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Comparative foliar micromorphological studies of some *Bauhinia* (Leguminosae) species

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Abstract: A comparative foliar epidermal micromorphology of 5 species of *Bauhinia* L. belonging to family Leguminosae (Fabaceae) was conducted with a view to elucidating their taxonomic significance and presenting complementary data to aid in the identification of the species. The epidermal cells in all species were polygonal with straight walls except in *B. tomentosa* L., which had an undulating outline. The stoma types were mainly anisocytic, anomocytic, and paracytic. Among the 5 species, *B. blakeana* L. was hypostomatic with anisocytic and paracytic stomata. Stomatal indices, frequencies, and sizes were determined. The frequency of stomata differed markedly between different species, with the highest frequency in *B. malabarica* L. and the lowest in *B. tomentosa* L. Trichomes were either unicellular or multicellular or both. *B. malabarica* L. can be identified by its unicellular hooked trichome. Based on the characteristic features, a diagnostic key for identification of the studied species was prepared.

Key words: *Bauhinia*, epidermal characters, stomata, trichomes

1. Introduction

The importance of micromorphological features for the taxonomic consideration of Angiosperms is now well established (Ramayya, 1972; Tomlinson, 1979; Ogundipe & Akinrinlade, 1998; Parveen et al., 2000). Micromorphological parameters of different plant parts have been used as aids in the taxonomical recognition of species (Kathiresan et al., 2011). The foliar epidermis is one of the most noteworthy taxonomic characters from a biosystematic point of view, and taxonomic studies of a number of families are conducted on the basis of the leaf epidermis (Bhatia, 1984; Jones, 1986).

The taxonomic relevance of the foliar epidermal characters of angiosperms has been well documented (Parveen et al., 2000; Yasmin et al., 2009; Celka et al., 2006; Zou et al., 2008). The leaf epidermal cells are of significant taxonomic importance; the length and width are regarded as useful aids in distinguishing varieties with similar flowering dates in perennial rye grass (Wilkins & Sabanci, 1990). Other characters with useful variation in epidermal cells include structure, orientation, undulation of the anticlinal wall, and curvature of the periclinal wall. Character size, distribution, and frequency of stomata have been found to be specific to some taxa and are used as significant parameters in taxonomy as well as in phylogeny (De Bary, 1884; Solereder, 1908; Metcalfe & Chalk, 1950; Stace, 1965; Paliwal, 1969; Ahmed, 1979; Rajagopal, 1979).

Bauhinia L. is an extremely variable genus of shrubs and medium-sized or large trees of more than 200 species in the subfamily Caesalpinioideae of the large flowering family Leguminosae (Fabaceae), with a pantropical distribution. The genus, named after the twin Bauhin brothers, is characterised by bilobed leaves with a cleft at the apex that forms 2 rounded lobes. From the base, the veins spread out fan-wise, and the leaf is more or less folded along the centre rib. *Bauhinia* is also known as mountain ebony or simply orchid tree and kachnar in India and Pakistan. *Bauhinia* trees typically reach a height of 6–12 m, and their branches spread 3–6 m outwards. The lobed leaves are usually 10–15 cm across. The 5-petaled flowers, generally in shades of red, pink, purple, orange, or yellow, are 7.5–12.5 cm in diameter and are often fragrant. The tree begins flowering in late winter and often continues to flower into early summer (Cooke, 1903).

Some of the *Bauhinia* species have a long history of traditional and medicinal applications. The entire *B. purpurea* L. plant has been used in cases of dropsy, rheumatism, convulsions, delirium, and septicaemia (Asolker et al., 2000). *B. purpurea* L. possesses potential antiproliferative and antioxidant activities (Zakaria et al., 2011). Various extracts of the leaves of *B. racemosa* L. have

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been studied to develop a new pharmaceutical drug for the prevention of enteric infections (Dahikar et al., 2011). The stem bark of *B. racemosa* L. is astringent and used in the treatment of headache, fever, skin diseases, tumour, blood diseases, dysentery, and diarrhoea (Prakash and Khosa, 1976).

