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Morpho-anatomical variation in some accessions of *Moringa oleifera* Lam. from Northern Nigeria

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Variations in the leaf morphological and anatomical features of *Moringa oleifera* Lam. accessions from Northern Nigeria have been reported. The wide range of uses of this naturalised crop in Nigeria has in recent time witnessed increasing demand of its foliar product in nutritional, medical and ecological applications. The upsurge demand for this product needs to be balanced with new lines of improved performance to meet the supply chain of the country and beyond. To achieve this, the present study was aimed at setting the foundation of variability analysis based on morphology and anatomical features of the leaf to detect promising cultivars for mapping out of future breeding schemes of this multi-purpose crop. The generated data were analysed with NTSYS pc software, and the plant accessions clustered into six groups irrespective of area of collection. Out of these accessions, 16BAU from north-east part of Nigeria gave the highest leaf length measurement of 60.3 cm while 18 KGGM had the lowest value of 35.3 cm and each were formed in separate clusters of IV and III respectively.

Key words: Leaf, morphology, stomata, variation, *Moringa oleifera.*

INTRODUCTION

Moringa oleifera Lam. commonly named horse-radish tree, drumstick tree, mothers best friend, Indian ben etc, is one of the thirteen known species belonging to the family Moringaceae with only one genus *Moringa*. It is a fast growing drought resistant tree or shrub. It originated from sub-Himalayan tracts of Northern India, distributed worldwide in the tropics and sub-tropic (Olson, 2002). The Moringaceae is one of the 15 families that produce mustard oil (glucosinolates), and related to other mustard oil plants like Brassicaceae, Caricaceae etc (Chase et al., 1998). In West Africa, the family is represented by 10 species whereas in Nigeria the plant is represented by the only species of *M. oleifera* (Keay, 1989).

M. oleifera is a multi-purpose plant with a tremendous variety of potential uses, and recently attracted the attention of several authors (Tsaknis et al., 1999; Olson, 2002; Muluvi et al., 2004). According to Fuglie (2001) it is used in different ways that improves man and his environmental well being. These uses include in alley – farming, animal forage, vegetable, biogas, dye, honey clarifier, medicine, ornamental, pulp, water purification, edible oil (Tsakins et al., 1999; Makkar and Becker, 1996; Foidl et al., 2001; Folkard et al., 2001; Bosch, 2004). In recent years the leaf of this plant with its rich micronutrient contents like zinc, iron and vitamin A has been used in treating malnutrition in children and improving the diets of lactating mothers in some African countries. Being a local resource, its use in checking child undernutrition would mean saving the meagre African financial resources in counter funding which depend on imported solutions. The oil extracted from the seed is used in lubricating delicate machines, cosmetics, perfume and pharmaceutical industries. Anbarassan et al. (2001) recently looking at the possibility of using the ben oil (up to 30 to 42% per seed kernel) as a bio-fuel considering the present high cost of crude oil in the International markets. In water purification, Foidl et al. (2001) reported that up to 99% of colloids can be removed from dirty (turbid) water and works more effectively than the imported alum (aluminium sulphate). Higher turbidity levels are associated with high levels of water borne disease–causing microorganisms Yakasai (2008) observed that the recent International food crisis

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Table 1. Locations of *M. oleifera* accessions measured for anato-morphological traits.

