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Full Length Research Paper

COMPARATIVE STUDY ON THE EPIDERMAL FEATURES OF TWELVE UNDER-UTILIZED LEGUME ACCESSIONS.

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Seeds of six species of twelve accessions of miscellaneous legumes were obtained from the germplasm unit of the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria. The seeds were planted into plots of 5m long, spaced 1 meter apart at the botanical garden of the University of Agriculture, Abeokuta (UNAAB), Ogun State, Nigeria.

Leaves of the plants were assessed for their genetic and phylogenetic relatedness through diagnostic epidermal studying of the cell shapes, anticlinal wall types and stomatal characteristics at the abaxial and adaxial surfaces. The cell shapes, anticlinal wall types and the stomatal characteristics revealed some correlations among the studied taxa. All the species were amphiostomatic possessing stomata on both the abaxial and adaxial surfaces. Proportions of the stomata distributions among the taxa were 75% (paracytic), 16.67% (anomocytic) and 8.3% (anisocytic) at both surfaces. There was no accession with diacytic stoma. Cell shapes were 75% polygonal, 25% irregular at the abaxial surfaces and 66.67%, 33.33% irregular and polygonal respectively at the adaxial surface. The prevalent anticlinal wall type was curved (41.67%) followed by curved/slightly straight (33.3%) and slightly straight (25%) at both surfaces. Result of the epidermal features of the studied taxa revealed some diagnostic characteristics that could be used for taxonomic decision.

Keywords: Epidermal features, underutilized legumes, phylogenetic, amphiostomatic, anomocytic, diacytic, anisocytic, polygonal.

INTRODUCTION

The term legume is applied broadly to all plants of the pea and bean family (Leguminosae) which comprises the Caesalpinaceae (Senna family), Mimosaceae (Locust bean family) and Pappillonacea (comprising about 10 tribes) (Flowering plants of the world, 1978; Apyanwu, 1981). Legume is a simple dry fruit formed from a superior monocarpus pistil; the pericarp dehisces at maturity longitudinally along both sides to liberate the seeds therein. The seeds are usually arranged along one of the margins of the fruits (Brenan, 1963).

The family Leguminosae embraces a large group of dicotyledonous plants having their fruits as pods, which may be round or flat, sometimes winged, straight or curved, of variable length, fibrous or fleshy and which often split open at maturity. The flowers are mostly complete and irregular. The calyx has five, more or less unequal and partially united sepals, and these flowers are usually hermaphroditic. The leaves are usually alternate, pinnately compound or trifoliolate (King, 1963).

Grain legumes have become a major component of grain – based farming systems in many parts of theworld. In terms of world economy and plant utility, grain

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legumes are grouped into two as major and minor species (Aykroye and Doughty, 1969). The major species include the industrial legumes such as soybean and groundnut, which are extremely important in the world economy. Others are common beans (*Phaseolus vulgaris),* chickenpea *(Cicer arietenum),* and pea *(Pisum sativum).* Minor species exist in a wide range of diversity either as cultivated or wild species across various regions of the world and are usually cultivated by the traditional farmers. The wild species of the minor grain legumes include kersting groundnut *(Kerstingiella geocarpa),* marama bean *(Tylosema esculentum)* (Aykroiye and Doughty, 1969). The minor grain legumes have also been referred to as miscellaneous, neglected, underutilized, under-cultivated or lesser – known legumes (Aremu et al., 2006; Omiogun et al., 2001, Adeboye, 2008).

The miscellaneous legumes are the minor grain legumes that have received very little research attention when compared with the major grain legumes such as cowpea and soybean. This neglect has lead to the loss or genetic erosion of the germplasm of many of the minor legumes. Most of the research efforts on miscellaneous legumes improvements have been on chemical composition and nutritional values. Majority of the representative miscellaneous legumes have not witnessed considerable research attention over the years (Bandoin and Mergeai, 2001). Consumption of legumes has been highly correlated with reduced mortality resulting from coronary heart disease (Aremu et al, 2006). Studies have also been carried out on the *invitro* multi-enzyme digestibility of flowers proteins of six varieties of African Yam bean (Oshodi et al., 1995; Okigbo, 1973; Evans and Boulter, 1974). Apata and Ologhobo, (1990) have also reported on the nutritional values of African yam bean.

