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VARIABILITY STUDIES AS INFERRED FROM LEAF MORPHO-STOMATAL FEATURES IN *Moringa oleifera* LAM. FROM NORTHERN NIGERIA.

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ABSTRACT

The increasing demand for the Moringa oleifera product needs to be complimented with new improved cultivars with high performance to meet the supply chain of the country. To achieve this, the present study was aimed at studying the variability among 21 ecotype of M. oleifera based on morphology and leaf anatomy to detect promising cultivars for mapping out of future breeding schemes of this important crop. The generated data were analysed with the NTSYS pc software, and the 33 plant accessions were clustered into five groups irrespective of area of collection. Significant variations were observed in the leaf morphological and anatomical parameters of the accessions such that on clustering, region unspecific were observed in clusters I, II and III indicating a high possibility of exchange of genetic information between samples from far and closer geographical locations since they are not completely isolated. From the analysed accessions, 26BDMKT from north-west part of Nigeria forms a single cluster (IV) and gave the highest leaf length measurement of 61.2cm.

KEYWORDS: Leaf, Morphology, Stomata, Variation and *Moringa oleifera*

INTRODUCTION

The “Miracle tree” is a popular common name given to *Moringa oleifera* Lam., and it is receiving greater attention nowadays because of its multiple uses to humans and all other component of the ecosystem. It is a fast growing drought resistant shrub belonging to the family Moringaceae, originally from sub-Himalayan tracts of India. It grows very well in northern Nigerian states, producing flowers throughout the year even without irrigation. Moringa farming aimed at leaf production for human consumption, and is a highly profitable venture for farmers who have access to irrigation. They include monoculture at a high tree density on small surfaces and various agroforestry patterns, such as intercropping with cereals or vegetables, mixed orchards and live hedges around cotton fields. Many rural and semi-urban homes also grow the crop in backyards and on farmlands for boundary demarcation.

M. oleifera particularly in terms of vegetable source is usually tied to the tradition of its growing societies. Whereas in Asia the fruits or pods are the most important part for nutrition, the leaves (including some flowers) are preferred in Africa (Bosch, 2004). In Sudan the flowers are sometimes eaten as a vegetable, added to sauces or used to make tea. Generally, Fuglie (2001)

confirmed that leaves, flowers, roots and immature pods of the moringa tree are edible and they form part of tradional diets in many countries of the tropics and sub-tropics. As a source of nutrition, moringa leaves probably rank as the best of all tropical vegetable.It grows throughout the developing world and has already been used by programmes to reduce child malnutrition in Senegal (Fuglie, 2001) and India source. Available reports also confirmed the efficacy of *M. oleifera* seed powder in turbid water clarification for safe domestic use and consumption (Muyibi and Evison, 1995; Ndabiagengesere and Narasial, 1998; Foidl *el al*; 2001). This alternative source from natural moringa seeds is germane not only in ensuring supply of clean healthy portable water source but, even a panacea in improving developing world diverse monetary expenditures in other sectors of social responsibilities.

Since most of the seed sources of the crop commonly found in Nigeria were introduced, the need arises for collection of ecotypes to analyse genetic diversity, and select best provenances based on products-leaves, seeds, green pods etc. For the overall improvement of the crop, Bosch (2004) observed that apart from Indian breeding programmes, very little breeding has been achieved so far, and none in Africa. Moringa is a highly cross pollinated tree, and this leads to a high heterogeneity of forms and yields within each species (Fuglie, 2001). Saint Sauveur (2001) observed that moringa's genetic diversity is one of the many problems farmers encountered when they plant the seeds. Even in small farms, differences in growth rate, age at first fruiting and yield can make production difficult. On a larger scale such as in Tanzania and Nicaragua, heterogeneity causes high production costs as some trees must be removed and others are eliminated naturally by their competitors. Similarly, Nduwayezu *et al*. (2007) observed differences in performance i.e. survival, diameter and height of growth when *M.oleifera* seed kernel sizes and early growth parameters were compared from different parts of southeast Botswana.

