INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
09–052/MHB/2009/11–3–279–284
http://www.fspublishers.org

Full Length Article

Diversity of Foliar Trichomes and Their Systematic Relevance in the Genus *Hibiscus* (Malvaceae)

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ABSTRACT

Qualitative and quantitative micromorphological characters, distribution and systematic relevance of both glandular and eglandular foliar trichomes in species of genus *Hibiscus* (Malvaceae) including *H. caesius, H. mutabilis*, *H. rosa-sinensis*, *H. sabdariffa*, *H. schizopetalus*, *H. syriacus* and *H. trionum* were characterized. Diversity of these epidermal appendages were separated into eight main types. Glandular capitate trichomes were the most abundant on both leaf surfaces of all the examined taxa. *H. mutabilis* was discrete in having multicellular uniseriate columna as a prominent feature of its abaxial leaf surface, whereas *H. schizopetalus* and *H. sabdariffa* could be demarked due to the twisted or *Spirogyra*-like appearance of their eglandular trichomes. In summary, the variation in morphology and distribution of foliar trichomes emerged as an important supportive taxonomic tool in delimiting species of genus *Hibiscus.*

Key Words: Leaf epidermal anatomy; Trichomes; Capitate glands; *Hibiscus*

INTRODUCTION

Genus *Hibiscus* is a polymorphic, comprising over 200 species of trees, shrubs and herbs, which are widely distributed in tropical and subtropical regions (Bailey, 1950; Bates, 1965; Beers & Howie, 1992). The genus exhibits considerable taxonomic complexity, and is so heterogeneous that it is hard to identify any distinguishing features; although 5-celled capsules and 5-capitate stigmas are essentially constant (Fryxell, 1997; Alam *et al*., 2006). Hochreutiner (1900) lately revised the genus, recognized 197 species and subdivided the genus into 12 sections. This is the only comprehensive grouping of the genus to date on the global basis. Some of these sections have been intensely studied (Wilson, 1994; Small, 2004), but still other sections are patently artificial and have unclear or not yet understood boundaries. The infrageneric classification of genus *Hibiscus* is a problem and needs much attention (Fryxell, 1997).

Flora of Pakistan is highly diverse and is reported to be represented by nearly 6000 species of flowering plants, occurring mainly in the Northern and North Western parts of Pakistan (Munsif *et al*., 2007). The genus *Hibiscus* in Pakistan is represented by 18 taxa, both cultivated and wild (Abedin, 1979).

Presence of various types of glandular and eglandular trichomes is a characteristic feature of genus *Hibiscus.* Scientific interest in plant trichomes is based on their functional and taxonomic importance and on the economic usefulness of some trichome-generated products. Trichomes may serve a variety of defensive and physiological functions. Leaf trichomes have been shown to reduce insect herbivory in a number of plant species (Levin, 1973; Marquis, 1992; Van Dam & Hare, 1998; Elle *et al*., 1999; Romeis *et al.*, 1999; Hare & Smith, 2005). For plants in xeric habitats, epidermal appendages reflect light and can reduce transpiration rate (Ehleringer, 1984; Larcher, 2001).

The micromorphological characteristics of foliar trichomes have played an important role in plant systematics, especially of particular groups at generic and specific levels (Hardin, 1979). Such type of studies in the field has fascinated plant morphologists and systematists towards the diversity of trichome features (Yan-Ming & Ru-Wen, 1993). It has been suggested by early investigators that the presence or absence of peltate hairs and their form, size and colour could be used in distinguishing between genera and species of plants (Cooper, 1931; Spring, 2000). The trichome types are not only useful in the identification of the two species, but also their corresponding parts, thus being important in pharmacognosy, archaeobotany, paleobotany and agronomy (Rao & Ramayya, 1977).

Adedeji and Dloh (2004) described the comparative foliar anatomy of ten species in the genus *Hibiscus*, but they concentrated on the general anatomy and added only few sentences about foliar trichomes without any morphological descriptions and anatomical measurements, secondly most of the taxa considered in the present work were not included in their studies. The present study was conducted with a view to investigate the detailed micromorphology and distribution of both glandular and glandular foliar trichomes

To cite this paper: Shaheen, N., M. Ajab, M.Q. Hayat and G. Yasmin, 2009. Diversity of Foliar trichomes and their systematic relevance in the genus *Hibiscus* (Malvaceae). *Int. J. Agric. Biol.*, 11: 279–284

and their systematic relevance in the seven selected species of genus *Hibiscus* L.

