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Research Article

PHYLOGENETIC AND SYSTEMATIC VALUE OF LEAF EPIDERMAL CHARACTERISTICS IN SOME MEMBERS OF NIGERIAN FABACEAE

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Abstract

This study was undertaken at the Botanical Garden of Biological Sciences Department, Kogi State University, Anyigba with the aim of assessing the systematic and phylogenetic relevance of leaf epidermal attributes in the 10 selected species of Fabaceae. Stomata, trichomes and epidermal cell attributes were taken from adaxial and abaxial leaf surfaces. Results obtained in this study revealed that all the 10 plant species considered possess hypo-amphistomatic leaf condition, paracytic stomata type, polygon and irregular shape epidermal cells(on the abaxial surface) which points to their common ancestry. All the analyzed leaf epidermal traits considered on the adaxial and abaxial surfaces showed significant variations among the 10 studied plant species which indicates that genetic diversity exists among members of Fabaceae for their delimitation. It was also observed that all the plants with tree habit considered in this study (i.e *Delonix regia, Parkia biglobosa, Senna siamea, Daniella oliveri* and *Caesalpinia pulcherrima*) lack stomata on the adaxial surfaces which strongly suggest that absence of stomata on the adaxial surface may be peculiar to Legumes with such habit. Cluster analysis revealed 2 major clusters and 2 sub-clusters with the first cluster comprising only *Senna siamea* and *Caesalpinia pulcherrima* which confirms their close phylogenetic relationship. Variations in trichomes, stomata and epidermal attributes were obvious and could be used to resolve systematic and phylogenetic problems in this family.

Keywords: Systematics; Phylogenetic Relationships; Hypo-amphistomatic; Paracytic; Fabaceae.

Introduction

The Fabaceae, commonly known as the legume pea or bean family is a large economically important family of flowering plants. The plant group according to Natarajan (2014) is the third largest land plant family that comprised approximately 730 genera and 19,400 species with a broad distribution and widely recognised importance. Many genera in this family are extremely widespread, while others are endemic to single countries. The family has diversification in most major land biomes, ranging from arid to wet tropical grassland and coastal regions. Fabaceae in the opinion of Burnham and Johnson (2004) is the most common family found in tropical rainforest and dry forests of America and Africa.

Legumes have often been associated with alleviation of poverty worldwide and consumption of beans according to Javaid *et al.* (2004) is a cheap way of maintaining nutritional requirements. Generally, legumes are known to contain very low cholesterol and fat. Beans are also good source of vitamin B, folic acid, potassium, magnesium and fibre (Bruneau *et al.*, 2008). However, members of the family Fabaceae are unique in that they naturally fix nitrogen in the soil. This natural process of nitrogen fixation proves to be one of the most unique characteristics of legumes (Hossain and Komatsu, 2014).Many tree legumes according to Kokilavani and Rajendiran (2015) have been reported to be useful plant species for agroforestry, soil restoration and erosion control in many countries.

Plant classification has been a subject of discussion among plant taxonomists and systematists over the years (Abdulrahman *et al.*, 2011; Agbolade *et al.*, 2011; Ogunkunle and Oladele, 2008). Plants are classified as soon as new evidence arises and this will be a continuous exercise over some years to come. The use of leaf epidermal characters in the taxonomy of Angiosperms is on the increase and has been practiced for decades (Okeke *et al.*, 2011; Yasim *et al.*, 2009).Abdulrahman *et al.* (2011) reported that before the end of 19th century, taxonomists were confined to features of reproductive organs, as floral characters were considered to provide the most valuable characters to depict taxonomic affinities. Ahmad *et al.* (2014) now opined that of all the non-reproductive organs, the leaf is the most widely used in plant taxonomy while Das *et al.* (2003) described the leaf epidermis as the second most important character after cytology for resolving taxonomic and evolutionary problems. The epidermis of the leaves constitutes a dynamic barrier between the plant's internal and external environment (Kokilavani and Rajendiran., 2015). The use of leaf epidermal features in systematic botany is now popular just like the use of other

makers like DNA sequence and chemical compositions (Nnamani and Nwosu, 2015; Usama, 2015).

