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Assessment of Genetic Biodiversity of Nigerian Cashew (*Anacardium occidentale* L.) Accessions using Morphological and Anatomical Traits

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Abstract
The genetic diversity and relationship among 20 accessions of cashew were evaluated in order to provide useful information for selecting good yield of cashew germplasm that will produce nuts of high marketable value. The principal component analysis (PCA) revealed that morphological features such as leaf length, leaf width and petiole length; as well as anatomical attributes such as stomatal complex type, stomatal density and stomatal index accounted for most of the variation. Leaf anatomical traits portrayed higher genetic similarity (93.65%) than the morphological features (50.00%) among the cashew accessions based on the unweighted pair group method of arithmetic averages (UPGMA), which grouped the 20 accessions into two main clusters and several sub-clusters; thereby signifying polymorphism among accessions. The different sub-clusters of cashew accessions suggest that genetic variation occurred among them. The study portrayed that accessions OGD 003, KMB 004, SOB 005, OGD 004, OGD 002 and UNI 002 showed potentials for selection using the morphological traits such as leaf length, leaf breadth, petiole length and nut weight; while accessions UNI 003, UNI 004, SOB 003, OGD 003, KMB 003 and KMB 004 showed potentials for selection using the anatomical attributes such as stomatal density, stomatal index, stomatal complex types and moderate stomatal size due to their high genetic variation. This information will be beneficial for tree improvement programme in cashew nuts.

Keywords: Cashew, Accessions, Morphological traits, Anatomical traits, Genetic variation

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Introduction
Cashew (*Anacardium occidentale* L.), is an economic tree crop in the family Anacardiaceae with about 75 genera and 700 species (Nakasone and Paull, 1998). The genus *Anacardium* contains 8 species that are native to the coastal parts of north eastern Brazil, Bolivia, Peru and West Indians (Samal *et al.,* 2003). The cashew tree is a drought resistance, broad-leaf, evergreen with dense foliage that thrive well with pH of 5.5 to 6.5 (Aliyu, 2007). Cashew plant is economically important due to its industrial, medicinal and nutritional values (Akinwale, 2000; Achal, 2002; Akash *et al*., 2009; Abitogun and Borokini, 2009; Olife *et al.,* 2013). Cashew industry had played an important role in the realization of the economic development of many of the African states, and has been one of the veritable platforms for the achievement of the United Nations Millennium Development Goals (MDGs) through economic empowerment of small holder farmers and rural women, employment generation and small-medium scale industrialization especially in the rural areas (Azam-Ali and Judge, 2000). Cashew is not only one of the

export commodity crops in Nigeria but a major source of livelihood for many smallholder farmers especially in the Southern and Middle belt regions of Nigeria (Akinhanmi and Akintokun, 2008; Olife *et al.*, 2013). Numerical taxonomic techniques, especially cluster analysis and principal component analysis method had been employed by researchers to compute the degree of genetic variation in cashew accessions using morphological, biochemical and molecular markers (Mneney *et al*., 2001; Chipojola *et al*., 2009; Dasmohapatra *et al*., 2014). Cashew production could be inhibited by several problems ranging from unavailability of good quality planting material, high susceptibility to pests and diseases, inadequate knowledge on management and genetic diversity of the present germplasm, among other factors (Shomari, 2002). Research into the genetic diversity of this species will provide opportunity for breeders to develop new and improved cultivars with desirable characteristics for tree improvement; that would include both farmers-preferred traits (yield potential and large seed) and breeders preferred traits (pest and disease resistance). Breeding of cashew is mostly based on selection of useful phenotypic (i.e., morphological) and agronomic traits, and these attributes have been used as a potent tool in the classification of cultivars; which continues to be the first step in the studies of genetic relationship in most breeding programmes (Van Beuningen and Busch, 1997; Mneney *et al.*, 2001). Hence, it becomes necessary to improve on this pattern, by quantifying the extent of variation among cashew germplasm in Nigeria by adding other taxonomical traits. The present investigation is therefore, aimed at providing information on the genetic diversity and relationship of cashew accessions in Ilorin, North Central part of Nigeria using morphological and anatomical traits.

Materials and Methods

Field Survey and Collection of Plant Materials

Field trips were undertaken from some selected areas, namely Sobi, Ogidi, Unilorin and Kambi in Ilorin, Kwara State, Nigeria from July 2018 to September, 2019. Five trees of cashew were selected randomly from each of the four locations in Ilorin. Each sample was given an accession number as described by Muchugl *et al*. (2008). Five accessions were taken from each of the four locations (Table 1; Plate 1).

Authentication and Documentation of Plant Materials

The identification of the specimens was authenticated at the Herbarium of the Department of Plant Biology, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria. All details related to each plant species was recorded in the field notebook indicating the collectors name, place and date of collection, habit, name of species and its description were also attached to the mounting sheet and were documented at Ilorin University Herbarium.

Determination of Morphological Parameters

Quantitative morphological data such as leaflet length, leaflet width, petiole length and petiole width were taking using standardized metre rule. The nuts were dried after their collection from the field; and the weight for each nut was taken with beam balance. The fruit colour was examined physically. Sample size of 30 was used for each parameter using the modified methods of Kolawole (2017).

Table 1: List of Accessions of Cashew Tree Four Locations in Ilorin

S/NO	Accession	Location
1	SOB 001	Sobi
2	SOB 002	Sobi
3	SOB 003	Sobi
4	SOB 004	Sobi
5	SOB 005	Sobi
6	UNI 001	Unilorin
7	UNI 002	Unilorin
8	UNI 003	Unilorin
9	UNI 004	Unilorin
10	UNI005	Unilorin
11	OGD 001	Ogidi
12	OGD 002	Ogidi
13	OGD 003	Ogidi
14	OGD 004	Ogidi
15	OGD 005	Ogidi
16	KMB 001	Kambi
17	KMB 002	Kambi
18	KMB 003	Kambi
19	KMB 004	Kambi
20	KMB 005	Kambi

Isolation of Leaf Epidermal Layers

Three leaves from each of the 20 accessions of cashew plant were collected from the studied locations and taken to the laboratory for anatomical studies. Leaf segments of an area of 1cm square from each specimen was cut from identical regions of the leaf samples, usually from the mid-way between the apex and base of the leaf lamina including the margin. Macerations were carried out by immersing the leaf segments in a concentrated solution of trioxonitrate (v) acid for some hours as described by AbdulRahaman and Oladele (2003). The upper (adaxial) and the lower (abaxial) surfaces were separated with dissecting needle and forceps and were rinsed with clean water. A small portion of each macerated epidermal layer was placed on a slide and stained in 1% aqueous solution of safranin for 3-5 minutes. Excess stain was rinsed off with clean water and mounted in glycerine for microscopic observation using binocular light microscope fitted with Amscope Camera (Model MU 1000) at a magnification of ×2000.