S/no	Accession code no	Location	Latitude	Longitude
1	1DTJG	Dutse, Jigawa State	11º44'N	9º17'E
2	2JHJG	Jahun, Jigawa state	12º06'N	9º25'E
3	3ARKB	Argungun, Kebbi State	12º26'N	4º40'E
4	4KFKD	Kafanchan, Kaduna State	11º34'N	8º18'E
5	5ZRKD	Zaria, Kaduna State	11º09'N	7º39'E
6	6GZKN	Gezawa, Kano State	12º14'N	8º04'E
7	7TWKN	Tudun-wada, Kano State	12º01'N	8º34'E
8	8DMKT	Dutsin-ma, Katsina State	12º27'N	7º29'E
9	9KFKT	Kafur, Katsina State	11º39'N	7º42'E
10	10DDSK	Dogon daji, Sokoto State	12º06'N	6º19'E
11	11GRSK	Goronyo, Sokoto State	13º27'N	5º40'E
12	12BDZM	Bodinga, Zamfara State	11º58'N	9º97'E
13	13WNZM	Wanzamai, Zamfara State	15º18'N	6º92'E
14	14NMAD	Numan, Adamawa State	9º26'N	12º18'E
15	15JMBA	Jama'are, Bauchi State	11º38'N	9º52'E
16	16BAU	Bauchi, Bauchi State	10º30'N	10º0'E
17	17GMB	Gombe, Gombe State	10º17'N	11º10'E
18	18KGGM	Kaltungo, Gombe State	9º38'N	11º05'E
19	19MLTR	Malobe, Taraba State	7º47'N	10º13'E
20	20MMYB	Mamado, Yobe State	11º42'N	11º05'E
21	21DMT	Damaturu, Yobe State	12º53'N	11º13'E

that anchored principally on biofuels, rising food prices, global warming and food production with consequent community concentration on Moringa leaf affordability, nutrition value and availability. Also, the raw materials research and development council of Nigeria (RMRDC) having realise the potentials of this crop, suggested during a national conference on the crop (November, 2010) the need to define the taxonomic attributes of the crop for its improvement. Bosch (2004) observed that apart from Indian breeding programmes, very little breeding has been achieved so far. He further observed that no breeding work has been undertaken in Africa.

In Nigeria with wide range of uses of moringa products, most research efforts are focused mainly on medicine (Ezeamuzie et al., 1996), anatomical identification of plant fragment (Jayeola, 2010), moringa anti-viral activity (Okoye et al., 2010) moringa seed and water treatment (Aho and Agunwamba, 2010) but, not targeted towards the improvement of this important plant. A prerequisite of any genetic improvement programme is a focus in morphological, anatomical and genetic variability in the local germplasm so that breeding strategies can be mapped out. Morphological and anatomical characters of plants have been used by many authors in plant identification (Sapir et al., 2002; Agbagwa and Ndukwu, 2004; Noraini and Cutler, 2009; Soladoye et al., 2010; Sharma et al., 2010). Taxonomic identification has been the basis on which plant breeding effort are founded such that diagnostic characters are assigned to specific or varietal parentage. In the light of the above fact, the present study was conducted to analyze morphological and leaf anatomical features of some accessions of *M. oleifera* with a view of setting the foundation for the improvement of this multi-purpose crop for improved foliar yield.

MATERIALS AND METHODS

Collection of plant material and morphological studies

Plant leaf materials were collected during field trips to the locations listed on Table 1, all in Sudan and Guinea savannah of northern Nigeria between March, 2007 and February, 2008. In foliar morphological studies, characters of whole leaf length (cm), leaflet length (mm), leaflet width (mm) and leaflet area (mm^2) were recorded using 50 randomly selected foliar materials from two plant stands in a population of each accession as suggested by Radford et al. (1974). Mean values of the morphological characters measured were calculated and standard error determined.

Anatomical studies

For anatomical studies, fresh leaves was fixed for 24 h 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 min. 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). Camera lucida drawings were made from the epidermal peels taken from the FAA-fixed materials. Stomatal length and width were measured at ×40 objective of the light microscope as described by Baker and Silverton (1982) using an average of 50 randomly selected guard cells.

Both the morphological and anatomical data were subjected to analysis using the NTSYS-pc vs 2.2e software package which provides an effective method for multivariate data analyses (Rohlf, 2009). The data were standardised and a SIMINT study was performed and analysed with SAHN module to compute the various association between the plant samples in this study.

