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Micromorphological Studies of Four Fuel Wood Yielding Tropical Leguminous Plants

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Abstract: Leaf epidermal micro-morphology of four-fuel wood yielding tree legumes viz., *Albizia lebbeck* Benth, *Cassia fistula* Linn, *Acacia nilotica* Willd and *Tamarindus indica* Linn has been investigated. The epidermal cells in each case were irregular in shape, being larger in slow growing species i.e. *A. nilotica* and *T. indica*. The stomata were hypostomatic, anomocytic and /or paracytic types with frequency, size and stomatal index higher in fast growing species i.e. *C. fistula* and *A. lebbeck*. The trichomes present only in fast growing species, were glandular or non-glandular, unicellular, straight or curved. These leaf epidermal characters can also be utilized as markers for fast growing fuel wood plants apart from taxonomical consideration of the taxa.

Key words: Micromorphology, leaf epidermis, fuel wood yielding plants, tree legumes

INTRODUCTION

Epidermal morphology of the leaf has been extensively used recently in emphasizing the relationship and ontogeny of different taxa. According to Chandra *et al*. (1969) the significance of epidermal morphology and arrangement was a helpful tool in phylogenetic and taxonomic consideration. Indeed the angiospermous foliar epidermis is an easily studied character that showed the sufficient diversity of details (Kannabiran and Krishnamurthy, 1972). The importances of epidermal characters of leaves in angiosperms have been reviewed by Stace (1984). Several authors have shown the importance of epidermal features in recognition of different taxa of plants (Hagerup, 1953; Stebbins and Jain, 1960; Borril, 1961; Davis and Heywood, 1973; Tomlinson, 1959; Nyawuame and Gill, 1990a, 1990b, 1990c, 1991; Ogundip and Olatunji, 1991a, 1991b; Edeoga, 1991; Edeoga and Ikem, 2001; Parveen *et al*., 2000).

The size, distribution and frequency of stomata have been reported to be specific to the taxa below the family and the stomatal characters were very significant parameters in the angiosperm taxonomy and phylogeny (Ahmad, 1979; De Bary, 1984; Karatela and Gill, 1986; Saikia and Dey, 1987; Mukherjee *et al*., 2000). Leguminosae is a very large family of angiosperm and has been anatomically studied by different workers from different point of view including foliar epidermis also (Metcalfe and Chalk, 1950; Kothari and Shah, 1975; Gill and Karatela, 1982; Leelavathi and Ramayya, 1983; Karatela *et al*., 1990; Ogundipe and Akinrinlade, 1999). However, a little work has been done on fuel wood yielding tree legumes, in respect of epidermal micro-morphology for taxonomic consideration as well as for identification of markers in relation to fast growing habit. As such, the present paper will reveal the leaf epidermal micro-morphology of four-fuel wood yielding tree legumes, viz *Cassia fistula*, *Albizia lebbeck*, *Acacia nilotica* and *Tamarindus indica*.

MATERIALS AND METHODS

Leaves of plant species belonging to four investigated taxa, among which *Albizia lebbeck* Benth. and *Cassia fistula* Linn. being fast growing and *Acacia nilotica* Willd. and *Tamarindus indica* Linn. being slow growing, have been collected from the area in and around Santiniketan, West Bengal. The study samples were selected from distal, central and proximal areas of the leaves of investigated plant species. These leaf samples were first boiled with absolute alcohol for 10-15 min and after decanting the alcohol, the leaf materials were again boiled in chloral hydrate solution for 10 mins. These treated leaf samples were then mounted in chloral hydrate solution and observed under compound light microscope.

For scanning electron microscope (SEM) study, method adopted by Mukherjee (1986) has been followed. The leaf samples were taken and washed in distilled water, fixed in 3% glutaraldehyde in 0.02 M phosphate buffer of pH 6.9, dehydrated through an ethyl alcohol series, critical point dried, sputter coated with gold and observed with a Hitachi S-530 SEM at Burdwan University, West Bengal.

