes edulis*

	NOL	L.L	L.B	L.B/R	Int.D	FLW	FSW	FRW	DLW	DSW	DRW	PH	C.D
L.L	0.15												
L.B	-1.00**	0.95**											
L.B/R	0.31	0.78**	0.50**										
Int.D	-0.15	0.68**	0.41**	0.12									
FLW	0.89**	0.18	-0.36**	0.37*	-0.04								
FSW	0.1	0.19	0.19	0.11	0.09	-0.29							
FRW	-0.14	-0.27	-0.2	0.09	-0.49**	-0.19	0.3						
DLW	0.99**	0	-0.26	0.19	-0.13	0.95**	-0.17	0.05					
DSW	0.32	0.36*	0.02	0.32	0.11	-0.07	0.89**	0.38*	0.03				
DRW	-0.24	-0.45*	-0.42*	-0.04	-0.19	-0.18	0.17	0.89*	-0.03	0.23			
PH	0.25	0.80**	1.0**	-0.14	0.48**	0.39*	-0.1	-1.00*	0.41*	-0.17	0.70**		
C.D	-1.00**	1.00**	1.00**	1.00**	1.00**	1.00**	1.00**	1.00**	1.00**	1.00**	1.00**	1.00**	1.00
Biomass	0.53**	0.1	-0.25	0.3	-0.04	0.24	0.67**	0.61**	0.40*	0.85**	0.54**	-0.24	**

*, ** Significant differences at 0.05 and 0.01 levels of probability respectively

NOL = Number of leaves; L.L = leaf length; L.B. = leaf breadth; L.B/R= leaf breadth ratio; Int.D = internode distance; FLW = fresh leaves weight; FSW = fresh stem weight; FRW= fresh stem weight; FRW= fresh root weight; DLW = dry leaves weight; DSW = dry stem weight; DRW = dry root weight; PH = plant height; C.D = collar diameter; Biomass = biomass production.

Table 4: Phenotypic correlation coefficients between plants characters evaluated among thirty Accessions of *Dacryodes edulis*

	NOL	L.L	L.B	L.B/R	Int.D	FLW	FSW	FRW	DLW	DSW	DRW	PH	C.D
L.L	-0.02												
L.B	-0.21	0.44											
L.B/R	0.18	0.35	-0.33										
Int.D	-0.13	0.51	0.25	0.04									
FLW	0.86**	0.04	-0.08	0.26	-0.06								
FSW	0.07	0.12	0.1	0.08	0.08	-0.25							
FRW	-0.11	-0.19	-0.13	0.09	-0.35	-0.17	0.28						
DLW	0.89**	-0.06	-0.01	0.15	-0.12	0.94**	-0.14	-0.04					
DSW	0.2	0.24	0	0.23	0.09	-0.07	0.89**	0.36	0.02				
DRW	-0.16	-0.29	-0.24	-0.02	-0.13	-0.15	0.16	0.85	-0.03	0.23			
PH	0.12	0.63**	0.46**	-0.11	0.46**	0.19	-0.06	0.64**	0.19	-0.09	-0.38		
C.D	-0.04	0.76**	0.46**	0.42*	0.75**	0.04	0.16	-0.31	0.07	0.15	-0.13	0.94**	
Biomass	0.47**	0.04	-0.09	0.22	-0.03	0.3	0.65**	0.57**	0.44*	0.83**	0.53**	-0.13	-0.01

*, ** Significant differences at 0.05 and 0.01 levels of probability respectively

NOL = Number of leaves; L.L = leaf length; L.B. = leaf breadth; L.B/R= leaf breadth ratio; Int.D = internode distance; FLW = fresh leaves weight; FSW = fresh stemweight; FRW= fresh stem weight; FRW= fresh root weight; DLW = dry leaves weight; DSW = dry stem weight; DRW = dry root weight; PH = plant height; C.D = collar diameter; Biomass = biomass production