Plate 1: Photograph of some Cashew Nuts Collected across the Locations

Determination of Anatomical Parameters

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The following anatomical attributes such as stomatal complex types and their frequencies, stomatal density, stomatal index, stomatal size, epidermal density, anticlinal cell wall pattern, epidermal cell shape, stomatal length and stomatal width were determined. Sample size of 30 was used for each of the parameters. Frequency of each stomatal complex type was expressed as percentage occurrence of each stomatal complex type relative to all occurrences (AbdulRahaman and Oladele, 2003). The stomatal complex type and shape of the epidermal cell were identified using the terminologies of Dilcher (1974). Measurements were taken for stomatal length and stomatal width with the aid of micrometer eye-piece graticule. The mean stomatal density was determined as the number of stomata per square millimeter based on the entire leaf surface. That is, number of stomata in 0.152mm^2 field of view (Stace, 1965). The mean stomatal index was determined as the number of stomata per square millimeter divided by number of stomata plus number of ordinary epidermal cells per square millimeter multiplied by 100

(Wilkinson, 1979). The mean stomata size of each of the specimen was determined as the product of length and breadth of guard cell multiplied by Franco's constant of 0.79 (Franco, 1939; Wilkinson, 1979). The shape of epidermal cell was determined according to the terminology used by Dilcher (1974) and Metcalfe and Chalk (1988).

Statistical Analysis

The data on the quantitative and qualitative traits was subjected to cluster analysis to conduct similarity estimates using unweighted pair group method of arithmetic averages (UPGMA) in multivariate statistical package (MVSP); and principal component analysis (PCA) using PAST version 3 software. The data on quantitative traits were subjected to analysis of variance (ANOVA) using Statistical Packages for Social Sciences (SPSS) version 20.0 software (Bailey, 1995) for comparison of means; while the means with significant difference were separated using Duncan's Multiple Range Test (Duncan, 1955).

Results

The quantitative morphological traits of 20 accessions of cashew shown in Table 2 revealed significant variation at $p \leq 0.05$ within and between the various accessions of cashew accessions across all the studied locations. Accessions KMB 004 and OGD 003 had the highest variation in leaf length; accession KMB 004 had the highest variation in leaf width; accession SOB 002 was prominent in petiole length; accessions SOB 003, SOB 005, UNI 002, OGD 002, OGD 004 and KMB 004 had the highest variation in petiole width, while accession KMB 001 was prominent in nut weight. The qualitative morphological features such as the fruit (apple) colour also varied across the locations (Table 2).

The leaf epidermal features for the accessions of cashew are shown in Tables 3 and 4; while the leaf epidermal structures are shown on plates 4-7. The results from the leaf anatomical study showed variations among the accessions. The leaves of the cashew accessions were hypostomatic (i.e. presence of stomata only on the abaxial surface). The stomatal complex type observed was brachyparacytic with frequency of 100.00% in all the accessions. The epidermal cell shapes were irregular and isodiametric; while the anticlinal cell walls were wavy and curved. The quantitative anatomical traits showed that accessions SOB 001, SOB 002, KMB 004 and KMB 005 had the highest variation in stomatal length; accession KMB 002 was prominent in stomatal width; accession UNI 004 was prominent in stomatal density; accession OGD 005 had the highest variation in stomatal index; accession KMB 005 had the highest variation in stomatal size; accessions OGD 001 and UNI 003 were prominent in epidermal length; accession OGD 001 was prominent in epidermal breadth; while accession UNI 004 had the highest variation in epidermal density.

The cluster analysis produces a hierarchical classification of the morphological traits (Figures 1) and anatomical traits (Figure 3) of cashew accessions based on similarity matrix; and the dendrogram from Euclidean distance of unweighted paired group method average divided the 20 cashew accessions into two major clusters and several sub-clusters for both morphological and anatomical traits.

The Principal Component Analysis (PCA) variance for morphological characters of the cashew accessions revealed that the first three (3) components accounted for 82% of the total variation among the 20 cashew accessions (Table 5); while the PCA variance for anatomical characters of the cashew accessions portrayed that the first three (3) components accounted for 74% of the total variation (Table 5); thereby contributing greatly to the separation of the accessions. The PCA biplot revealed spatial distribution of the accessions into four quadrants based on their morphological traits (Figure 2) and anatomical traits (Figure 4). However, those accessions in the same quadrant were closely related due to their similar traits; Similarly, the accessions in the same quadrant that are nearer to each other are more closely related than those farther from each other; while those accessions from different quadrants are genetically different from one another.

Table 2: Morphological Features of Accessions of Cashew Plant in Ilorin

S/N	Accession	Leaf Length (cm)	Leaf Width (cm)	Petiole Length (cm)	Petiole Width (cm)	Nut Weight (g)	Fruit Colour
1	SOB 001	18.23^{fg}	11.73^{abc}	1.95^{bcde}	0.33^{abc}	8.90^{bc}	Yellow
2	SOB 002	16.30^{gh}	10.50^{bc}	2.50^{a}	0.33^{abc}	7.50^{bcd}	Yellow
3	SOB 003	16.25^{gh}	9.38^{bcd}	1.50^{fgh}	0.38^{a}	9.70^{ab}	Red
4	SOB 004	20.80^{cdef}	10.53^{bc}	1.83^{fgh}	0.35^{ab}	5.00^{cd}	Red
5	SOB 005	23.93^{ab}	14.60^{a}	1.90^{cdef}	0.38^{a}	8.50^{bc}	Yellow
6	UNI 001	18.58^{efg}	11.78^{abc}	1.18^{h}	0.30^{abc}	10.20^{ab}	Red
7	UNI 002	18.45^{efg}	12.55^{ab}	1.98^{bcd}	0.38^{a}	10.50^{ab}	Red
8	UNI 003	20.13^{cdef}	10.68^{bc}	1.23^{gh}	0.33^{abc}	9.50^{abc}	Yellow
9	UNI 004	20.23^{cdef}	12.15^{ab}	2.05^{bc}	0.33^{abc}	7.30^{bcd}	Yellow
10	UNI 005	19.10^{def}	10.98^{bc}	1.60^{defg}	0.25^{c}	7.40^{bcd}	Yellow
11	OGD 001	15.20^{h}	9.83^{bcd}	1.40^{gh}	0.30^{abc}	4.60^{d}	Orange
12	OGD 002	23.93^{ab}	14.78^{a}	1.85^{cdef}	0.38^{a}	5.70^{cd}	Yellow
13	OGD 003	24.50^{a}	12.13^{ab}	1.58^{efg}	0.30^{abc}	8.50^{bc}	Yellow
14	OGD 004	21.90^{bc}	13.28^{a}	1.35^{gh}	0.38^{a}	6.90^{c}	Red
15	OGD 005	21.38^{cd}	11.88^{abc}	2.28^{ab}	0.33^{abc}	10.00^{ab}	Yellow
16	KMB 001	19.93^{cdef}	12.58^{ab}	1.38^{gh}	0.33^{abc}	11.00^{a}	Red
17	KMB 002	18.70^{efg}	11.88^{abc}	1.85^{cdef}	0.30^{bc}	9.00^{abc}	Red
18	KMB 003	21.75^{bc}	12.73^{ab}	1.58^{efg}	0.33^{abc}	8.90^{bc}	Red
19	KMB 004	24.50^{a}	14.90^{a}	1.80^{cdef}	0.38^{a}	4.80^{d}	Orange
20	KMB 005	20.88^{cde}	11.28^{abc}	1.83^{cdef}	0.33^{abc}	7.80^{bcd}	Yellow

