

Full Length Research Paper

Implication of foliar epidermal features in the taxonomy of *Abutilon* Mill. (Malvaceae)

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The micro morphology of both adaxial and abaxial leaf surfaces of seven species of the genus *Abutilon* Mill. (Malvaceae) viz., *Abutilon bidentatum* A. R. rich, *Abutilon fruticosum* Guill. & Perr., *Abutilon indicum* (L.) Sweet, *Abutilon molle* (Wild.) Sweet, *Abutilon muticum* (Del. Ex. DC.) Sweet, *Abutilon pakistanicum* Jafri & Ali and *Abutilon theophrasti* Medic was investigated using light microscopy to explore and evaluate the taxonomic implication of foliar epidermal features particularly that of glandular and eglandular trichomes. Leaves of all the seven species were observed to be amphistomatic and amphitrichomic. The ordinary epidermal cells were commonly polygonal with straight walls, or less often irregular in shape, with slightly undulating walls (abaxial surface of *Abutilon muticum* and *Abutilon theophrasti*). Two types of stomata, anisocytic and diacytic were generally more concentrated on abaxial surface. An indumentum of variable density and texture formed of wide variety of glandular and eglandular trichomes on both surfaces was characteristic of the genus. Seven diverse morphological forms of foliar trichomes were recorded. The trichome complements of each of the seven species studied were unique and thus, can easily be used as an important supportive taxonomic character to delimit each.

Key words: *Abutilon*, Foliar trichomes, taxonomy.

INTRODUCTION

Abutilon Mill., originally named by Miller (1754), is one of the larger and most difficult genera of the family Malvaceae without a solid, up-to-date reversionary treatment (Fryxell, 1997). Over 150 species are distributed in the tropics and subtropics of both the hemispheres (Abedin, 1979). The genus is delimited from most other genera in Malvaceae by the lack of an epicalyx, by its mericarps that lack wings, have an endoglossum, and generally show dorsal dehiscence, and by the sub-entire to markedly serrate leaves (Esteves and Krapovickas, 2002). Sweet (1826) segregated several species out of Linnaeus's broadly conceived *Sida* by distinguishing uniovulate species referable to *Sida* from multi-ovulate species treated as *Abutilon*. Subsequently many genera have been segregated out of *Abutilon* (Fries, 1908; Hochreutiner, 1913; Krapovickas, 1970; Kearney, 1949; Bates; 1973, 1978; Fryxell, 1997; Marticorena, 2001). The residual genus however is still very heterogenous

and is in need of critical revisionary treatment (Fryxell, 2002). The infrageneric classification of the genus is poorly understood. The genus has been variously subdivided into sections and subsections by a number of workers, but only for relatively limited geographical areas (Presl, 1830-1835; Grisebach, 1859-1864; Schumann, 1891; Mattei, 1915; Fryxell, 1988).

The Foliar epidermis is one of the most significant taxonomic characters from the biosystematic point of view and the taxonomic studies of a number of families were made on the basis of leaf epidermis (Bhatia, 1984; Stace, 1984; Jones, 1986; Baranova, 1992). Although the epidermal anatomy of the leaves of a number of Malvaceae species has been described (Inamdar and Chohan, 1969; Ramayya and Rao, 1976; Rao and Ramayya, 1977; Adedeji and Dloh, 2004; Rudgers et al., 2004; Celka et al., 2006) the emphasis was on general anatomical features, ontogeny of stomata, herbivore resistance traits or gross morphology of trichomes. Detailed study on comparative micromorphology of foliar epidermis and their systematic relevance within the same genus are however very scarce. Adedeji and Dloh (2004)

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

Species	Locality	Voucher specimen number
<i>A. bidentatum</i>	Kallar Kahar, Said Pur	07647 (ISL!), 39911 (ISL!)
<i>A. fruticosum</i>	Parachinar, Kohat	58537 (ISL!), 55207 (ISL!)
<i>A. indicum</i>	Islamabad, Rawalpindi	119367 (ISL!), 119047 (ISL!)
<i>A. molle</i>	Rawalpindi	07612 (ISL!)
<i>A. muticum</i>	Makran, Quetta	44811 (ISL!), 117998 (ISL!)
<i>A. pakistanicum</i>	Nazimabad, Karachi	119360 (ISL!), 118006 (ISL!)
<i>A. theophrastii</i>	Jhelum	119206 (ISL!)

described the comparative foliar anatomy of ten species in the genus *Hibiscus* L. (Malvaceae), but only few aspects on foliar trichomes were discussed. This paper details the quantitative epidermal morphology of *Abutilon* Mill. and its relevance to the delineation of species.

