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Leaf Epidermal Micromorphology as Diagnostic Features in Accessions of *Sesamum indicum* L.

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Key words: *Sesamum indicum*, stomata, epidermal cells, taxonomy, anticlinal cell-wall patterns

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Abstract: Fresh leaves of twelve accessions of *S. indicu*m were studied to examine their stomatal features, epidermal cell shapes and anticlinal cell-wall patterns which may be used for the delimitation of the accessions. The twelve accessions of *S. indicum* studied have amphistomatic leaves. The 3 types of stomatal complex types were observed namely, anisocytic, tetracytic and anomocytic. Accession Adaw-ting (improved) has the smallest stomatal size (26.39±0.34 µm) with highest stomatal density (79.08±1.47 mm^2) while accession Adaw-wula possessed the largest stomatal size (74.31±1.99 µm) with lowest stomatal density (20.80±0.58 mm^2), the exception was found in accession Adaw-ting whose stomatal size is larger (64.03±1.11 µm) but with higher stomatal density (71.54±1.47 mm^2). Wavy, curve or undulate anticlinal wall patterns with irregular and or isodiametric epidermal cell shapes were observed. The high variation in stomatal complex types suggests that these accessions probably have various capacities to conserve water. The leaf micro morphological features were found to be good diagnostic and additional tool in identification as well as nomenclature of the accessions of *S. indicum*.

INTRODUCTION

Sesame (*Sesamum indicum* L.) is known as the king of oil seeds in Vietnam due to the high oil content (50-60%) of its seed (Pham *et al*., 2010; Weiss, 1971). Prasad and Gangopadhyay (2011) described *Sesamum* as the “Queen of oilseeds” because it contains excellent qualities of seed oil with long shelf life, protein, calcium, phosphorous, oxalic acid and remarkable antioxidant function, resisting the oxidation. A total of 3.66 million tons of sesame produced is produced in the world, out of which Asia and Africa account for 2.55 and 0.95 million tons respectively. In Africa, Nigeria is the third largest producer after Sudan and Uganda with about 165.1 ha (harvested area) that produced 83 tons Farri. With wide range of uses of sesame products, most research efforts are focused mainly on culinary and traditional medicines, its nutritive, preventive and curative properties, seed characterization (Suhasini, 2006) agro-morphological identification (Georgiev *et al*., 2011), seeds oil (Pham, 2011; Prasad and Gangopadhyay, 2011) but not targeted towards the characterization of this plant using stomata, trichomes and epidermal cells features.

Essiett *et al.* (2012) reported that taxonomists lately realized the importance of microscopic features of the leaf epidermis and recently taxonomic monographs are now considered incomplete without them. Mbagwu *et al.* (2008) suggested that leaf epidermis possesses micro-morphological attributes that are of potential taxonomical significance even at the genus and species level. Epidermal features have become widely studied from three main perspectives which include ontogenetic, phylogenetic and taxonomic perspectives. Adegbite (1995) stated that stomata structures are under strong and highly integrated genetic control and their modification could reflect evolutionary trend. Abdulrahaman and Oladele (2004) envisaged the use of trichomes and stomatal features in some Nigerian vegetables to be of use, serving as indices of plants water requirement. Plant epidermal features are mildly influenced by environmental conditions and are of high structural diversity (Barthlott, 1981 and Abdulraham and Oladele, 2005). These leaf epidermal micro morphological characters are also reliable in taxonomy and systematics (Stace, 1965, Martin and Juniper, 1970, Abubakar and Yunusa, 1998; Ogunkunle and Oladele, 2000; Alege *et al*., 2013).

The present work therefore focused on the leaf epidermal microscopy of twelve accessions of *S. indicum* to facilitate their taxonomic stand. Emphasis was on the stomata, epidermal cells and anticlinal walls diagnostic features because of their importance in systematics. These features were used in this study to delimit these accessions.

MATERIALS AND METHODS

Collection of materials: Fresh leaves of twelve sesame accessions namely Ex-Gombe 6, Kenana 4, Lale-duk, Ex-Gombe 5, Ex-Sudan, Adaw-wula, Adaw-ting (improved), Adaw-ting, Ex-Gombe 4, Ex-Gombe 1, Ex-Gombe 3 and Ex-Gombe 2 were used in this study. The samples were collected from the Research Farm and the laboratory work was carried out at the Biology main Laboratory, Abubakar Tafawa Balewa University, Bauchi, Nigeria.

