Congruence Between Morphological and Molecular Characterization of Nigerian Coconut Cultivars.

[1*]Ahanon, M. J., [2]Emede, T. O., [2]Nwaoguala, C.N.C., [1]Okoye, M. N., [1]Odufale, O. O., [1]Ovie, S., [1]Imasuen, J. A., [1]Okereke, A. C. and [1]Adejoro, S. O.

[1]Nigerian Institute for Oil Palm Research (NIFOR), Benin City, Edo State

[2] Dept. of Crop Science, Faculty of Agriculture, University of Benin, Benin City, Edo State

*Corresponding author's phone number and e-mails: 08067801050; ahanonmonday@yahoo.com,

Abstract

This study evaluated the congruence of grouping patterns from morphological and molecular data to assess their effectiveness in revealing genetic relationships among Nigerian coconut cultivars. Accurate grouping is crucial for plant breeders to make informed decisions on parent selection and breeding strategies. We conducted a morphological analysis on 12 cultivars using 30 fruit traits, and a molecular analysis on a subset of 8 cultivars using SSR markers. Morphological data were analyzed using hierarchical cluster analysis (SPSS), while molecular data were analyzed with UPGMA (MEGA6 software); the results from both were visualized in dendrograms. The analyses revealed a strong congruence between the two methods' clustering patterns. For example, both methods consistently grouped the 'MYD' and 'Edo Tall' cultivars, as well as the 'MYD' and 'CGD' cultivars. These findings suggest that integrating both morphological and molecular techniques can provide higher precision for parent selection, thus improving the efficiency of breeding programmes..

***Keywords**: Coconut cultivars, Genetic relationships, Morphological characterization, Molecular markers, SSR markers, Cluster analysis,*

INTRODUCTION

Coconut (*Cocos nucifera* L.) is a perennial tropical plant species of the *Arecaceae* family, one of the most important in the Monocotyledoneae class. It is a diploid with 32 chromosomes (2n = 32). It is a perennial tree with adventitious roots. The coconut species includes two main varieties, tall and dwarf. A cross between the dwarf and the tall coconut produces the hybrid coconut.

The coconut palm sustains the livelihood of millions of people in coastal regions of the tropics and subtropics (Ohler, 1999; Fontes *et al*., 2003). Philippines, Indonesia, India, Sri Lanka, Thailand, Tanzania, Brazil and Malaysia are the main coconut producing countries, in decreasing order of importance (FAO, 2005).

Morphological traits continue to be the first step in the studies of genetic relationships in most breeding programmes (Cox and Murphy, 1990; Van Beuningen and Busch, 1997). Zizumbo-Villarreal and Pinero (1998) used morphological traits to study the pattern of variation in Mexican coconut gene pool.

Molecular markers are nucleotide sequences and can be investigated through the polymorphism present between the nucleotide sequences of different individuals. Insertion, deletion, point mutations, duplication and translocation are the basis of these polymorphisms. Molecular markers concern the DNA molecule itself and, as such, are considered to be more reliable. The use of molecular markers as tool for selection of cultivars will provide greater precision, reduce number of breeding cycles, speed up crop improvement and make potential planting materials available to farmers in a short period of time.

Cluster analysis is a valuable tool in plant breeding offering several benefits: genetic diversity assessment, grouping similar genotypes, parent selection, population structure analysis, marker-trait association, breeding programme optimization, germplasm management. Cluster analysis, enhances plant breeding efficiency, accuracy, effectiveness, ultimately contributing to crop improvement and food security.

Therefore, the objective of this study was to compare the grouping pattern of morphological and molecular data cluster analysis in indigenous and exotic coconut cultivars selected from NIFOR coconut gene pools.

MATERIALS AND METHODS

Location of Experiment

The experiment was conducted at the Nigerian Institute for Oil Palm Research (NIFOR), Benin City, Edo State, which lies between Latitude 6°60'N and 6°66'N and Longitude 5°41'E and 5°87'E and altitude 149m above sea level. The area also lies within the humid rain forest agro-ecological zones of Nigeria.