The family Fabaceae is classified into three sub-families which according to Bruneau *et al.* (2008) include Caesapinoideae, Mimosoideae and Papilionoideae. Many studies including molecular and morphological evidences supported the origin of Fabaceae through a monophyletic origin (Lewis *et al.*, 2005). The monophyletic origin of Fabaceae has also been supported not only by the degree of interrelations shown by different groups within the family but also by all the recent phylogenetic studies based on DNA sequences (Klitgard and Bruneau, 2003). Alege *et al.* (2014) observed that the family Fabaceae has followed two major lines of evolution and their taxonomy isstill debated because of the extreme morphological variability and ambiguous boundaries between members of this family. They therefore called for urgent multidimensional approach to revising the family Fabaceae. Aworinde andFawibe (2014) also affirmed that many research works have been done on the physiology, chemotaxonomy and medicinal uses of members of Fabaceae family but information on the anatomy and taxonomy is still very scanty. This study was therefore undertaken to assess the systematic and phylogenetic relationships of 10 selected species of Fabaceae using leaf epidermal attributes.

Materials and Methods

Collection of Materials

The seeds of *Vigna unguiculata, Arachis hypogeae, Glycine max, Vigna subterranea,* and *Phaseolus vulgaris* were bought from Anyigba market, Kogi State and planted in the Botanical Garden of Department of Biological Sciences, Kogi State University, Anyigba. On the other hand, all the five trees considered in this study (i.e *Delonix regia, Parkia biglobosa, Senna siamea, Daniella oliveri*and *Caesalpinia pulcherrima*) were located in Anyigba. Leaves from the planted crops and the trees (at different positions on the plant) were harvested at vegetative maturity (flowering stages) for leaf epidermal study. Identification of each plant species was carried out at the Department of Biological Sciences, Kogi State University, Anyigba. The plants were arranged as shown in Table 1 and considered as such throughout this study.

Leaf Epidermal Studies

Fresh leaves were collected from each of the 10 selected plant species. Each leaf was painted with fingernail polish on both the adaxial and abaxial surfaces and allowed to dry. After drying, short clear cellophane tape was firmly pressed over the dried nail polish on the surface according to the method of Mbagwu *et al.*(2007). Epidermal strips were taken from the median portion of matured leaves, stained in alcoholic Safranine and mounted in 50% glycerine jelly for microscopic examination. Epidermal strips from both the adaxial and abaxial surfaces were prepared and mounted separately. Photomicrographs of good preparations were taken at a magnification of ×400 objective. The length and breadth of epidermal cells and stomata apparatus were measured with micrometer eyepiece graticule. The number of stomata and epidermal cells were taken and recorded. Ten epidermal peels were mounted for each leaf surfaces while observations and measurements were made from 30 microscope fields of focus at ×40 objectives.

Data Analysis

Data obtained from each leaf epidermal attributes on both the adaxial and abaxialsurfaces were subjected to Analysis of Variance (ANOVA) and means with significant difference separated using Duncan Multiple Range Test (DMRT).The Stomata Index (S.I) was estimated for the leaf surface using the formulae described by Wilkinson (1979) as below:

$$\text{Stomata Index (S.I)} = S/S+E \times 100$$

Where:

S = Number of stomata per unit area and

E = Number of Epidermal cells in the same unit area.