Mean with the same letters along the column are not significantly different at $P \leq 0.05$

Table 3: Abaxial Stomatal Features of Accessions of Cashew Plant in Ilorin

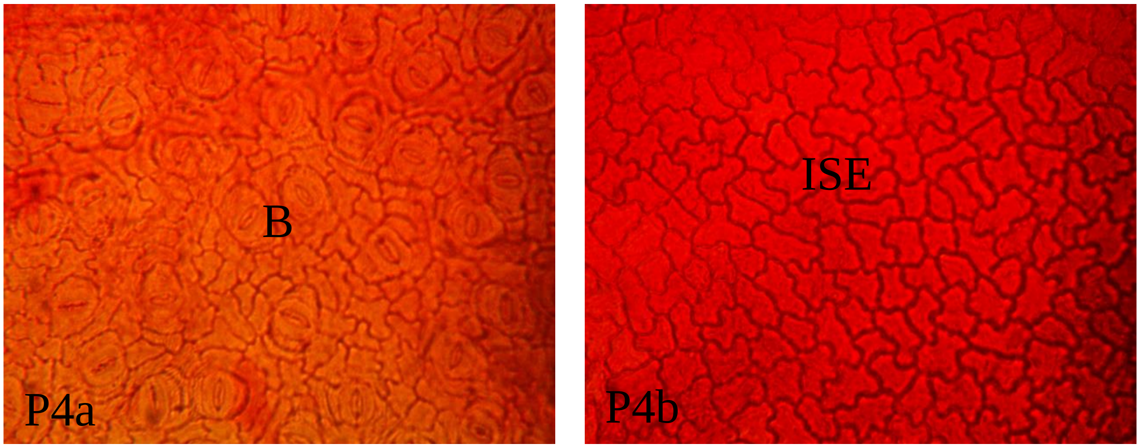
S/N	ACC	SCT	FREQ. (%)	SL (µm)	SW (µm)	SD (mm^{-2})	SS (µm^{2})	SI (%)
1	SOB 001	Brachyparacytic	100.00	9.20^{a}	7.00^{ab}	94.74^{bcd}	37.00^{a}	27.46^{bc}
2	SOB 002	Brachyparacytic	100.00	9.20^{a}	3.80^{d}	90.79^{bcd}	22.00^{cd}	37.31^{ab}
3	SOB 003	Brachyparacytic	100.00	7.40^{bc}	4.00^{d}	101.32^{ab}	23.63^{cd}	29.91^{bc}
4	SOB 004	Brachyparacytic	100.00	8.20^{ab}	7.00^{ab}	81.58^{bcd}	35.42^{ab}	36.95^{ab}
5	SOB 005	Brachyparacytic	100.00	7.20^{bc}	3.80^{d}	86.84^{bcd}	26.26^{d}	26.52^{bc}
6	UNI 001	Brachyparacytic	100.00	6.60^{bcd}	4.40^{cd}	107.89^{ab}	31.92^{cd}	39.16^{a}
7	UNI 002	Brachyparacytic	100.00	7.60^{bc}	6.40^{abc}	127.63^{ab}	31.89^{bc}	29.63^{bc}
8	UNI 003	Brachyparacytic	100.00	5.20^{d}	3.80^{d}	100.00^{ab}	19.21^{e}	37.32^{ab}
9	UNI 004	Brachyparacytic	100.00	6.00^{cd}	3.80^{d}	133.22^{a}	24.15^{d}	35.55^{ab}
10	UNI 005	Brachyparacytic	100.00	7.60^{bc}	5.20^{bcd}	96.05^{bcd}	30.16^{bc}	38.15^{ab}
11	OGD 001	Brachyparacytic	100.00	7.40^{bc}	4.80^{cd}	82.89^{bcd}	33.70^{cd}	27.07^{bc}
12	OGD 002	Brachyparacytic	100.00	7.40^{bc}	5.60^{abcd}	67.11^{cd}	31.16^{bc}	25.88^{bc}
13	OGD 003	Brachyparacytic	100.00	7.00^{bc}	5.00^{bcd}	62.89^{cd}	30.11^{bc}	17.43^{c}
14	OGD 004	Brachyparacytic	100.00	6.00^{cd}	4.00^{d}	67.05^{cd}	29.32^{d}	25.61^{bc}
15	OGD 005	Brachyparacytic	100.00	6.20^{cd}	4.40^{cd}	114.47^{ab}	29.89^{d}	39.33^{a}
16	KMB 001	Brachyparacytic	100.00	7.60^{bc}	4.40^{cd}	73.68^{cd}	33.16^{cd}	36.93^{ab}
17	KMB 002	Brachyparacytic	100.00	8.20^{ab}	7.40^{a}	98.68^{bcd}	36.71^{a}	30.20^{ab}
18	KMB 003	Brachyparacytic	100.00	7.40^{bc}	4.40^{cd}	110.53^{ab}	24.25^{cd}	32.08^{ab}
19	KMB 004	Brachyparacytic	100.00	9.20^{a}	5.00^{bcd}	119.74^{abc}	34.58^{ab}	30.94^{ab}
20	KMB 005	Brachyparacytic	100.00	9.20^{a}	7.00^{ab}	94.74^{bcd}	37.08^{a}	29.88^{bc}

