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Full Length Research Paper

Foliar epidermal anatomy and its systematic implication within the genus *Sida* L. (malvaceae)

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Micro morphological investigations of the foliar epidermal anatomy, particularly the diversity and distribution of glandular and eglandular trichomes on leaves of *Sida alba* L., *S. alii* S. Abedin var. alii, *S. cordata* (Burm. F.) Brss, *S. mysorensis* Wight and Arn, *S. ovata* Forssk. *S. spinosa* L and *S. yunnanensis* S.Y.Hu have been carried out to assess the systematic relevance of epidermal features and trichome diversity within the genus *Sida* L. Configuration of epidermal cells is polygonal to irregular with smooth or undulating outline. Leaves are amphistomatic and amphitrichomic. Six diverse morphological types of glandular and eglandular foliar trichomes are described. Stellate and peltate types of trichomes are characteristically found in all taxa studied. *S. alii* and *S.spinosa* are distinct in having long-stalked and short-stalked capitate trichomes. *S. mysorensis* is characterized by multicellular uniseriate trichomes, but these are not observed in other taxa of genus *Sida*. The results of foliar epidermal anatomy, especially type, distribution and structure of foliar trichomes are of high systematic value in this genus of family Malvaceae.

Key words: Foliar anatomy, trichomes, capitate glands, malvaceae.

INTRODUCTION

The genus *Sida* L. is larger and one of the more complex genera of the family Malvaceae. More than 1000 names have been published in the genus, although recent estimates acknowledge only 150-250 species are distributed in tropics and subtropics of both Hemispheres. Even this collection may be too high. There is much synonymy (Fryxell, 1985). In Pakistan it is represented by 12 specific and infraspecific taxa (Fryxell, 1997; Abedin, 1979). The genus Sida has been heterogeneous from the beginning because Linnaeus and his successors tended to put into *Sida* any member of the Malvaceae whose fruits were not capsular and lacked an involucel (Fryxell, 1997). Many species that were originally placed in *Sida* have been relegated to other genera and this remaining group was subdivided by Fryxell (1985) into 11 sections. Many taxa are occasionally recognized on the basis of variation in the inflorescence. However, a close study of herbarium specimens has shown variation in the inflorescence to be continuous; so any division on this basis is not justified (Naqshi et al., 1988). Variation in the inflorescence of *S. cordata* sometimes gives the illusion of the presence of at least two taxa (Abedin, 1979). The difference between *S. alba*, *S. spinosa* and *S. alnifolia* seems to be little. In fact Riedle (1976) cited *S. alba* and *S. alnifolia* as synonyms of *S. spinosa*. Fryxell (1978) earlier emphasized the morphology of the mericarps and of calyx in delimiting taxa. Study of African material has demonstrated that there are superficially similar species that differ most obviously in particulars of mericarp morphology. More information is needed on variation within populations, particularly in mericarp morphology to evaluate the status of the awnless species of *Sida,* as there are indications that both awned and awnless mericarps can occur on the same plant, thereby suggesting a possible reduction in species numbers (Tang et al., 2007). Hence, it is felt that the time is opportune to begin a new treatment of *Sida* Fryxell, 1985).

The leaf epidermis is generally a valuable character for the classification and delimitation of species and genera, and/or for the discussion of relevant phylogenetic problems (Stace, 1984; Jones, 1986; Baranova, 1972). Although the epidermal anatomy has been described in

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Table 1. List of the species investigated, with location of voucher specimen arranged in alphabetical order of the taxa.

S/N	Species	Locality	Voucher No.
1	*Sida alba* L.	Abbottabad, Gujar Khan & Lahore	07536, 07540 & 22411
2	*S. alii* S. Abedin var. alii	Jhelum, Rawalpindi & Islamabad	71404, 71775 & 07036
3	*S. cordata* (Burm. F.) Brss.	Quetta, Hazara, Lahore, Ponch, Rawalpindi, Muzaffrabad & Attock	71771, 41566, 44802, 83296, 07605, 84675 & 113645
4	*S. mysorensis* Wight & Arn.	Rawalpindi & Sialkot	103361 & 71772
5	*S. ovata* Forssk.	Kohat, Attock & Lahore	07598, 51127 & 55217
7	*S. Spinosa* L.	Rawalpindi, Jhelum, Mir Pur Khas & Chakwal	66166, 74415, 66101 & 74417
6	*Sida yunnanensis* S.y.Hu	Rawalpindi, Islamabad & Muzaffrabad	41555, 38882 & 41554

Table 2. Diversity of foliar trichomes within the genus *Sida* L.