Coconut Cultivars Evaluated and Experimental Design

Morphological study was carried out on fruit components of 48 coconut genotypes from twelve coconut cultivars in Table 1. Four genotypes (palms) per cultivar were randomly selected and used for this study. Four nuts per palm were collected as samples for two years and used for morphological studies.

Data Collection for Morphological Studies

Data collection was carried out following the method described by Ohler (1999). The fruits selected were

weighed and measured using the following materials and apparatus: digital weighing balance, digital vernier callipper, measuring tape, measuring glass cylinder, and hand refractometer. The descriptors specified by International plant genetic resources institute in manual on standardized research techniques in coconut breeding (IPGRI, 1995) were used. Thirty (30) morphological characteristics for 48 coconut genotypes were evaluated: weight of the fruit, weight of the husk (mesocarp), weight of the nut or kernel, weight of split nut, weight of the shell, weight of the seed, weight of the water or liquid endosperm, weight of the meat or solid endosperm, length of fruit, longitudinal and transverse circumference of the fruit, length of nut, longitudinal and transverse circumference of the nut, thickness of the husk or mesocarp, shell thickness, meat thickness, volume of water, sugar content of water, percentage of mesocarp in fruit, percentage of shell in fruit, percentage of weight of water in fruit, percentage of meat in fruit, percentage of volume of water in nut, percentage of nut in fruit, percentage of shell in nut, percentage of weight of water in nut, percentage of meat in nut, percentage of seed in nut and percentage of seed in fruit.

Morphological Data Analysis

Cluster analysis was used to determine natural groups from the cultivars studied Diversity among the 48 genotypes for 30 characters was classified by using cluster analysis. The output of cluster analysis was visualized using dendrogram which showed the relationships among all the cultivars.

Molecular Sample collection

Fresh leaf samples were collected from the spear (unopened) leaf of sixteen (16) coconut genotypes at NIFOR Main Station, Benin City (Table 2). Each sample was placed in a separate labelled locked polythene bag placed in an airtight container with ice chips before it was transferred into cold storage (-80^{o}C) at the facility of Bioscience Center, International Institute of Tropical Agriculture (IITA), Ibadan for molecular analysis.

Molecular data analysis

For the purpose of amplification, 20 SSR primers (Table 3) were synthesized at Inqaba Biotec, West

Table 1: List of coconut cultivars sampled for morphological traits analysis

S/N	Cultivar Name	Variety	Origin
1	Badagry Tall (BAT)	Tall	Badagry, Nigeria
2	Edo Tall (EDO)	Tall	Edo, Nigeria
3	Kwara Tall (KWT)	Tall	Kwara, Nigeria
4	Eastern Tall (EST)	Tall	Eastern Nigeria
5	Malayan Green Dwarf (MGD)	Dwarf	Malaysia
6	Malayan Orange Dwarf (MOD)	Dwarf	Malaysia
7	Malayan Yellow Dwarf (MYD)	Dwarf	Malaysia
8	Chowghat Green Dwarf (CGD)	Dwarf	India
9	MGD x WAT (HYB)	Hybrid	Badagry, Nigeria
10	MGD x WAT (GDT)	Hybrid	Edo, Nigeria
11	MYD x WAT (YDT)	Hybrid	Edo, Nigeria
12	Exotic Tall (EXO)	Tall	Cote d'Ivoire

WAT = West African Tall

Table 2: List of coconut cultivars sampled for molecular (DNA) analysis

S/N	CULTIVAR NAME	ORIGIN	NUMBER OF PALMS SAMPLED	SAMPLE NUMBER
1	Edo Tall (EDO)	Nigeria	2	1 and 2
2	Malayan Green Dwarf (MGD)	Malaysia	2	3 and 4
3	Malayan Orange Dwarf (MOD)	Malaysia	2	5 and 6
4	Malayan Yellow Dwarf (MYD)	Malaysia	2	7 and 8
5	Chowghat Green Dwarf (CGD)	India	2	9 and 10
6	Malayan Green Dwarf x WAT (GDT)	Nigeria	2	11 and 12
7	Malayan Yellow Dwarf x WAT (YDT)	Nigeria	2	13 and 14
8	Malayan Yellow Dwarf x VTT (YDV)	Ghana	2	15 and 16
	Total		16	