RESULTS

The leaf morphological features of the *M. oleifera* accessions from northern Nigeria are shown in Table 2. The highest leaf length range ((58.85-61.75) cm and leaflet length (34.55-37.25) cm were recorded by 16BAU and 5ZRKD respectively, while the highest leaflet width (1.85-1.87) mm by 12BDZM. The accession showing the highest leaf area in all the samples studied was 2JHJG (3.45 mm^2). The accession collected from Argungun in Kebbi State (3ARKB) gave the highest stomatal length (30.8 µm) on its adaxial surface, while lowest stomatal width was shown by 6GZKN (14.1 µm) on its abaxial surface. The highest and lowest epidermal cell width of 32.6µm/16.8 µm in the adaxial/abaxial surfaces was found in 16BAU and 2JHJG as well 3ARKB respectively. The epidermal wall pattern shows considerable variations ranging from straight to undulate or deeply sinuous among the accessions studied. For instance, the adaxial surfaces of samples from Dutse, Jigawa State (Figure 2a and b) are straight walled while their abaxial surface are sinuous and undulate respectively. Straight and undulate epidermal cell walls were also observed in the accession from Bauchi (16BAU) in addition to having the highest leaf length among all other accession (Figure 2g and h). A remarkable distinguishable feature of seed wing striation was found only in accession 10DDSK in addition to the possession of straight epidermal cell wall patterns on upper and lower leaf surfaces respectively (Figure 2e and f).

The analysis of the values of morpho-anatomical features of the accessions from northern Nigeria with NTSYS-pc software based on UPGMA method indicated six cluster groupings (Figure 1). Cluster I is comprised of 1DTJG, ARKB, 2JHJG, 17GMB and 20MMYB, while cluster II constitute accession number 5 from Zaria. Cluster III constitute the group with the highest number of accessions totalling 12 while one accessions each makes up cluster V and VI. It was observed that the grouping does not take into account the variations in area of collection of the samples studied.

DISCUSSION

The general appearance of the leaves of *M. oleifera* in all of the accessions as alternate, composite, bipinnate, with 2 to 6 pairs of opposite pinnae bearing opposite leaflet in 3 to 5 pairs suggest it to be an inherent character. Similarly, possession of terminal leaflet that is larger, more or less glaborous, ovate to elliptic, rounded at apex and base, with the petiole being pubescent and generally with mean leaf sizes considerably variable even among samples from same location, agrees with the descriptions of (Verdcourt, 1982; Keay, 1989; Arbonnier, 2004).

Leaf morphological and anatomical sizes after clustering as in this work have shown region non-specificity groupings in all clusters, indicating a high possibility of exchange of genetic information between samples since they are not completely isolated. It was also a known fact that *M. oleifera* is both selfed and cross pollinated, with the former ensured by wind. Since these accessions where from northwestern and northeastern Nigeria and grouped together, may suggest the possibility of human transfer of seed source across states. The implication of these, suggest a basis of morphological differences exhibited by the accessions albeit the fact that they are collected from different geographical locations.

Changes in plant morphological features in terms of variation have been attributed to small-scale evolutionary processes, a view supported by the works of Serebrayanaya and Shipunov (2009) in different species of plants. In addition to this, leaf size variation in taxonomy forms a basis for evolutionary changes in plants; also it can modify the distribution of leaf biomass between support and functional tissues (Xu et al., 2008). Plants are generally agreed to have high phenotypic plasticity, and therefore one may not exclude the possibility of the observed differences in terms of leaf sizes being caused by direct influence of environmental conditions. But, some authors (Linhart and Grant, 1996; Widen and Schienmann, 2006) observed that phenotypic plasticity from differences in morphological sizes can coincide with genetic variation.

The mature stomatal complex in all of the accessions were anomocytic with amphistomatic distribution, confirming the earlier work of Gill et al. (1985) who reported similar stomatal morphology. Appearance of these stomata was at the same level with the adjacent epidermal cells. However, variation in terms of stomatal sizes exists between the accessions as they have been collected from different geographical locations. 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. Similar seed source from close pedigree may also account for same clustering as it is easy to transfer them for cultivation to the nearest farmlands than distantly located remets. Recently (Maghsoudi and Maghsoudi, 2008) reported that variation in stomatal characteristic was found to correlate with variation of transpiration and yield of *Triticum aestivum*.