MATERIALS AND METHODS

Dried leaves of representative specimens of the genus *Abutilon* Mill. in herbarium of Quaid-i-Azam University Islamabad Pakistan (Table 1) were used for anatomical studies. Leaf samples were prepared according to the Clark's (1960) technique as modified by Cotton (1974). Dried leaves were placed in boiling water for few minutes until unfolded. 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 to 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 LM. 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 quantitative and qualitative micro morphological features of foliar epidermis of *Abutilon* Mill. is presented in Tables 2 and 3. Epidermal cells were polygonal in shape, mostly with smooth walls; sometimes the epidermal cells with slightly undulating walls were also observed in *Abutilon molle* and *Abutilon theophrasti*. Though the leaves are amphistomatic and amphitrichomic, the stomata and trichomes were observed to be more concentrated on the abaxial surface. Largest stomatal complex was observed on abaxial leaf surface of *Abutilon bidentatum*, 45 (55 ± 3.53) 65 × 25 (33.2 ± 3.12) 41 µm. The distinguishing characters of taxonomic value include the morphological variation, distribution and anatomical measurements of the foliar trichomes. The trichome complements of each of the seven species studied were unique and thus, can be used as an impor-

tant supportive taxonomic character to delimit each. Solereder (1908), Metcalf and Chalk, (1950); Ramayya, 1962; Uphof (1962), Inamdar (1967), Patel and Inamdar (1972), Inamdar and Patel (1973), Ramayya and Rao (1976), Gangadhara and Inamdar (1977), Rao and Ramayya (1977), Healy et al., (1986), Yan- Ming and Ru-Wen (1993), Celka et al. (2006) emphasized the taxonomic implication of trichomes. Seven diverse morphological forms of trichomes were observed on both leaf surfaces, which were placed in five major categories. Stellate trichomes and peltate glands were found characteristically in all the taxa under study. However, the density, number of ray cells and their relative length and thickness in the stellate trichomes were found variable across the species. The number of ray cells for stellate trichomes in *A. bidentatum*, *Abutilon fruticosum* and *Abutilon pakistanicum* was 4-9, 8-9 and 5-8 respectively. The stellate trichomes with more than twelve ray cells were found common in *Abutilon indicum*, *A. molle* and *A. theophrasti*. *A. muticum* was distinct in having thickest ray cells of their stellate trichomes, 25 (39.17 ± 5.38) 60 µm (Figure 8), whereas longest ray cells of up to 750 µm were observed in *A. fruticosum* (Table 3). Presence of stellate trichomes has been reported in the leaves of a number of Malvaceous plants. In *Malva alcea* L. the most frequent were 4-5 branched hairs which covered about 90% of the plant (Celka et al., 2006). Inamdar and Chohan (1969a) while reporting the distribution of trichomes on different organs in *Hibiscus rosa-sinensis* L. (Malvaceae) from India described the 2-8 armed trichomes as the common one. *Pavonia serenna* (Malvaceae) was characterized by the indumentum composed of stellate trichomes with 5-12 arms (Esteves, 1994). *A. theophrasti* could be demarked from the rest of the taxa under study by having multicellular and uniseriate trichomes (Figure 1). These trichomes were roughly conical in shape with obtuse apex and were composed of 10-12 cells in height.

Flask shaped trichomes, as observed in *A. bidentatum*, *A. indicum*, *A. molle* and *A. muticum* were morphologically distinct from one another. Those of *A. bidentatum* and *A. indicum* (Type-II) were multicellular (4-5 cells in height) with broader basal portion and a longer and thinner apical cell forming a prominent neck like portion

Table 2. Diversity of foliar trichomes within the genus *Abutilon* Mill.