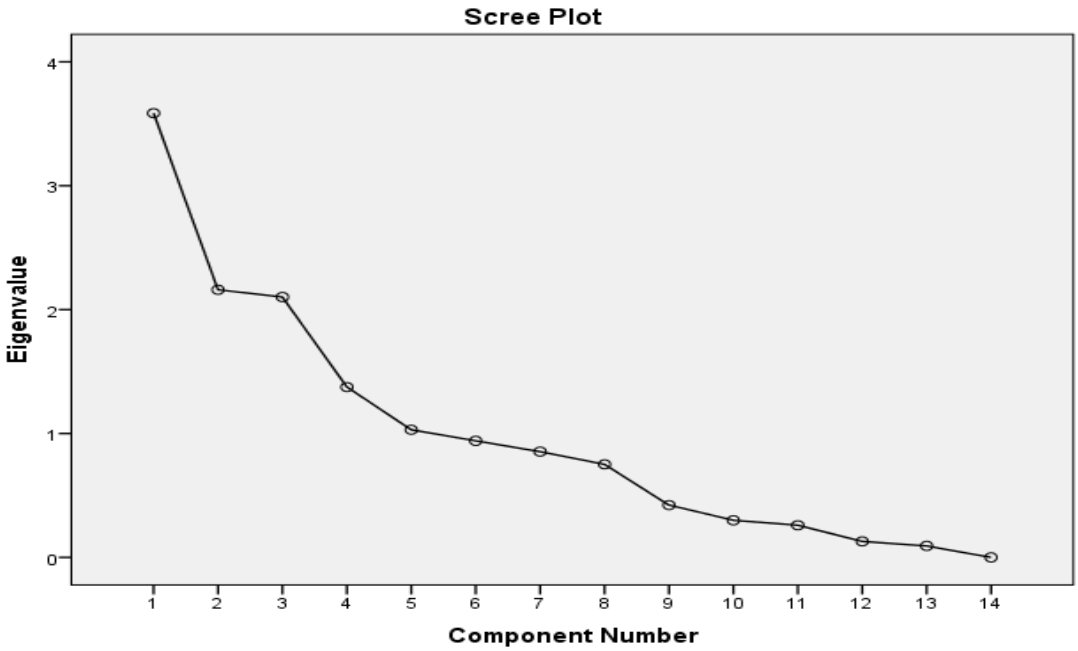

Figure 1: The scree plot showing the Eigen value and the character component of 30 *Dacryodes edulis* accessions

Table 5: The first five component axes and the scores of the major characters

Characters	PC1	PC2	PC3	PC4	PC5
Number of leaves	0.34	-0.43	0.08	-0.15	0.03
Leaf Length	0.07	0.06	0.45	0.5	-0.03
Leaf Breadth	0.06	0.08	0.42	-0.14	-0.14
Length/ Breadth Ratio	0.05	-0.14	0.02	0.77	0.06
Internode Dist	0.06	0.26	0.46	-0.03	-0.03
Fresh leaf wt	0.36	-0.44	0.08	-0.07	-0.02
Fresh stem wt	0.02	0.07	0.02	0.02	0.91
Fresh root wt	0.31	0.4	-0.19	0.16	-0.17
Dry leaf wt	0.41	-0.32	-0.01	0.02	-0.06
Dry stem wt	0.3	0.23	-0.13	0	0.26
Dry root wt	0.31	0.38	-0.24	-0.02	-0.18
Plant Height	0.14	0.19	0.44	-0.26	0.09
Collar diameter	0.17	0.1	0.26	-0.06	-0.01
Biomass accumulation	0.48	0.13	-0.16	0.01	0.07
Eigen value	3.58	2.16	2.1	1.37	1.03
% of total variation	25.61	15.43	15.1	9.82	7.36
Cumulative %	25.61	41.04	56.05	65.05	73.23

Bolded: Significant contribution to the total variation (scored above 0.3)

Table 6: Means and Standard Deviation (SD) of Major Morphological characters of four *Dacryodes edulis* group using Ward Linkage clustering procedure

	CLUSTERS			
	I	II	III	1V
Characters	**5, 10,12, 14,16, 11, 19, 20, 22, 24**	**1, 3, 7, 9, 17, 18, 23, 25,28, 29, 30**	**2, 4, 15, 27**	**6, 8, 13, 21, 26**
No of leaves	16.95±1.41	17.96± 2.70	16.47 ±2.18	**18.84 ±2.49**
Leaf length	11.25 ±1.01	**11.84 ±0.33**	9.96±0.94	10.41±1.19
Leaf breadth	5.13 ±0.63	**7.98±2.12**	5.61±0.42	5.66 ±0.61
Leaf length|breadth ratio	**2.22 ±0.01**	1.70 ±0.09	1.84 ±0.18	1.87 ±0.16
Internode distance	5.31±0.27	**6.11 ±0.56**	4.88 ±0.74	5.14±0.76
Fresh leaf weight	4.70 ±0.39	4.61±0.84	4.24 ±0.53	**5.07±0.80**
Fresh stem weight	**20.58 ±0.38**	4.19±0.60	4.30 ±1.65	7.42 ±1.50
Fresh root weight	4.05 ±0.10	3.34 ±0.90	3.83 ±0.43	**5.52 ±1.27**
Dry leaf weight	2.46±0.51	2.25 ±0.07	2.13 ±0.23	**2.86±0.39**
Dry stem weight	1.77±0.26	1.77±0.33	1.80±0.99	**4.24±1.36**
Dry root weight	1.94±0.13	1.56±0.59	1.83±0.28	**2.89 ±0.89**
Plant height	23.00±1.27	**25.45±3.00**	20.89±1.95	22.22±1.90
Collar diameter	5.12±0.06	**5.74±0.41**	4.90 ±0.42	5.30 ±0.25
Biomass	6.16 ±0.12	5.58±0.84	5.75±1.18	**9.99±1.00**