The aim of the current study is to carry out detailed diagnostic leaf anatomical studies on six species of twelve accession of miscellaneous legumes including: Bambara groundnut (*Vigna subterranea*; accession numbers Tvsu1126 and TVsu1415)/ Mung bean(*Vigna mungo;* accession numbers TVm12 and TVm13), rice beans (*Vigna angularis;* accession numbers TVa1 and TVa1173),green gram (*Vigna radiata* ; accession number TVr145 and TVr1001), lablab bean (*lablab purpureus;* accession numbers TLn21 and TLn29) and Africa yam bean (*Sphenostylis stenocarpa);* accession number TSs137 and TSs156) with a view to revealing their diagnostic comparative leaf epidermal features that could be used for taxonomic grouping.

MATERIALS AND METHOD

Epidermal Peeling

A small portion of the fresh mature leaves of the species were obtained from the transverse section of each leaf with the aid of a razor blade. The leaf portions were thereafter soaked in concentrated solution of trioxonitrate (v) acid in embryonic dishes for a period of one hour. Appearance of air bubbles on the surface of the leaf fragments indicated their suitability for peeling. The leaf fragments were transferred into water contained in a petridish with a pair of forceps to strip off thin slices of epidermis of the leaves into water in a petridish. Upper and lower epidermises were carefully and completely isolated from the mesophyll using pair of fine forceps and dissecting needles. The epidermal peels were cleared with a camel hair brush and rinsed in distilled water for five minutes.

Staining

The epidermal peels were later stained in aqueous safranil for five minutes and each membrane was dehydrated by passing through different concentrations of alcohol (30%, 50%, 70% and 95%) for about 15 minutes each. After dehydration, the peels were later stained in aqueous fast green for three seconds and then cleared in xylene for about 15 minutes. The peels were then transferred into clove oil for 15 minutes and again cleared in xylene for another 15 minutes.

Mounting and Photomicrographying

The membranes were mounted on clean slides with DPX mountant. The slides labeled appropriately and examined under the light microscope. Good photographs of the micro-morphological features were taken at a magnification of X400 using a photomicroscope with installed digital camera optics.

RESULT

Results of the leaf epidermal features of the six species of twelve accessions of the miscellaneous legumes studied revealed similarities and differences both at the adaxial and abaxial surfaces. Summary of the cell shapes, anticlinal wall types and the stomatal characteristics with their percentages is presented in Tables 1 and 2

The most common stomatal type among the miscellaneous legumes studied was paracytic (75%), followed by anomocytic (16.67%), anisocytic (8.3%) and none was diacytic both at the abaxial and adaxial surfaces. The plants also possessed anticlinal wall types at both surfaces and in the same proportion. 41.67% had curved anticlinal wall, 25% were slightly straight while the rest (33.3%) were curved/slightly straight. The cell shapes of the studied taxa differed at both surfaces (adaxial and adaxial). 75% irregular and 25% polygonal

Table 1: Summary of Stomata, Cell Shape and Anticlinal wall types of the miscellaneous legumes studied (Adaxial)

	Characters	Accessions	Percentages
Stomatal type	Anomocytic	TVsu1126,TVsu1415	16.67
	Anisocytic	TVm13	8.33
	Paracytic	TVa1,TVa1173,TVm13,TLn29,TLn21, TSs137,TSs156, TVr145,TVr1001	75
	Diacytic	-	0
Cell shape	Irregular	TVa1,TVa1173,TVm12,TVm13, TLn21,TLn29,TSs137,TSs156, TVsu1415.	75
	Polygonal	TVsu1126,TVr145,TVr1001	25
Anticlinal	S.S	TVm13,TLn29,TVsu1415	25
	Curved	TVm12,TLn21,TSs137,TSs156,TVr1001	41.67
	Curve/ Slightly straight	TVa1,TVa1173,TVsu1126,TVr1001	33.33

Table 2: Summary of Stomata, Cell Shape and Anticlinal wall types of the miscellaneous legumes studied (Abaxial)