Kotresha and Seetharam (1995) performed epidermal studies in some species of *Bauhinia* L. Lusa and Bona (2009) conducted comparative morphological and anatomical analyses of *B. forficata* L. and *B. variegata* L. However, not much work has been done on the micromorphology of *Bauhinia*. Hence, in the present study micromorphological aspects of 5 species of *Bauhinia* L. were examined.

2. Materials and methods

Fresh leaves of the different species of *Bauhinia* were collected during March and April in 2010 and 2011 from Junagadh Agricultural University, Junagadh (Gujarat, India) and Vadodara (Gujarat, India). The source of each species is listed below:

Species	Locality
B. racemosa	Lal Dhori hill (Junagadh)
B. blakeana	Campus of Junagadh Agricultural University
B. malabarica	Motibaug, Junagadh Agricultural University
B. tomentosa	Opp. Hansa Mehta Library garden, MSU Baroda
B. purpurea	Science faculty garden, MSU Baroda

All species were identified with help of *Gujarat Flora* (Shah, 1978) and *The Flora of the Presidency of Bombay* (Cooke, 1903) except *B. blakeana*, which is a hybrid between *B. variegata* and *B. purpurea*, grown in Junagadh.

To obtain epidermal surfaces, portions of trimmed leaf samples were soaked in Jeffrey's fluid for 24 h at 58 °C in an oven. Upper and lower epidermises were separately stripped off gently with the help of needles and forceps. The epidermal peels were washed thoroughly with water 2–3 times, stained in 0.05% aqueous toluidine blue in 1% borax, mounted in 50% glycerine, and observed under a light microscope. Microphotographs were taken using a digital camera fitted onto a Leica DME microscope.

Qualitative and quantitative features of epidermal cells, stomata, and trichomes from 10 different peels were assessed under uniform magnification (Salisbury, 1927).

3. Results

The following account is based on the epidermal characters of 5 species of *Bauhinia*. Qualitative and quantitative micromorphological features of *Bauhinia* species are presented in Tables 1, 2, and 3. All epidermal cells are polygonal with straight anticlinal wall patterns; however, in *B. tomentosa*, the anticlinal walls are irregular and wavy with an undulating outline. In *B. racemosa*, the epidermal cells have anticlinal walls and anisocytic and anomocytic stomata (Figure 1); *B. blackeana* has anticlinal walls and anisocytic and paracytic stomata (Figure 1); *B. malabarica* has an anticlinal wall pattern with anisocytic stomata (Figure 1); in *B. tomentosa*, irregular, undulating epidermal walls are found with paracytic stomata (Figure 1); and *B. purpurea* has anticlinal walls and paracytic and anisocytic stomata (Figure 1). The cell length and breadth also varied among species, with the maximum length and

Table 1. Micromorphological features of epidermal cells and their dimensions.

Taxa	Leaf surface	Epidermal cell shape and nature of cell wall	Epidermal cell length (μm)	Epidermal cell width (μm)	Epidermal cell frequency (mm^2)
B. racemosa	upper	polygonal, straight	28.79	25.06	601
	lower	rectangular, straight	26.67	26.66	530
B. blakeana	upper	polygonal, straight, some beaded	33.4	30.93	517
	lower	polygonal, straight	42.84	20.88	539
B. malabarica	upper	polygonal, straight, beaded	24.5	30.32	430
	lower	polygonal, straight	23.96	29.7	400
B. tomentosa	upper	irregular, undulated	60.56	66	78
	lower	irregular, undulated	59	69	76
B. purpurea	upper	polygonal, beaded	26.48	27.58	432
	lower	polygonal, straight	34.8	28	496

Table 2. Micromorphological features of stomata and their dimensions.