Epidermal peels of the leaf surfaces of the accessions were made using the method of Metcalfe and Chalk (1988). The abaxial and adaxial surfaces of the leaves surfaces were carefully separated by using dissecting needle and forceps after being rinsed in tap water. Alternatively, the epidermal surfaces were sectioned with razor blade (free-hand section) and placed on microscope slides. The preparations were stained with 1% safranin and 50% glycerol or Formalin Acetic Acid (FAA) and observed under a light microscope (Abdulrahaman and Oladele, 2005) as amended by Zhigila *et al*. (2015).

Using 35 fields of view at ×40 objective as quadrats, the numbers of subsidiary cells per stoma was noted to determine the frequency of the different stomatal complex types and was expressed as percentage occurrence of such complex type based on all occurrences (Obiremi and Oladele, 2001). Terminologies for naming stomatal complex types followed those of Dilcher (1974). The stomatal densities were determined as the number of stomata per square millimeter (Stace, 1965). The stomata observed were viewed with the light microscope and were calculated in unit area using the Stomatal Index (SI) formular as: $SI = S/S+E\times100$ where S and E mean numbers of stomata and epidermal cells respectively within the particular area under investigation. The mean stomatal sizes of the accessions were measured by using an eye piece micrometer graticule as the product of Length of stomata, Breadth of stomata and K where K = Franco's constant = 0.78524 as described by Wilkinson (1979).

Statistical analysis: All data were processed using Analysis of Variance (ANOVA), Duncan's Multiple Range Test (DMRT) and the construction of the dendrogram. Computer software used was IBM SPSS version 20. A probability value of 0.05 was used as bench mark for significant difference between parameters.

RESULTS AND DISCUSSION

Leaf epidermis of sesame accessions were studied anatomically (Fig. 1-4). Focus was on such leaf micro morphological features like stomata, epidermal cells and anticlinal wall patterns. The twelve accessions of *S. indicum* studied have amphistomatic leaves, i.e., having stomata on both surfaces. This has confirmed the work Alege *et al.* (2013). Three types of stomatal complex types were observed in the twelve accessions of *S. indicum* namely, anisocytic, tetracytic and anomocytic. Anisocytic type was the most common occurring on both surfaces of all the accessions (Table 1). Homogenous stomatal complex types were observed on both adaxial and abaxial surfaces of accessions Ex-Gombe 6, Lale-duk and Ex-Sudan and on the abaxial surfaces of accessions Adaw-ting (improved) and Adaw-wula with anisocytic types having 100% frequency (Table 1 and Fig. 2). Ex-Gombe 2 and Adaw-ting showed three types of stomata-anisocytic, tetracytic and anomocytic (Fig. 3) and anisocytic and tetracytic (Fig. 4) respectively. These observations indicate that adawa-ting exhibit xeromorphic features than the other varieties which have stomata with three or more subsidiary cells per stoma. Abdulrahaman and Oladele (2004) reported that a large number of subsidiary cells per stoma may be responsible for a more precise and rapid regulation of stomatal opening process.

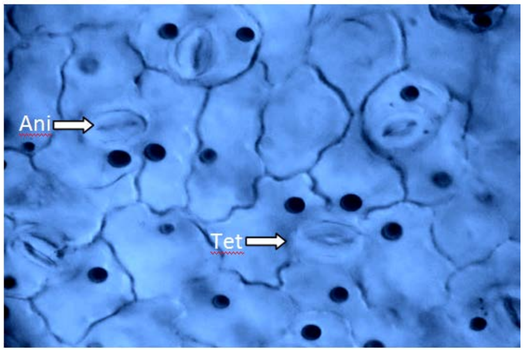

Fig. 1: Abaxial surface of kenana 4 showing anisocytic (ani) and tetracytic (tet) stomata ×600