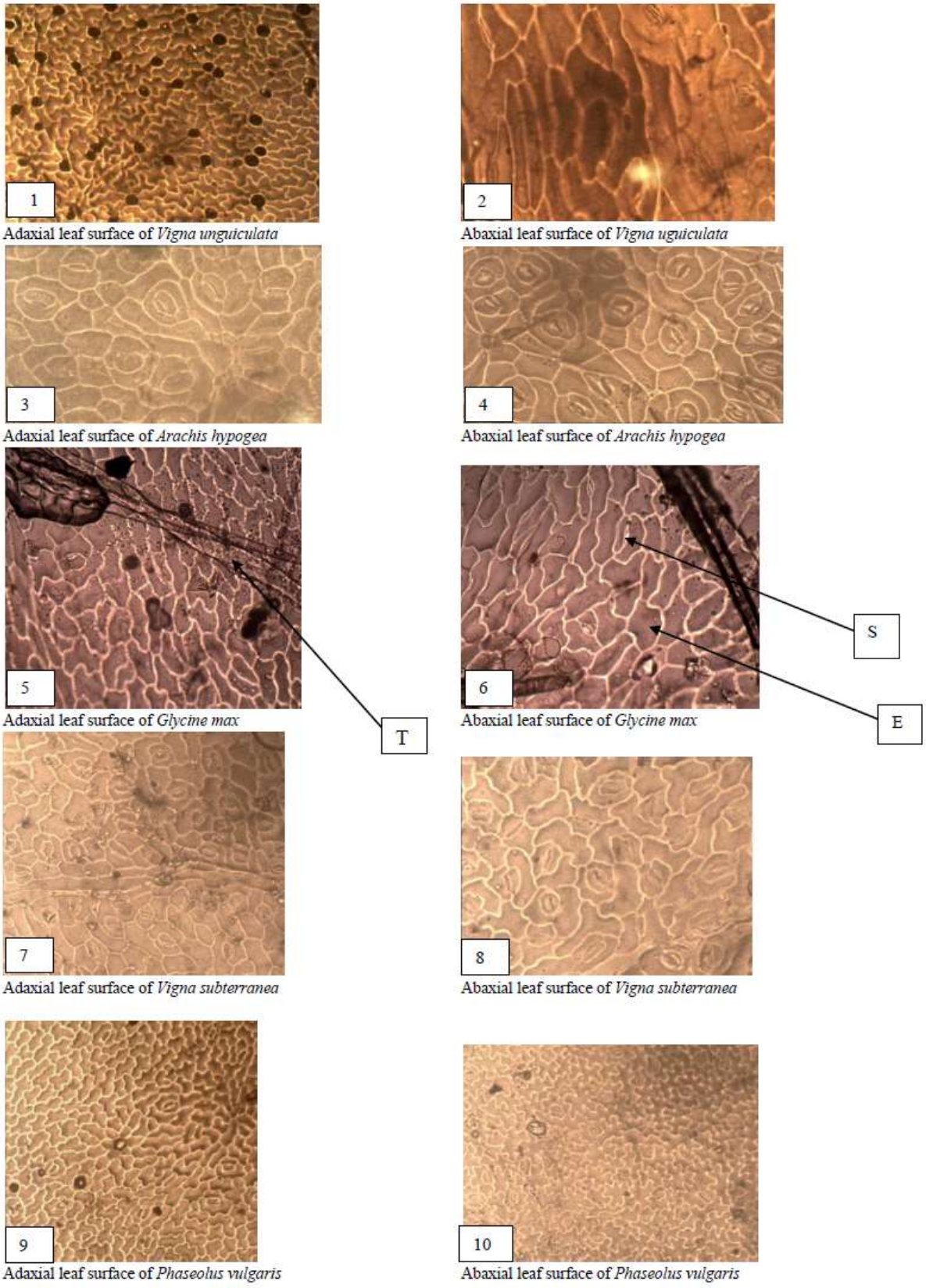
Dendogram (hierarchical cluster) was constructed using the Unweighted Pair Group Method Average (UPGMA). All computations were done using SPSS V16 window software.

Table 1: Brief Description of the 10 Plant Species Studied.