The variability of epidermal cell wall patterns may be used to differentiate these accessions of *M. oleifera* from northern Nigeria. Gill and Nyawuame (1990) were able to established phylogenetic relationships among the

Table 2. Means (and standard error) of the morpho-antomical features of the accession of *M. oleifera* from northern Nigeria.

Character	1DTJG	2JHJG	3ARKB	4KFKD	5ZRKD	6GZKN	7TWKN	8DMKT	9KFKT	10DDSK	11GRSK	12BDZM	13WNZM	14NMAD	15JMBA	16BAU	17GMB	18KGGM	19MLTR	20MMYB	21DMT
LL)(cm)	43.1 (1.2)	40.7 (1.18)	39.5 (1.5)	36.8 (1.32)	35.9 (1.35)	52.1 (1.25)	42.5 (1.83)	55.2 (1.06)	39.5 (1.18)	52.8 (1.23)	50.5 (1.09)	48.5 (1.33)	49.1 (1.85)	45.3 (1.12)	49.8 (1.78)	60.3 (1.45)	53.6 (1.15)	35.3 (1.04)	45.5 (1.31)	46.1 (1.52)	48.6 (1.48)
LTL)(mm)	2.31 (0.01)	2.24 (0.02)	2.08 (0.01)	1.78 (0.09)	2.04 (0.57)	2.31 (0.1)	1.83 (0.08)	2.31 (0.07)	1.85 (0.07)	2.28 (0.08)	2.08 (0.12)	1.93 (0.01)	2.56 (0.15)	2.53 (0.11)	1.24 (0.08)	1.36 (0.5)	1.67 (0.05)	2.05 (0.12)	1.54 (0.08)	1.45 (0.03)	2.07 (0.03)
LLW)(mm)	1.15 (0.01)	1.54 (0.02)	1.23 (0.01)	1.19 (0.09)	1.43 (0.57)	1.48 (0.1)	1.21 (0.08)	1.38 (0.07)	1.56 (0.07)	1.43 (0.08)	1.35 (0.12)	1.86 (0.01)	1.52 (0.15)	1.36 (0.11)	1.23 (0.08)	1.41 (0.5)	1.21 (0.05)	1.32 (0.12)	1.35 (0.08)	1.61 (0.03)	1.08 (0.03)
LA(mm2)	2.26	3.45	2.56	2.07	2.92	3.42	2.08	3.19	2.89	3.26	2.81	3.59	3.89	3.44	1.53	1.92	2.02	2.71	2.08	2.33	2.24
ECW(μm)□	22.5 (0.06)	23.7 (0.93)	23.7 (0.93)	30.7 (0.05)	29.3 (0.54)	27 (0.36)	27.6 (0.61)	28.3 (0.5)	29.4 (0.72)	30.3 (0.52)	26.7 (0.32)	25.3 (0.45)	30.4 (0.47)	29.6 (0.64)	22.5 (0.68)	32.6 (0.56)	19.3 (0.7)	25 (0.62)	25.2 (0.58)	23.5 (0.46)	24.6 (0.62)
SL(μm))□	29.8 (0.7)	30.8 (0.46)	30.8 (0.46)	22.3 (0.51)	201.5 (0.34)	24.3 (0.36)	23.6 (0.33)	23.8 (0.33)	22.3 (0.48)	24.5 (0.51)	20.9 (0.56)	24.9 (0.56)	18.1 (0.62)	21.7 (0.56)	23.3 (0.48)	28.6 (0.48)	31.3 (0.66)	26.7 (0.68)	24.8 (0.36)	30.3 (0.66)	25.4 (0.48)
SW(μm)□	25 (0.7)	24.9 (0.46)	24.9 (0.46)	24.7 (0.51)	24.8 (0.34)	23.9 (0.36)	21.7 (0.33)	19.4 (0.33)	19.6 (0.48)	24.9 (0.51)	20.4 (0.56)	24.3 (0.56)	25.9 (0.62)	24.9 (0.56)	19.9 (0.48)	20.8 (0.48)	22.9 (0.66)	20.8 (0.68)	19.8 (0.36)	25.4 (0.66)	20.7 (0.48)
SI□	1.21	4.05	1.59	3.06	10.3	4.93	3	5.72	3.98	2.35	2.56	2.24	6.49	3.05	6.17	2.86	2.78	2.68	2.35	4.58	5.81
ECW*(μm)	20.1 (0.61)	16.8 (0.88)	16.8 (0.88)	27.7 (0.38)	28.3 (0.57)	25.1 (0.46)	26 (0.41)	28.3 (0.48)	26.2 (0.64)	25.6 (0.59)	24.5 (0.4)	18.8 (0.4)	24.5 (0.45)	22.5 (0.5)	19.3 (0.39)	28 (0.7)	18.3 (0.41)	20.6 (0.58)	16 (0.7)	13.2 (0.44)	21.5 (0.36)
SL*(μm)	26 (0.43)	26.7 (0.59)	26.7 (0.59)	19.4 (0.32)	18.9 (0.5)	21.8 (0.44)	20.5 (0.48)	20.8 (0.48)	19 (0.49)	21.2 (0.48)	18 (0.58)	21.6 (0.36)	14.8 (0.7)	18.8 (0.48)	20.2 (0.56)	22.1 (0.56)	26.4 (0.41)	22.2 (0.58)	21.3 (0.61)	24.2 (0.56)	22.1 (0.5)
SW*(μm)	20.5 (0.36)	20 (0.43)	20 (0.43)	14.5 (0.56)	17.9 (0.58)	14.1 (0.48)	15.5 (0.52)	15.8 (0.41)	17.6 (0.63)	14.8 (0.53)	19.9 (0.49)	20.2 (0.48)	21.1 (0.49)	19.2 (0.52)	18 (0.56)	18.8 (0.56)	20.2 (0.57)	18.4 (0.62)	16.7 (0.7)	20.7 (0.65)	18.4 (0.7)
SI*	18.1	20.1	18.1	13.2	25	26.3	24.6	22.5	24.7	25	25	26.1	22.7	24.5	24	24.1	22.1	24.9	22.9	24.1	24