MATERIALS AND METHODS

Fresh leaves collected from different locations of Pakistan and dried leaves of representative specimens of genus *Hibiscus* in Herbarium of Quaid-i-Azam University Islamabad, Pakistan, listed in Table I, were used for anatomical studies. Vouchers of fresh collection were deposited in the herbarium. Dried leaves were first 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 Clark (1960) modified by Cotton (1974). The leaves were placed in 88% lactic acid for softening and kept hot in boiling water bath (Model, Memmert-91126-FRG, Germany) for about 30 to 40 min. Slides of both abaxial and adaxial surface of leaf were prepared and mounted in clean 88% lactic acid. Both qualitative and quantitative micromorphological characteristics of foliar trichomes were observed and micrographs 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 Pyne (1978) and Harris and Harris (2001). However, simple self-explanatory terms are added to identify the specific types of trichomes.

RESULTS AND DISCUSSION

The data of qualitative and quantitative anatomical features of *Hibiscus* L. is presented in Tables II and III, respectively. Eight major types of glandular and eglandular foliar trichomes have been observed. Features of hairs are broadly regarded as useful for establishing the systematic relations within the family Malvaceae (Walas, 1959; Iljin, 1974; Ramayya & Rao, 1976; Inamdar & Chohan, 1983; Dorr, 1990). Such view was also submitted by Bayer and Kubitzki (2003), who used eglandular trichomes in characterizing Malvales. Taxonomy of *Malva alcea* complex is based primarily on the morphological structure of the hairs and corolla petals (Celka *et al.*, 2006). In the South Indian genera, peltate scale is the characteristic of *Thespesia* Solander ex Corrêa and *Cullenia* Wight. On the same basis, the two genera were delimited from the rest of the Malvaceae (Ramayya & Rao, 1976).

Glandular capitate trichomes with unicellular stalk and multicellular uniseriate or biseriate head are characteristic of all taxa studied (Fig. 1), which serve as taxonomic feature at the generic level in Malvaceae but are found effective to a lesser degree at infrageneric level in delimiting *Hibiscus* spp. Simple unicellular conical trichomes are observed in all taxa under study except *H. syriacus* but differ a great deal in density and micromorphology (Fig. 2). Very few intermixed with forked and stellate trichomes are observed on adaxial and abaxial surfaces of *H. rosa-sinensis* and H. *schizopetalus*, whereas in *H. caesius* and *H. sabdariffa* these are the only nongladular trichomes and are present along the veins only.

Although the epidermal anatomy has been described in the leaves of a number of Malvaceae species (Inamdar & Chohan, 1969; Ramayya & Rao, 1976; Rao & Ramayya, 1977; Adedeji & Dloh, 2004; Celka *et al*., 2006), but the

Fig. 1. Capitate glandular trichome on adaxial surface of *H. rosa-sinensis* (100X)

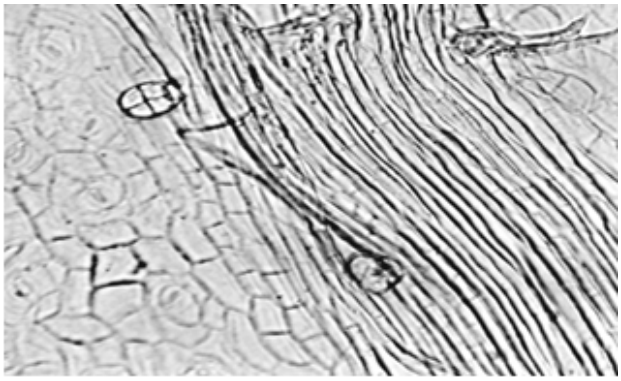


Fig. 2. Eglandular conical trichome on abaxial surface of *H. sabdariffa* (100 X)