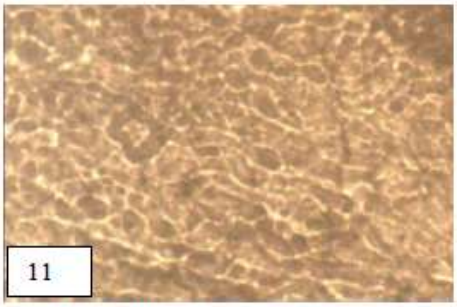
S/N	Common Name	Botanical Name	Habit	Sub-Family
1	Cowpea	*Vigna unguiculata*	Herb	Faboideae
2	Groundnut	*Arachis hypogaea*	Herb	Faboideae
3	Soybeans	*Glycine max*	Herb	Faboideae
4	Bambara nut	*Vigna subterranean*	Herb	Faboideae
5	Beans	*Phaseolus vulgaris*	Herb	Faboideae
6	Locust bean	*Parkia biglobosa*	Tree	Mimosoideae
7	Balsam tree	*Daniella oliveri*	Tree	Caesalpinioideae
8	Flamboyant tree	*Delonix regia*	Tree	Caesalpinioideae
9	Cassod tree	*Senna siamea*	Tree	Caesapinioideae
10	Pride of Barbados	*Caesalpinia pulcherrima*	Tree	Caesalpinioideae

Result and Discussion

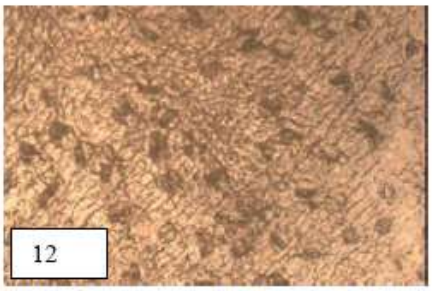
Table 2 shows the summary of leaf epidermal attributes for the ten selected plant species studied while Tables 3 and 4 show the mean measurements for the leaf epidermal attributes on the adaxial surface and abaxial surfaces for the ten studied plant species respectively. Plates 1 to 20 present the photomicrographs of the adaxial and abaxial leaf surfaces for the ten studied plants.



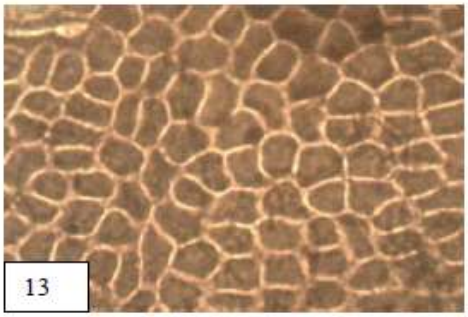
Plates 1 (1-10): Photomicrographs of Adaxial and Abaxial Leaf Surfaces for the Ten Plant species Studied at ×400.
[E- Epidermal cell, S- Stomata, T- Trichome]



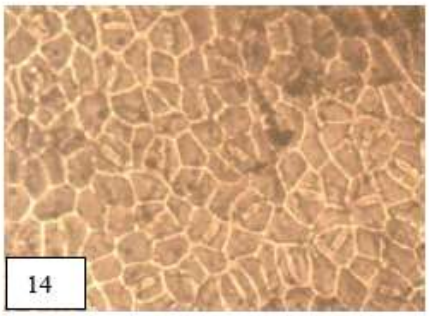
Adaxial leaf surface of *Parkia biglobosa*



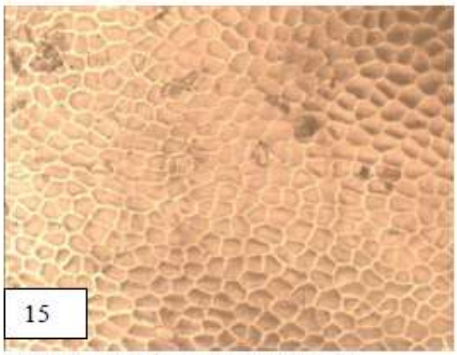
Abaxial leaf surface of *Parkia biglobosa*



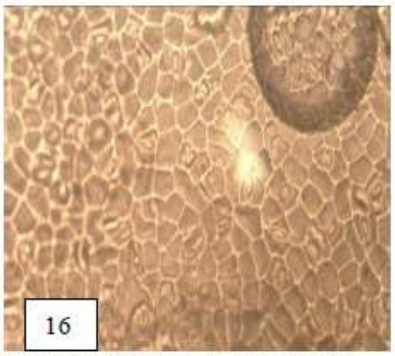
Adaxial leaf surface of *Daniellia oliveri*