Taxa	Leaf surface	Stoma type	Stomatal frequency (mm^2)	Guard cell length (μm)	Guard cell breadth (μm)	Subsidiary cell length (μm)	Subsidiary cell breadth (μm)	Stomatal index (%)
B. racemosa	upper	absent						
	lower	anisocytic & anomocytic	14	14.54	3.8	21.76	17.48	16
B. blakeana	upper	anisocytic & paracytic	8	10.3	3	20.98	11	11
	lower	anisocytic & paracytic	24	12.77	3.32	25.43	12.82	23
B. malabarica	upper	absent						
	lower	anisocytic	30	13.26	2.8	26.2	13.22	24
B. tomentosa	upper	absent						
	lower	paracytic	13	12.2	3	54	60	8
B. purpurea	upper	absent						
	lower	paracytic & anisocytic	18	12	5.1	26.5	17.5	19

Table 3. Micromorphological features of trichomes and their dimensions.

Taxa	Leaf surface	Trichome type	Trichome frequency (mm^2)	Trichome length (μm)	Trichome breadth (μm)	Trichome index
B. racemosa	upper	absent				
	lower	unicellular, covering	3	56	14.35	1.53
B. blakeana	upper	absent				
	lower	multicellular, uniseriate unicellular, covering	11	89	13.7	7.6
B. malabarica	upper	multicellular, uniseriate, unicellular, hooked	2	89	10.8	1.89
	lower	multicellular, uniseriate, unicellular, covering	9	100	11.3	11
B. tomentosa	upper	unicellular, covering	11	90	10.9	18
	lower	unicellular, covering	15	96		20
B. purpurea	upper	absent				
	lower	multicellular, uniseriate, covering	1	87	13.1	0.51

breadth appearing in *B. tomentosa* (60.56 μm and 66 μm, respectively), minimum length in *B. malabarica* (23.96 μm), and minimum breadth in *B. blakeana* (20.88 μm). *B. tomentosa* differs from the other species by its irregularly shaped epidermal cells with undulating walls. All the other species had polygonal epidermal cells with straight or beaded walls, with the exception of the lower epidermis of *B. racemosa*, which had rectangular epidermal cells.