WAT = West Africa Tall, VTT = Vanuatu Tall

Africa Ltd, Africa Genomics Company, South Africa. SSR primers used were sourced from Kamaral *et al*. (2014) and Yong *et al.* (2013). The molecular analysis was carried out at the facility of Bioscience Center, International Institute of Tropical Agriculture (IITA), Ibadan.

Table 3: Properties of SSR primer sequences synthesized for genotyping of coconut cultivars

S/no	Primers	Sequences	GC content (%)	Melting Temperature (Tm)
1	CAC 06	F: TGTACATGTTTTTTGCCCAA	35	45.63
		R: CGATGTAGCTACCTTCCCC	57	53.25
2	CAC 08	F:TGTACATGTTTTTTGCCCAA	45	49.73
		R:CGATGTAGCTACCTTCCCC	45	49.73
3	CAC 23	F:TGAAAACAAAAGATAGATGTCAG	30	48.14
		R:GAAGATGCTTTGATATGGAAC	38	48.50
4	CAC 68	F:AATTATTTTCTTGTTACATGCATC	25	47.13
		R:AACAGCCTCTAGCAATCATAG	42	50.45
5	CNZ51	F:CTTTAGGGAAAAAGGACTGAG	42	50.45
		R:ATCCATGAGCTGAGCTTGAAC	47	52.40
6	WCYZ2470	F:GGGAATAACAAAGCAGCAAAAC	40	51.11
		R:CCCCTACTAATGTCCACCAGAA	50	54.84
7	WCYZ9240	F:GGGAATAACAAAGCAGCAAAAC	42	50.45
		R: GAGAGAGTCGTCAGGAAGAGGA	54	56.70
8	WCYZ8097	F:GAGGAGAGAGAGAAAGGAAGGG	54	56.70
		R: CATGGTTGATGAGAAACACCTC	45	52.97
9	WCYZ3075	F: GGAATGGGACTTGGAATTGATA	40	51.11
		R: TGAACTTACAACAATGAGCGGA	40	51.11
10	WCYZ8231	F: GTGCCAGGTCATGTGAGGATA	52	54.36
		R: TCGGTATTGTGCTTGGTTGATA	40	51.11
11	WCYZ2928	F: TCCTCACATTCCTCTTCGTCTT	45	52.97
		R: ATTCCTCCAGCGATGTACTTGA	45	52.97
12	WCYZ2116	F: GAAGAAAGATGAGAATGGCTCG	45	52.97
		R: GTGGGGAGATTACGAGTACGAC	54	56.70
13	WCYZ5080	F: CTGAGGAGACCGCCTACAAG	60	55.88
		R: GGTGATGATTGCGATGGTAGT	47	52.40
14	WCYZ4825	F: TTCTTTTGCTATCGAGTACCGC	45	52.97
		R: AGCTAGGTGAGGAAGTTGGTGA	50	54.84
15	WCYZ8483	F: AGGCAGACAGGTTTATCCAGAA	45	52.97
		R: CATTGTCACCTCCTCCTCCTC	57	56.31
16	WCYZ8277	F: GCTCCAGTATCTCTGTCCCCT	57	56.31
		R: TGATGAATCGGTTGACTATCCA	40	51.11
17	WCYZ6681	F: AAAGAAAGAAAGACGCATGGAC	40	51.11
		R: GGCTCGTATTAGGGTTAGGGTT	50	54.84
18	WCYZ8374	F: ACGTCAACCAGAGTTTATTGGC	50	54.84
		R: ATGGTAATGGTAATGGCAAAGG	40	51.11
19	WCYZ1753	F: AATAACTAGCGGCAGAAGAAGC	45	52.97
		R: CTTGCAGAAAAGGGATTTGAAG	40	51.11
20	WCYZ9119	F: GAGATGAGGTAGAGGAGGAGCA	54	56.70
		R: GAGTAATAGAGGCGATGGCAAG	50	54.84