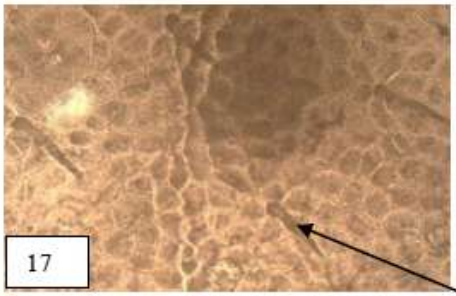
Abaxial leaf surface of*Daniellia oliveri*



Adaxial leaf surface of *Delonix regia*



Abaxial leaf surface of *Delonix regia*



Adaxial leaf surface of *Sennasiamea*

Abaxial leaf surface of *Sennasiamea*

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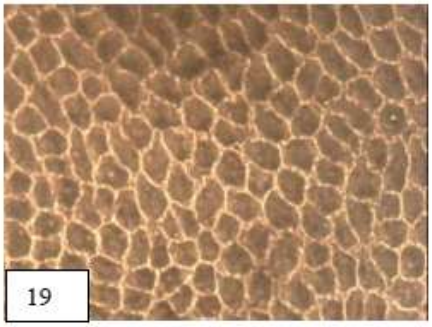
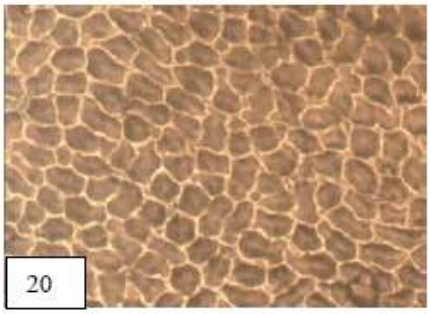


Adaxial leaf surface of *C. pulcherrima*



Abaxial leaf surface of *C. pulcherrima*

Plates 1 Contd. (11-20): Photomicrographs of Adaxial and Abaxial Leaf Surfaces for the Ten Plant species Studied at ×400.
[E- Epidermal cell, S- Stomata, T- Trichome]

Table 2: Summaries of Some Foliar Epidermal Attributes on the Adaxial and Abaxial Surfaces of the Ten Selected Fabaceae Studied.

Plant species	Leaf Surface	Shape of Epidermal Cells	Epidermal Wall Pattern	Stomata Type	Leaf Condition
Vigna unguiculata	Abaxial	P, I	U	Paracytic	Hypoamphistomatic
	Adaxial	P, I	S, C	Nil	
Arachis hypogaea	Abaxial	P, I	S, C	Paracytic	Hypoamphistomatic
	Adaxial	P, I	S, C	Paracytic	
Glycine max	Abaxial	P, I	S, C	Paracytic	Hypoamphistomatic
	Adaxial	P, I	S, C	Paracytic	
Vigna subterranean	Abaxial	P, I	S, C	Paracytic	Hypoamphistomatic
	Adaxial	P, I	S, C	Paracytic	
Phaseolus vulgaris	Abaxial	I	U	Paracytic	Hypoamphistomatic
	Adaxial	P, I	S, C	Paracytic	
Parkia biglobosa	Abaxial	P, I	S, C	Paracytic	Hypoamphistomatic
	Adaxial	P, I	S, C	Nil	
Daniella oliveri	Abaxial	P, I	S, C	Paracytic	Hypoamphistomatic
	Adaxial	P, I	S, C	Nil	
Delonix regia	Abaxial	P, I	S, C	Paracytic	Hypoamphistomatic
	Adaxial	P, I	S, C	Nil	
Senna siamea	Abaxial	P, I	S, C	Paracytic	Hypoamphistomatic
	Adaxial	P, I	S, C	Nil	
Caesalpinia pulcherrima	Abaxial	P, I	S, C	Paracytic	Hypoamphistomatic
	Adaxial	P, I	S, C	Nil	

Key:P- Polygonal, I – Irregular, S – Straight, C – Curve, U - Undulating.

Table 3: The Mean Measurements for the Leaf Epidermal Attribute on the Adaxial Surface for the Ten (10) Members of Fabaceae Studied.