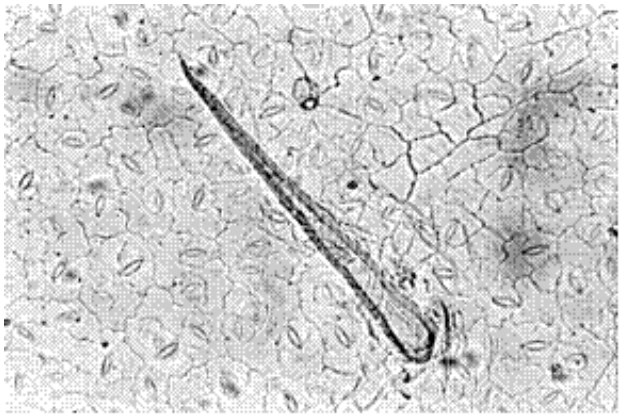


Fig. 3. Disc shaped multicellular peltate scales Type-I on abaxial surface *of H. schizopetalus* (100X)

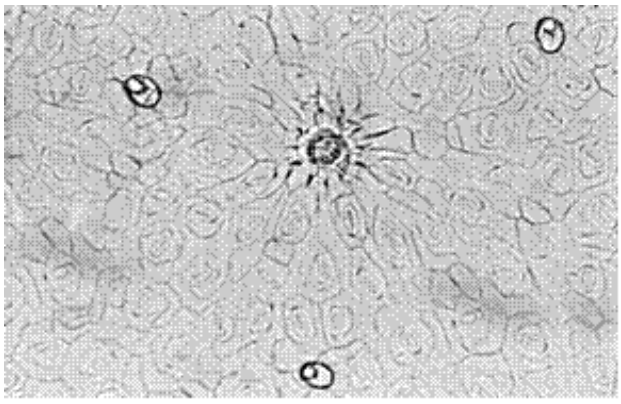


Table I. List of species investigated, with location of voucher specimens arranged in alphabetical order of taxa

Species	Fresh/preserved	Locality	Voucher specimen No./Accession No.
H. caesius	Fresh	Islamabad/ Rawalpindi/Attock	ISL.H1 to ISL.H3
H. mutabilis	fresh	Wah Cantt, Rawalpindi & Islamabad	ISL.Hm1 to Hm3
H. rosa-sinensis	fresh	Wah Cantt, Islamabad, Jehlum, Haripur	ISL.Hr1 to Hr4
H. sabdariffa	preserved	Rawalpindi, Attock& Sialkot	07720, 07723, 07719
H. schizopetalus	fresh	Kohat, Attock & Lahore	ISL.Hs1 to ISL.Hs3
H. syriacus	preserved	Rawalpindi, Jhelum, Mir Pur Khas & Chakwal	72350, 75405, 07639, 07692
H. trionum	preserved	Swat, Quetta & Muzaffrabad	07744, 07743, 06745

Table II. Diversity of foliar trichomes in *Hibiscus* species

Type of Trichome	Description	Distribution
Conical	Unicellular, axillary elongated, broad at the base and tapering above (Fig. 2)	Present on both abaxial and adaxial surfaces of *H. rosa-sinensis*, *H. caesius*, *H. trionum*, *H. schizopetalus*, *H. mutabilis* and *H. sabdariffa* in varing density
Forked	Two unicellular ray cells present in the same cell cavity (Fig. 9)	Very few are observed in *H. schizopetalus*, *H. mutabilis*, *H. rosa-sinensis* and *H. trionum*
Stellate	Formed of considerable number of unicellular ray cells held together in the center.(Fig. 8)	Well distributed on both abaxial and adaxial leaf surfaces of *H. rosa-sinensis* and *H. mutabilis* whereas very few are observed in *H. schizopetalus*
Capitate glandular trichome	Staked Trichome with a multicellular slightly elongated oval shaped head (Fig.1)	Distributed on both leaf surfaces of all taxa examined
Multicellular uniseriate columna	Erect, formed of single row of cells. No. of cells vary between 5 and 15, basal cells are smaller with slightly circular out line while upper 1 or two cells are much elongated with apical opening.(Fig. 10)	Exclusively observed in *H. mutabilis*
Clavate capitate trichome	Multicellular, uniseriate, Smaller than the Pillar form trichome but slightly elongated the glandular capitate trichome and with obtuse apex.(Fig. 6, 7,12)	On both leaf surfaces of *H. rosa-sinensis* and *H. caesius*
Flask shape trichome	Unicellular or multicellular having swollen base and narrowing upwards, forming a neck like portion (Fig. 11).	Few are observed in *H. trionum*
Peltate scales	Type-I. multicellular disk like central portion surrounded by a circle of modified epidermal cells (Fig. 3) Type-II. Unicellular cup like with broad apical opening(Fig. 4, 5)	Cup shaped in *H. sabdariffa*, *H. mutabilis* and *H. trionum* whereas multicellular disk shaped in *H. rosa-sinensis* and *H.schizopetalus*

