Studies on the foliar epidermal architecture of some Indian Cassiinae (Caesalpiniaceae)

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ABSTRACT

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It has been observed that there is great degree of variation in the shape, size and structure of epidermal cells in different species and also on the two surfaces of same leaf of Cassiinae. The size of the epidermal cells in tree species is smaller than those in herbaceous species. This may be due to age, environmental condition or different position of leaf on the shoot. The basic type of stomatal apparatus recorded is paracytic type, particularly holoparacytic and various gradations towards anomocytic condition such as staurocytic, stephanocytic, brachiparacytic and hemiparacytic conditions have been observed in different species. Many such variations have been observed even on the same surface of the leaf in *Senna siamea* and *Senna surattensis*. It appears that these gradations in stomatal architecture have been derived by longitudinal and transverse divisions of one or both the subsidiary cells of the paracytic stomata. The stomatal architecture in the genus also suggests the meso or xero-mesomorphic nature of taxa. The stomatal frequency is greater in the tree species,. The stomatal index varies in different species but it is nearly constant in a species growing under different ecological conditions and hence bear taxonomic implication. Variation has also been reported in the structure of trichomes.

Key-words: Foliar epidermal architecture, Cassiinae, Caesalpiniaceae, India.

INTRODUCTION

Subtribe Cassiinae of family Caesalpiniaceae was established by raising the genus *Cassia* L. to the level of subtribe and elevating the previous subgenera to the generic rank. It includes three genera, viz. *Cassia* L., *Senna* Mill. and *Chamaecrista* Moench., comprising about 600 species of trees, shrubs and herbs, distributed in tropics and subtropics (Irwin & Turner 1960).

Morphological variability of the epidermal characteristics, particularly stomatal complex, the number, form and arrangement of subsidiary cells associated with stomatal guard cells, have shown that epidermal architecture can provide valuable taxonomic and systematic evidence in angiosperms (Linsbauer 1930, Florin 1931, 1951, Tomlinson 1947; Metcalfe & Chalk 1950, Pant & Kidwai 1964, Baranova 1972, 1992, Wilkinson 1979, Upchurch 1984, 1995; Kotresha & Seetharam 1995, 2000, Kong 2001, Carpenter 2005). Epidermal micromorphology shows considerable variation at various level of taxa. These features are taxonomically significant in determining phenetic relationship at even generic level (Parveen & Pullaiah 2008). The detailed epidermal micromorphology of the genus *Cassia* was studied by Okpon (1969a, b), Pandey (1970), Shah and Gopal (1971), Leelavati (1976), Reddy and Shah (1978) and Kotresha and Seetharam (2000). They reported that the species differ in form and size of the epidermal cells, nature of anticlinal walls and form and structure of hairs.

In the present investigation, the foliar epidermal features of ten species belonging to two genera (*Cassia* and *Senna*) of subtribe Cassiinae have been studied and compared to find out their importance in identification and taxonomic delimitation of closely related taxa. The characteristic distribution of stomata, type of stomatal complex, stomatal frequency, stomatal index and trichome features have been studied to

resolve the taxonomic riddles of some closely related species of the genera *Cassia* and *Cenna*. In addition, the effect of environmental factors on the distribution of stomata in different species of the genus, growing in ecological niches of Allahabad, are also studied.

MATERIAL AND METHOD

The epidermal peels of fresh leaflets of 10 species, collected from different localities of Allahabad were studied. These species are *Cassia fistula* L., *Cassia javanica* L., *Cassia nodosa* Buch-Ham. ex Roxb., *Cassia renigera* Wall. ex Benth., *Senna alata* (L.) Roxb., *Senna obtusifolia* (L.) Irwin & Barneby., *Senna occidentalis* (L.) Roxb., *Senna siamea* (Lam.) Irwin & Barneby., *Senna surattensis* (Burm.f.) Irwin & Barneby. and *Senna tora* (L.) Roxb. The leaflets were cut into suitable pieces. The mid-portion of mature leaflets are soaked in aqueous sodium hypochlorite solution. The epidermal peels of both abaxial and adaxial surfaces were taken out by stripping off or by scrapping the segments with blade. The peeled pieces were washed in water and stained in 1% aqueous safranin and mounted in 50% glycerine. Several samples of every species were selected. The preparations were examined under microscope and photographs of the epidermal peels were taken with the help of Nikon micro photographic apparatus at 150x and 450x magnifications. Epidermal cell frequency, epidermal cell size, stomatal architecture and their size, stomatal frequency, stomatal indices and trichomal features were studied. The stomatal index of different species was calculated by the expression used by Salisbury (1928).