S/No.	Type of trichome	Description	Distribution
1	Peltate glands	Sessile, unicellular, cup shaped or rounded with narrow apical opening.	Recorded in all the seven species investigated
2	Capitate	Trichomes with simple unicellular base and rounded to oval shaped or slightly elongated multicellular and uniseriate head.	Present in all the taxa investigated except <i>A. fruticosum</i> and <i>A. pakistanicum</i>
3	Flask-Shaped	Three diverse morphological are identified Type-I. Unicellular, elongated, basal portion slightly dilated gradually narrowing upwards with an apical opening. Type-II. Basal swollen portion multicellular, with unicellular neck like portion having an apical opening. Type-III. Similar to Type I, but multicellular and unlike Type II, all cells are almost of the same size.	<i>A. bidentatum</i> , <i>A. molle</i> , <i>A. muticum</i> <i>A. bidentatum</i> and <i>A. indicum</i> Exclusively observed in <i>A. molle</i>
4	Stellate	More than two ray cells held together in the same cell cavity, quite variable in number of ray cells and their relative length and thickness.	Characteristically found in all the taxa investigated
5	Multicellular and uniseriate	Multicellular and uniseriate filament formed of a single row of 4-5 cells.	Recorded in <i>A. theophrasti</i> only

with an apical opening (Figures 4, 5 and 6). Flask shaped trichomes (Type-I) of *A. molle* and *A. muticum* were unicellular (Figures 3 and 7). Type-III, observed in *A. molle* was multicellular (Figure 2). Type-III observed in *A. molle* was multicellular (Figure 2). Unicellular flask shaped trichomes (Type-I) and multicellular trichomes (Type-III) observed in the present study are quite similar to those reported by Shaheen et al., (2009) on leaves of *Hibiscus trionum* L. (Malvaceae) and described as sub-type-I and sub-type-II of flask shaped trichomes respectively. *A. fruticosum* and *A. pakistanicum* could be demarked from the rest of taxa by the complete absence of capitate trichomes. Inamdar et al., (1983) described only stellate and simple unicellular trichomes in *A. indicum* and *A. muticum*, whereas capitate trichomes were described in *A. indicum* only.

The delimitation of trichome types of angiosperm is problematic due to frequent integration of one type into the other and due to lack of knowledge about the total types of trichomes in the Phylum (Ramayya, 1962). It is often intricate to assign a proper term to describe an observed hair due to differences in hair classification in the published literature (McClerry, 1907; Esau, 1977; Malinowski, 1980; Broda, 1982; Payne, 1978; Judd et al., 1999). Much confusion in terminology of stellate trichome is observed in literature. Inamdar and Chohan (1969a) described a multicellular trichome having 2-8 arms surrounded by 8-10 epidermal cells as stellate trichome.

Ramayya and Rao (1976) followed Inamdar and Chohan while describing the details of stellate hair observed in *Hibiscus micranthus* L., but Rao and Ramayya (1977) pointed out that the change in the number of ray cells from one trichome form to other constitutes a qualitative difference and considered such forms to represent different trichome types. Yan- Ming and Ru-Wen (1993) placed only trichome having more than five ray cells in the category of stellate trichomes and those having two to four ray cells were discussed under the caption of 2-4 armed trichomes (Shaheen et al., 2009). Celka et al., 2006 placed 2-branched hair in a separate class of bifurcate hair whereas stellate trichomes were further classified on the basis of number of ray cells as 3-branched, 4-branched, 6-branched and 8-branched. The details on micro morphological features of the foliar epidermis of *Abutilon* Mill. may serve as a useful taxonomic tool to delineate the species studied (Figure 9 and 10).

Key to the species of *Abutilon* Mill.

- 1a. Epidermal cells irregular in outline with slightly undulating walls multicellular and uniseriate trichomes (Type III) present.....*A. theophrasti*
- 1b. Epidermal cells are rectangular to polygonal in shape on both surfaces or irregular on abaxial surface multicol-

Table 3. Quantitative foliar epidermal features of investigated taxa of *Abutilon* Mill.