S/N	Type of trichomes	Description	Species
1	Conical	Axillary elongated, broad at the base and tapering above	Present in combination with forked and stellate trichomes on adaxial surface of *Sida cordata* and on both leaf surfaces of *Sida yunnanensis*
2	Forked	Two ray cells present in the same cell cavity	Present in all *Sida* L. species on both leaf surfaces
3	Stellate	Formed of considerable number of ray cells held together in the center. Ray cells may be slightly ascending or spreading along the surface of leaf	Present in all *Sida* L. species on both surfaces
4	Peltate glands	Sessile, cup shaped or rounded with narrow apical opening	Present in all *Sida* L. species
5	Capitate glands	Multicellular and uniseriate having a basal cell , unicellular or multicellular stalk and a terminal more or less rounded or slightly elongated head or gland cell	*S. alii* S. Abedin var. alli and *S. spinosa*
6	Multicellular and seriate	Type I; Single row of 13 cells forming a filament with broad base and gradually narrowing upwards	Common on abaxial surface of *S. mysorensis*
		Type II; a broad basal portion of 5 cells and upper elongated beak like portion with or without transverse constriction	

the leaves of a number of Malvaceae species (Inamdar and Chohan, 1969; Adedeji and Dloh, 2004; Rudgers et al., 2004; Celka et al., 2006), the emphasis was on general anatomical features, ontogeny of stomata, antiherbivore resistance traits or gross morphology of trichomes. Little published work deals with the detailed comparative micro morphological characteristics of foliar epidermis and their systematic relevance within the same genus. The present investigation was undertaken with a view to fill in this gap in our knowledge.

MATERIALS AND METHODS

Dried leaves of representative specimens of genus *Sida* L. in Herbarium of Quaid-i-Azam University Islamabad Pakistan, listed in Table 1 was used for anatomical studies. Dried leaves were placed in boiling water for few minutes to soften until they became unfolded and were ready for epidermal scrapping. Leaf samples were prepared according to the modified method of Cotton (1974), who followed Clark's (1960) technique. The leaves were placed in a tube filled with 88% lactic acid kept hot in boiling water bath (Model, Memmert-91126-FRG, Germany) for about 30 - 40 min. Lactic acid softens the leaf due to which it was possible to scrap the leaf surface with sharp scalpel. Slides of both abaxial and adaxial surface of leaf were prepared and mounted in clean 88% lactic acid. Both qualitative and quantitative micro morphological characteristics of foliar epidermis were observed using light microscope. Micro histological photographs of both surfaces were taken by Nikon (FX-35) Camera equipped light microscope. Basic terminology used in trichome classification and description is that suggested by Harris and Harris (2001). However simple self explanatory terms are added to identify the specific types of trichomes.

RESULTS AND DISCUSSION

The data of quantitative and qualitative anatomical features of *Sida* L. is presented in Table 2-5. The ordinary epidermal cells of the leaves of *Sida* L. were commonly polygonal or irregular in form with merely curved to

Table 3. Quantitative foliar epidermal features within the genus *Sida* L.