According to Fred (2001), proximity in plants does not always equate with genetic similarity and species need to be considered on a case by case basis. Four major varieties on of *M.oleifera* were identified in Kenya based on colour and length of pod (Odee *et al.,* 2001). Makkar and Becker (1996) reported the existence of many different varieties of *M .oleifera* whose kernels taste from sweet to very bitter. Muluvi *et al.* (2004) recommended the need to define seed source in order to design planting schemes for maximum cross-fertilization among unrelated clones and minimize selfing among related ramets. Based on all these observations, the aim of this work is to study the variations in morphological and anatomical features of some accessions of *M. oleifera* from northern Nigeria with a view of its future improvement.

MATERIALS AND METHODS

Collection of Plant Material and Morphological Studies

Plant leaf materials were collected during field trips at the locations listed in Table 1, all in Sudan and Guinea savannah of northern Nigeria between March, 2007 and February, 2008.

Measurements of foliar morphological features were made based on the procedures of Radford *et al*. (1974). Mean values of the morphological characters measured were calculated and standard error determined.

Anatomical studies

For anatomical studies, fresh leaves were fixed for 24 hrs in formalin-acetic-acid (FAA) and preserved in 70% ethanol. Peels were obtained using forceps with the fixed material in a water-filled petri dish, cleared with a camel hair brush and rinsed in distilled water for 5 mins. The resultant epidermal peels were mounted on clean slides stained with safranin solution and mounted in glycerine (Hilu and Randall, 1984) then, examined under an Olympus Light Microscope (HSC 447591 Model). Stomatal length and width were measured at x40 objective of the light microscope as described by Baker and Silverton (1982) using an average of 50 randomly selected guard cells.

The leaf morphological features and stomatal measurements were then transformed using the resample module of the software, STANDARDISED and analysed using the SIMINT module of the NTSYSpc vs 2.2e software package (Rohlf, 2009). This analysis generated a dissimilarity matrix of the individual stomatal variation of each of the operational taxonomic unit (OTU) with respect to one another. The matrix values from the stomatal characters were then clustered at a cophenetic correlation of the distance matrix and a tree constructed based on the unweigheted pair group method with arithmetic mean (UPGMA).

Table 1: *Moringa oleifera* accessions acquired and their source

S. No.	Accession	Place of collection (acquisition)	GPS
1.	JHJG	Jahun, Jigawa State, Nigeria (2)	12º06'N 9º25'E
2.	GWKB	Gwandu, Kebbi State, Nigeria (2)	12º16'N 6º33'E
5.	KFKD	Kafanchan, Kaduna State, Nigeria (2)	11º34'N 8º18'E
6.	GZKN	Gezawa, Kano State, Nigeria (1)	12º14'N 8º04'E
7.	TWKN	Tudunwada, Kano State, Nigeria (2)	12º01'N 8º34'E
8.	DMKT	Dutsin-ma, Katsina State, Nigeria (2)	12º27'N 7º29'E
9.	KFKT	Kafur, Katsina State, Nigeria (2)	11º39'N 7º42'E
10.	DDSK	Dogondaji, Sokoto State, Nigeria (1)	12º06'N 6º19'E
11.	GRSK	Goronyo, Sokoto State, Nigeria (1)	13º27'N 5º40'E
12.	BDZM	Bodinga, Zamfara State, Nigeria (2)	11º58'N 9º97'E
13.	WNZM	Wanzamai, Zamfara State, Nigeria (2)	15º18'N 6º92'E
14.	NMAD	Numan, Adamawa State, Nigeria (3)	9º26'N 12º18'E
15.	JMBA	Jamaare, Bauchi State, Nigeria (2)	11º38'N 9º52'E
16.	BAU	Bauchi, Bauchi State, Nigeria 2)	10º30'N 10º0'E
17.	GMB	Gombe, Gombe State, Nigeria (1)	10º17'N 11º10'E
18.	KGGM	Kaltingo, Gombe State, Nigeria (1)	9º38'N 11º05'E
19.	MLTR	Malobe, Taraba State, Nigeria (2)	7º47'N 10º13'E
20.	MMYB	Mamado, Yobe State, Nigeria (2)	11º42'N 11º05'E
21.	DMT	Damaturu, Yobe State, Nigeria (1)	12º53'N 11º13'E