Stomatal Index (SI) = S / (E+S) × 100; S = Number of stomata in a unit area; E = Number of epidermal cells in the same unit area

Terminology: Terminology pertaining to stomata is very specialized and has been used consistently. It is necessary to define the terms used here. Stoma (Stomata) refers to stomatal pore and a pair of guard cells that form it. Stomatal complex refers to the stomata plus any specialized epidermal cells (adjacent to it). These definitions have been followed by majority of authors (Metcalfe & Chalk 1950, Esau 1953, Pant 1965, Stace 1965, Van Cotthem 1970, Baranova 1983, 1992). In the present study, the term contact cells has been adopted for the specialized or nonspecialized subsidiary cells (used by Upchurch 1984, Carpenter 2005), adjacent to the stomata. The following terminology has been used for stomatal complexes reported in various species of the genus.

1. Paracytic: Characterized by one or two lateral subsidiary cells oriented parallel to the guard cells (Dilcher 1974, Carpenter 2005). a. **Holoparacytic:** If the two subsidiary cells are well differentiated and lie parallel to the long axis of the pore (Dilcher 1974); b. **Brachiparacytic**: If the subsidiary cells are weakly differentiated or with one or both the subsidiary cells are notably short (Carpenter 2005); and c. **Hemiparacytic**: If the stomata are surrounded by single weakly differentiated subsidiary cell (Dilcher 1974).

2. Staurocytic: Stomata surrounded by four, (some times three or five) similar subsidiary cells more or less radially elongated (Von Cotthem 1970)

3. Stephanocytic: It comprises a more or less well defined rosette of four or more weakly specialized subsidiary cells (Baranova 1992).

OBESERVATION

Observations on the epidermal characters of 10 species under Cassiinae growing in the ecological niches of Allahabad District are shown in Tables 1 and 2 and Plates 1 and 2.

Epidermal cell complex: The epidermal cells on the two surfaces of pinna are dissimilar in shape, size and thickness of the anticlinal walls. They are either

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Plate 1

1-15. Stomatal Complex. 1. *Cassia obtusifolia* (abaxial), 2. *Cassia obtusifolia* (adaxial), 3. *Cassia tora* (abaxial), 4. *Cassia tora* (adaxial), 5. *Cassia occidentalis* (abaxial), 6. *Cassia occidentalis* (abaxial), 7. *Cassia occidentalis* (abaxial), 8. *Cassia occidentalis* (adaxial), 9. *Cassia occidentalis* (adaxial), 10. *Cassia alata* (abaxial), 11. *Cassia alata* (adaxial), 12. *Cassia siamea* (abaxial), 13. *Cassia surattensis* (abaxial), 14. *Cassia surattensis* (adaxial), 15. *Cassia fistula* (adaxial). Bar: 25µm; Magnification: x450.