Taxa	Ordinary Epidermal cells L. x W. µm Min. (Mean±S.E) Ma.		Stomata L. x W. µm Min. (Mean±S.E) Ma.		L. Sto. opening µm Min. (Mean±S.E) Ma.		Stomatal Complex L. x W. µm Min. (Mean±S.E) Ma.	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
Sida alba	25 (41.67± 3.80)50 x 15(20 ± 1.54) 25	30 (41.67± 3.33) 52 x 15 (19.167 ± 1.54) 25	25 (25± 0) x 15 (15± 0)	25 (25± 0) x 15 (15± 0)	15 (15± 0)	15 (15± 0)	35 (37.5 ± 1.12)40x30 (33.67± 1.17) 36	30 (33.14± 1.14)37 x 30 (32.5 ± 1.12) 35
S. alli	15 (31.87± 4.52) 50 x 15 (17.5 ± 1.12) 20	20 (38.57± 5.09) 58 x 15 (16.17 ± 1.30) 20	20 (20 ± 0) x 10 (10 ± 0),	20 (20 ± 0) x 10 (10 ± 0)	10 (10 ± 0)	10 (10 ± 0)	35 (40.71± 2.54) 50 x 35(35 ± 0)	30 (42.5 ± 2.14) 50 x 40(40 ± 0)
S. cordata	35 (45.71± 3.67) 60 x 20 (22.5 ± 1.118) 25	30 (46.5 ± 5.62) 64 x20 (22 ± 1) 25	20 (22.5 ± 1.12) 25 x 15 (17.5 ± 1.12)20	20 (21.67± 1.05) 25 x 15 (17.5 ± 1.12) 20	10 (13.3 ± 1.05) 15	10 (13.5 ± 1.11) 16	30 (39.17± 2.39) 45 x 30 (38.33 ± 2.11) 45	32 (43.67± 3.33) 55 x 30 (39.17± 2.39) 45
S. mysorensis	20 (32.86± 4.48) 50 x 15 (17.5 ± 1.12) 20	25 (40 ± 4.88) 60 x 20 (33.33± 4.41) 50	15 (17.5 ± 1.12) 20 x 20 (22.5± 1.12) 25	15 (17.5 ± 1.12) 20 x 18 (22.17± 1.30) 25	10 (12.5 ± 1.12) 15	10 (11.67± 1.05)15	30 (32.5 ± 1.12) 35 x 40 (42.83± 1.01) 45	30 (32.33± 1.05) 35 x 42 (45.33± 0.95) 48
S. ovata	30 (38.33± 2.47) 45 x 25 (25± 0)	30 (37.8± 2.24) 45 x 25 (26 ± 0.45)27	15 (15± 0) x 7.5 (7.5± 0)	15 (15± 0) x 7.5(7.5± 0)	10 (10± 0)	10 (10± 0)	40 (42.5 ± 1.12) 45 x 40 (40± 0)	40 (42.5 ± 1.12) 45 x40 (41.64± 0.69) 42.5.
S. Spinosa	25 (33.33± 2.79) 40 x 20 (32.14± 3.42)45	25 (35 ± 3.41) 45 x 25 (30 ± 1.82) 35	20 (20± 0) x 10 (10± 0)	20(20± 0) x 10 (10± 0)	10 (10± 0)	10 (10± 0)	35 (37.5 ± 1.12) 40 x 30(31.67± 1.05)35	35 (38.33± 1.67) 45 x30 (32.5 ± 1.12) 35
S. yunnanensis	35 (44.5 ± 3.25)57.5 x 20 (25 ± 1.82) 30	30 (44.28± 3.85) 60 x 20 (26.14± 1.92) 33	20 (20± 0) x 15 (15± 0)	20 (20± 0) x 16 (16± 0)	10 (10± 0)	10 (10± 0)	35 (44.17± 2.39)50 x30 (39.25± 2.43) 45.5	35 (45.43± 2.34) 53 x25 (37.17± 3.61) 48

L. length, W. width, Min. minimum, Ma. maximum, S.E. standard error, L. sto. opening length of stomatal opening.

nearly straight walls, often thickened (Figures 6-10). Stomata are anomocytic, paracytic, diacytic and aniso-cytic. The differences in most of the anatomical features were of little taxonomic importance to delimit different taxa under study with certainty. The foliar trichomes of *Sida* however possess a remarkable diversity and provide a great deal of systematic evidence; there were six main types. Typically peltate, stellate and forked trichomes were present in all the species investigated and were accompanied by either conical hair (*S. cordat* and *S. yunnanensis*), by stalked capitate trichomes (*S. alii* and *S. spinosa*) or by multicellular and uniseriate tri-chomes (*S. mysorensis*).

Features of hairs are broadly regarded as useful for establishing the systematic relations within the family Malvaceae (Walas, 1959; ILjin 1974; Ramaya and Rao, 1976; Inamdar et al., 1983; Dorr, 1990). Such view was also submitted by-Kubitkzi and Bayer (2003) who used the character of eglandular trichomes in characterizing Malvales.