Taxa investigated	Ordinary Epidermal cells L. x W. μ m		Stomata L. x W. μ m		L. Sto. opening μ m		Stomatal Complex L. x W. μ m	
	Min. (Mean \pm S.E) Ma.		Min. (Mean \pm S.E) Ma.		Min. (Mean \pm S.E) Ma.		Min. (Mean \pm S.E) Ma.	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
<i>A. bidentatum</i>	20 (35 \pm 5.70) 50 x 18 (23.6 \pm 2.64)30	22.5 (36.25 \pm 5.76) 55x15(21 \pm 2.91) 30	25 (25 \pm 0) x 15 (15 \pm 0)	25 (25 \pm 0) x 15 (15 \pm 0)	15(17.5 \pm 1.12) 20	15(17.5 \pm 1.12) 20	40 (49 \pm 2.91) 55 x 30 (32.5 \pm 1.12) 35	45 (55 \pm 3.53) 65 x 25 (33.2 \pm 3.12) 41
<i>A. fruticosum</i>	20 (31 \pm 3.67) 40 x 15 (16.67 \pm 1.05) 20	20 (30 \pm 3.16) 45 17 (18.62 \pm 1.31)22.5	20 (20 \pm 0) x 15 (15 \pm 0)	20 (20 \pm 0) x 16 (16 \pm 0)	12.5(14 \pm 0.61) 15	12.5 (15.3 \pm 0.83) 17	30 (34 \pm 2.45) 40 x25 (31.67 \pm 1.6) 35	35 (40.4 \pm 2.27) 45 x 25.5 (33.3 \pm 2.15) 37
<i>A. indicum</i>	26.5 (32.3 \pm 2.83) 40 x 15 (22.3 \pm 2.13) 26.5	25 (30 \pm 2.24) 35 x 13 (22.6 \pm 2.87) 30	20 (20 \pm 0) x12.5 (13.8 \pm 0.56) 15	21(21 \pm 0) x 14 (14.6 \pm 0.24) 15	15 (16 \pm 0.45) 17	15 (16 \pm 0.45) 17	30 (40 \pm 2.74) 45 x 20 (25.2 \pm 2.24) 30	30 (38.6 \pm 3.74) 48 x 22 (26.4 \pm 1.57) 30
<i>A. molle</i>	20 (31.6 \pm 3.88) 40 x 16 (24 \pm 2.11) 30	20 (34.4 \pm 4.76) 45 x15 (20 \pm 1.70) 25	17 (22 \pm 1.84)25x 12.5 (17.5 \pm 1.58) 20	15 (21.67 \pm 1.67) 25 x 12.5(17.0 \pm 1.36) 20	10 (11.14 \pm 0.40) 12	12 (15.2 \pm 0.92) 17	25 (25 \pm 0) x 30 (35 \pm 1.82) 40	22 (41.5 \pm 4.81) 54 x 32(37.4 \pm 1.66) 40
<i>A. muticum</i>	20 (34 \pm 4.30) 45 x 10 (15 \pm 2.24) 20	22 (34.67 \pm 5.16) 51 x 10(15 \pm 2.23) 20	20(20 \pm 0) x 10 (10 \pm 0)	17(17 \pm 0) x11(11 \pm 0)	12.5 (13.8 \pm 0.56) 15	12.5 (13.8 \pm 0.56) 15	35 (38 \pm 1.22) 40 x 35 (39 \pm 1.87) 45	30 (41 \pm 3.06) 42 x 30 (36.8 \pm 2.39) 44
<i>A. pakistanicum</i>	15 (25.4 \pm 2.94) 32 x 20 (23.2 \pm 1.32) 26	15(27.167 \pm 3.51)35 x20 (22.5 \pm 1.12)25	12.5 (14 \pm 0.61) 15 x10 (12 \pm 1.22) 15	12.5(14 \pm -0.61)15 x7.5 (11.4 \pm 1.68) 15	10 (11.4 \pm 0.58) 12. 5	10 (12.4 \pm 0.64) 13..5	30 (35 \pm 1.84) 40 x 40 (51.67 \pm - 3.07) 60	30 (35 \pm 2.23) 40 x 40 (50.83 \pm 3.51) 60
<i>A. theophrasti</i>	25 (38.33 \pm 4.216) 50 x 20 (25 \pm 1.290) 30	22 (37.17 \pm 4.20)48 x20 (25 \pm 1.29) 30	20 (22.5 \pm 1.12) 25x15 (18 \pm 1.22) 20	25 (25 \pm 0) x 15 (18.8 \pm 1.7) 20	15 (18 \pm 1.225) 20	15 (18.2 \pm 1.32) 21	35 (40 \pm 2.73) 50 x 25 (35.6 \pm 4.4) 45	37 (45.5 \pm 2.48) 53 x 27 (41.1 \pm 4.4) 50.5