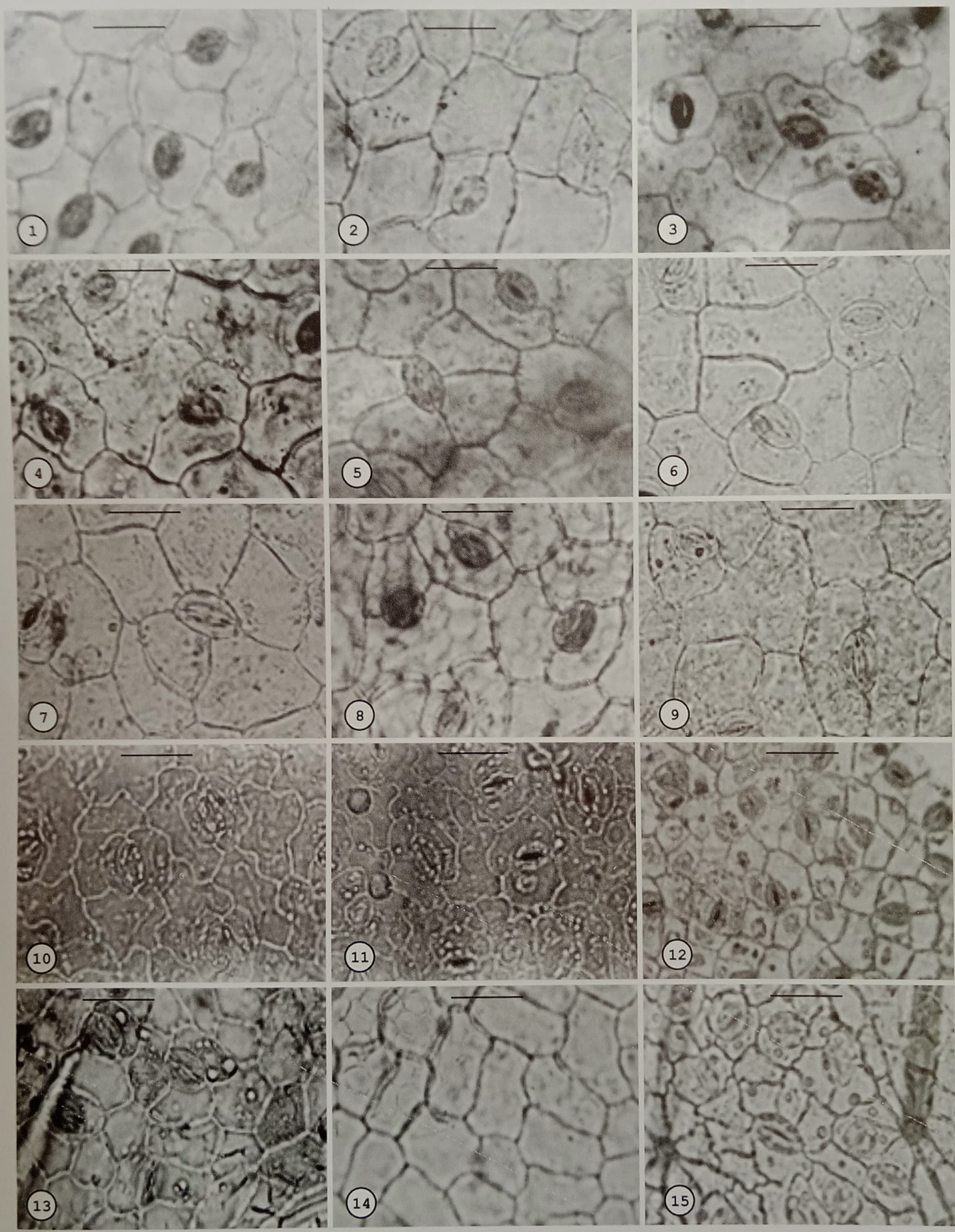

Plate 1

Table 1. Epidermal and trichomal features of some species of Cassiinae

Taxa	Type of epidermal cell wall		Average size of epidermal cell (µm)				Trichome		Size (µm)
	Abaxial surface	Adaxial surface	Abaxial surface		Adaxial surface		Occurrence	Form	
			Length	Width	Length	Width			
C. fistula	Polygonal, anticlinal wall undulate or straight	Polygonal, anticlinal wall straight	±20	±10	±18	±14	Densely dispersed on both the surfaces	Unicellular, hair base cells distinct	50-152
C. javanica	Polygonal, anticlinal wall straight	Polygonal, anticlinal wall straight	±14	±9	±13	±13	Sparsely dispersed on both surfaces	Multicellular, hair base cell distinct.	40-150
C. nodosa	Irregular, anticlinal wall sinuous	Irregular, anticlinal wall straight	±19	±11	±17	±9	Numerous on both surfaces	Multicellular, hair base cells not distinct.	47-183
C. renigera	Polygonal, anticlinal wall straight	Polygonal, anticlinal wall straight	±18	±10	±13	±9	Numerous, multicellular on both surfaces	Multicellular, hair base cells not distinct	55-203
S. alata	Irregular, anticlinal wall sinuous, cells papillose, papillae frequent and prominent	Irregular, anticlinal wall sinuous, cells papillose, papillae less frequent	±34	±21	±40	±16	Sparsely dispersed on both surfaces	Unicellular, hair base cells distinct	30-110
S. obtusifolia	Irregular, anticlinal wall undulate	Irregular, anticlinal wall undulate or straight	±48	±23	±45	±25	Numerous on both surfaces	Multicellular, hair base cells distinct.	120-370
S. occidentalis	Polygonal, anticlinal wall straight or slightly undulate	Polygonal, anticlinal wall straight or slightly undulate	±35	±24	±61	±30	Scanty, confined to the leaf margin of both surfaces	One to several cell, glandular or non glandular	155-396
S. siamea	Polygonal, anticlinal wall straight, cells papillate	Polygonal or rectangular, anticlinal wall straight	±18	±13	±31	±20	Numerous on both surfaces	Multicellular, hair base cells distinct	45-140
S. surattensis	Polygonal, anticlinal wall straight or undulate	Polygonal, anticlinal wall straight or slightly undulate	±30	±15	±35	±18	Present on lower surfaces	Multicellular, hair base cells distinct	53-170
S. tora	Irregular, anticlinal wall slightly undulate	Irregular, anticlinal wall slightly undulate	±44	±22	±57	±26	Numerous on both surfaces	Multicellular, hair base cells not distinct	160-395