DISCUSSION

Genetic variability is an inherent character that largely influences the quality attributes and survival fitness of crops. Scarce information is found on the genetic diversity of *Dacryodes edulis* in Nigeria. Providing such information is essential for the improvement of this long gestation and multipurpose fruit tree. Significant differences observed among the Pear genotypes for most of the traits (except plant height) in this study indicated variation in the performance of the genotypes. This is in agreement with the findings of Atangana *et. a*l., 2002 and Kengue, 2002. Therefore, there is potential for selection among the genotypes.

The existence of wide morphological variations among the genotypes was supported by principal component analysis, which indicates that the overall diversity observed could be explained by the first five Eigen vectors. The major contributing characters were fairly distributed among leaf production, leaf length, fresh leaf weight, fresh root weight, and dry root weight. Characters such as leaf breadth, leaf length/breadth ratio,

fresh stem weight, dry leaves weight, dry stem weight and plant height were also important to the variations observed. The major role of morphological traits in phenotypic variation is consistent with the works on *Irvingia gabonensis* (Atangana *et al*., 2002) and *D. edulis* (Waruhiu *et al*., 2004).

Cluster analysis was employed to observe the genetic relationship among the thirty *D. edulis* genotypes. Knowledge of genetic similarity between genotypes is useful in any breeding program because it facilitates efficient sampling and utilization of germplasm resources. The breeder can use genetic similarity information to make informed decisions regarding the choice of genotypes to cross for the development of segregating populations or to facilitate the identification of diverse parents to cross in hybrid combinations in order to maximize the expression of heterosis (Hemeida *et al*., 1998).

Significant phenotypic and genotypic correlation coefficients observed between most of the plant characters evaluated in the study and biomass accumulation indicated that the phenotypic association will be a good index for the genotypic association. Also, the differences in magnitude and direction as well as a greater number of significant character relationships for genotypic correlations relative to phenotypic correlations further confirm the influence of environmental indices on genetic (traits) expression. It is known that phenotypic character expression incorporates both genotypic and environmental effects. Therefore the non-significant phenotypic correlation between any two characters, relative to its significant genotypic counterpart, is indicative of appreciable environmental effects. Generally, however, genotypic correlation between biomass accumulation and some of the characters (collar diameter and number of leaves) indicated their importance in tree breeding.

This pattern of clustering indicated that there was no association between eco-geographical distribution of genotypes and genetic diversity as genotypes selected under diverse locations, got clustered together. This kind of genetic diversity might be due to differential adoption, selection criteria, selection pressure and environment (Vivekananda and Subramanian, 1993). This indicates that genetic drift produce greater diversity than the geographic diversity (Selvakumar *et al*., 1989; Singh *et al*., 1996).This also is in agreement with the study of Josephine *et al*., 2015, who reports that the genetic diversity detected in *Dacryodes edulis* was intra-population. The use of cluster analysis and Principal component analysis (PCA) also revealed close genetic relationships among the populations. Consequently, these results clearly illustrated the contribution of gene flow to genetic differences among populations. In low input farming systems, gene flow can be considered a function of pollen flow and seed exchange, which, in different ways, are influenced by natural and human selection pressures. *D. edulis* is a dioecious species (Iseri and Temple, 2000; Kengue, 2002) but there are individuals who carry both female and male flowers, and individuals who bear hermaphrodite flowers and this therefore favors self-pollination. *D. edulis* fruits and seedlings are also usually exchanged between relatives, neighbors, communities and/or villages facilitating seed dispersal (Tabuna, 1999; Degrande *et al*., 2013; Takoutsing *et al*., 2013).

CONCLUSIONS AND RECOMMENDATION

The result from this study clearly indicates that there existed genetic variability among the thirty accessions of *Dacryodes edulis* genotypes used in this study as observed by wide range of variations among the characters evaluated. The overall diversity observed were explained by the first five eigen vectors which accounted for percentage of the total variations..

The cluster analysis classified the accessions into four distinct clusters. There were similarity among FOR2, FOR4, FOR15 and FOR27 which were different from FOR15, FOR 1, FOR 14, FOR 20, FOR 28 and FOR 22 used in the study and this offers availability for genetic diversity.

Significant phenotypic and genotypic correlation coefficients observed between most of the plant characters evaluated in the study and biomass accumulation indicated that these characters would be good index for the genotypic association.

This pattern of clustering indicates that there was no association between eco-geographical distribution of genotypes and genetic diversity as genotypes selected under diverse locations, got clustered together.

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