Stellate trichome is characteristic of entire family Malvaceae (Gamble. 1935; Hutchinson, 1959; Metcalfe and Chalk, 1950; Solereder, 1908) and its details as observed in *Sida* L. reveals that trichome is variable in number of ray cells and their relative length and thick-ness. Number of ray cells in a single trichome ranges between 4-8 but in *S. alba*; it was up to 20. Longest; 200 (277.5 ± 19.09) 325 µm and thickest 30 (35 ± 1.82) µm 40 ray cells were observed in *S. cordata*, associated with lowest number of ray cells that is, 3-4 rarely 5. The lowest length of ray cells recorded in this study was for *S. alba* (25-125 µm). Capitate trichome with or without osculum (apical opening) were absent in most of the species of *Sida*; however such trichomes were observed in *S.*

Table 4. Quantitative data for glandular foliar trichomes in *Sida* L.

Taxon	Peltate H. x W. µm Min. (Mean ± S.E) Ma.	Capitate H. x W. µm Min. (Mean ± S.E) Ma.				Mu. and Uni. L. x W. µm Min. (Mean ± S.E) Ma.	
	Type-II	Type-I	Type-II	Type-III	Type-IV	Type-I	Type-II
Sida alba	15 (30 ± 2.58) 40 x 20 (26.67 ± 1.67) 30	-		-		-	-
S. alli.	25 (38.33 ± 3.07) 45 x 30 (33.33 +/- 1.05) 35			75 (77.5 ± 1.12) 80 x 33 (34 ± 1.3)37	-	-	-
S. cordata	20 (22.5 ± 1.12) 25 x 20 (22.5 ± 1.12) 25	-		-		-	-
S. mysorensis	25 (30 ± 1.82) 35 x 25 (27.5 +/- 1.12) 30	-		-		350 (350+/-0) x 40 (40+/-0)	200 (200+/-0) x 50 (50+/-0)
S. ovata	15 (20 ± 1.82) 25 x 25 (31.67 ± 2.47) 40	-		-		-	-
S. Spinosa	25 (28.33 ± 1.05) 30 x 15 (24.17 ± 2.39) 30	100 (100 ± 0) x 40 (40 ± 0)	100 (108.33 ± 2.79 x 15 (18.33 ± 1.05)20		30 (36.67 ± 1.67)40 x 25 (27.5 +/- 1.12) 30	-	-
S.yunnanens	10 (20.83 ± 2.39) 25 x 20 (22.5 ± 1.12) 25	-		-		-	-

H. height, W. width, L. length. Min. minimum, Ma. Maximum, S.E. standard error, Mu. and Uni multicellular and uniseriate.

spinosa and *S. alii*, S. Abedin var. alii.

Peltate glands Type-II, were found quite variable in their shape, that is cup-shaped, ball like with narrow apical pore or pitcher shaped. Anatomical measurements were rather uniform especially those of width (Table 4) and were found effective to a lesser degree in distinguishing between *Sida* species.

S. cordata was most closely related to *S. yunnanensis* having both stellate and simple unicellular conical trichomes. A conical trichome is composed of only one cell and is surrounded by a ring of 7-9 modified epidermal cells (Figure 5). These surrounding cells nourish and support the trichome (Celka et al., 2006). Both taxa could be radially differentiated on the basis of distribution and anatomical measurements of unicellular conical trichomes. These were thicker and longer in case of *S. cordata* than in *S. yunnanensis* and formed a comparatively dense cover on adaxial surface of *S. cordata*. The measured length of conical trichome in *S. cordata* was up to 475 µm and width of basal part was 30 (35 ± 1.82) 40 µm while in *S. yunnanensis* the observed length was up to 150 µm and width of basal part was 10 (17.5 ± 3.35) 25 µm.