Binary data was then generated for each primer sets using 1 (presence of positive amplification at a particular band size) and 0 (absence of positive amplification at a particular band size). The generated binary data was then used to create a data matrix which was analyzed using the Powermarker V2.35 software (Liu and Muse, 2005). Genetic diversity parameters such as major allele frequency, gene diversity and polymorphic information content were then generated using the Power-Marker software. The genetic relationship among treated samples was also estimated by constructing a dendrogram through unweighed pair group method with arithmetic means [UPGMA] using the mega6 software (Tamura, 2013).

RESULTS

Cluster analysis based on morphological traits

The dendrogram resulting from cluster analysis is shown in Figure 1. The cluster analysis classified the cultivars into four distinct groups. Cluster I included cultivars from Cote d'Ivoire (EXO-1, EXO-2, EXO-3 and EXO-4), Malaysia (MYD-2 and MYD-3) and Nigeria (GDT-2, GDT-3, GDT-4, EDO-3, EDO-4 and EST-4). Cluster II had cultivars from Malaysia (MOD-1, MOD-2, MOD-3, MOD-4, MYD-1, MYD-

4, MGD-1 and MGD-3), India (CGD-1, CGD-2, CGD-3 and CGD-4) and Nigeria (GDT-1, YDT-1, YDT-2, YDT-3, YDT-4, EDO-1, KWT-1 and KWT-4). Cluster III had only 2 genotypes: MGD-2 from Malaysia and EDO-2 from Nigeria. Cluster IV consisted of cultivars from Malaysia (MGD-4) and Nigeria (HYB-1, HYB-2, HYB-3, HYB-4, EST-1, EST-2, EST-3, BAT-1, BAT-2, BAT-3, BAT-4, KWT-2 and KWT). In this analysis, it was observed that the groupings of the coconut cultivars within the different clusters were not consistent with their place of origin and variety.

Analysis based on SSR Markers

SSR Polymorphism

Seventeen out of the twenty SSR markers were polymorphic. They generated a total of 52 amplified alleles with a mean number of 3.059 alleles per locus (Table 4). The number of allele per locus varied from 2 to 5. CAC 06, CNZ51 and WCYZ8097 markers had the highest number (5) of alleles per locus respectively. The major allele frequency per locus ranged from 0.313 (CNZ51) to 0.938 (WCYZ2470) with a mean of 0.614. Mean gene diversity for all loci was 0.492 with a range of 0.117 (WCYZ2470)

Table 4: Allelic polymorphism of 17 SSR markers

Marker	NA	MAF	GD	PIC
CAC_06	5.000	0.625	0.563	0.525
CAC_08	3.000	0.813	0.320	0.294
CAC_23	3.000	0.625	0.508	0.428
CAC_68	4.000	0.688	0.484	0.443
CNZ_51	5.000	0.313	0.750	0.708
WCYZ2470	2.000	0.938	0.117	0.110
WCYZ9240	3.000	0.625	0.531	0.468
WCYZ8097	5.000	0.375	0.695	0.642
WCYZ3075	2.000	0.688	0.430	0.337
WCYZ8231	2.000	0.625	0.469	0.359
WCYZ2928	2.000	0.563	0.492	0.371
WCYZ2116	2.000	0.875	0.219	0.195
WCYZ5080	2.000	0.688	0.430	0.337
WCYZ4825	2.000	0.688	0.430	0.337
WCYZ8483	4.000	0.438	0.672	0.612
WCYZ6681	3.000	0.438	0.648	0.575
WCYZ1753	3.000	0.438	0.602	0.516
Mean	3.059	0.614	0.492	0.427

MAF = Major Allele Frequency, NA = Number of Allele,
GD = Gene Diversity, PIC = Polymorphic Information Content

– 0.750 (CNZ51). The SSR markers had mean polymorphism information content (PIC) of 0.427 with a range of 0.110 (WCYZ2470) to 0.708 (CNZ51).