	Characters	Accessions	Percentages
Stomatal type	Anomocytic	TVsu1126,TVsu1415	016.67
	Anisocytic	TVm13	8.33
	Paracytic	TVa1,TVa1173,TVm13,TLn29,TLn21, TSs137,TSs156, TVr145,TVr1001	75
	Diacytic	-	0
Cell shap e type	Irregular	TVa1,TVm12,TVm13, TLn21,TLn29,TSs137,TSs156,TVsu1415	66.67
	Polygonal	TVsu1126,TVr145,TVr1001	33.33
Antic linal wall type	Slightly straight	TVm13,TLn29,TVsu1415	25
	Curved	TVm12,TLn21,TSs137,TSs156,TVr1001	41.67
	Curve/Slightly straight	TVa1,TVa1173,TVsu1126,TVr1001	33.33

were recorded at the abaxial surface while 41.7% irregular and 33.33% polygonal were observed for the cell shapes at the adaxial surface. The species and the accessions displayed similarities and differences in their epidermal characters. Species belonging to the same genera appeared to have common features which justify their classification into the same genus. TVsu,TVa and TVr of the genus *Vigna* all had paracytic stomata both at the abaxial and adaxial surfaces. Similarly, accessions of the same species were also found to be similar in their epidermal features display. TVa1, TVa1173 (*Vigna angularis),* TVr145, TVr1001 (*Vigna radiata),* TLn21, TLn29 (*Lablab purpureus) and* TSs137, TSs156 (*African yam bean)* had paracytic stomata; also TVsu1126 and TVsu1145 *(Vigna subterranea)* had anomocytic stomata. However, species of the same genera and accessions belonging to the same species also displayed at least little variability in their other epidermal features, which further lend credence to their classification as not being completely and entirely similar. Although, TVm12 and TVm13 are two accessions belonging to the same species, variabilities were observed in their epidermal features display. While TVm13 had paracytic stoma TVm12 had anisocytic stoma.TVm13 was observed to have slightly straight anticlinal wall while TVm12 had curved anticlinal wall. The plants revealed discernible similarities and differences that could be used for taxomonic decision. Structures of the various stomata types and cell shapes of the studied plants are presented in Figures 1, 2, 3, 4, 5, and 6.

Discussion

Result of the epidermal features of the studied taxa revealed some diagnostic characteristics that could be used for taxonomic decision. Features that separated the species and the accessions from one another is in line with the earlier works of Okwulechi and Okoli (1999); Edeoga and Emeka (2000). They both used comparative morphology of different species in establishing relationship among various taxa. The cell wall and cell shape with the stomatal type revealed correlations among the studied taxa. All the species were amphistomatic; possessing stomata on both the abaxial and adaxial surfaces. Only three accessions namely;

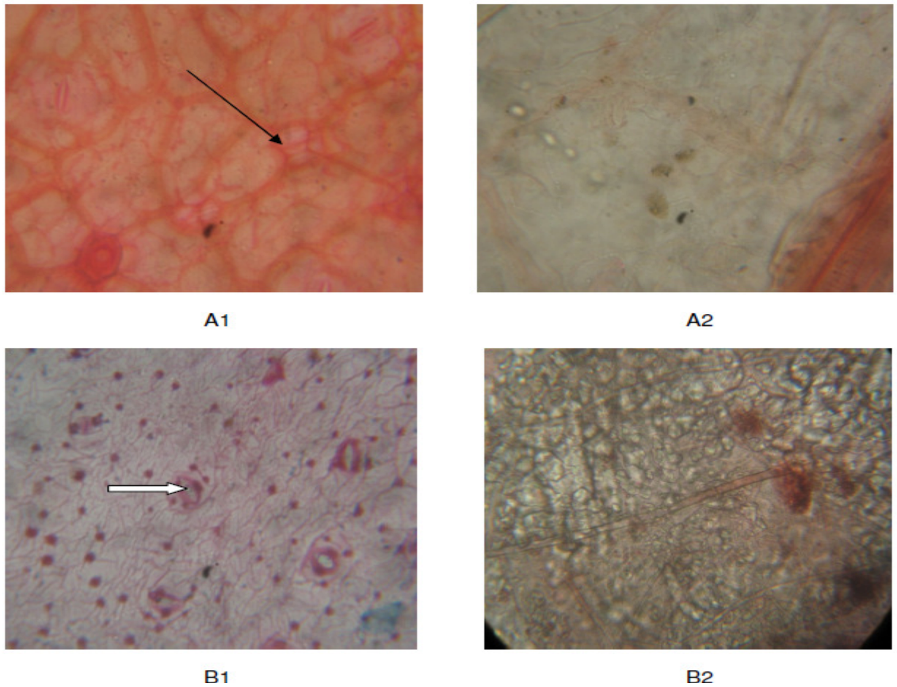

Figure 1: A1= Abaxial surface of (TVa1) showing paracytic stomata and irregular cell.
A2= Adaxial surface of (TVa1) showing paracytic stomata and polygonal cell.
B1= Abaxial surface of TVa1173) showing paracytic stomata and polygonal cell.
B2= Adaxial surface of TVa1173) showing paracytic stomata and polygonal cell.