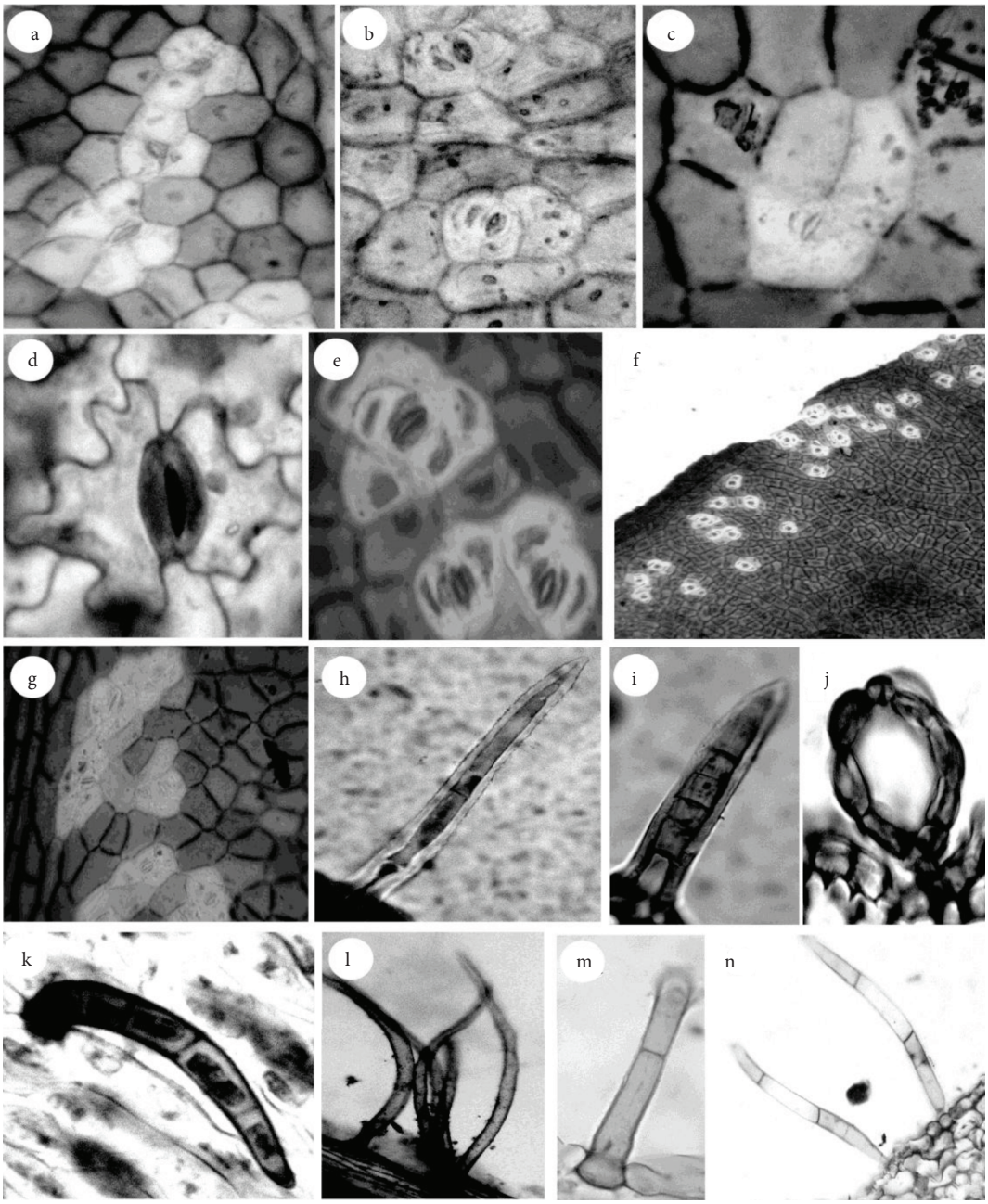

Figure 1. Stomata and trichomes. **a-** *B. racemosa*: anisocytic and anomocytic stomata, b- *B. blakeana*: anisocytic and paracytic stomata, c- *B. malabarica*: anisocytic stomata, d- *B. tomentosa*- paracytic stomata, e- *B. purpurea*: paracytic and anisocytic stomata, f- *B. purpurea*: stomata crowded at margins, g- *B. purpurea*: stomata grouped near midvein, h- *B. racemosa*: multicellular covering trichome, i- *B. blakeana*: multicellular covering trichome, j- *B. blakeana*: multicellular covering trichome, k- *B. malabarica*: hooked and multicellular trichome, l- *B. tomentosa*: unicellular covering trichome, m- *B. purpurea*: multicellular covering trichome, n- *B. purpurea*: multicellular covering trichome.

All 5 species are hypostomatic except *B. blakeana*, which is amphistomatic. All species have paracytic and anisocytic stomata except *B. racemosa*; here, the paracytic type of stoma was absent, and only anomocytic and anisocytic stomata were present (Figure 1). *B. blakeana* had an abnormal type of stoma with only a single subsidiary cell or 2 adjacent stomata with common subsidiary cells (Figure 1). In *B. malabarica* and *B. tomentosa* the subsidiary cells were normal (Figure 1). In *B. purpurea* distribution of stomata was restricted and typically

crowded near the leaf margin and the midvein (Figure 1). There was great variation in the stomatal index (Table 2), with the highest in *B. malabarica* (24.65%) and the lowest in *B. tomentosa* (8%). Variations in the length and breadth of subsidiary cells and guard cells were also recorded. The highest subsidiary cell length and breadth were observed in *B. tomentosa* (54 µm and 60 µm, respectively) while the lowest was found in *B. racemosa* (21.76 µm and 17.48 µm, respectively). The highest guard cell length was seen in *B. racemosa* (14.54 µm) and the lowest in *B. blakeana* (10.30 µm). Guard cell breadth was highest in *B. purpurea* (5.1 µm) and lowest in *B. malabarica* (2.8 µm) (Table 2).

