J. Orchid Soc. India, 28: 47-59, 2014 ISSN 0971-5371

# FOLIAR FEATURES IN SOME INDIAN ORCHIDS: SUBTRIBE HABENARIINAE (ORCHIDEAE, ORCHIDOIDEAE)

A Chattopadhyay[1], Promila Pathak, and K C Mahant[2]

*Orchid Laboratory, Department of Botany, Panjab University, Chandigarh, 160 014, India*
*[1]Present Address: Faculty of Medicine, Kuwait University, P.O. Box-24923, Safat 131 10, Kuwait*
*[2]Present Address: Botany Department, Government College Nerwa - 171 210, Chopal, Distt. Shimla, H.P., India*

## Abstract

Studies on leaf, epidermal features and anatomy were conducted in some Indian orchids belonging to the subtribe Habenariinae (Orchideae, Orchidoideae). In most of the presently studied species, thin and smooth cuticle was present on both leaf surfaces; it was, however, striated and rugulose in *Habenaria commelinifolia*, *H. ensifolia* and *Herminium lanceum*. Adaxial epidermal cells were thin walled, elongated, hyaline and generally distinctly larger than abaxial cells ranging from 1.1 times in *Habenaria edgeworthii* to 6.38 times size in *Herminium lanceum*. Prominent epidermal papillae were also observed on laminar edges in some of the presently studied species (*Habenaria edgeworthii* and *H. monorchis*). Well developed outer cuticular ledges of stomata were observed as xeromorphic trait, in present species. Though a typical hypodermis was absent in most of the presently studied species, hyaline raphide idioblasts were found irregularly scattered below epidermis on both the laminar surfaces in *Habenaria edgeworthii* and *H. pectinata*. Mesophyll comprised thin-walled parenchymatous cells, with abundant Intercellular spaces. Crystalliferous raphide idioblasts and isodiametric water storage cells were found scattered in mesophyll. In most of the presently studied species, xylem and phloem were usually well distinguishable in midrib bundles but indistinct in small laminar bundles. Sometimes phloem was found intermingled with parenchymatous cells and more than one xylem patch was observed in *Habenaria edgeworthii*, *H. ensifolia*, and *H. pectinata*. Ecological significance of leaf, epidermal and anatomical characters in this subtribe is also discussed, in this paper.

## Introduction

THE IMPORTANCE of anatomical studies, in predicting ecological amplitude and phylogenetic relationships, is well documented in plants. Despite the fact that orchids are considered amongst the most highly ecologically adapted plants, only a few attempts have so far been made to study these features at tribe and subtribe level in Orchidaceae, and to understand the ecological anatomy of this group (Kaushik,1983). Members of presently studied Habenariinae are mostly terrestrial plants with spheroid or oblong root-stem tuberoids. Stems are usually slender with soft herbaceous non-articulate leaves arranged spirally or scattered. Leaves are sometimes basal and clustered. Inflorescence in this subtribe is terminal and simple, bearing one to many flowers. Flowers are resupinate, and the lip usually has a basal spur. Number of pollinia varies from two to four. Pollinia are sectile, and exine of pollen grains is laevigate to baculate-pilate. Members of this subtribe are also characterized by convex, entire or bilobed, and long-stalked stigma (Dressler, 1993). Species are pantropical and distributed in Asia, Africa, Europe, and North America. Twenty four genera and 932 species are listed under this sub-tribe (Dressler, 1993). The largest genus *Habenaria* is represented by 600 species followed by *Cynorkis* with 125 species. However, the genus *Herminium* comprises of about 35 species.

Studies on leaf, epidermal features and anatomy, though made in a few species at tribe and subtribe level in subtribe Habenariinae (Das and Paria, 1992; Inamdar, 1968; Kaushik, I983; Samuel and Bhat, 1994; Singh, 1981; Stern,1997 a;Vij *et al.*,1991; Williams, 1979), detailed information of these features in relation to their habitat is however, still lacking in majority of taxa belonging to this subtribe. To bridge this information gap, presently, an attempt was made to study the leaf, anatomy and epidermal features of ten orchid species belonging to sub-tribe Habenariinae with a view to assessing their taxonomic and ecological significance.

## Materials and Methods

Seven species of *Habenaria, i.e., H. clavigera* (Lindl.) Dandy, *H. commelinifolia* (Roxb.) Wall. ex Lindl., *H. edgeworthii* Hook. f. ex Collet, *H. ensifolia* Lindl., *H. intermedia* D. Don, *H. latilabris* (Lindl.) Hook. f. and *H. pectinata* (J. E. Sm.) D.Don and three species of *Herminium*, namely *Herminium lanceum* (Thun. ex Sw.) Vujik, *H. monophyllum* (D. Don) P. F. Hunt and Summerh., and *H. monorchis* ( L.) R.Br. were included in the present study. The species included under the scope of present study were collected from different climatic zones of Shimla Hills of NorthWest Himalayan region. The data regarding their habitat, ecology, and altitude, *etc*. were recorded for each species. Table

Received : October 15, 2013; Accepted : December 30, 2013

Table 1. Source of collection, habit, habitat, and ecology of presently studied orchid species.