RESULTS AND DISCUSSION

A total of five clusters were revealed as in (Fig. 1), with cluster I comprising of twelve OTUs which specifically identified with (15.2 - 32.08) µm stomatal length on their upper surfaces. Cluster III is represented with the highest number of 18 OTUs making it possible that proximity in terms of area of collection of these samples may account for their clustering in a group with the likelihood of pollen transfer within few distances to ensure uniformity in genetic traits. Notably, two of the clusters i.e. IV and V being region specific, comprising of individual OTUs collected from the north-western States of Katsina and Bauchi respectively. The later had the highest leaf length (61.2cm).

The leaf epidermal peels of all the accessions as observed under the light microscope indicated the appearance of anomocytic stomata and unicellular trichomes, confirming the earlier work of Gill *et al.* (1985). Anomocytic stomata are usually surrounded by four or more subsidiary cells that are similar in structure with other common epidermal cells (Fig. 2). Distribution of these

stomata in all the accessions is amphistomatic in which stomatal presence were on both the adaxial and abaxial surfaces. Anomocytic stomata were common to closest ancestors (i.e. extinct genus of *Dressiantha*) of moringaceae within the brassicales clade (Soltis *et al.,*2000). This may confirm the fact that phylogenetically, moringaceae were actual relatives of the brassicaceae since stomatal complexes are produced by the direct influence of the genetic information of the parentage lineage. Flint and Moreland (1946) also observed the pattern of stomatal distribution and structures features which differed among varieties of sugar cane, as a result of growth rate, turgidity of tissues, exposure to light etc.

Epidermal cell wall pattern shows variation both at intra and inter accession levels, for instance, accession 10GWKB has straight to sinous wall patterns on adaxial and abaxial surfaces. While accession 66MLTR has straight to undulate wall patterns on their surfaces respectively (Figs. 2a, b and c, d). However, straight to undulate were observed on the surfaces of 29KFKT (Figs. 2e, f). Individual measurements of stomatal characters of all the OTUs are shown in Table 3, indicating the average values and the standard error using ocular micrometer. The lowest epidermal cell width (14.00µm) is shown by the abaxial surface of 53JMBA and the highest (34.80µm) by 29KFKT on its adaxial surface. Foliar epidermal characters of *Cola* species from Nigeria were analysed (Goji and Ayodele, 2005) using mature stomatal types, epidermal cell width, anticlinal cell wall pattern as well as cell shape, and these were able to differentiate the plants at species level. From our findings, the epidermal cell wall not only varied among accessions from same climatic zone but, even among samples from the same location.

In conclusion, the accessions from Dutsin-ma, Katsina State (26DMKT) showed the highest leaf length (61.2cm) and represented by a single cluster (IV) in the dendrogram (Fig. 1). Other variable parameters were also shown by the different accession analysed confirming the existence of variability among these *M. oleifera* samples from northern Nigeria. Thus, chances now exist that the crop may likely be improved after mapping out appropriate breeding schemes.

Table 2: Leaf morphological features measurements (±SE) of the *M. oleifera* accessions