Plant Secies	Number of Epidermal Cells (µm)	Length of Stomata (µm)	Breadth of Stomata (µm)	Length of Epidermal cells (µm)	Breadth of Epidermal cells (µm)	Number of Trichomes (µm)	Number of Stomata(µm)	Stomata Index (%)
Vigna unguiculata	99.67^{b}	0.00^{a}	2.32^{c}	1.33^{e}	0.00a	0.00^{a}	36.03^{d}	0.00
Arachis hypogaea	86.73^{b}	16.00^{f}	2.71^{d}	1.21^{d}	1.13^{c}	0.59^{d}	0.00^{a}	15.57
Glycine max	70.83^{a}	2.33^{c}	3.15^{e}	1.51^{f}	1.12^{c}	0.45^{b}	1.17^{b}	3.18
Vigna subterranean	69.77^{a}	10.80^{e}	3.29^{e}	1.52^{f}	1.18^{d}	0.53^{c}	0.00^{a}	13.40
Phaseolus vulgaris	70.07^{a}	5.63^{d}	3.93^{f}	1.46^{f}	1.18^{d}	0.50^{c}	0.00^{a}	7.44
Parkia biglobosa	117.10^{e}	0.00^{a}	1.92^{b}	0.76^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00
Daniella oliveri	201.43^{f}	0.00^{a}	1.47^{a}	0.94^{c}	0.00^{a}	0.00^{a}	0.00^{a}	0.00
Delonix regia	120.33^{e}	0.00^{a}	1.92^{b}	0.78^{ab}	0.00^{a}	0.00^{a}	0.00^{a}	0.00
Senna siamea	254.10^{g}	0.00^{a}	1.50^{a}	0.90^{bc}	0.00^{a}	0.00^{a}	5.80^{c}	0.00
Caesalpinia pulcherrima	285.57^{h}	0.00^{b}	1.57^{a}	0.81^{ab}	0.00^{b}	0.00^{a}	0.00^{a}	0.00
SED	25.37	1.79	0.27	0.10	0.17	0.08	3.57	-

SED- Standard Error of Deviation

Table 4: The Mean Measurements for the Leaf Epidermal Attribute on the Abaxial Surface for the Ten (10) Members of Fabaceae Studied.

Plant Secies	Number of Epidermal Cells(µm)	Length of Stomata(µm)	Breadth of Stomata(µm)	Length of Epidermal cells (µm)	Breadth of Epidermal cells(µm)	Number of Trichomes(µm)	Number of Stomata(µm)	Stomata Index (%)
Vigna unguiculata	251.40^{g}	1.22^{e}	0.79^{f}	0.99^{a}	0.50^{a}	0.73^{b}	19.63^{ab}	7.24
Arachis hypogaea	112.63^{c}	1.15^{cd}	0.50^{c}	2.91^{fg}	1.02^{g}	0.60^{b}	20.73^{ab}	15.94
Glycine max	93.80^{ab}	0.85^{b}	0.58^{d}	2.78^{f}	1.22^{h}	2.40^{c}	18.13^{a}	16.20
Vigna subterranean	90.40^{a}	1.20^{de}	0.63^{e}	2.99^{g}	1.25^{hi}	0.00^{a}	19.80^{ab}	17.97
Phaseolus vulgaris	110.07^{bc}	1.14^{cd}	0.60^{de}	3.53^{h}	1.31^{i}	0.00^{a}	22.37^{cd}	16.89
Parkia biglobosa	128.07^{f}	1.11^{c}	0.52^{c}	1.09^{a}	0.59^{b}	0.00^{a}	20.63^{ab}	13.87
Daniella oliveri	325.01^{h}	0.70^{a}	0.32^{a}	1.25^{b}	0.69^{f}	0.00^{a}	56.63^{f}	14.84
Delonix regia	127.43^{c}	0.85^{b}	0.42^{b}	1.09^{a}	0.62^{bc}	0.00^{a}	53.70^{ef}	29.65
Senna siamea	521.83^{j}	0.88^{b}	0.32^{a}	1.40^{e}	0.63^{bc}	4.30^{d}	51.60^{e}	9.00
Caesalpinia pulcherrima	410.30^{i}	0.73^{a}	0.43^{b}	1.10^{a}	0.61^{bc}	0.00^{a}	22.63^{d}	5.23
SED	48.60	0.06	0.05	0.32	0.10	0.46	5.13	-