Mean with the same letters along the column are not significantly different at $P \leq 0.05$. ACC= Accession; SL - Stomatal Length; SW- Stomatal Width; SS- Stomatal Size; SD- Stomatal Density; SI- Stomatal Index; SCT- Stomatal Complex Type

Table 4: Epidermal Cell Features of Accessions of Cashew Plant in Ilorin

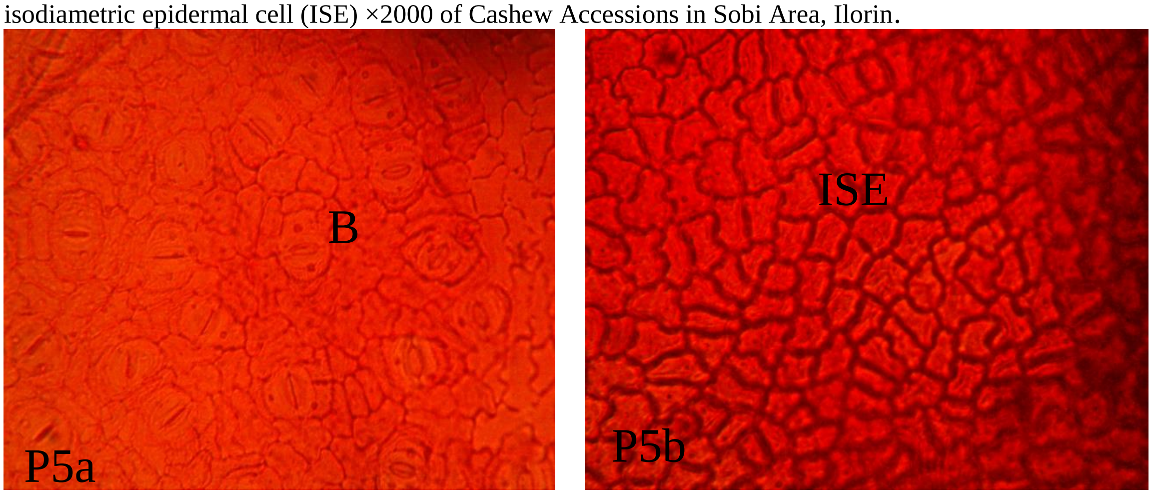
S/N	Accession	Leaf Surface	Epidermal Cell Shape	Anticlinal Cell wall Pattern	Epidermal Length (µm)	Epidermal Width (µm)	Epidermal Density (mm-^{2})
1.	SOB 001	Abaxial	Isodiametric	Wavy	9.40^{def}	3.80^{cd}	300.00^{a}
		Adaxial	Isodiametric	Wavy	10.79^{b}	3.97^{c}	315.00^{b}
2.	SOB 002	Abaxial	Isodiametric	Wavy	8.40^{def}	3.40^{d}	285.00^{b}
		Adaxial	Isodiametric	Wavy	9.20^{bc}	3.60ef	288.92c
3.	SOB 003	Abaxial	Isodiametric	Wavy	8.20^{def}	3.60^{d}	310.55b
		Adaxial	Isodiametric	Wavy	8.25^{bcd}	3.50ef	315.05c
4.	SOB 004	Abaxial	Irregular	Wavy	8.20^{def}	4.00^{bcd}	302.49^{a}
		Adaxial	Irregular	Wavy	8.95^{bcd}	4.84d	321.80a
6.	UNI 001	Abaxial	Irregular	Wavy	9.60^{def}	4.60^{abcd}	318.42^{a}
		Adaxial	Irregular	Wavy	8.32^{bcd}	4.52^{de}	312.50^{b}
7.	UNI 002	Abaxial	Isodiametric	Wavy	14.60^{ab}	5.60^{a}	332.89^{a}
		Adaxial	Isodiametric	Wavy	14.86^{a}	7.94^{ab}	350.88^{a}
8.	UNI 003	Abaxial	Isodiametric	Wavy	7.80^{f}	4.20^{bcd}	274.21^{c}
		Adaxial	Isodiametric	Wavy	9.20^{bc}	5.10^{cde}	308.44^{b}
9.	UNI 004	Abaxial	Isodiametric	Wavy	11.60^{bcd}	4.80^{abcd}	338.16^{a}
		Adaxial	Isodiametric	Wavy	14.25^{a}	5.74^{c}	355.90^{a}
10.	UNI 005	Abaxial	Isodiametric	Wavy	10.80^{cdef}	4.20^{bcd}	313.16^{a}
		Adaxial	Isodiametric	Wavy	15.66^{a}	4.12^{def}	310.00^{b}
11.	OGD 001	Abaxial	Isodiametric	Wavy	15.60^{a}	6.00^{a}	315.79^{a}
		Adaxial	Isodiametric	Wavy	9.86^{bc}	8.59^{a}	325.50^{a}
12.	OGD 002	Abaxial	Irregular	Wavy	11.20^{cde}	5.40^{abc}	273.16^{c}
		Adaxial	Irregular	Wavy	10.22^{b}	5.45^{a}	291.48^{c}
13.	OGD 003	Abaxial	Irregular	Wavy	14.00^{abc}	4.60^{abcd}	317.11^{a}
		Adaxial	Irregular	Wavy	14.86^{a}	4.92^{cdef}	330.00^{a}
14.	OGD 004	Abaxial	Isodiametric	Curved	11.40^{bcd}	4.80^{abcd}	301.32^{a}
		Adaxial	Isodiametric	Curved	12.29^{ab}	5.84^{c}	290.66^{c}
15.	OGD 005	Abaxial	Irregular	Curved	11.40^{bcd}	4.60^{abcd}	307.89^{a}
		Adaxial	Irregular	Curved	14.59^{a}	7.26^{ab}	338.25^{a}
16.	KMB 001	Abaxial	Irregular	Wavy	7.80^{f}	3.40^{d}	313.16^{a}
		Adaxial	Irregular	Wavy	7.82c	4.10d^{ef}	314.22^{b}
17.	KMB 002	Abaxial	Isodiametric	Wavy	8.20^{def}	4.00^{bcd}	294.74^{a}
		Adaxial	Isodiametric	Wavy	8.20^{bcde}	5.50^{cd}	294.90^{c}
18.	KMB 003	Abaxial	Irregular	Wavy	8.20^{def}	4.40^{abcd}	309.21^{a}
		Adaxial	Irregular	Wavy	8.50^{bcd}	6.20^{bc}	316.26^{b}
19.	KMB 004	Abaxial	Isodiametric	Wavy	7.40^{f}	4.00^{bcd}	290.58^{b}
		Adaxial	Isodiametric	Wavy	7.50^{cd}	6.00^{bcd}	280.22^{c}
20.	KMB 005	Abaxial	Isodiametric	Wavy	9.20^{def}	3.80^{cd}	296.05^{a}
		Adaxial	Isodiametric	Wavy	10.50^{b}	5.14^{cde}	304.10^{b}