S/no.	Accession no.	Leaf length (cm)	Leaflet length (mm)	Leaflet width (mm
1.	4JHJG	50.6±1.15	1.65±0.035	1.36±0.520
2.	6JHJG	34.7±1.08	2.11±0.040	1.08±0.048
3.	10GWKB	51.6±1.46	2.43±0.070	1.20±0.420
4.	11GWKB	52.1±1.79	2.14±0.096	1.37±0.500
5.	13KFKD	44.8±1.87	1.86±0.083	1.63±0.480
6.	15KFKD	42.3±1.63	1.98±0.086	1.35±0.015
7.	17GZKN	46.3±1.15	1.99±0.088	1.12±0.059
8.	20TWKN	50.2±2.11	2.11±0.012	1.03±0.250
9.	21TWKN	48.3±1.81	1.85±0.065	1.40±0.016
10.	24DMKT	45.8±1.37	2.53±0.125	1.36±0.059
11.	26DMKT	61.2±1.95	2.77±0.195	1.50±0.083
12.	28KFKT	40.6±1.26	2.01±0.015	1.06±0.350
13.	29KFKT	38.7±1.93	2.34±0.030	1.71±0.590
14.	31DDSK	49.7±1.05	1.96±0.017	1.08±0.058
15.	33GRSK	40.6±1.25	1.86±0.310	1.09±0.071
16.	36BDZM	53.0±1.92	2.03±0.124	1.48±0.105
17.	37BDZM	39.5±1.25	2.35±0.950	1.06±0.056
18.	40WNZM	44.8±1.56	1.93±0.032	1.07±0.210
19.	41WNZM	36.7±1.09	1.58±0.540	1.29±0.380
20.	43NMAD	48.2±1.24	1.90±0.413	1.63±0.025
21.	47NMAD	49.2±1.52	2.08±0.157	1.41±0.301
22.	48NMAD	50.7±1.80	2.41±0.306	1.33±0.045
23.	52JMBA	50.6±1.43	1.06±0.073	1.41±0.310
24.	53JMBA	55.2±1.31	1.83±0.450	1.06±0.063
25.	55BAU	58.4±1.08	1.45±1.030	1.02±0.093
26.	56BAU	52.7±1.93	1.08±0.069	1.11±0.084
27.	61GMB	50.5±1.46	1.04±0.810	1.17±0.411
28.	64KGGM	38.4±1.82	1.63±0.043	1.56±0.132
29.	66MLTR	48.0±1.81	2.35±0.075	1.41±0.064
30.	67MLTR	39.8±1.73	1.70±0.130	1.55±0.049
31.	70MMYB	37.5±1.21	2.05±0.081	1.21±0.421
32.	72MMYB	52.3±1.09	1.47±0.093	1.83±0.092
33.	74DMT	50.5±1.53	1.75±0.340	1.21±0.350