Table III. Quantitative characteristics of foliar trichomes in *Hibiscus* species

Taxon	Glandular trichomes					Eglandular trichomes			
	Capitate	Peltate glands		Multicellular Uniseriate Columna	Flask shaped	Clavate	Stellate		Conical
	H x W µm	Type-I H x W µm	Type-II diameter µm	H x W µm	H x W µm	H x W µm	No of r.c.	s.r.c L x W µm	L x W µm
H. caesius	45-50 x 30-35	_	_	_	_			-	325-550 x 30-55
H. mutabilis	33-45 x 25-32	12.5-17.5 x 25-37.5	_	410-500 x 30-40	_		5-15	87.5-200 x 10-15	100-110 x 25-30
H. rosa-sinensis	35-42.5 x 25-32.5	_	15-30	_	_	62.5-100 x 25	3-7	95-170 x 7.5-22.5	62.5-135 x 7.5-15
H. sabdariffa	35-47.5 x 22.5-30	15-25 x 17.5-30	_	_	_			-	157.300 x 37.5-50
H.schizopetalus	35-50 x 25-35	_	15-35	_	_		4 - 8	125-335 x 25-30	50-275 x 25
H. syriacus	30-35 x 20-25	_	_	_	_			-	-
H. trionum	37.5-52 x 27.5-35	15-42.5 x 15-30	_	_	525-105 x 30-40		3 -7	360-700 x 20-30	350-700 x 20-30

H. height W. width L. length No of r.c. number of ray cells s.r.c. single ray cell

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 micromorphological characteristics of foliar trichomes and their systematic relevance within the same genus. Adedeji and Dloh (2004) described the comparative foliar anatomy for ten species in the genus *Hibiscus* L. but they concentrated on the general anatomy and added only few sentences about foliar trichomes without any morphological descriptions and anatomical measurements and secondly most of the taxa considered in the present work were not included in their studies. To the best of our knowledge the present study represents the first detailed comparative analysis of leaf trichome morphology and anatomical measurements in *Hibiscus* L. species.

In *Hibiscus*, both leaf surfaces are covered with

different types of trichomes forming an indumentum of variable texture and density (Table II). Peltate scales observed in *Hibiscus* spp. are of two main types. Type-I, multicellular and disc-shaped (Fig. 3), surrounded by a circle of modified epidermal cells, which is different from type-II, unicellular and cup-shaped (Fig. 4 & 5). *H. schizopetalus* and *H. rosa-sinensis* share the presence of clavate capitate trichomes (Fig. 6 & 7) and multicellular disc-shaped peltate scales (type-I), whereas type-II is observed in *H .mutabilis*, *H. sabdariffa* and *H. trionum. H. schizopetalus*, *H. rosa-sinensis*, *H. mutabilis and H. trionum* are closely related having stellate, forked and conical trichomes as their eglandular epidermal appendages. Stellate trichomes (Fig. 8), a characteristic feature of family Malvaceae (Solereder, 1908; Gamble, 1935; Metcalfe & Chalk, 1950; Hutchinson, 1969) are variable in number of ray cells and their relative length and thickness. Anatomical measurements indicate the presence of longest ray cells in *H. trionum* (360-700µm), whereas shortest length is observed in *H. rosa-sinensis* (95-170 µm). Number of ray cells in a single trichome ranges from 3-8 but in *H. mutabilis* it is up to 15. Presence of stellate trichomes has been reported in the leaves of a number of Malvaceous plants. In *Malva alcea* L. the most frequent are 4-5 branched hairs, which cover about 90% of the plant (Celka *et al*., 2006). Inamdar and Chohan (1969), while reporting the distribution of trichomes on different organs in *H. rosa-sinensis* (Malvaceae) from India described the 2 to 8-armed trichomes as the common one. *Pavonia serenna* (Malvaceae) is characterized by the indumentum composed of stellate trichomes with 5-12 arms (Esteves, 1994).