SED- Standard Error of Deviation

All the ten species considered in this study possess hypo-amphistomatic leaf condition (having more stomata on the abaxial surface than the adaxial surface), paracytic stomata type, polygon and irregular shape of epidermal cell on the abaxial surface (except in *Phaseolus vulgaris* with only irregular shaped epidermal cells), smooth and curve epidermal wall (Plate 1 and Table 2).Those characteristics common to all the ten leguminous plants considered in this study are fixed among members of the family Fabaceae which therefore points to common ancestry. This observation indicates that these attributes are unique to leguminous plants irrespective of habit of the plant. Agbolade *et al.* (2011) and Arunadevi *et al.* (2015) reported the valuable use of foliar anatomical studies in revealing the interrelationship and evolution trends in some selected underutilized legumes and Rudiaceae family respectively.

Although the desmocytic stomata type and presence of stomata on the adaxial surface coupled with lack of stomata on the abaxial surface reported by Aworinde and Fawibe (2014) on *Daniella oliveri* contradicts our report on the plant in this study.

All the analyzed leaf epidermal traits considered on the abaxial and adaxial surfaces showed significant variations among the ten studied plant species (Tables 3 and 4) which is an indication that genetic diversity exists among members of Fabaceae family for their delimitation. It can also be observed from table 3 that all the trees considered in this study (i.e*Delonix regia, Parkia biglobosa, Senna siamea, Daniella oliveri*and *Caesalpinia pulcherrima*) lack stomata on the adaxial surfaces which is an indication that absence of stomata on the adaxial surface may be a peculiar trait in Legumes with tree habit. Though, stomata were lacking also on the adaxial surface of*Vigna unguiculata*which suggests that absence of stomata may be used to delimit this plants from other leguminous plants with herb habit under taxonomic confusion.

Generally, the relatively high Stomata Indices (S.I) on the abaxial surface compared to the adaxial surface is common to all the ten Fabaceae studied (Table 3 and 4). This observation may have taxonomic importance and ecological implication on transpiration among members of this family. This agrees with the earlier report of Esseitt *et al.* (2012) that the role of Stomata Index in systematics to separate plant species cannot be over-emphasized.

Cluster analysis revealed 2 major clusters and 2 sub-clusters with the first cluster comprising of only *Senna siamea* and *Caesalpinia pulcherrima* (both from the sub-family Caesalpinoidea) while the remaining 8 (i.e. *Delonix regia, Daniella oliveri, Parkia biglobosa, Vigna unguiculata, Vigna subterranea, Phaseolus vulgaris, Arachis hypogea* and *Glycine max*) occupied the other cluster. This observation supports the polyphyletic origin of Fabaceae reported by Bruneau *et al.*(2008) especially evolution along two major lines reported by Alege *et al.*(2014) but opposes the monophyletic origin proposed by Van Den Bosch and Stacey, (2003). Also, the clustering of *Arachis hypogaea, Glycine max, Vigna subterranea* and *Phaseolus vulgaris* (all Faboidea) together in the same sub-cluster is a strong indication that these four plant are genetically and phylogenetically close and should be retained in the same sub-family Faboidea. The grouping of *Vigna unguiculata* and *Daniella oliveri* together (in different sub-families) indicates that *Vigna unguiculata* may be the link between Faboidea and Caesalpinoidea. The clustering of *Senna siamea* and *Caesalpinia pulcherrima*is consistent with the report of Alege *et al.* (2014) which further confirms their close phylogenetic relationship and origin. Also, clustering of *Vigna subterrenae* and *Arachis hypogaea* in the same group (Fig. 2) further confirms the earlier report of Alege *et al.* (2014) that the 2 plant species are very close phylogenetically.