Table 3: Stomatal measurements of the *M. oleifera* accessions

Accession no.	Surface	ECW (µm)	SL (µm)	SW (µm)	SI	ECWP
4JHJG	U	22.1±0.59	29.3±0.59	24.8±0.49	6.02	st
	L	14.5±0.57	25.0±0.48	19.8±0.42	22.11	un
6JHJG	U	22.0±0.62	35.3±0.58	24.8±0.51	2.29	st
	L	15.0±0.67	28.9±0.80	20.2±0.40	20.1	un
10GWKB	U	26.9±0.68	31.3±0.78	25.8±0.44	4.08	st
	L	17.9±0.54	30.0±0.64	20.9±0.44	23.3	st
11GWKB	U	22.8±0.69	29.5±0.51	25.8±0.49	3.08	st
	L	15.0±0.47	26.1±0.46	20.8±0.39	25.00	st
13KFKD	U	30.7±0.50	22.3±0.51	24.7±0.73	3.06	st
	L	27.9±0.53	19.4±0.32	14.5±0.56	13.17	un
15KFKD	U	27.6±0.41	22.6±0.51	19.5±0.48	5.17	st
	L	19.8±0.70	20.1±0.44	15.6±0.21	19.08	st
17GZKN	U	27.1±0.58	22.2±0.43	22.9±0.83	2.06	st
	L	24.1±0.60	24.1±0.44	14.0±0.44	25.89	un
2OTWKN	U	28.4±0.48	24.1±0.53	18.7±0.48	2.76	st
	L	27.0±0.56	22.3±0.43	14.0±0.60	25.25	s
21TWKN	U	28.3±0.54	26.9±0.46	27.1±0.70	6.08	st
	L	26.7±0.78	21.2±0.67	15.9±0.73	23.67	st
24DMKT	U	32.8±0.65	26.3±0.40	22.0±0.71	2.69	st
	L	26.0±0.61	19.7±0.41	15.5±0.41	21.19	s
26DMKT	U	30.6±0.42	24.3±0.32	18.8±0.60	4.54	st
	L	26.9±0.81	20.1±0.25	16.0±0.52	22.71	un
28KFKT	U	30.3±0.85	24.5±0.40	22.3±0.83	2.06	st
	L	28.9±0.70	20.1±0.53	13.4±0.41	21.91	un
29KFKT	U	34.8±0.74	25.9±0.46	25.1±0.70	3.06	st
	L	26.6±0.53	21.8±0.45	14.9±0.42	24.97	un
31DDSK	U	31.5±0.41	23.6±0.51	24.7±0.67	2.73	st
	L	18.0±0.79	19.4±0.42	15.3±0.88	24.01	st
33GRSK	U	31.8±0.73	23.9±0.48	23.1±0.60	2.27	st
	L	28.0±0.57	20.3±0.44	14.9±0.52	23.67	un
36BDZM	U	25.3±0.45	24.9±0.56	24.3±0.56	2.24	st
	L	18.8±0.40	21.6±0.36	20.2±0.48	26.07	st
37BDZM	U	26.4±0.49	24.6±0.54	21.7±0.70	4.35	st
	L	21.4±0.44	18.6±0.58	19.1±0.64	20.91	un
40WNZM	U	28.8±0.62	23.9±0.39	24.6±0.42	6.46	st
	L	24.8±0.48	20.3±0.56	19.6±0.44	22.59	st
41WNZM	U	26.2±0.39	24.2±0.70	25.0±0.71	2.91	st
	L	18.2±0.52	21.5±0.54	18.4±0.39	20.87	st
43NMAD	U	26.6±0.37	22.1±0.36	24.4±0.56	4.75	st
	L	23.1±0.51	19.1±0.48	20.6±0.49	23.28	s
47NMAD	U	21.6±0.64	24.3±0.70	22.4±0.44	2.25	st
	L	20.1±0.48	21.6±0.48	18.0±0.58	22.91	st

Table 3: *Continued*

Accession no.	Surface	ECW (µm)	SL (µm)	SW (µm)	SI	ECWP
48NMAD	U	18.6±0.65	30.0±0.76	22.3±0.49	3.05	st
	L	16.1±0.61	24.0±0.75	20.7±0.76	21.21	s
52JMBA	U	24.3±0.58	26.5±0.62	22.6±0.36	5.33	st
	L	20.6±0.49	24.1±0.59	19.5±0.48	23.89	st
53JMBA	U	16.9±0.68	30.2±0.82	25.1±0.37	4.47	st
	L	14.0±0.54	23.5±0.72	18.8±0.56	24.05	st
55BAU	U	20.6±0.63	18.0±0.48	17.0±0.56	2.67	st
	L	19.6±0.58	16.6±0.48	15.8±0.56	24.85	s
56BAU	U	22.8±0.61	28.3±0.46	23.9±0.46	5.50	st
	L	14.6±0.59	27.0±0.78	19.8±0.52	24.09	st
61GMB	U	22.0±0.58	29.9±0.64	22.2±0.71	4.45	st
	L	15.4±0.62	28.5±0.57	20.2±0.44	22.31	un
64KGGM	U	22.4±0.56	26.5±0.67	24.8±0.44	4.23	st
	L	17.8±0.64	25.3±0.34	20.5±0.48	24.27	st
66MLTR	U	27.5±0.52	25.1±0.70	26.6±0.64	2.85	un
	L	18.3±0.72	23.2±0.68	20.5±0.52	24.45	un
67MLTR	U	25.1±0.68	28.8±0.68	25.0±0.42	6.81	st
	L	15.5±0.50	25.7±0.85	21.3±0.58	24.76	un
70MMYB	U	18.7±0.58	24.3±0.44	21.2±0.62	2.28	st
	L	17.0±0.66	21.5±0.80	19.1±0.68	24.36	st
72MMYB	U	21.3±0.55	29.5±0.77	23.6±0.59	2.66	st
	L	18.5±0.36	24.4±0.45	19.1±0.39	23.29	s
74DMT	U	26.4±0.56	21.7±0.40	24.8±0.56	3.34	st
	L	20.5±0.60	19.1±0.59	20.1±0.70	23.97	st