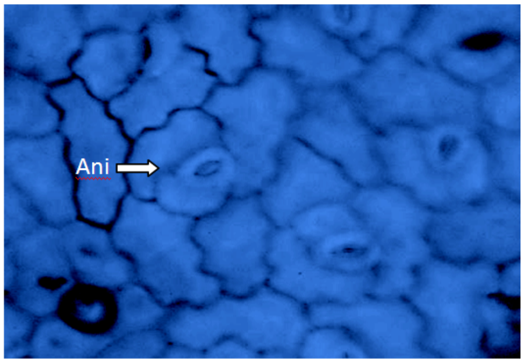

Fig. 2: Abaxial surface of ex-Sudan showing anisocytic stomata ×600

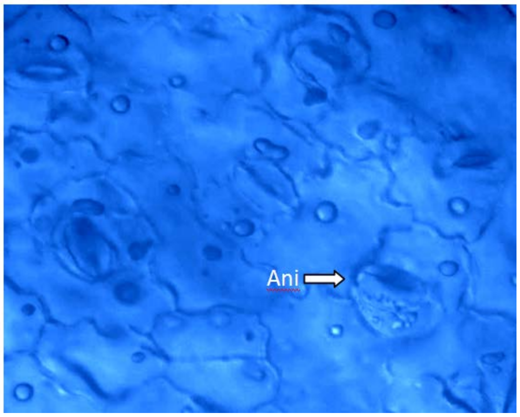

Fig. 3: Adaxial surface of Adaw-ting showing anisocytic stomata ×600

Duncan Multiple Range Test (DMRT)-one-way ANOVA revealed that there was no significant difference between the stomatal densities of accessions Ex-Gombe 6, Ex-Sudan, Adaw-wula, Adaw-ting, Ex-Gombe 4 and Ex-Gombe 2. The stomatal density was highest on the

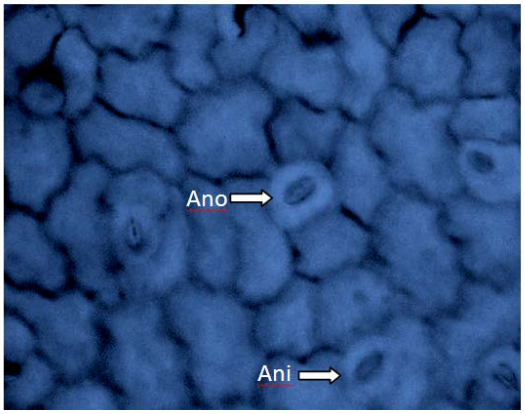

Fig. 4: Abaxial surface of Ex-Gombe 2 showing anisocytic and anomocytic (ano) stomata ×600

adaxial surface of accession Ex-Gombe1 (80.01 mm^2) and lowest on adaxial surface of accession Lale-duk and Ex-Gombe 5 (20.80 mm^2). DMRT of the stomatal sizes in the twelve taxa were shown in Table 1. However, the stomatal sizes of accession Adaw-wula was the highest (74.31 µm), Adaw-ting (improved) and Ex-Gombe 1 show the lowest (26.40 µm). Stomatal size is often correlated with stomatal density such that small stomata give high density and large stomata give low density (Metclafe and Chalk, 1988; Abdulrahaman and Oladele, 2004). This statement is in line with the findings from this work where accession Adaw-ting (improved) has the smallest stomatal size with highest stomatal density while accession Adaw-wula possessed the largest stomatal size with lowest stomatal density, the exception was found in accession Adaw-ting whose stomatal size is larger but with higher stomatal density (Table 1).

The stomatal index which indicates the proportion of stomata relative to leaf surface is also a reliable taxonomic character. This is because Abdulrahaman and Oladele (2004) reported that stomatal index is independent of the changes in epidermal cells size brought about by environmental factors. The stomatal index was generally highest on the adaxial surface of accession Ex-Gombe 6 (78.92%) and lowest on the abaxial surface of accession Ex-Gombe 2 (26.04). This shows that stomata occupied larger proportion of the leaf surface in the former and smaller proportion in the latter (Table 1).