Mean with the same letters along the column are not significantly different at $P \leq 0.05$



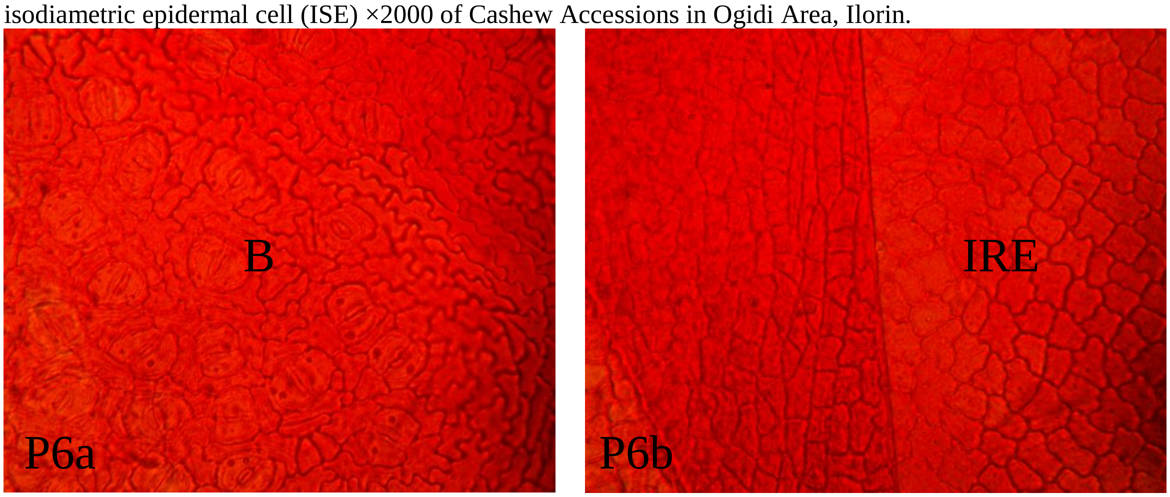
(a) abaxial (b) adaxial

Plate 4: Surface View of Leaf Epidermis showing brachyparacytic stomatal complex type (B) and isodiametric epidermal cell (ISE) ×2000 of Cashew Accessions in Sobi Area, Ilorin.



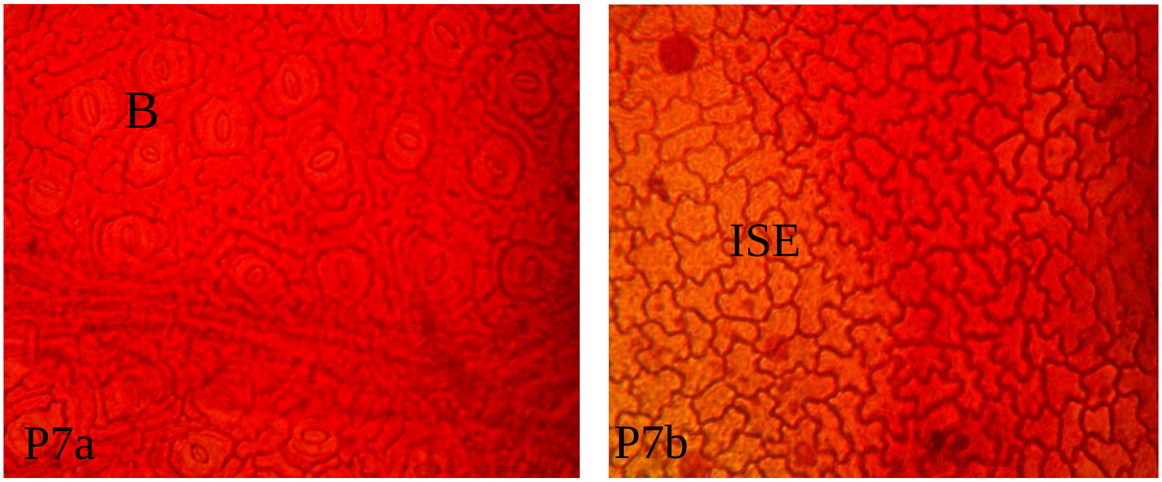
(a) abaxial (b) adaxial

Plate 5: Surface View of Leaf Epidermis showing brachyparacytic stomatal complex type (B) and isodiametric epidermal cell (ISE) ×2000 of Cashew Accessions in Ogidi Area, Ilorin.



(a) abaxial (b) adaxial

Plate 6: Surface View of Leaf Epidermis showing brachyparacytic stomatal complex type (B) and (IRR) Irregular epidermal cell ×2000 of Cashew Accessions in Unilorin Area, Ilorin.



(a) abaxial (b) adaxial

Plate 7: Surface View of Leaf Epidermis showing brachyparacytic stomatal complex type (B) and isodiametric epidermal cell (ISE) ×2000 of Cashew Accessions in Kambi Area, Ilorin.