S. spinosa and *S. alii* stayed apart from the rest of species by having glandular capitate trichome as their distinguishing feature. Unlike peltate trichomes, presence or absence and if present the size and structure of these trichomes could be used as a strong supporting character to distinguish *Sida* species. Capitate trichomes generally consist of rounded to pear shaped heads of one to two cells supported by stalks of variable length (Ascensao et al., 1999). The present study indicates the presence of two types of capitate trichomes, long-stalked capitate trichomes (Type-I and II) in *S. spinosa* and short-stalked capitate trichomes (Type-III) in *S. spinosa* and in *S. alii* (Figure 3). Multicellual peltate glands present on abaxial surface of *S. ovata* (Figure 4). Long-stalked capitate trichomes in present study were comparable to the long-stalked capitate trichome described by Ascensao et al. (1999) and glandular multicellular trichome type-I by Jacob et al., (2003). Long-stalked capitate trichome in *S. spinosa* could be clearly differentiated into two main types, Type-I with prominent apical pore or osculum (Figure 8) common on adaxial surface of leaf and Type-II without apical pore (Figure 6), more common on abaxial surface. Short-stalked capitate trichomes found in *S. alii* have unicellular stalk and multicellular slightly elongated head with

Table 5. Quantitative data for eglandular foliar trichomes in *Sida* L.

Taxon	Stellate L. x W µm Min. (Mean ± S.E) Ma.		Conical L. x W. µm Min. (Mean ± S.E) Ma.	Forked Min. (Mean ± S.E) Ma.
	N.r.c	S.r.c		
Sida alba	4 - 20	75 (103.33 ± 9.72) 125 x 10 (12.5 ± 1.12)15	-	50 (94.17 ± 11.14)120 x 10 (12.5 ± 1.12) 15
S. alli	8 - 15	45 (90 ± 11.47) 125 x 7.5 (8.83 ± 0.46) 10	-	40 (71.57 ± 9.53) 100 x7 (9 ± 0.52) 10
S. cordata	3 - 5	250 (294 ± 14.09) 325 x 30 (35 ± 1.82) 40	250 (355 ± 39.05) 475 x 30 (35 ± 1.82) 40	200 (277.5 ±19.09) 325 x 30 (35 ± 1.82) 40
S. mysorensis	8 - 10	125 (175 ± 17.68) 225 x 7 (8.33 ± 0.56) 10	-	125 (161 ± 13.08) 200 x 7 (7 (9 ± 0.52) 10
S. ovata	8 - 12	75 (95 ± 7.30) 125 x 20 (22.5 ± 1.12) 25	-	75 (75 (103.33 ± 9.72) 125 x 20 (22.5 ± 1.12) 25
S. Spinosa	5 - 7	45 (211.67 ± 44.58) 350 x 7.7 (8.95 ± 0.39)10	-	50 (138.33 ± 31.13) 250 x 7 (8.5 ± 0.56) 10
S. yunnanensis	4 - 10	45 (79.17 ± 10.28) 115 x 5 (16.67 ± 3.80) 25	60 (101.67 ± 12.95) 150 x 10 (17.5 ± 3.35) 25	45 (65.5 ± 6.73) 80 x 10 (15 ± 1.82) 20

L. length, W. width, Min. minimum, Ma. Maximum, S.E. standard error, N.r.c. number of ray cells, S.r.c single ray cell,

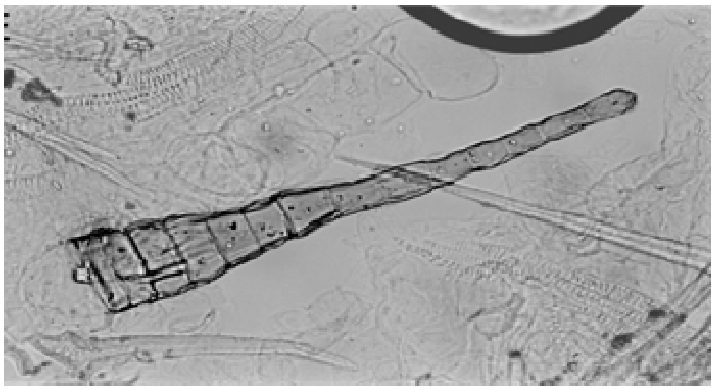

Figure 1. Multicellual and uniseriate trichome type-I on abaxial leaf surface of *S. mysorensis* (200X).