Table 3. Contd

Taxa investigated	Peltate. H x W μ m	Capitate H x W μ m	Flask-Shaped H. x W. μ m			Stellate L. x W. μ m	Mu. and Uni. L. x W. μ m	
	Min.(Mean \pm S.E)Ma.	Min.(Mean \pm S.E)Ma.	Min. (Mean \pm S.E.)Ma.			Min. (Mean \pm S.E.) Ma.	Min. (Mean \pm S.E) Ma.	
	Type-II	Type-V, subtype-II	Type-I	Type-II	Type-III	N.r.c	S.r.c	
<i>A. bidentatum</i>	5 (19.17 \pm 3.27)25 x 15 (19.17 \pm 0.83) 20	25 (30 \pm 1.82) 35 x 20 (20 \pm 0)	-	100(126.2 \pm 6.7145) x 25 (27.5 \pm 0.9)30	-	4-9	125 (190 \pm 16.09) 250 x 5 (8.75 \pm 1.06) 15	-
<i>A. fruticosum</i>	15 (20.83 \pm 1.54) 25 x 10 (12.5 \pm 1.12) 15	-	-	-	-	8-9	150 (421.428 \pm 91.19) 750 x 5 (20 \pm 5.32) 40	-
<i>A. indicum</i>	15 (15 \pm 0) x 20 (20 \pm 0)	25 (27.5 \pm 1.12) 30 x 20 (20 \pm 0)	125(125 \pm 0) x 20(20 \pm 0)	120(135.8 \pm 3.2140)x20 (22.8 \pm 1.0)25	-	5-more than 12	125 (230.72 \pm 33.94)350 x 20 (22.5 \pm 1.12) 25	-
<i>A. molle</i>	10 (13.33 \pm 1.05 x 15 (21.67 \pm 1.67)25	35 (49.167 \pm 3.27) 55 x 20 (23.33 \pm 1.05) 25	120(120 \pm 0) x 25(25 \pm 0)	-	80(82.5 \pm 1.185)x 20(20 \pm 0)	4-more than 12	225 (385.5 \pm 36.09) 600 x 15 (20.84 \pm 1.54) 25	-
<i>A. muticum</i>	15 (17.5 \pm 1.12) 20 x 15 (17.5 \pm 1.12) 20	15 (20 \pm 1.82) 25 x 15 (16.67 \pm 1.05) 20	175 (191.67 \pm - 5.27) 200 x 25 (25 \pm -0)	-	-	5-12	225 (347.5 \pm 42.59) 485 x 25 (39.17 \pm 5.38) 60	-
<i>A. pakistanicum</i>	10 (10 \pm 0) x 20 (20 \pm 0)	-	-	-	-	5-8	125 (201.67 \pm 24.55)300 x 5 (11.67 \pm 1.67) 15	-
<i>A. theophrasti</i>	10 (12.5 \pm 1.12) 15 x 20 (22.5 \pm 1.12) 25	20 (26.67 \pm 1.67) x 20 (23.33 \pm 1.05) 25	-	-	-	4 - more than 12	200(283.3 \pm 22.05)350 x 10 (15.8 \pm 2.01) 20	175(255 \pm 32.02) 350 x 20 (23.33 \pm 1.0) 25



Figure 1. Multicellular uniseriate trichome on adaxial surface of *A. theophrasti* (200X).



Figure 4. Capitate trichome on abaxial surface of *A. molle* (200X).

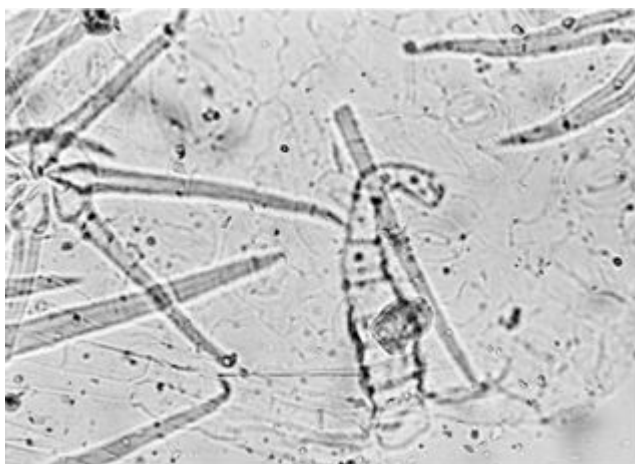


Figure 2. Flask shaped trichome (Type-III) on abaxial surface of *A. molle* (200X).