RESULTS

The following observations were based on the leaf epidermal characters including stomatal features of four investigated leguminous tree taxa.

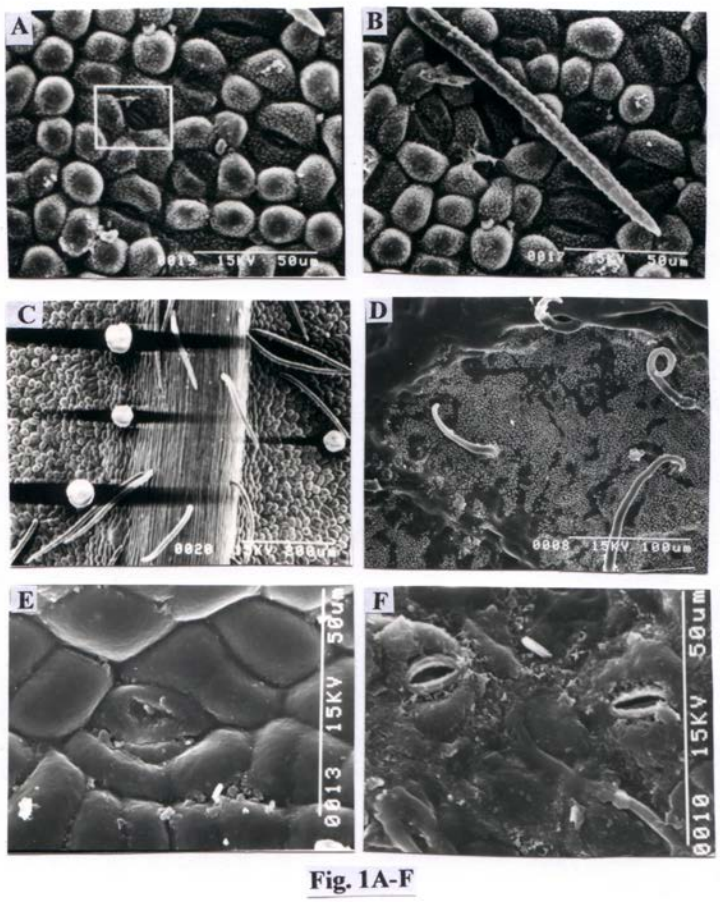

Fig. 1: SEM study of leaf epidermis showing stomatal and trichome features
- A: Anomocytic stomata in *Albizia lebbeck* (abaxial surface)
- B: Single non-glandular trichome in *Albizia lebbeck* (abaxial surface)
- C: Glandular and non-glandular trichomes in *Albizia lebbeck* (abaxial surface)
- D: Epidermal trichomes of *Cassia fistula* (adaxial surface)
- E: Anomocytic stomata in *Acacia nilotica*
- F: Paracytic stomata of *Tamarindus indica*

Epidermal cell characters: Table 1 indicates the data on epidermal cell characters of abaxial and adaxial surfaces of leaf samples. The shape of epidermal cells in all the samples from both the surfaces is only one type i.e. irregular. Regarding the cellular dimension, epidermal cells were larger in adaxial surface than those of abaxial surface in all cases. Here the average size of the cells varied from 19.9 X 10.7 μ in *Tamarindus indica* to 15.2 X 8.4 μ in *C. fistula* on adaxial side and 14.7 X 8.9 μ in *T. indica* to 12.07 X 6.93 μ in *C. fistula* on abaxial side of epidermis. In general, epidermal cells, considering both the surfaces, were larger in *Acacia nilotica* and *T. indica* than in *C. fistula* and *Albizia lebbeck*. Frequency was always high on abaxial surface compared to adaxial surface. Difference in frequency between adaxial and abaxial surface was higher in *C. fistula* and *A. lebbeck* than in A. *nilotica* and *T. indica*. Palisade ratio was higher in A. *nilotica* and *T. indica* than in *C. fistula* and *A. lebbeck*.