polygonal with straight or slightly undulate anticlinal walls (*C. fistula*, *C. javanica*, *C. renigera*, *S. occidentalis*, *S. siamea* and *S. surattensis*) or of irregular shape with markedly sinuous anticlinal walls (*C. nodosa*, *S. alata*, *S. obtusifolia*, *S. tora*). The average size of the abaxial cell ranges from 14 × 9 µm in *C. javanica* to 48 × 23 µm in *S. obtusifolia*, and adaxial cell ranges from 13 µm, in C. *renigera* to 61 × 23 µm in *S. occidentalis*. The epidermal cells may be papillose with single prominent papillae or idioblast in some cells of perennial species (*C. fistula, C. nodosa, C. renigera, S. alata, S. siamea* and *S. surattensis*) but absent in

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Plate 2

1-6. Stomatal Complex. 1. *Cassia fistula* (adaxial), 2. *Cassia nodosa* (abaxial), 3. *Cassia javanica* (abaxial), 4. *Cassia renigera* (abaxial), 5. *Cassia siamea* (adaxial), 6. *Cassia fistula* (adaxial); 7-15. Epidermal Trichome. 7. *Cassia obtusifolia* (adaxial), 8. *Cassia alata* (abaxial), 9. *Cassia surattensis* (abaxial), 10. *Cassia siamea* (adaxial), 11. *Cassia renigera* (abaxial), 12. *Cassia javanica* (abaxial), 13. *Cassia nodosa* (abaxial), 14. *Cassia fistula* (abaxial), 15. *Cassia tora* (abaxial). Bar: 25µm; Magnification: Figures 1 to 6 x450; Figures 7 to 15 x150.