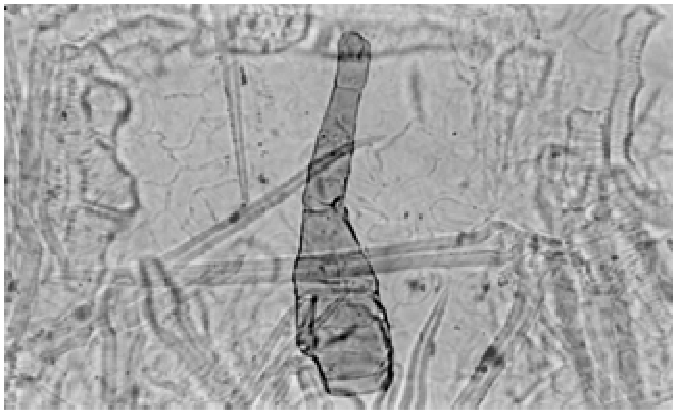

Figure 2. Multicellual and uniseriate trichome type-II on abaxial leaf surface of *S. mysorensis* (200X).

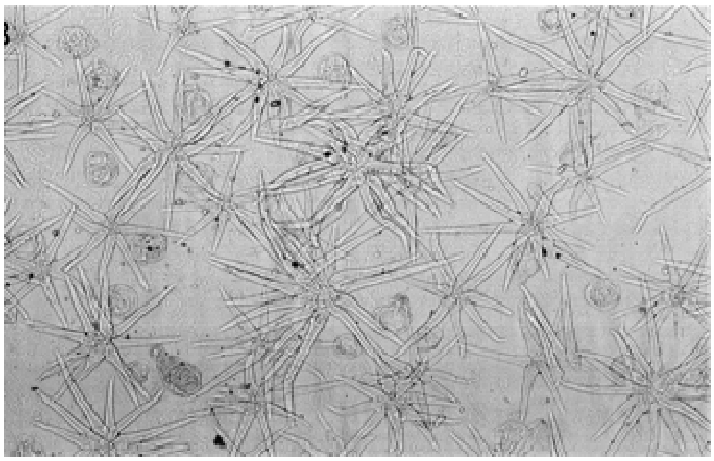

Figure 3. Short-stalked capitate trichome on abaxial leaf surface of *S. alii* (100X).

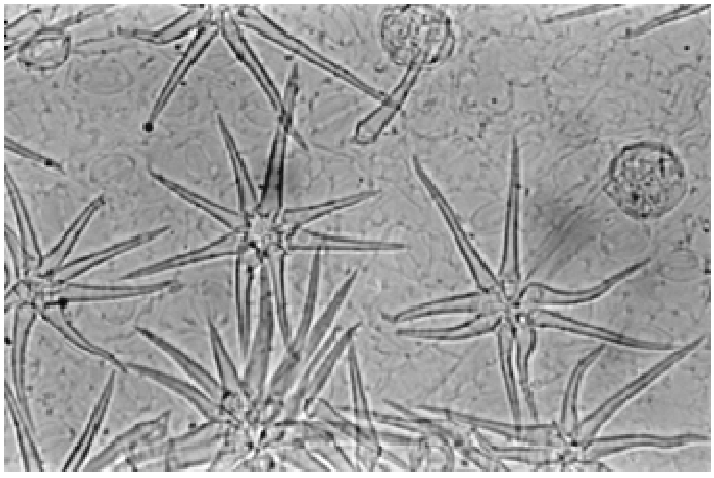

Figure 4. Multicellual peltate glands on abaxial surface of *S. ovate* (200X).

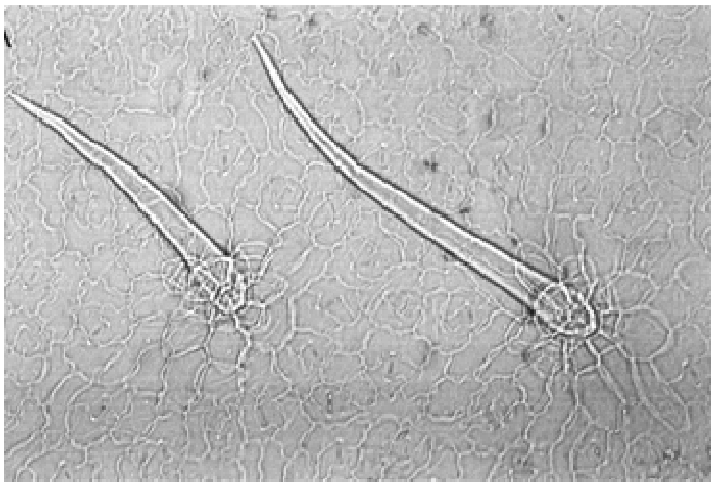

Figure 5. Adaxial leaf surface of *S. cordata* showing simple unicellular conical trichomes (200X).