LL= Leaf length, LTL= Leaflet length, LW= Leaflet width, LA = Leaf area, ECW= epidermal cell width, SL= stomatal length, SW= stomatal width, SI= stomatal index, □ Adaxial surface, * Abaxial surface.

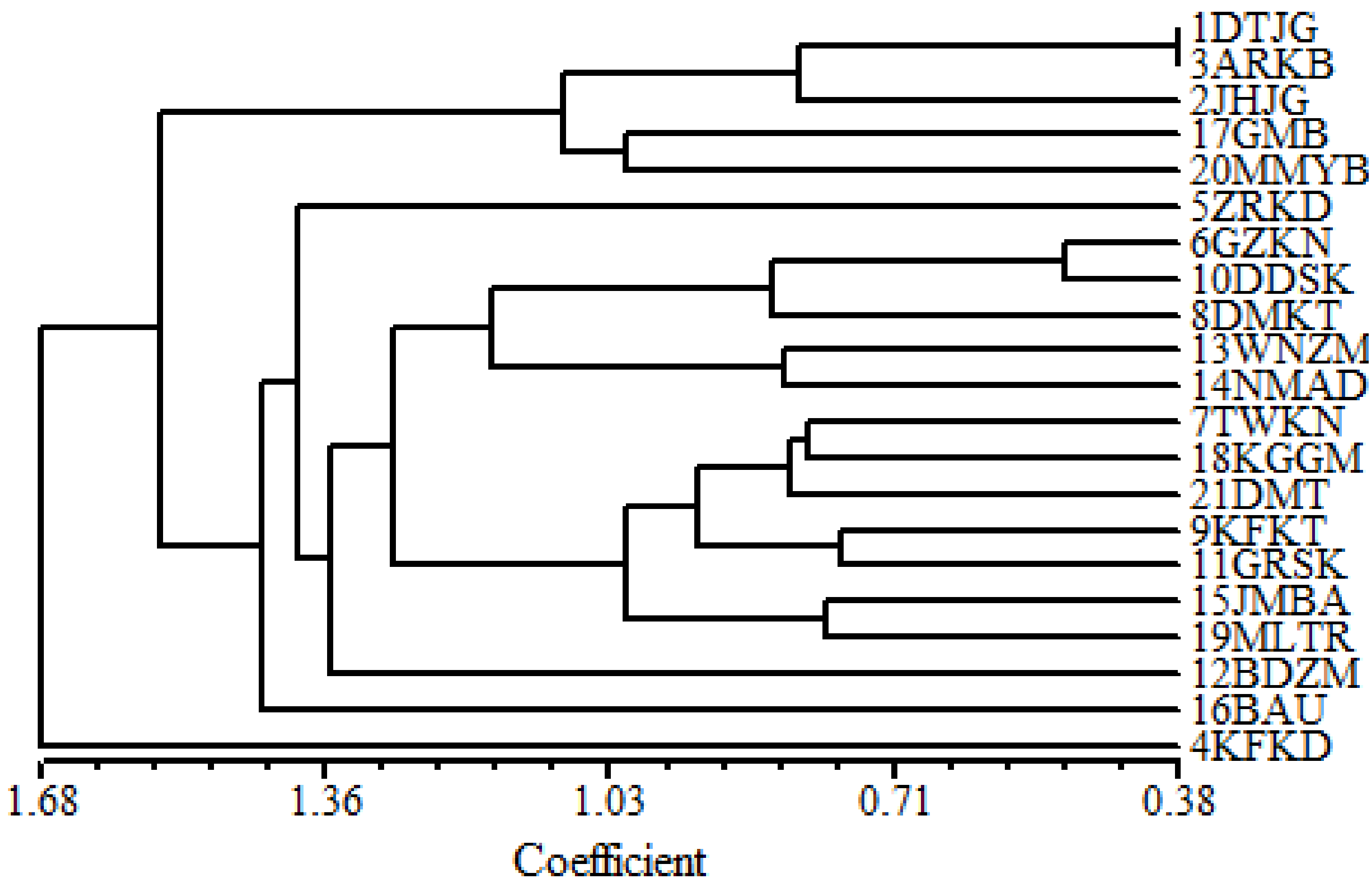

Figure 1. Dendogram of the *M. oleifera* accessions from northern Nigeria using SAHN module.

bicarpillatae following the analysis of the stomatal complexes and its ontogeny. The trichome appeared to be unicellular throughout the accessions, confirming the earlier studies of Gill et al. (1985), who reported similar trichome formation in *M. oleifera* collected from Jos, Nigeria. However, the consistent similitude of these structures in *M. oleifera* in all the accessions makes the trichome structure of not much significance in establishing variability of the samples.

Wide range of epidermal cell wall pattern existed among the studied accession, ranging from straight to undulate as well as sinuous. The epidermal cell wall not only varied among accessions from same climatic zone but, even among samples from the same location. Many factors can be attributed to these variations, which include the prevailing environmental conditions like high wind speed, soil moisture content which can increase cellular turgidity and hence more waviness. Similar findings were made in the *Pteris vittata* from China (Bondada et al., 2007). These therefore, suggests that to certain extent and with verifications from other taxonomic characters, the variations in leaf epidermal cell pattern as well as sizes can make contribution in delimiting these accessions of *M. oleifera* from northern Nigeria. However, Belhadj et al., (2007) have cautioned that ecotypes can be differentiated based on epidermal characters due to adaptation of the environment in which they grow. However, Mbagwu et al. (2007) confirmed closer affinity among *Citrus* species based on leaf edipermal cell wall variations.

In conclusion, the present analysis using morpho-antomical features of the *M. oleifera* accession from northern Nigeria suggest the existence of variation among the samples and may form a basis for the possibility of improving the crop. Specifically, the accession 16BAU showed the highest leaf length value of 60.3 cm and epidermal cell width of 32.6 µm on adaxial surface and was represented by a single accession in cluster IV. However, additional effort using other taxonomic markers like RAPD, ISSR, AFLP etc to support the present findings with a view to enhance the delimitation of the accessions of *M. oleifera* from northern Nigeria for mapping out of breeding schemes for improved yield is herby recommended.