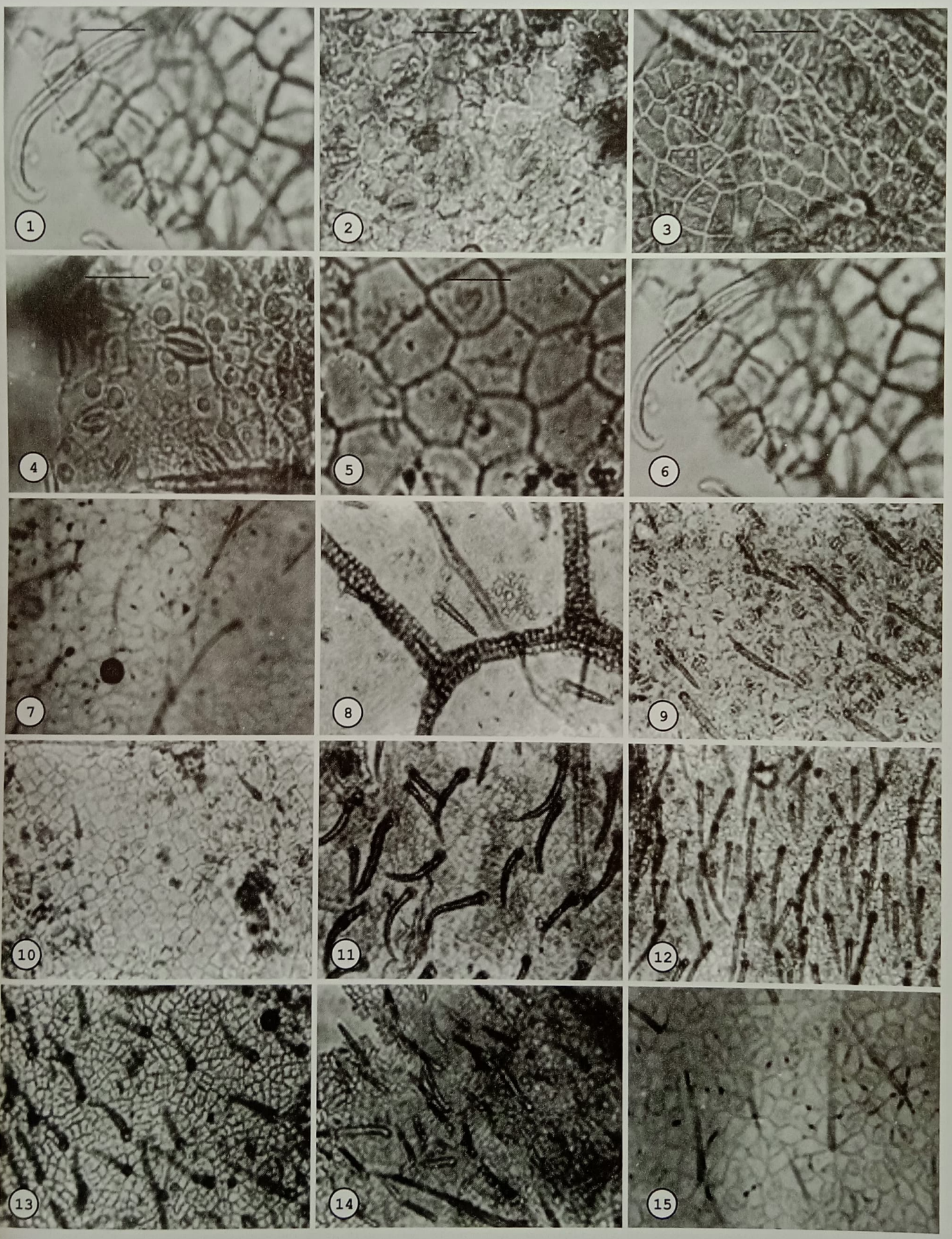

Plate 2

herbaceous species (*S. obtusifolia, S. occidentalis and S. tora*).

Trichome complex: The epidermal appendages called trichomes show wide variation in distribution and structure in different species of the subtribe. In some species, they are quite scant, being confined chiefly to the margin of the pinna, while in others, they may occur all over the leaf surface. The dense hairs are variable, one to several cells high, uniseriate and thick walled. The surfaces of the hairs are often ornamented. They may be horn-shaped, spindle-shaped, unicellular, or multicellular. The foot cells are generally rounded and swollen and they are inserted in a thick-rimmed pore. The hair base cells are usually distinct, but in some species, the difference in the epidermal cells is not very sharp.

Stomatal complex: Leaves examined are both hypostomatic and amphistomatic. The stomata are dispersed randomly over the whole abaxial surface in tree and shrub species and the whole abaxial and adaxial surface of herbaceous species. The basic stomatotype of the genus *Cassia* is paracytic type as reported earlier (Okpon 1969, Pandey 1970, Leelavati 1976).

During the present course of our studies, it was observed that the subtribe Cassiinae is heterostomatic, characterized by different stomatotypes on a single leaf but holoparacytic type prevails over other type. Apart from holoparacytic type other stomatotypes are surrounded by variable number of contact cells, which are generally indistinguishable or slightly distinguishable from the other epidermal cells and they appear to form a ring consisting of variable number of cells (3,4 or more)