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). Much confusion in terminology of stellate trichome is observed in literature. Inamdar and Chohan (1969) defined stellate trichome as a multicellular trichome having 2-8 arms surrounded by 8-10 epidermal cells. This was followed by Ramayya and Rao (1976), while describing the details of stellate hairs observed in *H. 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 the trichomes having more than five ray cells in the category of stellate trichomes and those having two to four ray cells are discussed under the caption of 2-4 armed trichomes. Celka *et al*. (2006) placed 2-branched hair in a separate category of bifurcate hairs whereas stellate trichomes were further classified on the basis of number of ray cells as 3, 4, 6 and 8 branched.

Among others, *H. schizopetalus* and *H. sabdariffa* can be clearly demarked by the twisted appearance of ray cells (Fig. 9) of their eglandular trichomes. Presence of conical trichomes and peltate scales in *H. sabdariffa* is in contrast

Fig. 4. Cup-shaped peltate scale Type-II on abaxial surface of *H. trionum* (100X)

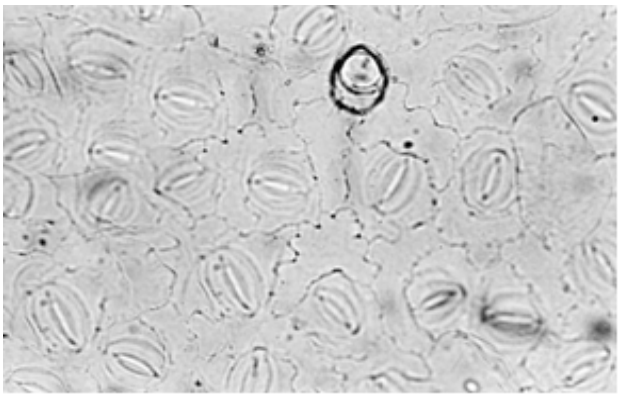


Fig. 5. Unicellular peltate scale tpe-II, surrounded by modified epidermal cells, on adaxial surface of *H. sabdariffa* (100X)

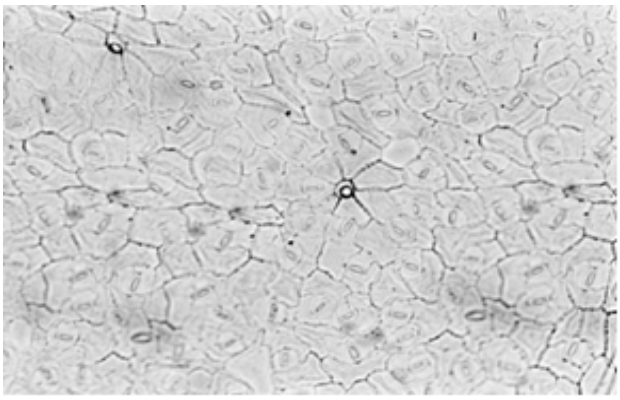
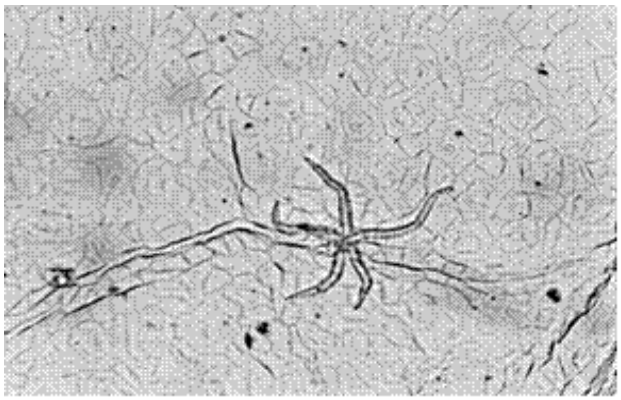


Fig. 6. Different developmental stages of a glandular clavate capitate trichome along the midrib on adaxial surface of *H. rosa-sinensis* (200X)

with the findings of Adedeji and Dloh (2004), who indicated the presence of only glandular capitate trichomes in the taxa. It is estimated that the difference may be due to different geographical localities. *H. mutabilis* is distinct in

Fig. 7. Clavate capitater trichome on abaxial surface of *H. rosa-sinensis* (200X)