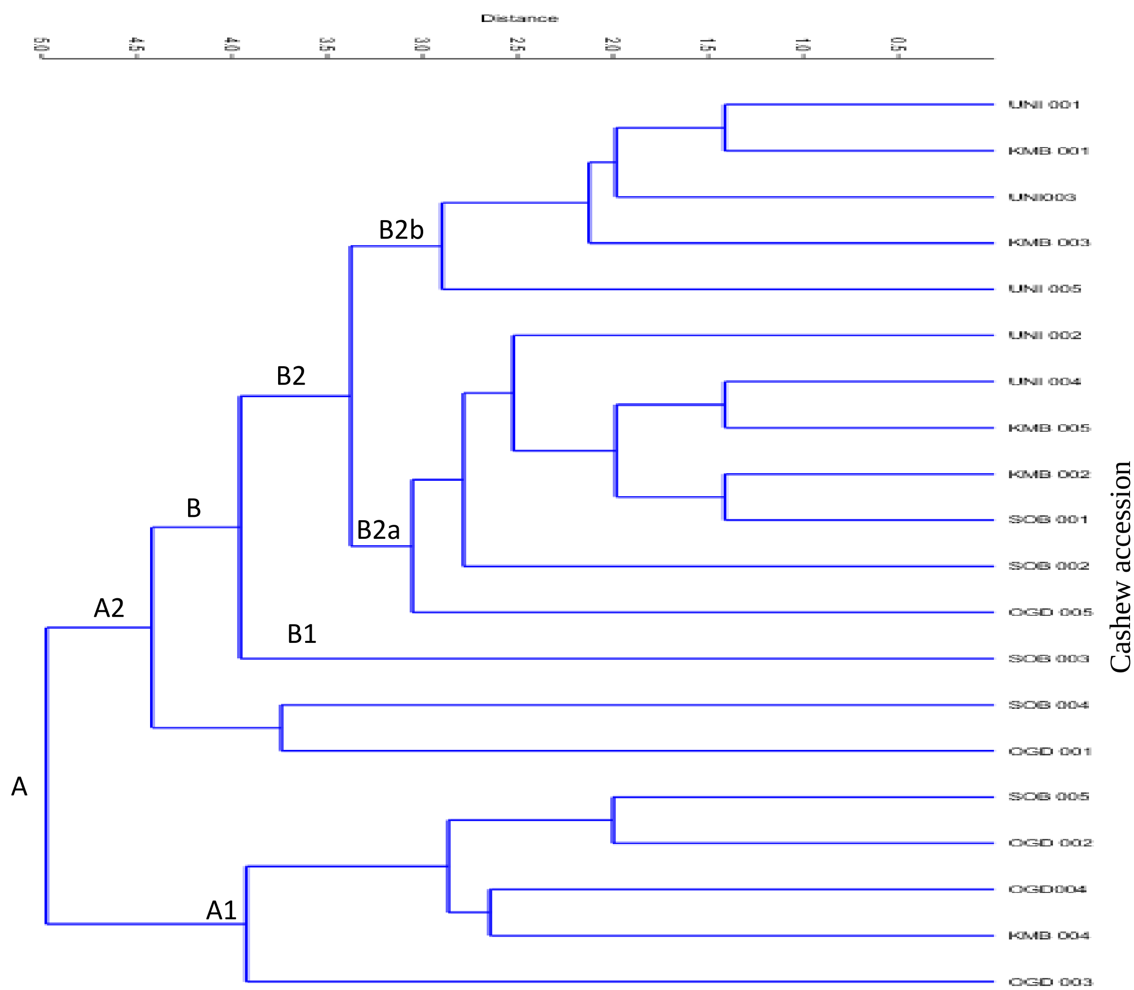


Figure 1: Dendrogram showing the relationship among the accessions of cashew plant based on morphological characters using UPGMA on selected locations in Ilorin.

Table 5: Principal Component Analysis Variance for Morphological Features of Accessions of Cashew Plant in Ilorin

Principal Component	Eigenvalue	% Variance
1.	3.6953	41.24
2.	2.02492	22.598
3.	1.69501	18.916
4.	0.978633	10.922
5.	0.35826	3.9982
6.	0.208412	2.3259

Table 6: Principal Component Analysis Variance for Leaf Anatomical Features of Accessions of Cashew Plant in Ilorin

Principal Component	Eigenvalue	% Variance
1.	5.11319	33.957
2.	3.67307	24.393
3.	2.51071	16.674
4.	1.26683	8.413
5.	0.808071	5.3664
6.	0.755508	5.0174
7.	0.524947	3.4862
8.	0.251535	1.6705
9.	0.11371	0.75515
10.	0.0403163	0.26774
11.	0	0

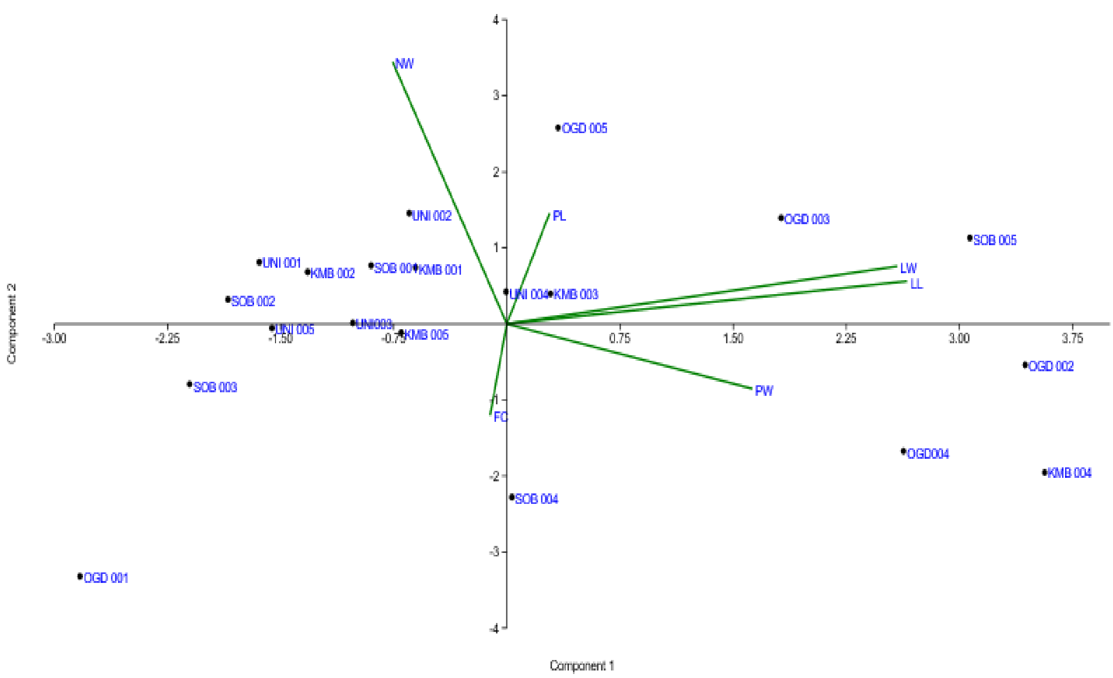


Figure 2: Principal Component Analysis (PCA) Biplot showing Relationship among the Accessions of Cashew Plant based on Morphological Characters using UPGMA on selected locations in Ilorin