Table 2: Stomatal features of some species of *Cassiinae*

Taxa	Stomatal type		Stomatal size (μm)				Stomatal index		Stomatal frequency	
	Abaxial surface	Adaxial surface	Abaxial surface		Adaxial surface		Abaxial	Adaxial	Abaxial	Adaxial
			Length	Width	Length	Width				
C. fistula	Holoparacytic, Stephanocytic, Brachyparacytic	-	±16	±12	-	-	±21	-	±328	-
C. javanica	Holoparacytic, Stephanocytic, Brachyparacytic	-	±18	±11	-	-	±15.4	-	±205	-
C. nodosa	Holoparacytic Staphanocytic, Brachyparacytic	-	±20	±11	-	-	±13.2	-	±123	-
C. renigera	Holoparacytic, Stephanocytic	-	±16	±10	-	-	±9.7	-	±103	-
S. alata	Holoparacytic	Holoparacytic	±16	±11	±16	±11	±19.3	±17	±125	±153
S. obtusifolia	Holoparacytic, Staurocytic	Holoparacytic	±17	±12	±14	±14	±21.7	±25.1	±151	±97
S. occidentalis	Holoparacytic, Stephanocytic	Holoparacytic, Staurocytic	±20	±14	±17	±14	±21.1	±18.4	±123	±99
S. siamea	Holoparacytic, Staurocytic, Brachyparacytic	-	±13	±9	-	-	+18.2	-	+353	-
S. surattensis	Holoparacytic Staurocytic, Hemiparacytic	-	±18	±12	-	-	±12.6	-	+176	-
S. tora	Holoparacytic, Staurocytic	Holoparacytic, Staurocytic	±16	±11	±15	±11	±17.8	+16.4	±125	±103

around the stoma and these stomatotypes have been recognized as staurocytic, stephanocytic, brachyparacytic and hemiparacytic types in different taxa of the genus (Plate 1, 2 and table 2).

The stomata are usually of oval shape with rounded poles. 'T' shaped thickenings are often seen at the poles. The guard cells have thin outer wall and moderately thickened inner wall. The stomata are of variable medium size, i.e. not more than 30 μm long but less than 20 μm wide being generally longer than wide. Stomatal size ranges from 13 × 9 μm on abaxial surface of *S. siamea* to 27 × 18 μm on the adaxial surface of *S. occidentalis*.

Stomatal index varies from ±12.6 on the abaxial surface of *S. surattensis* to ±25.1 on the adaxial surface of *S. obtusifolia* but it is nearly constant in a species growing under different ecological conditions. Stomatal frequency varies in different species and even in the plants of the same species, growing under different ecological conditions. The species, which are generally tree, show higher stomatal frequencies as compared to herbaceous or shrubby species. The highest stomatal frequency has been recorded in *C. fistula* (±353) and *S. siamea* (±328) planted over the national highways by the forest department.

DISCUSSION

Present studies on subtribe Cassiinae (*Cassia* L.) has revealed that the epidermal cells in the pinna of different species vary in size and form. They may be polygonal or irregular. The average length of epidermal cell is greater in herbaceous species as compared to shrub and tree species. In herbaceous species, the adaxial epidermal cells are longer than the abaxial cells, but no such correlation exists between the epidermal cells of the tree species. The anticlinal walls of epidermal cells are reported to be straight, undulated, and sinuous, in different species (Plate 1, 2, Table 1).

The undulated anticlinal wall has been observed on the both surfaces of *Senna obtusifolia*, *S. occidentalis* and *S. tora*. The sinuate type of anticlinal wall has been seen on the abaxial surface of *C. fistula*, *C. nodosa* and on the both surfaces of *S. alata*. The undulation may be related to the development of stresses during the differentiation of the leaf (Avery 1933) or due to hardening of differentiating cuticle (Watson 1962). The waviness may also be affected by environmental conditions prevailing during leaf development (Linsbauer 1930, Watson 1962).

Present observation shows that those species, which are generally tree, have smaller epidermal cell size (*S. siamea*, *C. fistula*, *C. javanica*). The decreasing cell size may be related with the increasing CO_2 concentration, drier air and drier soil and greater altitude. The herbaceous species growing mostly in humid or more shaded conditions have longer epidermal cells (Table 1).