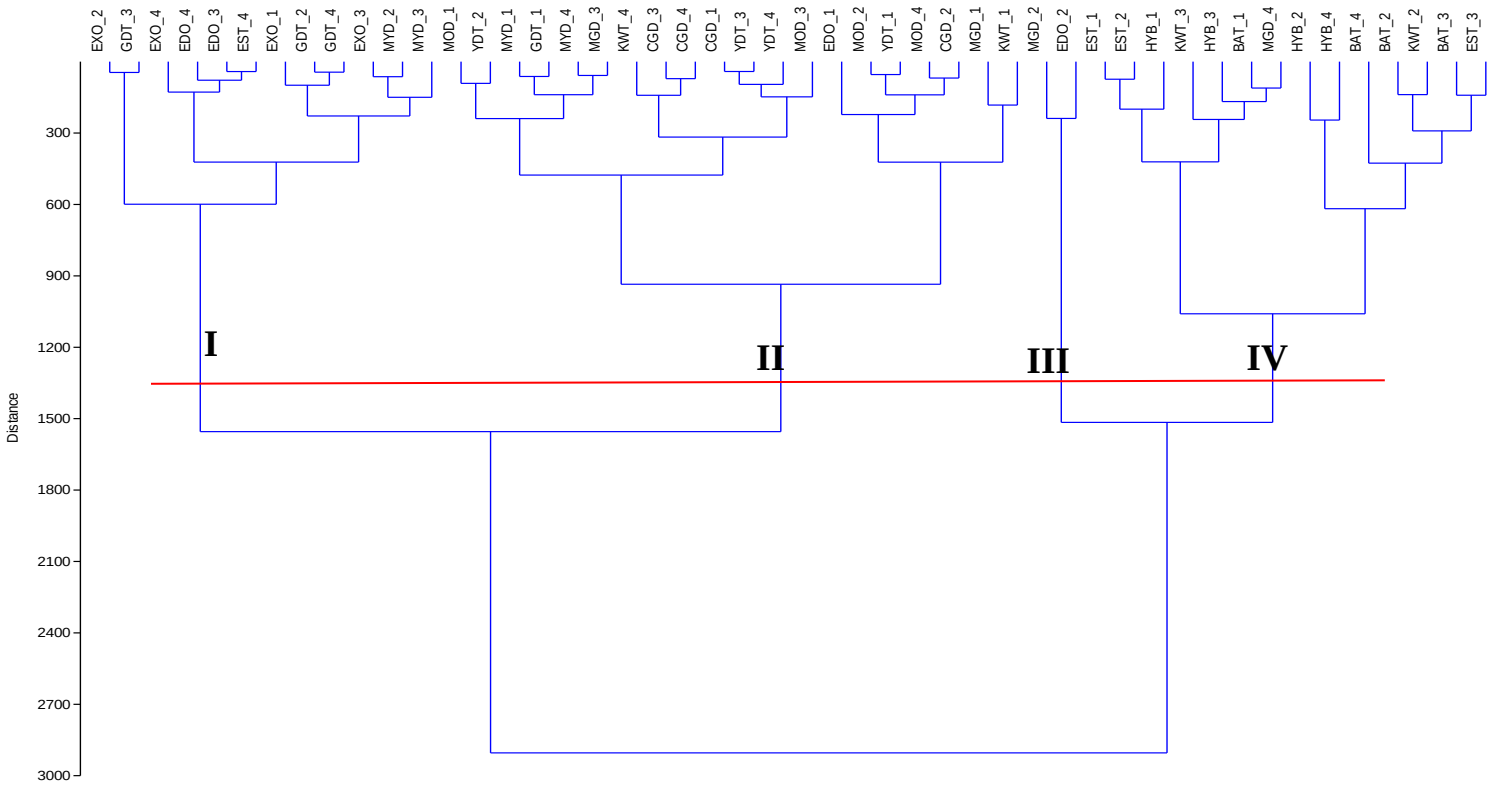
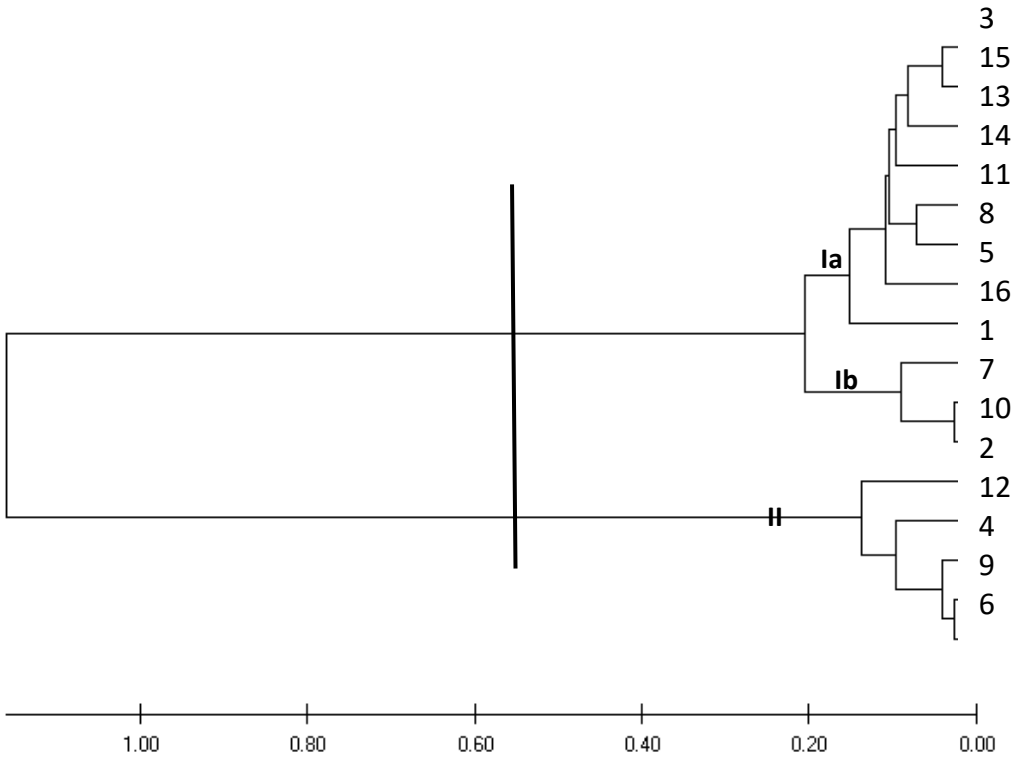