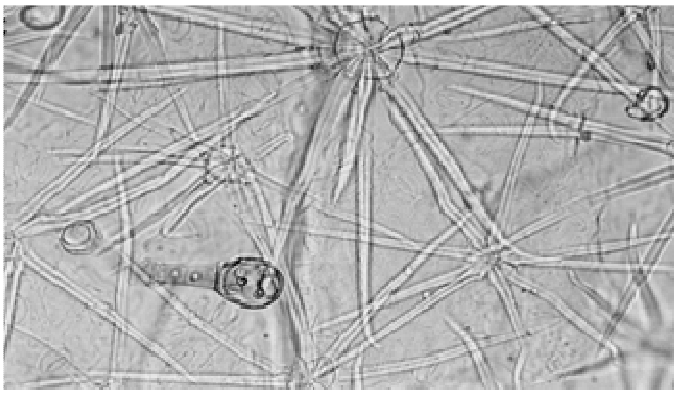

Figure 6. Long stalked capitate trichome and eglandular stellate trichome on abaxial leaf surface of *S. spinosa* (200X).

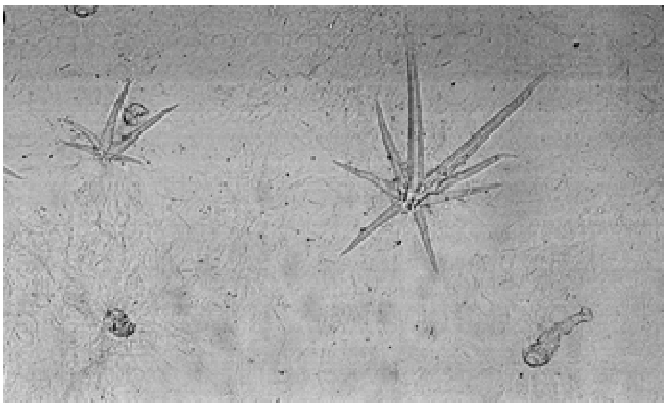

Figure 7. Adaxial surface of *S. spinosa* showing stellate and long stalk trichome (100X).

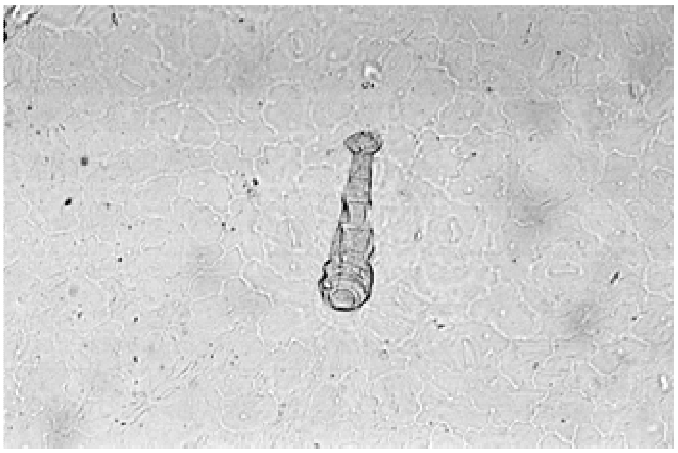

Figure 8. Adaxia leaf surface of *S. spinosa* with long stalked capitate trichome with prominent apical pore (200X).

prominent apical pore. Very rarely trichomes with one celled stalk and semicircular head, like “pileus” of a mushroom (Type-IV Brevicollate Capitate) were found on adaxial surface of *S. spinos* and were similar to those reported on stem of *Ajuga repans* (Akcin et al., 2006). Capitate trichomes in *Sida* species were quite different