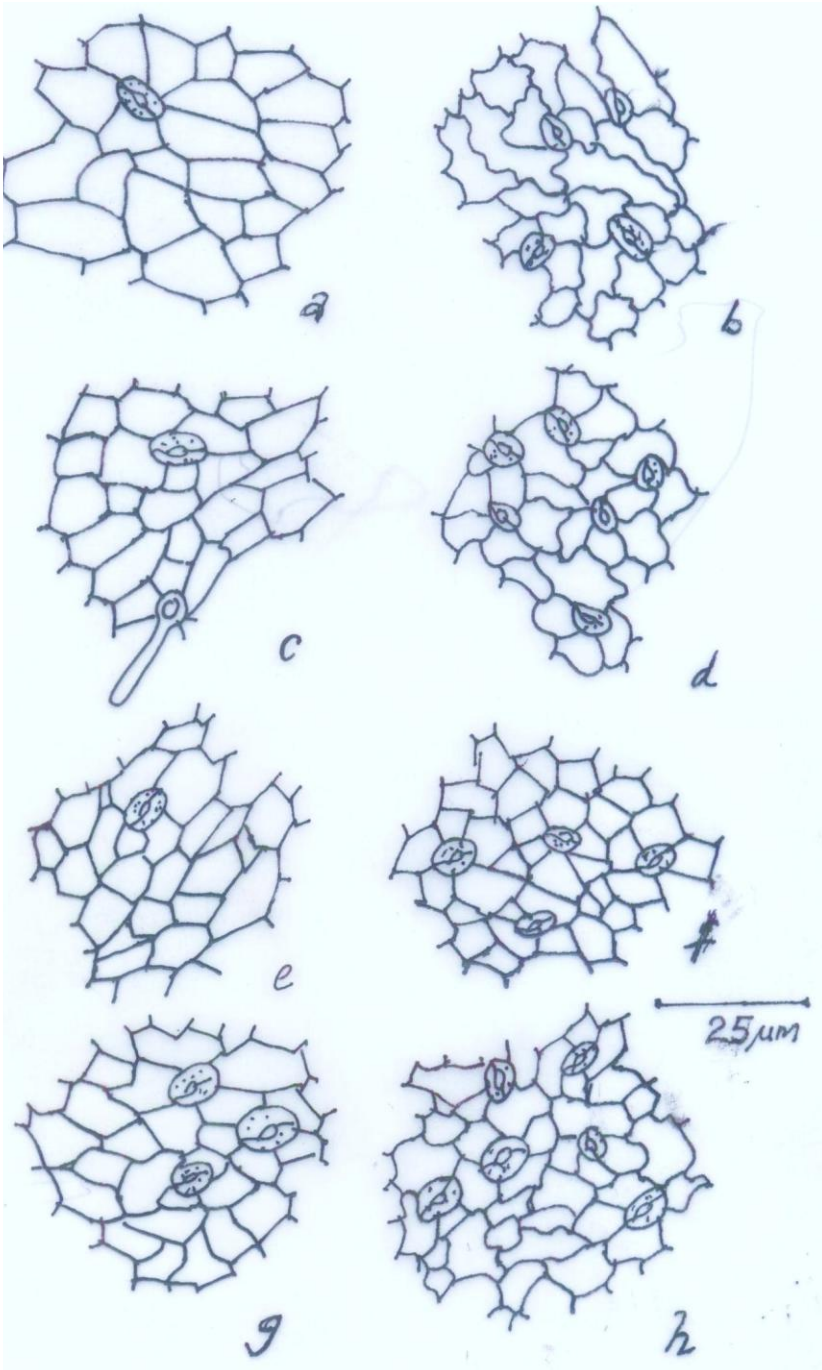

Figure 2a-h. Leaf epidermal features showing anomocytic stomata in adaxial and abaxial surfaces of (a and b) 1DTJG (c and d) 7TWKN with a unicellular trichome (e and f) 10DDSK and (g and h) 16BAU accessions.

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