Observed foliar trichomes were non-glandular and covering, and 2 types of trichomes could be distinguished: unicellular, uniseriate, covering and multicellular, uniseriate, covering. Trichomes were absent on the upper surfaces of the leaves in *B. racemosa* and *B. blakeana*. In *B. racemosa* there was a multicellular covering trichome (Figure 1), in *B. blakeana* a multicellular covering trichome (Figure 1), in *B. malabarica* a hooked and multicellular trichome (Figure 1), in *B. tomentosa* a unicellular covering trichome (Figure 1), and in *B. purpurea* a multicellular covering trichome (Figure 1). Multicellular trichomes are 2–4 celled and thick walled. In *B. malabarica*, multicellular and unicellular trichomes were present on both surfaces. In *B. blakeana* and *B. purpurea*, multicellular trichomes were seen only in the lower region.

Trichome frequency varied in all species. The highest frequency (15 per mm^2) was found in *B. tomentosa* and the lowest (1 per mm^2) in *B. purpurea* (Table 3). The length of the trichome varied in different species. It could be categorised into long trichomes (95 µm and above) and short trichomes (below 95 µm). The longest trichomes (100 µm) were observed in *B. malabarica* and the shortest in *B. racemosa* (56 µm). The breadth in *B. racemosa* was highest, and it was lowest in *B. malabarica*. The trichome index was also calculated for all species, and it showed variation among species, with the highest in *B. tomentosa* and the lowest in *B. purpurea*.

4. Discussion

The investigated taxa showed a number of characters in the epidermal cells, stomata, and trichomes. Boodle and Fritsch (1908) reported that the significance of differences in epidermis structure was in the shape of the cells or structure of the cell walls in some *Cassia* species. In this study the differences in the cell walls, which are of only 3 types (straight, wavy, and sinuate) have been confirmed. The present study found that epidermal cell walls in all *Bauhinia* species are polygonal and straight, except in *B. tomentosa*, where they are wavy and undulating.

Studies of stomata can have great taxonomic significance for the delimitation of different levels of taxa (Kothari & Shah, 1975). According to Carpenter and Smith (1975), variations in stomatal frequencies have taxonomic importance at a generic level. Patil and Patil (1987) investigated stomatal distribution, frequency, index, and size in the leaves of 11 species and varieties of *Chlorophytum* L. and showed that these characters were significant at the subgenus level. Carlquist (1961) emphasised the contribution stomatal size variation made in delimiting species within a genus. Major variations in stomatal frequencies of *B. malabarica* and *B. tomentosa* are also notable; the distribution of stomata is also very specific in *B. blakeana*, which has an amphistomatic condition (the other studied species are hypostomatic). *B. purpurea* also differs due to the crowded conditions near the leaf margins and veins. The ratio of average length and breadth of guard cells and subsidiary cells also showed some dissimilarity; thus, the species can be differentiated on the basis of all of the above-mentioned characters.

Trichome features are now considered important in taxonomic studies (Leelavathi & Ramayya, 1983). This study found that *Bauhinia* species have both long and short hairs, but the size and the morphology of the hair differ. In *B. racemosa* the hairs are unicellular, long, and taper to a pointed tip; in *B. malabarica* hairs are multicellular and hooked. Bannerje et al. (2002) studied the micromorphology of tree legumes and found that *Bauhinia variegata* does not have any trichomes on its epidermis. Thus, different species of the same genus may also be identified by their distinct trichome characters.

The reliability of epidermal characteristics as taxonomic indicators varies from one group of plants to another.

A diagnostic key has been prepared for the 5 species of *Bauhinia* studied, based on foliar micromorphological features.

1. Epidermal cell wall undulating ..... *B. tomentosa*
1. Epidermal cell wall straight 2
2. Stomata amphistomatic ..... *B. blakeana*
2. Stomata hypostomatic 3
3. Stomata crowded at margin ..... *B. purpurea*
3. Stomata distributed evenly 4
4. Trichome unicellular, straight, and tapering; very high frequency ..... *B. racemosa*
4. Trichome multicellular, uniseriate, and hooked .... *B. malabarica*

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