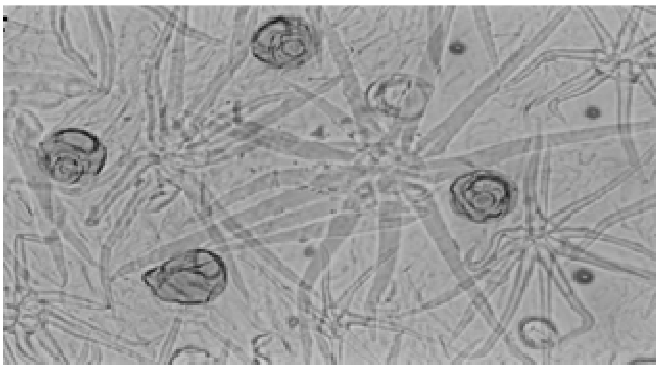

Figure 9. Peltate glands on abaxial leaf surface of *S. alba* (200X).

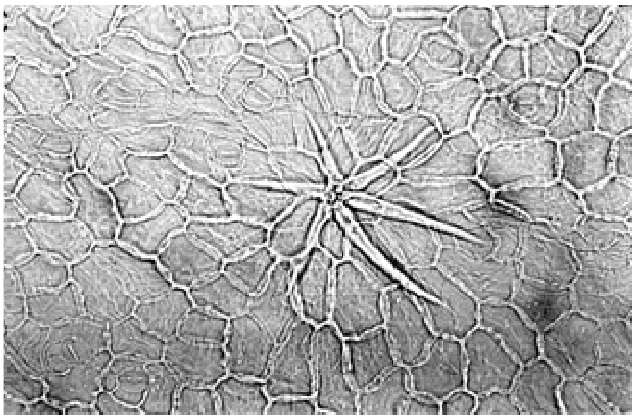

Figure 10. Stellate trichome on adaxial leaf surface of *S. yunnanensis* (200X).

from those reported by Inamdar and Chohan (1969) in *Hibiscus rosa-sinensis* (Malvaceae). It was interesting to note down that *S. spinosa* and *S. alii* also share the same stomatal size and length of stomatal opening (Table 3).

S. mysorensis could be demarked by the presence of multicellular uniseriate trichomes, not observed in other taxa investigated in present work. These were of two types; type-1, having a row of 13 cells , broad at the base and gradually narrowing upwards, while type-II, was with broad basal portion of 5 cells and upper elongated beak like portion with or without transverse constriction (Figures 1 and 2).

It is concluded that detailed comparative study of foliar epidermal features with particular emphasis on better terminology, morphology, micro morphology and distribution of various glandular and eglandular trichomes in *Sida* L may serve as a useful taxonomic tool.

Key to species

(1a) Width of abaxial epidermal cells is in the range of 20 (32.86 +/- 4.48) 50 µm, multicellular and uniseriate trichomes (Type-I and Type-II) present…… *S. mysorensis*

(1b) Abaxial epidermal cell's width is less than 50 µm, multicellular and uniseriate trichomes absent……..2

(2a) Width of adaxial epidermal cells is 15-20 µm, short stalked capitate trichomes Type-II with osculum are frequently seen…… *S. alli*

(2b) Adaxial epidermal cells are of different range, capitate trichomes without osculum are present……3

(3a) Stomata are of only diacytic type, width of stomata on both leaves surfaces is 7.5 µm……*S. ovata*

(3b) Stomata are anaomocytic, anisocytic, diacytic or paracytic type, width of stomata is more than 7.5 µm……...4

(4a) Length of stomatal opening on abaxial surface is in the range of 10 (13.5 +/- 1.11) 16 µm, unicellular peltate glands type III are present……..*S. cordata*

(4b) On abaxial surface, length of stomatal opening is less than 16 µm, peltate glands of type II are present……..5
(5a) Capitate trichomes without osculum are present, widths of adaxial epidermal cells are in the range of 20 (32.14 +/- 3.42) 45 µm…… .*S. spinosa*
(5b) Capitate trichomes absent, width of adaxial epidermal cells is less than 40 µm……6
(6a) Stomata are anomocytic, paracytic and anisocytic, length of stomatal opening on both leaf surfaces is 15 µm…… *S. alba*
(6b) Stomata mostly diacytic, some anisocytic, length of stomatal opening on both leaf surfaces is 10 µm……..*S. yunannensis*

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