KEY:
ECW - epidermal cell width
SL - stomatal length
SW - stomatal width
SI - stomatal index
ECWP- epidermal cell wall pattern

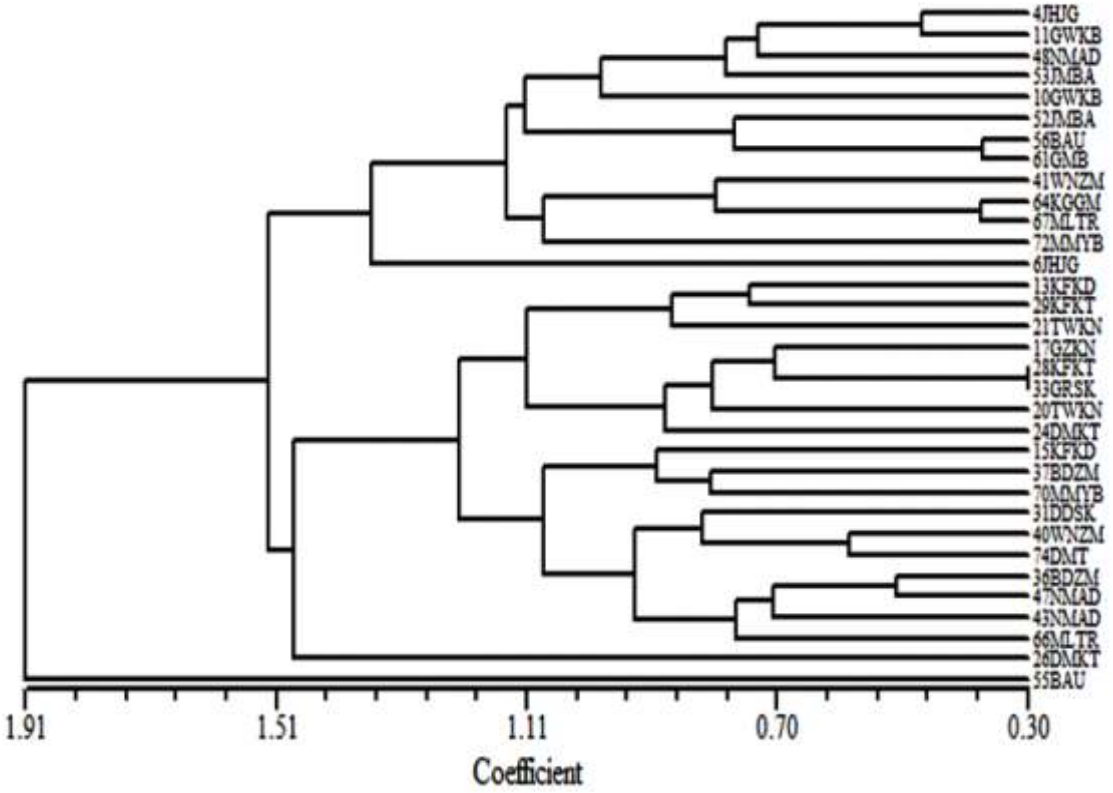

Figure 1 Cluster analysis of *Moringa oleifera* samples from northern Nigeria on Jaccard dissimilarity index using SAHN module