Chen *et al.* (2009) and Alege *et al.* (2013) reported that stomata density may depend on the environmental factor. Contrary to their observation, stomata index, stomata density and stomata size on both surfaces may be inherent characters as the accessions were subjected to the same treatment. This may have adaptive implication for water conservation in the studied plant since the leaf surfaces are responsible for loss of water through the

Table 1: Stomatal features in accessions of *S. indicum*

Accessions	Leaf surface	Stomatal complex type	Frequency (%)	Stomatal density (mm^2)	Stomatal length (µm)	Stomatal breadth (µm)	Stomatal size (µm)	Stomatal index (%)
Adaw-ting	Abaxial	Anisocytic	71.54	28.11±0.43[a]	12.11±0.43	6.71±0.33	64.03±1.11[a]	39.29±1.02
		Tetracytic	28.46					
	Adaxial	Anisocytic	60.30	76.05±9.65	12.04±0.23	5.98±0.02	63.90±1.04[a]	32.29±1.90
		Tetracytic	39.70					
Adaw-ting)	Abaxial	Anisocytic	66.20	67.20±3.43	6.60±0.24	5.00±0.45	26.39±0.34[c]	50.61±2.3
(improved		Tetracytic	33.80					
	Adaxial	Anisocytic	57.30	79.08±1.47	7.07±0.71	6.21±0.21	28.12±0.76[c]	72.33±2.29
		Tetracytic	42.70					
Adaw-wula	Abaxial	Anisocytic	10.00	33.40±2.56[a]	10.60±0.40	9.20±0.20	74.31±1.99[b]	61.69±0.80
	Adaxial	Anisocytic	59.11	29.60±0.51[a]	9.87±0.42	9.04±0.23	68.16±2.11[b]	72.21±2.11
		Tetracytic	40.89					
Ex-Gombe 1	Abaxial	Anisocytic	76.50	66.98±2.43	6.71±0.30	4.98±0.23	26.40±0.50[c]	65.11±2.13
		Tetracytic	23.50					
	Adaxial	Anisocytic	80.90	80.01±3.20	6.66±0.29	5.09±o.22	28.34±0.71[c]	77.38±4.01
		Tetracytic	19.10					
Ex-Gombe 2	Abaxial	Anisocytic	76.50	25.01±0.32	12.09±0.88	6.70±0.1	63.99±3.20[a]	26.04±0.90
		Tetracytic	17.80					
	Adaxial	Anomocytic	5.70	27.90±1.46[a]	11.98±0.12	6.44±0.21	66.19±2.90[ab]	25.72±1.19
Ex-Gombe 3	Abaxial	Anisocytic	53.80	67.44±2.65	7.00±0.57	5.03±0.65	27.82±0.64[c]	65.91±2.75
		Tetracytic	46.20					
	Adaxial	Anisocytic	54.40	78.11±1.76	6.74±0.42	6.22±0.67	28.10±0.92[c]	31.46±0.76
		Tetracytic	48.60					
Ex-Gombe 4	Abaxial	Anisocytic	79.54	24.87±0.66	12.56±0.71	6.80±0.31	67.14±2.02[ab]	30.33±1.01
		Tetracytic	20.46					
	Adaxial	Anisocytic	75.00	29.86±1.52[a]	11.22±0.27	6.72±0.46	64.54±0.57[a]	31.98±1.20
		Tetracytic	25.00					
Ex-Gombe 5	Abaxial	Anisocytic	62.00	33.00±1.64[a]	11.60±0.40	6.60±0.24	60.68±3.79[a]	38.07±0.55
		Tetracytic	38.00					
	Adaxial	Anisocytic	58.32	20.80±0.58	11.01±0.41	6.28±0.22	59.87±2.10[a]	31.11±0.51
		Tetracytic	41.68					
Ex-Gombe 6	Abaxial	Anisocytic	10.00	29.20±0.37[a]	10.20±0.80	8.40±0.75	68.66±1.76[ab]	63.28±1.52
	Adaxial	Anisocytic	10.00	61.60±3.37	9.98±0.40	7.82±0.48	71.10±1.32[ab]	78.92±0.84
Ex-Sudan	Abaxial	Anisocytic	10.00	34.20±1.11[a]	10.80±0.37	5.60±0.40	46.14±2.96[a]	37.74±1.54
	Adaxial	Anisocytic	10.00	24.60±2.67	10.11±0.24	6.39±0.48	48.01±1.39[a]	28.83±2.42
Kenana 4	Abaxial	Anisocytic	63.40	25.40±0.51	6.80±0.20	5.60±0.24	30.18±1.94[c]	28.17±0.99
		Tetracytic	36.60					
	Adaxial	Anisocytic	70.01	30.40±1.81[a]	7.01±0.37	6.12±0.42	28.29±1.22[c]	37.80±1.57
		Tetracytic	29.99					
Lale-duk	Abaxial	Anisocytic	10.00	24.40±0.40	12.60±0.24	6.80±0.37	67.47±2.82[ab]	35.43±2.51
	Adaxial	Anisocytic	10.00	20.80±0.86	11.92±0.22	7.00±0.30	66.87±1.22[ab]	31.12±1.54