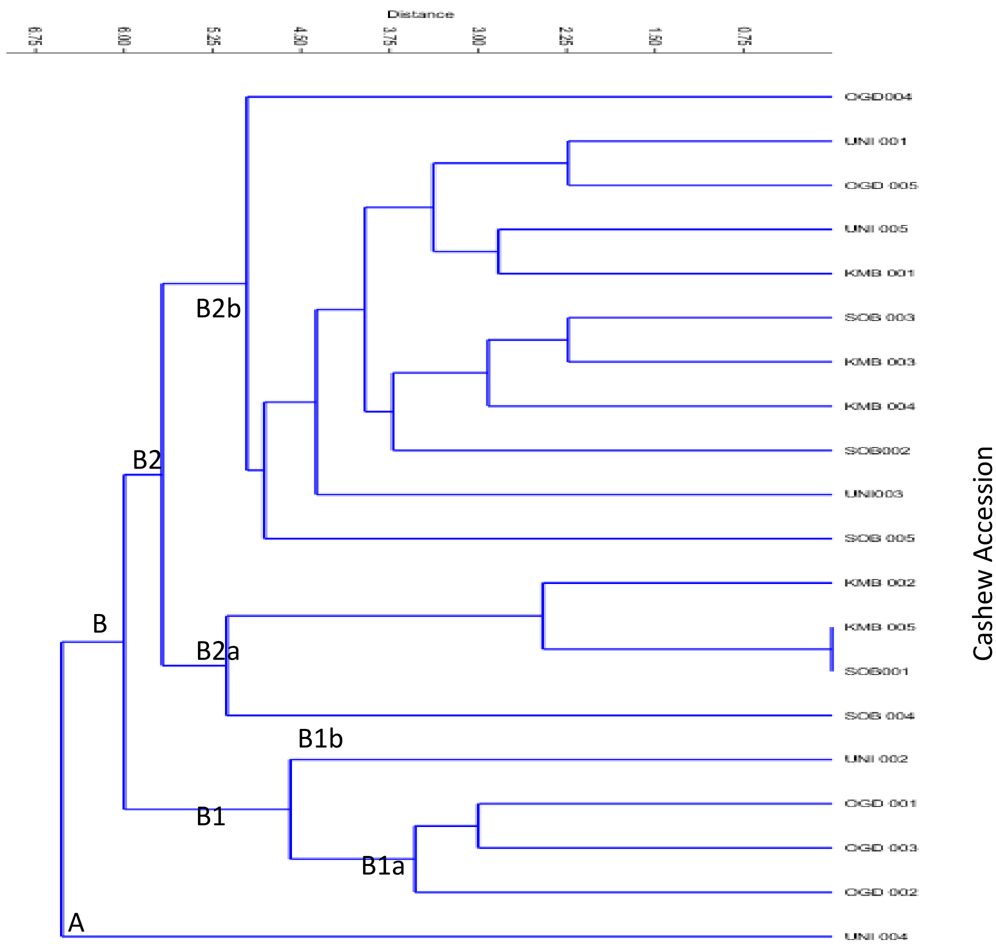


Figure 3: Dendrogram showing Relationship among the Accessions of Cashew Plant based on Leaf Epidermal Characters using UPGMA on selected locations in Ilorin

Discussion

The morphometric study of the cashew accessions revealed that the leaf length, leaf width and petiole length accounted for most of the variations; as compared with the outcomes from other traits. Therefore, these traits suggest genetic variation that could be useful for crop improvement in cashew plant. This finding conformed to the observations on some other species by earlier researchers (Sonibare *et al*., 2004; Soladoye *et al*., 2010; Kolawole *et al*., 2016; Jeruto *et al*., 2017 and Kolawole, 2017). However, these morphological differences could be attributed to factors such as soil type, ecological adaptation to sites and selection by man for good traits.

The leaf anatomical study showed that brachyparacytic stomatal complex types, stomatal density and stomatal index accounted for most of the variations as compared to others among the cashew accessions. These attributes suggest high genetic variations that can serve as benchmarks for selection of cashew accessions for crop improvement.

The occurrence of hypostomatic leaf nature corroborated with the outcomes of Iroka *et al*. (2014) on cashew species. The presence of brachyparacytic stomatal complex type in this study was contrary to the observation of Iroka *et al*. (2014) who reported diacytic stomatal complex type. The difference may be due to the habitat of the species. However, the occurrence of anatomical traits like brachyparacytic stomatal complex type, hypostomatic leaf nature, low stomatal index as well as small and moderate stomatal size in most of the accessions may be responsible for the survival of the cashew accessions in the dry region. This conformed to the observations of Oyeleke *et al*., (2004); AbdulRahaman and Oladele (2008) and Omolokun and Oladele (2010).

The dendrogram grouped the 20 cashew accessions into two major clusters at a distant (i.e. dissimilarity) coefficient of 50.00; which signified high similarity coefficient of 50.00% among the 20 cashew accessions in the various locations. This suggests that some of the cashew accessions are genetically related. This observation agrees with the finding of Chilopoja *et al*. (2009) who reported the ranged of 35 - 64% on cashew germplasm in Malawi; as well as Samal *et al*. (2003) who investigated India cashew germplasm. The first major cluster (A) consisting of 5 accessions OGD 003, KMB 004, OGD 004, OGD 002 and SOB 005 that were completely different from the rest of the accessions due to the size of their leaf; that is, possession of high leaf length and leaf breadth that might be genetically different from the rest. The second major cluster (B) consists of the remaining 15 accessions (OGD 001, SUB 004, SOB 003, OGD 005, SOB 002, SOB 001, KMB 002, KMB 005, UNI 004, UNI 002, UNI 005, KMB 003, UNI 003, KMB 001 and UNI 001) with representatives from all the locations. This suggests polymorphism and high genetic similarity among the accessions in the group. This corroborated with the observations of Chilopoja *et al*. (2009) and Dasmohapatra *et al.* (2014). However, accessions that were grouped together in the same clusters and sub-clusters revealed that they are genetically and phylogenetically close. This observation supports the findings of some workers who reported on other species (Sonibare *et al*., 2004 and Kolawole, 2017). Nevertheless, the different sub-clusters of the cashew accessions in the four different locations suggest that genetic diversity occurred among them.