In this study, numbers of epidermal cells on theabaxial surface (Table 3) have taxonomic and evolutionary implications in Fabaceae because plants with epidermal cells less than 250 on the surface clustered together in the same group (Fig. 2). This observation conforms to the earlier report of Alege *et al.* (2013) that epidermal cell attributes on the abaxial surface are of great importance than stomata traits for unraveling the phylogenetic origin of plants. Also Aworinde and Fawibe (2014) reported that epidermal cells and presence of trichomes were of great significance in determining the relationships among some members of Caesalpinoidaea. The findings in this study are therefore relevant to phylogenetics and systematics of members of the family Fabaceae.

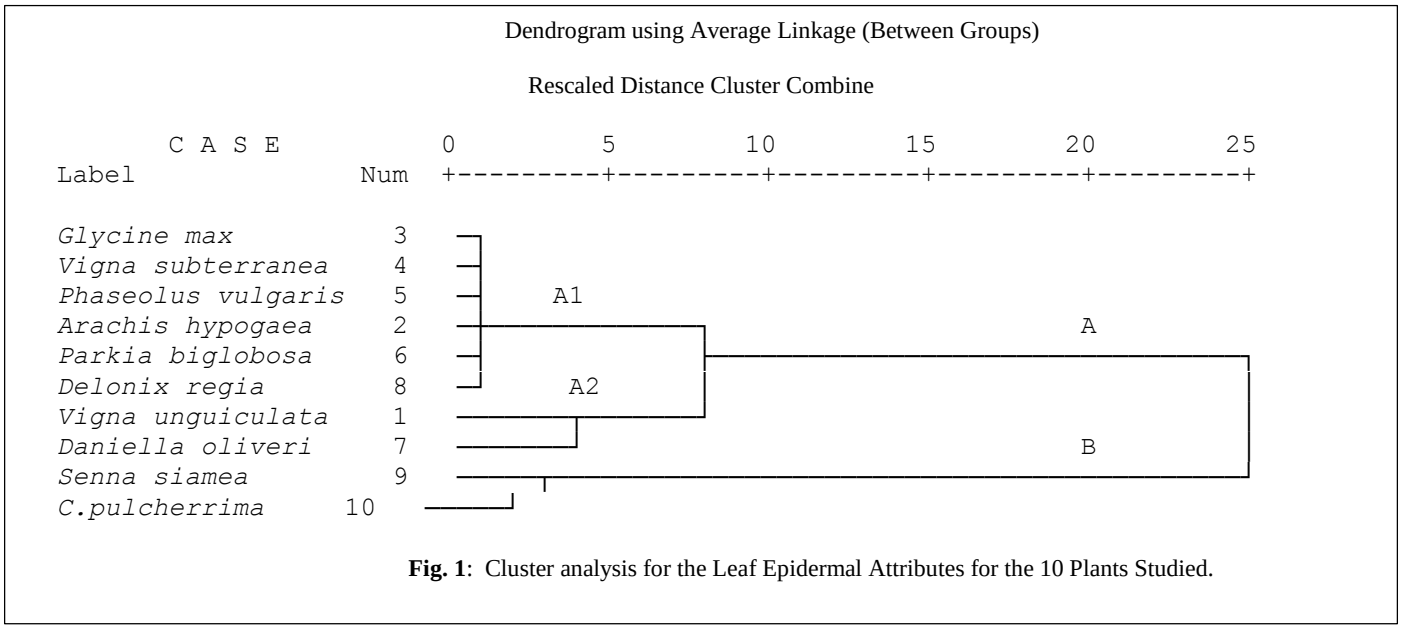


Fig. 1: Cluster analysis for the Leaf Epidermal Attributes for the 10 Plants Studied.

Conclusion and Receommendation

This study has revealed that hypo-amphistomatic leaf condition and paracytic stomata type among other characteristic are common features in Fabaceae which can be used for their delimitation from other families. The study also supported the polyphyletic origin of Fabaceae (especially evolution along two major lines) which therefore opposes the monophyletic origin proposed by some researchers. A larger number of species under the Fabaceae family should be considered from the perspective of leaf epidermal studies. Also, studies from other fields of Biology like cytology, palynology, phytochemistry and molecular biology are therefore recommended to complement the finding in this study.

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