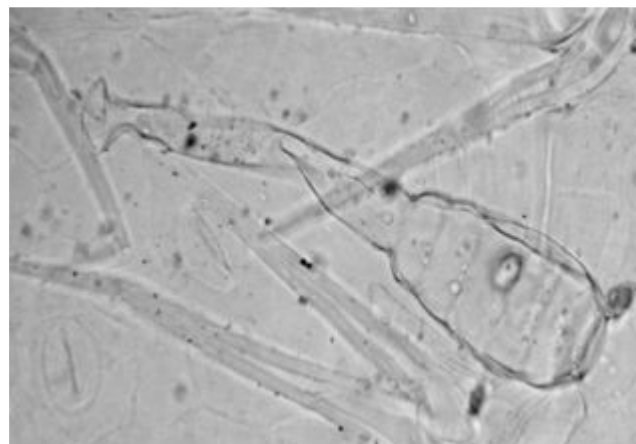


Figure 5. Flask shaped trichome (Type-II) on adaxial surface of *A. bidentatum* (200x).

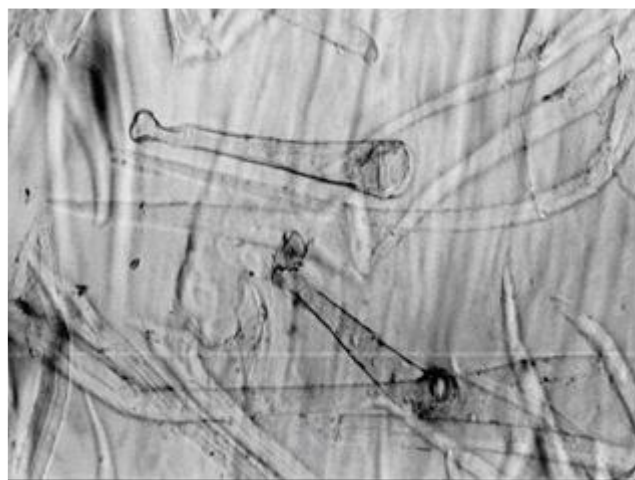


Figure 3. Flask shaped trichome (Type-I) on adaxial surface of *A. molle* (200X).

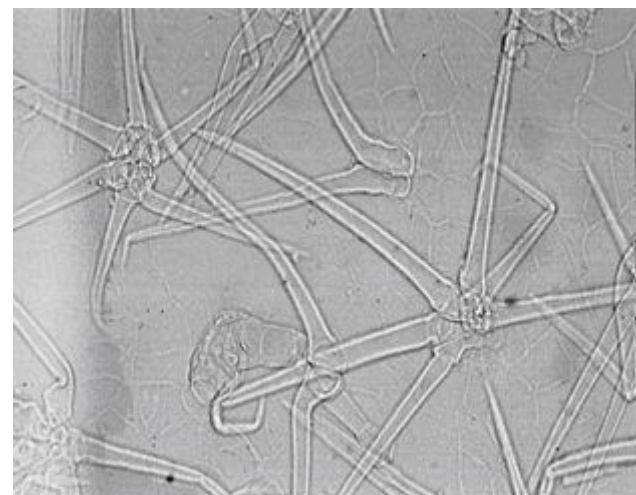


Figure 6. Stellate trichomes along with Flask shaped trichome (Type-II) on adaxial surface of *A. bidentatum* (100X).

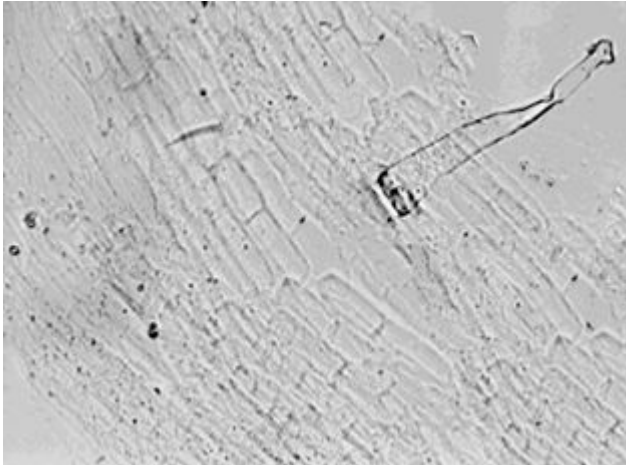


Figure 7. Flask shaped trichome (Type-I) on adaxial surface of *A. muticum* (200X).