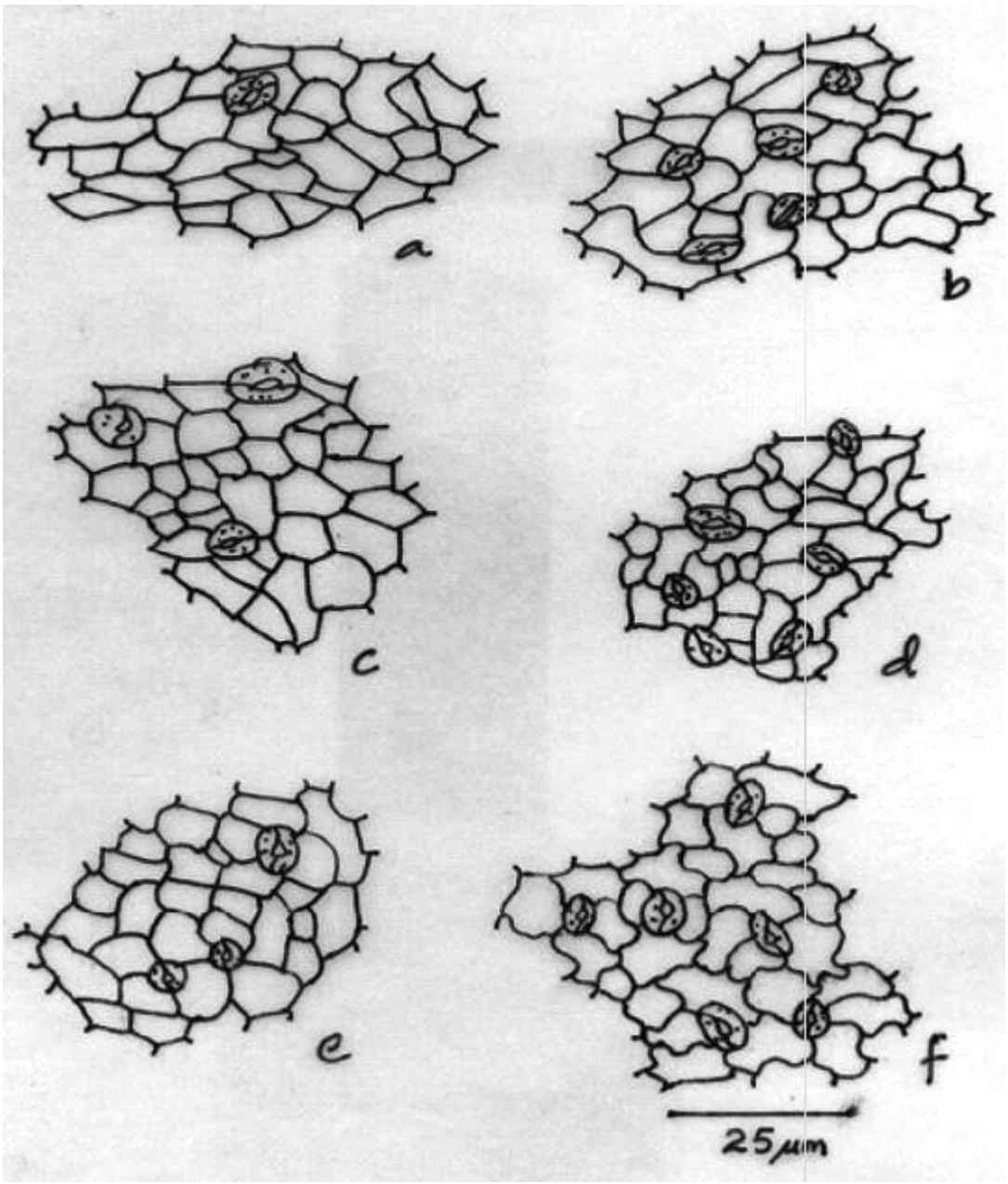

Figure 2. Leaf epidermal features showing anomocytic stomata in upper and lower surfaces of (a and b) 10GWKB (c and d) 66MLTR (e and f) 29KFKT

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