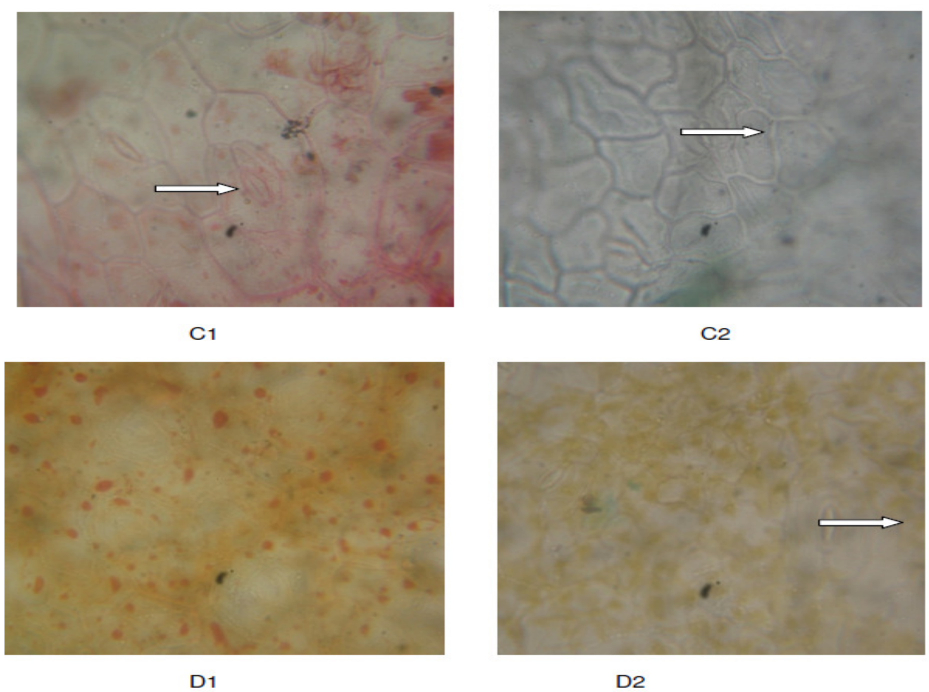

Figure 2: C1=Abaxial surface of (TVm12) showing anisocytic stomata and irregular cell.
C2= Adaxial surface of (TVm12) showing paracytic stomata and irregular cell.
D1=Abaxial surface of (TVm13) showing Paracytic stomata and irregular cell.
D2 =Adaxial surface of (TVm13) showing paracytic stomata and irregular cell.