Figure 1: Dendrogram of 48 coconut genotypes using single linkage cluster analysis

Exo-1 to Exo-4 = Exotic tall; GDT-1 to GDT-4 = MGD x WAT (GDT); YDT-1 to YDT-4 = MYD x WAT; MYD-1 to MYD-4 = Malayan yellow dwarf; CGD-1 to CGD-4 = Chowghat green dwarf; MGD-1 to MGD-4 = Malayan green dwarf; MOD-1 to MOD-4 = Malayan orange dwarf; HYB-1 to HYB-4 = MGD x WAT (HYB); BAT-1 to BAT-4 = Badagry tall; EST-1 to EST-4 = Eastern tall; KWT-1 to KWT-4 = Kwara tall; EDO-1 to EDO-4 = Edo tall.

The dendrogram derived from molecular analysis, showing relationship among the individual coconut palms studied is shown in Figure 2. There were two major clusters (I and II) and the first cluster was further divided into two sub-clusters (designated Ia and Ib). Cluster Ia had cultivars from Ghana (1 MYD x VTT), Malaysia (2 Malayan orange dwarf and 2 Malayan green dwarf), India (1 Chowghat green dwarf) and Nigeria (1 MYD x WAT and 1 MGD x WAT (GDT)). Cluster Ib had cultivars from Nigeria (1 Edo tall and 1 MYD x WAT) and Malaysia (1 Malayan yellow dwarf). Cluster II had cultivars from Nigeria (1 Edo tall and 1 MGD x WAT (GDT)), Ghana (1 MYD x VTT), Malaysia (1 Malayan yellow dwarf) and India (1 Chowghat green dwarf).