Stomatal features: Stomatal features for four leguminous species have been presented in Table 2. Here all the investigated taxa were found to be hypostomatic i.e. no stomata on the adaxial epidermal layer. Both the anomocytic and paracytic type of stomata were recorded. Anomocytic type was prominent in *A. lebbeck* (Fig. 1A) where as paracytic type was found in *C. fistula*, *A. nilotica* and *T. indica*, (Fig. 1E, 1F) although some anomocytic type of stomata was also recorded in *C. fistula*. Stomatal

Table 1: Epidermal cell characters of four investigated taxa

Taxa	Leaf surface	Epidermal cell shape	Epidermal cell length (µm)	Epidermal cell width (µm)	Number of epidermal cell/sq. mm	Palisade ratio
Cassia	Adaxial	Irregular	15.2±0.83	8.4±1.14	91.9±1.82	3.66±0.54
fistula	Abaxial	Irregular	12.07±1.13	6.93±0.816	120.39±2.91	
Albizia	Adaxial	Irregular	18.02±1.58	7.3±1.01	89.2±0.824	4.28±0.84
lebbeck	Abaxial	Irregular	14.23±1.00	6.91±0.91	168.41±1.58	
Acacia	Adaxial	Irregular	19.2±1.49	11.8±1.2	110.8 ±1.52	8.0±0.58
nilotica	Abaxial	Irregular	13.8±2.23	8.0±1.96	108.5 ±1.01	
Tamarindus	Adaxial	Irregular	19.9±1.22	10.7±1.67	108.7 ±1.25	6.76±1.56
indica	Abaxial	Irregular	14.7±1.67	8.9±1.35	126.4 ±1.42	

Table 2: Stomatal feaures of four investigated taxa

Taxa	Leaf surface	Stomatal type	Stomatal frequency	Stomatal length (µm)	Stomatal width (µm)	Stomatal length/ stomatal width	Stomatal index
Cassia fistula	Adaxial						
	Abaxial	Paracytic and rarely anomocytic	17.9±1.08	9.66 ±0.56	1.57 ±0.22	6.15 ±0.87	15.76±2.30
Albizia lebbeck	Adaxial						
	Abaxial	Anomocytic type	14.91±1.31	8.20±0.54	1.62±0.66	5.06±1.07	12.33±1.69
Acacia nilotica	Adaxial						
	Abaxial	Anomocytic and Paracytic type	7.03±1.93	6.01±0.62	1.52±0.28	3.95±1.21	9.00±2.11
Tamarindus indica	Adaxial						
	Abaxial	Paracytic type	9.4±1.73	5.03±0.55	1.9±0.31	2.64±1.14	9.89±1.92

Table 3: Trichome characters of four investigated taxa

Taxa	Occurrence	Type of trichome	Length of trichome (µm)	Width of trichome (µm)	No of trichome / sq mm
Cassia fistula	Both adaxial and abaxial	Non-glandular Simple unicellular			
		(i) long hair	49.5±1.21	5.2±0.56	2.42±0.85
		(ii) short hair	22.8±1.23	3.57±0.43	7.89±1.01
Albizia lebbeck	Both adaxial and abaxial	Simple unicellular non-glandular type	98.24±2.21	5.95±0.25	5.33±1.58
		glandular type	_	_	6.88±1.00
Acacia nilotica	Both adaxial and abaxial	_	_	_	_
Tamarindus indica	Both adaxial and abaxial	_	_	_	_

frequency varied from 7.03/mm^2 in *A. nilotica* to 17.9/mm^2 in *C. fistula*. Stomatal frequency was clearly low in case of *A. nilotica* and *T. indica* compared to *A. lebbeck and C. fistula*. Stomatal size was larger in case of *C. fistula* (9.66 X 1.57 µ) and *A. lebbeck* (8.20 X 1.62 µ) than in *A. nilotica* (6.01 X 1.52 µ) and *T. indica* (5.03 X 1.9 µ), because of higher length in the former two species. As a result length / width ratio was higher in these species. Stomatal index (ranging from 9.0 to 15.76) was also high in *A. lebbeck* and *C. fistula* than in *A. nilotica*, and *T. indica*.