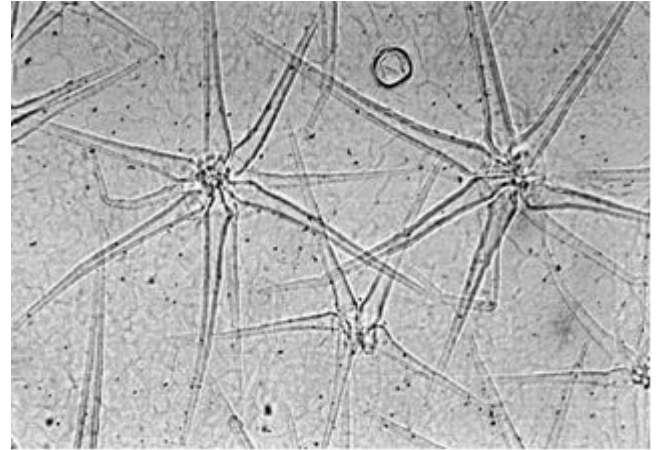


Figure 10. 5-6 armed stellate trichomes on adaxial surface of *A. indicum* (100X).

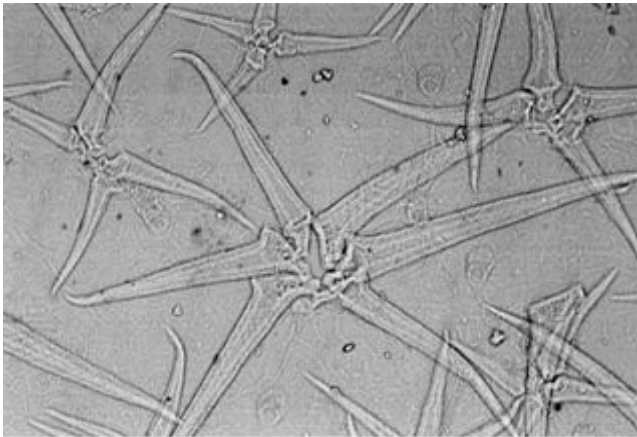


Figure 8. Stellate trichomes having thickest ray cell on adaxial surface of *A. muticum* (200X).

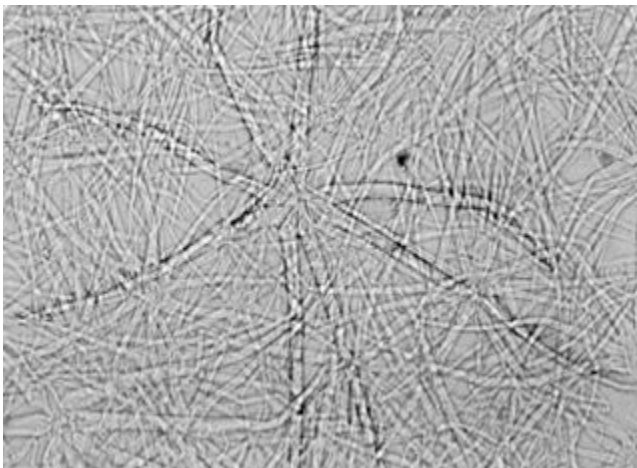


Figure 9. Stellate trichomes in many layers completely masking the underlying epidermal cells and stomata on abaxial surface of *A. Pakistanicum* (100X).

lular uniseriate trichomes (Type III) absent.....
2
 2a. Epidermal cells irregular to roughly rectangular in shape abaxially, height of capitate trichomes in the range of 35 (49.167 +/- 3.27) 55 µm.....
*A. molle*
 2b. Shape of epidermal cells mostly polygonal on both surfaces, height of capitate trichomes is of different range.....3
 3a. Width of flask shaped trichome is 25 (27.5±0.94491)30 µm, length of stomatal complex on abaxial surface 45 (55 ±3.53) 65x µm...
*A. bidentatum*
 3b. Width of flask shaped trichome less than 30 µm, length of stomatal complex on abaxial surface less than 65 µm.....4
 4a. Height of stellate trichomes in the range of 150-750 µm, among the glandular trichomes only peltate glands (Type II) present.....
*A. fuitico*
 4b. Height of stellate trichomes is of different range, glandular trichomes either capitate, flask shaped or peltate.....5
 5a. Stomata of anisocytic type, width of stellate trichomes varies from 25 to 60 µm.....*A. muticum*
 5b. Stomata anisocytic and diacytic, width of peltate glands less than 60 µm.....6
 6a. Number of ray cells in stellate trichomes 5-8, only peltate glands (Type II) present.....*A. pakistanicum*
 6b. Number of ray cells in stellate trichomes more than 12, capitate, flask shaped and peltate trichomes are frequently present

*A. indicum*

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