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1 and 2 = Edo tall, 3 and 4 = Malayan green dwart, 5 and 6 = Malayan orange dwarf, 7 and 8 = Malayan yellow dwarf, 9 and 10 = chowghat green dwarf, 11 and 12 = MGD x WAT (GDT), 13 and 14 = MYD x WAT, 15 and 16 = MYD x VTT.

DISCUSSION

Cluster analysis used to study genetic variability in the coconut cultivars revealed a wide range of genetic diversity. The maximum number of genotypes (twenty) fell in cluster II and these were mainly dwarf cultivars. Twelve fell in cluster I, two in cluster III and fourteen in cluster IV indicating that there was genetic divergence between the genotypes tested. According to Odewale *et al*., (2012), the distinctiveness of the dwarf cultivars which made them isolated first could be due to their being characterized by very high selfing intensity. Fatokun (1985), who carried out investigation on amaranth accessions, observed that the first accession to separate had the largest cotyledon and smallest total dry matter yield. Thus, cultivars with extreme values are naturally the first to be isolated from others. However, in this study, dwarf cultivars were mainly grouped in cluster II. The clustering pattern indicated that cultivars from different locations were grouped together which can be attributed to free exchange of breeding materials from one place to another (Mahasi *et al.*,2005).

Due to the fact that SSR markers have relative superiority in detecting DNA polymorphism (Varshney *et al*., 2005), the discriminative power of each SSR marker was investigated by calculating polymorphic information content (PIC).

Microsatellite markers have been reported to be useful in assigning inbred lines into known heterotic groups, especially for those accessions without clear and accurate pedigree records (Li *et al.*, 2002). The dendrogram grouping of the cultivars from different origin in the same cluster indicated lack of correlation between origin and genetic distance among the coconut cultivars. Similar result was also observed in maize (Barata *et al.*, 2006), (Mumm and Dudley 1994), which showed that grouping of inbreds based on molecular data do not always agree with the available pedigree information. According to (Barata *et al.*, 2006), this may be due to several possible factors, in addition to the fact that DNA markers may be affected by selection, drift and mutation. Incongruity may also be as a result of clustering process and the method used (Li *et al*., 2002). This was demonstrated where in some clusters, a cultivar that was related to two other cultivars fell in one of two separate clusters (Senior *et al.*, 1998). Information about the relationship among breeding materials and the inherent genetic variation is important in making choices of parents in breeding programmes. This is

significant in hybrid breeding where detection and utilization of heterotic patterns between different sources are important for genetic improvement success (Okoye *et al*., 2016). The analyses revealed a strong congruence between the two methods' clustering patterns. For example, both methods consistently grouped the 'MYD' and 'Edo Tall' cultivars, as well as the 'MYD' and 'CGD' cultivars. Also, in cluster ll of both techniques, Tall coconut from Edo Nigeria and Dwarf coconut from Malaysia were grouped together. The grouping of tall and dwarf coconut in the same cluster by the two techniques can be influence by factors such as: (1) Genetic Markers: the type and number of genetic markers used can impact the clustering results; (2) Population Structure: the genetic diversity and the population structure of the coconut accession being studied can also influence the clustering results; (3) Breeding History: the breeding history of the coconut accessions including selection for specific traits, can affect the genetic relationships between tall and dwarf coconuts.

CONCLUSION

In comparing morphological and molecular techniques in this study, it was observed that the two techniques failed to group coconut cultivars in Nigeria based on their places of origin and variety, and there was similarity in their clustering pattern.

Therefore, the authors suggest the used of both techniques in grouping coconut cultivars for hybridization during crop improvement programmes.

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