These observations serve to emphasize the variability of epidermal size not only with age and minor genetic variation but also with environment and position of the leaf on the shoot. The undulation in anticlinal wall in some species is related with increasing the surface area without any increase in volume. Much variation in undulation has been noted in various environmental conditions. The different degree of undulation has been observed in different species. It varies from deeply sinuous to straight walls even within the single species (*C. fistula*, *C. nodosa*, *S. surattensis*). It is apparent that the amplitude of the undulation increases with the increased shade and humidity (Watson 1962). The species growing in the sun, the epidermal cells become mature and rigid more quickly, so that the hardening of the cuticular membranes do not have an effect to such a great depth as in the shade.

In Cassiinae, the basic type of stomatal apparatus is paracytic particularly holoparacytic and various gradations toward anomocytic condition such as staurocytic, stephanocytic, brachyparacytic and hemiparacytic have been probably derived from holoparacytic condition. Jalan (1962) investigated the ontogeny of the stomata in *Schisandra grandiflora* where a similar arrangement of subsidiary cells as in most of the *Cassia* and *Senna* species, were observed. He found that due to the longitudinal or transverse division in one or both of the subsidiary cells, the stomata get surrounded by 3, 4, 5 or more cells. Thus staurocytic, stephanocytic, hemiparacytic and brachyparacytic condition characterized by more than two subsidiary

cells around stomata have arisen in the corresponding manner.

The same explanation may be also applicable for similar cell arrangement found in *Cassia* and *Senna*. It is reinforced by the fact that in *S. siamea* (Plate 2, figure 4) and *S. surattensis* (Plate 2, figure 6) and other species, staurocytic, stephanocytic and hemiparacytic conditions with three, four or many contact cell (subsidiary cells) in the same leaflet epidermis arises by transverse or longitudinal division of one or both the subsidiary cells. Brachyparacytic condition as observed in *S. siamea* (Plate 2, figure 4) and other species arises probably due to the overlapping of the epidermal cells at the poles over the lateral subsidiary cells either from one pole (3 subsidiary cells) or from both the poles (4 or 5 subsidiary cells). The paracytic and its various gradations express the mesomorphic and xero-mesomorphic nature of the taxa (Carr et al. 1986).

The stomatal frequency varies from 97 to 355 (Table 2). Stomatal frequency is greater on the abaxial surface of the amphistomatic leaves but relative frequency is greater in tree species as compared to the herbaceous species. The tree species planted over the highway such as *S. siamea* and *C. fistula* have highest stomatal frequency, 355 and 328 respectively as compared to red *Cassia* tree species like *C. javanica*, *C. nodosa* and *C. renigera* planted in the garden, which indicate that the stomatal frequency increases with increasing concentration of dust particles, automobile exhaust etc. Present result on the variation of stomatal frequency in the same species caused by ecological condition is supported by Salisbury (1928). According to him the highest stomatal frequency in the leaves of trees and shrubs as compared to herbaceous vegetations. The marginal species have greater frequency than the shade flora, since humidity tends to reduce the proportion of stomatal frequency.

The stomatal index is constant for a plant species growing in different ecological conditions as against the stomatal frequency because the latter tends to decrease or increase with high or low humidity respectively. Therefore, the stomatal frequency bears ecological significance as it may be taken as an indicator of environmental humidity while stomatal index may be taken as the taxonomical indicator for a particular plant species. In the present course of studies the stomatal index has been undertaken as a taxonomic criterion along with other epidermal features for delimitating various closely related species, *S. obtusifolia* and *S. tora*; *C. nodosa, C. javanica* and *C. renigera,* that exhibits marked difference in stomatal index (Table 2).

Epidermal trichomes (indumentum) exhibit great diversity of form, structure and function, some of which are taxonomically significant. The condition of the environment usually affects the degree of hairness but the type of hair is usually constant in a species. Unicellular hairs have been reported in *S. alata*, *C. fistula* and *S. occidentalis*, while other species show uniseriate, multicellular hairs with distinct or indistinct foot cells (Table 1 and Plate 2). The hairs are thick walled often ornamented with small tubercles. They may be horn-shaped (*S. occidentalis*), hook-shaped (*C. fistula*), spindle-shaped (*S. siamea*) or sickle-shaped with pointed apex (*S. tora*, *S. obtusifolia*, *C. nodosa,* etc.). Hairs have been found to be more abundant in greater sunlight and wind exposure, drier air, drier soil and higher altitude. It is evident from the present study that epidermal architecture can be used as an additional taxonomic tool in identification and delimitation of different taxa.

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