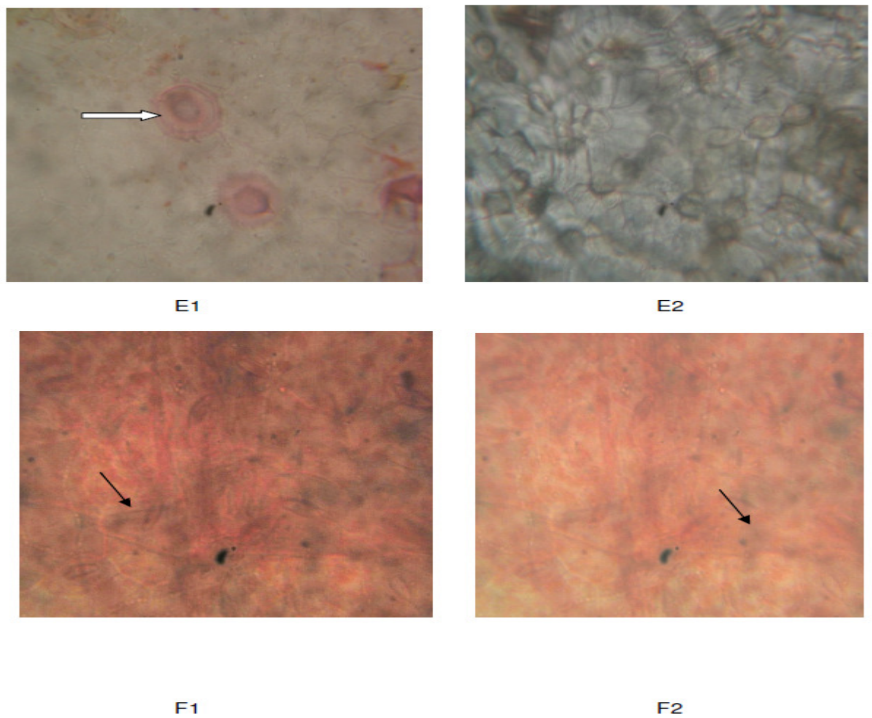

Figure 3: E1= Abaxial surface of (TLn21) showing anomocytic stomata and irregular cell.
E2= Adaxial surface of (TLn21) showing anomocytic stomata and irregular cell.
F1=Abaxial surface of (TLn29) showing paracytic stomata and irregular cell.
F2=Adaxial surface of (TLn 29) showing anomocytic stomata and irregular cell

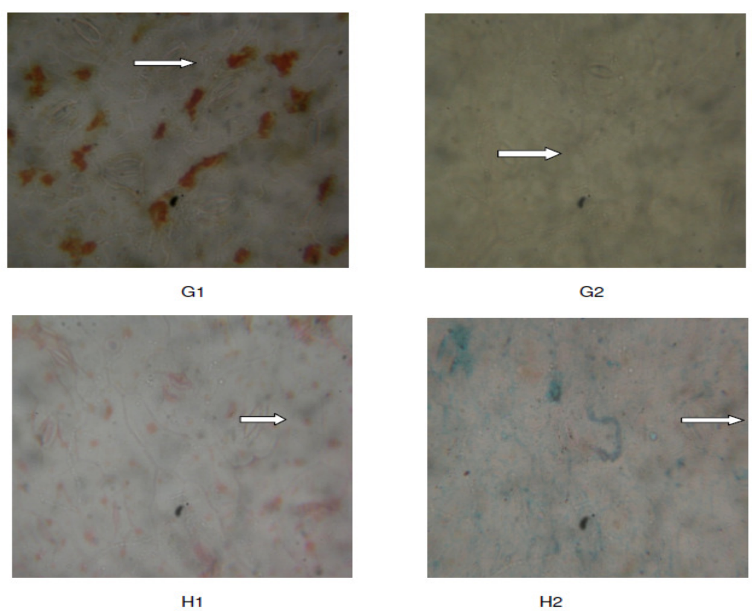

Figure 4: G1= Abaxial surface of (TSs137) showing paracytic stomata and irregular cell
G2=Adaxial surface of (TSs137) showing paracytic stomata and irregular cell.
H1=Abaxial surface of (TSs 156) showing anomocytic stomata and polygonal cell.
H2= Adaxial surface of (TSs156) showing anomocytic stomata and polygonal cell.