Fig. 8. 5-rayed stellate trichome on abaxial surface of *H. rosa-sinensis* (100X)



having multicellular uniseriate columna (Fig. 10). Similarly, *H. trionum* can be delimited from other taxa due to having unicellular cup-shaped peltate scales (type-II) as a prominent feature of both adaxial and abaxial leaf surfaces and by the presence of few multicellular or unicellular flask-shaped glandular trichomes along the veins (Fig. 11). Flask-shaped trichomes were exclusively observed in *H. trionum* and can be placed in two sub-types; sub-type-1 is unicellular, slightly broader at the base and narrowing upwards forming a neck like portion with apical opening, whereas sub-type-II, morphologically similar to type-I but multicellular and uniseriate. *H. syriacus* stays apart from the rest of the taxa under study by the absence of any eglandular foliar trichomes. Capitate glandular trichomes are the only representatives of the foliar epidermal trichomes.

CONCLUSION

Better terminology and detailed comparative study of morphology, micromorphology and distribution of various glandular and eglandular foliar trichomes in *Hibiscus* L. may serve as a valuable supportive taxonomic tool.

Fig. 9. Forked trichome with twisted appearance on abaxial surface of *H. schizopetalus* (100X)

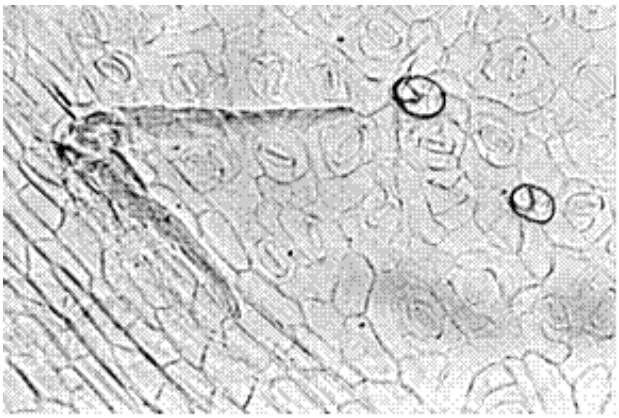


Fig. 10. Glandular multicellular uniseriate columna along with stellate trichome on abaxial surface of *H. mutabilis* (100X)

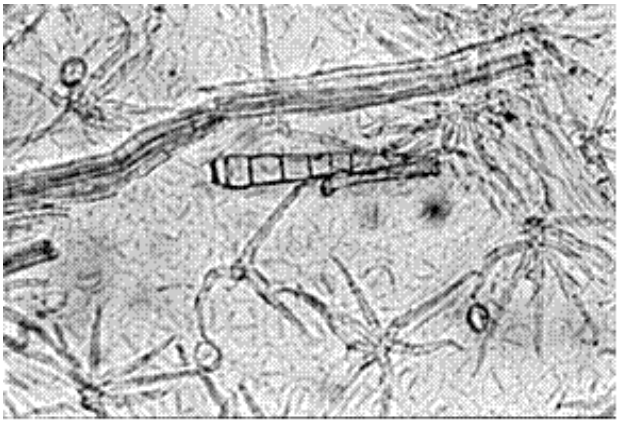
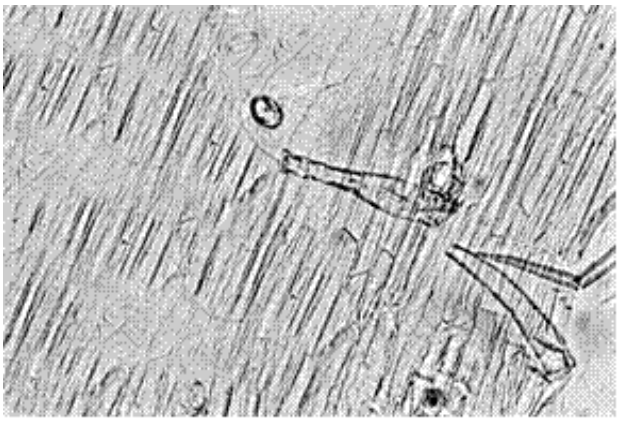


Fig. 11. Flask-shaped glandular trichome on adaxial surface of *H. trionum* (100X)



Acknowledgements. We are thankful to Higher Education Commission of Pakistan for funding this research work.

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(Received 11 February 2009; Accepted 19 February 2009)