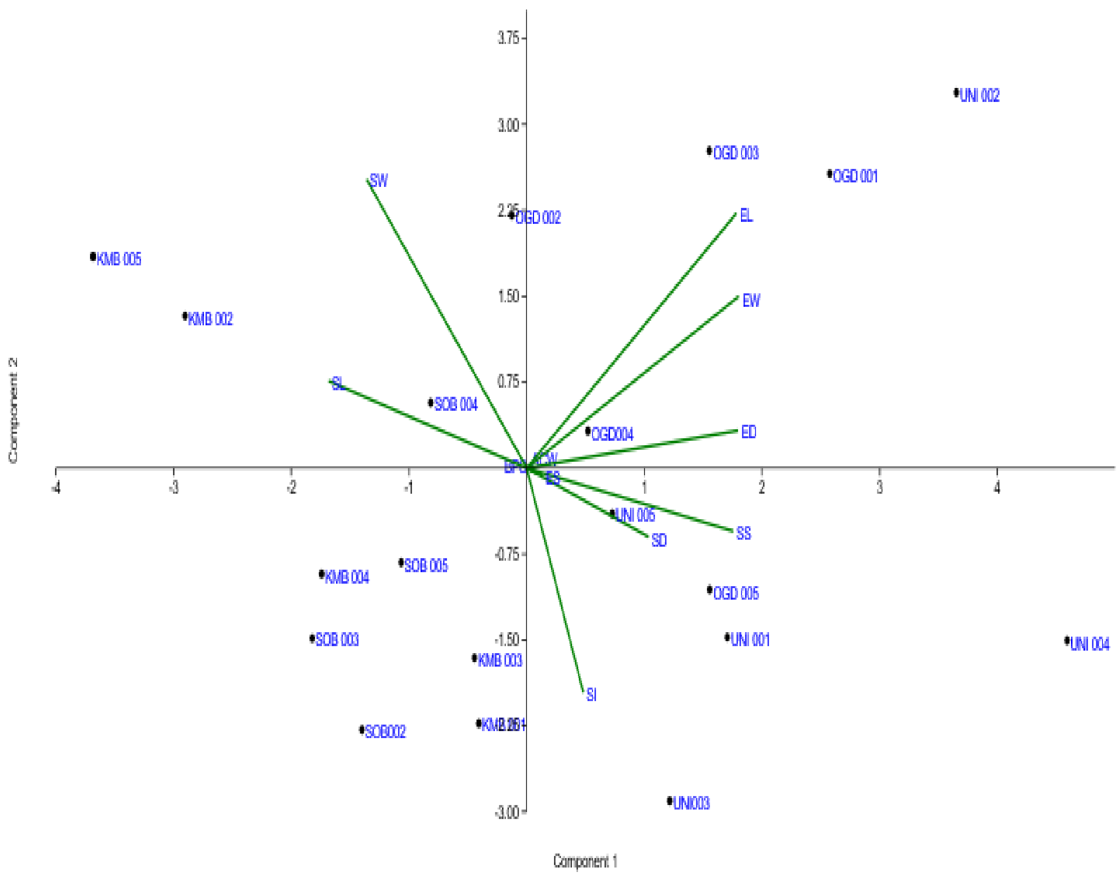


Figure 4: Principal Component Analysis (PCA) Biplot showing Relationship among the Accessions of Cashew Plant based on Leaf Epidermal Characters using UPGMA on selected locations in Ilorin

The Principal Component Analysis biplots for the morphological traits showed that accessions (i.e. OGD 005, OGD 003, SOB 005, KMB 003 and UNI 004) in the first quadrant were delimited from those in other quadrants due to possessions of high leaf length, leaf width, and petiole length; the accessions (i.e. OGD 002, OGD 004, KMB 004 and SOB 004) in the second quadrant differed from other those in other quadrants due to their high petiole width; the accessions (i.e. OGD 001, SUB 003, UNI 005, KMB 005 and UNI 003) in the third quadrant varied from accessions in other quadrants due to the presence of mostly yellow fruit colour; while the fourth quadrant comprised of accessions UNI 001, UNI 002, SOB 002, KMB 002, KMB 001 and SOB 004 that were delimited from those in other quadrants based on their large and medium nut weight. This showed that the distinct morphological traits from each quadrant could be used in separating the cashew accessions; which indicated genetic diversity. This observation is in line with the finding of Chilopoja *et al*. (2009).

The dendrogram constructed for the leaf anatomical features grouped the 20 cashew accessions into two major clusters at a distant (i.e. dissimilarity) coefficient of 6.50; which signified high similarity coefficient of 93.50% among the 20 cashew accessions in the various locations. This suggests that most of the cashew accessions are genetically related. The first major cluster (A) consisting of 1 accession UNI 004 which was completely different from the rest of the accessions due to the possession of the highest stomatal density and epidermal density. The second major cluster (B) consists of the remaining 19 accessions (i.e. OGD 002, OGD 003, OGD 001, UNI 002, SOB 004, SOB 001, KMB 005, KMB 002, SOB 005, UNI 003, SOB 002, KMB 004, KMB 003, SOB 003, KMB 001, UNI 005, OGD 005, UNI 001 and OGD 004) from all the locations. This suggests polymorphism and high genetic similarity among the accessions in the group. The various accessions may also be attributed to wide seed dispersal across the locations. However, the different sub-clusters suggest that genetic variations occurred in the accessions of the different locations. This observation supports the findings of some researchers who worked on other species (Bruneau *et al*., 2008; Alege and Shaibu, 2015).

The Principal Component Analysis biplots for the anatomical attributes revealed that accessions (i.e. UNI 002, OGD 001, OGD 003 and OGD 004) in the first quadrant were separated from those of other quadrants due to possessions of high epidermal length, high and moderate epidermal width, high epidermal density and mostly wavy anticlinal wall pattern; the accessions (i.e. OGD 005, UNI 001, UNI 004, UNI 003 and UNI 005) in the second quadrant varied from accessions in other quadrants due to their low stomatal index, high stomatal density, mostly moderate stomatal size and mostly isodiametric epidermal cell shape; the accessions (i.e. SOB 005, KMB 004, SOB 003, SOB 002, KMB 003 and KMB 001) in the third quadrant were delimited from those in other quadrants due to the presence of brachyparacytic stomatal complex type; while the accessions (that is, SOB 004, OGD 002, KMB 002 and KMB 005) in the fourth quadrant were delimited from those in other quadrants due to their moderate stomatal width and high stomatal length. This portrayed that the distinct anatomical traits from each quadrant could be used in separating the cashew accessions; which signified genetic diversity among them. Similar trend was noted by Kolawole (2017) on plant species of subtribe Cassinae.

Conclusion

The study concluded that the morphological and leaf anatomical traits showed potentials for selecting good yield of cashew accessions that will produce seeds of high marketable value. These traits may also serve as taxonomic tools for adequate delimitation and identification of the species within the genus. This study recommends a wider distribution and broad genetic base using molecular markers for cashew selection and breeding in Nigeria.

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