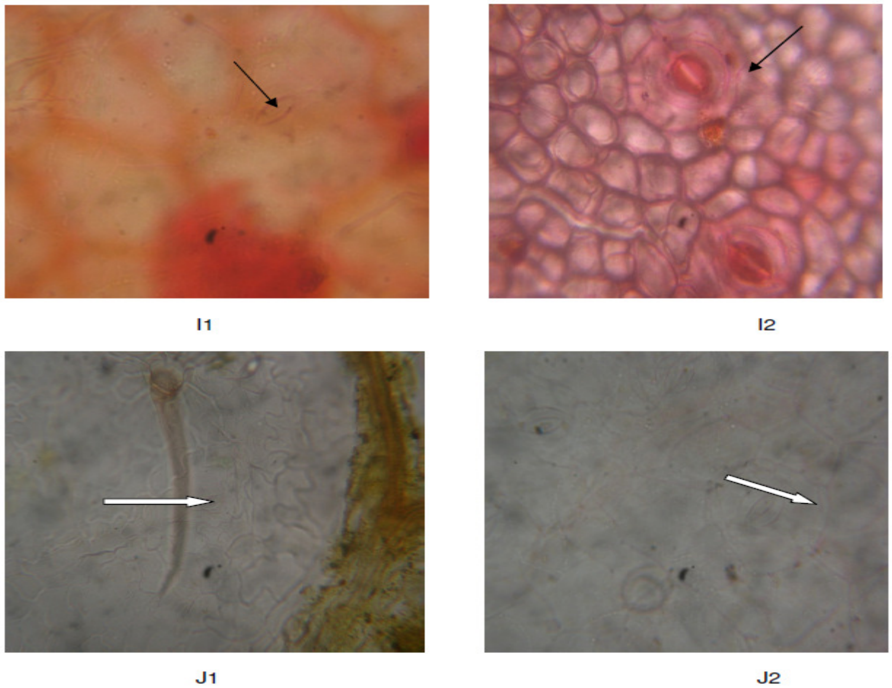

Figure 5:I1= Abaxial surface of (TVsu1126) showing paracytic stomata and irregular cell wall
I2= Adaxial surface of (TVsu1126) showing anomocytic stomata and polygonal cell wall
J1= Abaxial surface of (TVsu1415) showing paracytic stomata and polygonal cell wall
J2=Adaxial surface of (TVsu1415) showing paracytic stomata and polygonal cell wall

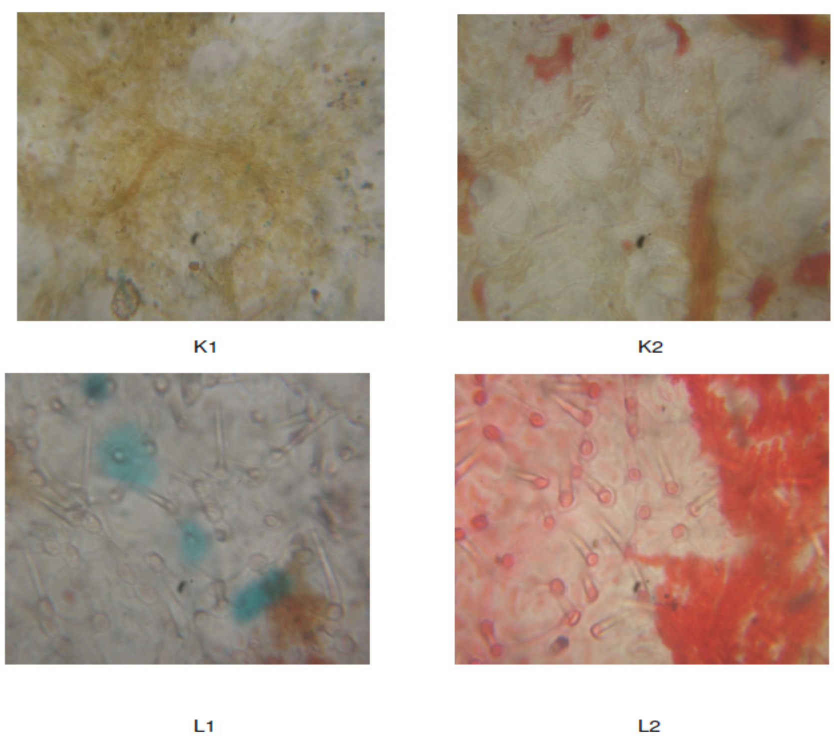

Figure 6:K1= Abaxial surface of (TVr145) showing paracytic stomata and irregular cell wall
K2= Adaxial surface of (TVr145) showing paracytic stomata and irregular cell wall
L1= Abaxial surface of (TVr 1001) showing anisocytic stomata and irregular cell wall
L2= Adaxial surface of (TVr 1001) showing anisocytic stomata and irregular cell wall

TVa1, TLn21 and TVr145 out of the twelve plants had trichomes on both surfaces. The possession of curved and slightly straight wall was associated with irregular cell shape. Stace (1965) suggested tat environmental conditions, such as humidity play a significant role in determining the pattern of anticlinal walls. The appearance of more stomata on the abaxial surface is probably and adaptation to water loss (Mbagwu et al., 2008). This is in agreement with Metacalfe and Chalk (1979); Mbagwu and Edeoga (2006) who both observed that stomata are usually more on the lower epidermis.

This study clearly presents characteristic epidermal features of six species of twelve accessions of miscellaneous legumes. Features observed are representatives of the genetic variabilities and similarities among these plants and could be used to establish relationship among the studied taxa.

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