Table 2: Epidermal cell characteristics in accessions *S. indicum*

	Epidermal Cell Characteristics			
Accessions	Leaf surface	Epidermal cell shape	Anticlinal wall pattern	Epidermal cell density
Adawa-ting	Abaxial	Irregular	Undulated	38.21±0.91
	Adaxial	Irregular	Undulated	35.06±1.12
Adawa-ting	Abaxial	Irregular	Undulated	33.60±3.06
(improved	Adaxial	Irregular	Undulated	39.00±0.70
Adawa-wula	Abaxial	Irregular	Wavy, curved	17.40±0.24
	Adaxial	Irregular	Wavy, curved	18.40±0.75
Ex-Gombe 1	Abaxial	Irregular	Wavy	19.12±0.67
	Adaxial	Irregular	Wavy	30.52±0.82
Ex-Gombe 2	Abaxial	Irregular	Wavy, curved	34.71±0.87
	Adaxial	Irregular	Wavy, curved	31.09±0.88
Ex-Gombe 3	Abaxial	Irregular	Wavy	27.86±1.24
	Adaxial	Irregular	Wavy	22.72±0.27
Ex-Gombe 4	Abaxial	Isodiametric, irregular	Wavy, curved	21.26±1.30
	Adaxial	Isodiametric, irregular	Wavy, curved	18.62±1.09
Ex-Gombe 5	Abaxial	Irregular	Wavy	53.60±2.18
	Adaxial	Irregular	Wavy	66.00±1.34
Ex-Gombe 6	Abaxial	Irregular	Wavy	17.20±1.16
	Adaxial	Irregular	Wavy	16.40±0.93
Ex-Sudan	Abaxial	Isodiametric, irregular	Curved	56.80±4.04
	Adaxial	Isodiametric, irregular	Wavy, curved	59.80±1.47
Kenana 4	Abaxial	Irregular	Wavy	68.20±3.26
	Adaxial	Irregular	Wavy	51.20±0.97
Lale-duk	Abaxial	Irregular	Wavy	47.80±3.26
	Adaxial	Irregular	Wavy	46.60±3.63

stomata (transpiration). The reduction in stomata size in the study of Rai and Mishra (2013) was attributed to adaptive response of the studied plants to avoid entry of harmful constituents of exhaust.

This study revealed the presence of isodiametric and irregular shapes of epidermal cells on both surfaces of accessions Ex-Gombe 4 and Ex-Sudan with curved and wavy anticlinal wall patterns. Only irregular shaped epidermal cell occurred on the surfaces of the rest of the accessions with wavy or undulating anticlinal wall patterns (Table 2 and Fig. 1-4). The highest average epidermal cell density was recorded on the abaxial surface of accession Kenana 4 (68.20 mm^2) and the lowest was recorded on the adaxial surface of accession Ex-Gombe 6 (16.40 mm^2). Wide range of epidermal cell wall pattern existed among the studied accessions According to Alege *et al.* (2013); epidermal cell characteristics are under strong genetic control, hence are stable traits and therefore proofed to be a better tool for the delimitation of sesame than stomata traits. Similar findings were made in *Pteris vittata* from China (Bondada *et al*., 2006) and *Moringa oleifera* accession from Northern Nigeria (Abubakar *et al*., 2011) and Mbagwu *et al.* (2008) confirmed closer affinity among Citrus species based on leaf epidermal cell characteristics. These therefore, suggests that to certain extent and with verification from other taxonomic characters, the variations in leaf epidermal cell pattern, epidermal cell shapes as well as epidermal cell density can make contribution in delimiting these accessions.

CONCLUSION

Leaf epidermis in accessions of *S. indicum* possesses micro-morphological attributes that are of potential taxonomical significance and proofed to be a better tool for the delimitation of sesame accessions. The existence of variation among the samples may also form a basis for the possibility of improving these accessions.

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