***Trichomes*:** Epidermal hairs or trichomes were only found on both epidermal surfaces of *A. lebbeck* and *C. fistula* while these were totally lacking in *A. nilotica*, and *T. indica* (Table 3). In *C. fistula* trichomes appeared as non-glandular, simple, unbranched and unicellular. Both long and short trichomes were recorded in *C. fistula* (Fig. 1D), former type being less in number compared to later. In *A. lebbeck* both non-glandular and glandular types of hairs were present. Non-glandular hairs were dispersed through-out the leaf surface but glandular hairs were restricted to the surroundings of the midrib (Fig. 1C). In C. *fistula*, apex of short hair was found to be coiled as well as hooked, while that of long hairs was only hooked (Fig. 1D). The trichomes of *A. lebbeck* were long, straight, unicellular and with tapering pointed apex (Fig. 1B)

DISCUSSION

The present study revealed that micro-morphological features of the leaf epidermis have a considerable taxonomic value in identification of these investigated taxa in consideration to their respective groups. But more importantly, this study yielded data on micro-morphological features that can well be considered as marker for fast growing habit. The investigated taxa showed a number of characters, some of which conformed to the features reported earlier by Metcalfe and Chalk (1950, 1979) and Leelavathi and Ramayya (1983). However, the epidermal features of these four taxa are described in more detail and contain some features, which were not reported before. Analysis of epidermal cell characters indicates that the cell size was always larger on adaxial side of leaves, which was a general observation made earlier by Solereder (1908). Interestingly, average cell dimension was higher in the slow growing legume species

(*A. nilotica* and *T. indica*) than in fast growing species (*A. lebbeck* and *C. fistula*). On the other hand, difference in frequency of epidermal cells between adaxial surface and abaxial surface was higher in fast growing plants.

Stomata have been considered as important unit of plant structure, physically, taxonomically and phylogenetically (Kasturi *et al*., 2001). Cuticular morphology and type of stomata in members of leguminosae is the valuable marker that could go a long way in distinguishing these taxa (Edeoga and Amayo, 2001). Metcalfe and Chalk (1950, 1979) reported that paracytic type of stomata for the members of the family Mimosaceae. But in present investigation stomata were anomocytic type in *A. lebbeck* and both anomocytic as well as paracytic type of stomata was recorded in case of *A. nilotica*. Stomatal frequency was found to be clearly higher in fast growing species than slow growing species. According to Carpenter and Smith (1975) the variations in stomatal frequencies have taxonomic importance also at generic levels. Carlquist (1961) emphasized the contribution of variation in stomatal size in delimiting species within a genus. Here the stomatal size was also larger in the two fast growing species than in the slow growing species. Rowson (1943, 1946) found that stomatal index values are very reliable in distinguishing between the leaves of co-generic species. Similarly, Edeoga and Osawe (1996) recognized the significance of stomatal index values in distinguishing between the leaves of closely related species. Here the stomatal index was also distinctively higher in two fast growing species than that of slow growing species.

The trichome features have now been considered in taxonomical study of the taxa (Leelavathi and Ramayya (1983). Interestingly, trichomes were totally absent in slow growing species while fast growing species have trichomes with distinctive features. Trichomes in these species were mostly non-glandular along with some glandular type in *A. lebbeck* only. Trichomes were of two types in respect of length only in *C. fistula*. However, in all cases trichomes were found to be unicellular, although multicellular non-glandlular hair has been reported earlier in *A. lebbeck* (Ogundipe, 1999).

In consideration, certain micro-morphological features of leaf epidermis can well be considered as features or markers that distinguish between fast growing and slow growing leguminous fuel wood tree species. Thus, higher stomatal frequency, size and stomatal index and presence of trichomes were characteristic features of fast growing species while slow growing species showed large epidermal cells.

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