| Species | Habit | Locality and altitude | | Habitat and ecology |
|---|---|---|---|---|
| | | Present collection | Altitudinal range (m) | |
| *Habenaria clavigera* (Lindl.) Dandy | T | Shimla<br>(Tara devi, 1,851m;<br>Summer hill, 2,100m) | 1,800-4,000 | exposed grassy slopes from low to high elevations |
| *H. commelinifolia* (Roxb.) Wall. ex Lindl. | T | Hamirpur<br>(Hamirpur, 800m); Kangra<br>(Palampur, 1,219m) | 300-1,200 | common under exposed open grasslands |
| *H. edgeworthii* Hook. f. ex Collet | T | Shimla (Tara devi, 1,851 m; Summer hill, 2,100m);<br>Solan (Kasauli, 1,927m);<br>Pithoragarh<br>(Sandev, 1,600m) | 1,500-3,000 | under semi shady to somewhat exposed grassy situations from low to high elevations road-embankments |
| *H. ensifolia* Lindl. | T | Shimla<br>(Tara devi, 1,851m;<br>Chail, 2,150m) | 500-3,200 | common under semi shady to somewhat exposed open grasslands, forest borders |
| *H. intermedia* D. Don | T | Shimla<br>(Tara devi,1,851m;<br>Summer hill, 2,100m,<br>Mashobra, 2,400m);<br>Pithoragarh<br>(Sandev, 1,600m) | 1,500-2,800 | common under semi shady to somewhat exposed grassy situations from low to high elevations |

**contd.**

Table 1. Source of collection, habit, habitat, and ecology of presently studied orchid species (contd.)

| Species | Habit | Locality and altitude | | Habitat and ecology |
|---|---|---|---|---|
| | | Present collection | Altitudinal range (m) | |
| *Habenaria latilabris* (Lindl.) Hook. f. | T | Shimla<br>(Tara devi, 1,851 m;<br>Summer hill , 2,100m);<br>Solan (Kasauli, 1,927m);<br>Pithoragarh<br>(Sandev, 1,600m) | 1,800-4,000 | semi shady to somewhat exposed grasslands and road-embankments. |
| *H. pectinata* (J. E. Sm.) D.Don | T | Shimla<br>(Tara devi,1,851m;<br>Summer hill, 2,100m,<br>Mashobra, 2,400m);<br>Solan (Kasauli, 1,927m) | 1,500-3,500 | semi shady to somewhat exposed grassy situations. humus laiden rocky slopes and forest borders |
| *Herminium lanceum* (Thun. ex Sw.) Vujik | T | Shimla<br>(Tara devi,1,851m;<br>Summer hill, 2,100m);<br>Solan (Kasauli, 1,927m);<br>Pithoragarh<br>(Didihat, 1,725m;<br>Sandev, 1,500m) | 1,500-3,000 | humus rich soil intermixed with litter and barks, common under shady to semi shady conditions |
| *H. monophyllum* (D. Don) P.F. Hunt and Summerh. | T | Shimla<br>(Tara devi, 1,851m) | 800-2,000 | exposed grassy and gravely slopes, moist humus laden rock, crevices of stones. |
| *H. monorchis* (L.) R.Br. | T | Kullu (Mahri, 3,500m),<br>Rohtang slopes (3,600m) | 2,700-4,000 | exposed grassy situations in the sub-alpine to alpine climates |

1. enlists their exact source of collection and other data in details.

Small pieces of leaves were preserved in FAA (Formaldehyde: Acetic acid: 50% Alcohol-1:1:18) and subsequently stored in 50% alcohol. Preserved plant material was dehydrated in TBA (Tertiary Butyl Alcohol) series, infilterated and embedded in paraffin wax and sectioned on rotary microtome at 10-15$\mu$m thickness following Kaushik (1983). The sections were double stained by safranin–fast-green combination (Johansen, 1940), mounted in DPX and observed under light microscope. For studying the epidermal peels, the fresh and/or stored mature leaves were stained, segmented and softened in 5% KOH solution for 45 minutes; leaf scrapings were made following Kaushik (1983), Williams (1975), stained in safranin, and mounted in 5% glycerine. Detailed anatomical studies were made and leaf epidermal characters such as shape, size and margin of epidermal cells; structural organisation, distribution, frequency, index of stomata; and structure of trichomes were studied. Stomatal types were identified based on the classification given by Rasmussen (1981). Photographs were taken using Olympus microscope model BH-2 with attached camera.

## Results and Discussion

All the presently studied species excepting for *Habenaria commelinifolia* and *Herminium monorchis* were collected from the temperate zone (1,501-3,000m). However, *Habenaria commelinifolia* was collected from sub-tropical zone (300-1,200m) and *Herminium monorchis* was procured from alpine zone (3,000-3,500m) (Table1). Though there is a considerable uniformity of leaf anatomy and epidermal features amongst different species, some discontinuities of such features were also observed. In the following paragraphs, some of the salient features are described and discussed in light of available information. Most of the presently studied species (*Habenaria edgeworthii, H. ensifolia, H. intermedia, H. latilabris, H. pectinata, Herminium lanceum*) were common in both exposed and semi exposed situations. However, *Habenaria clavigera, H. commelinifolia, Herminium monophyllum* and *H. monorchis* were found to grow on completely exposed situations. The leaves were spreading and nearly fleshy in all the presently studied species excepting for, in *Herminium monophyllum* and *H. monorchis*, where leaves were foliar and less succulent. Number of leaves varied from one (*Herminium monophyllum*) to many (*Habenaria clavigera*);most of the species were with 3 leaves (Table 2).

*Leaf Surface*

Epidermal Characters

Epidermis was comprised of non-sinuous rectangular to polygonal cells (Figs.1-5). In all the species, the adaxial epidermal cells were larger in size than abaxial epidermal cells, though to varied extents (Table 2). According to Vij *et al.* (1991), size pattern of epidermal cells vary with leaf orientation and these authors reported presence of larger adaxial epidermal cells in orchid taxa with spreading leaves. In *H. commelinifolia,* abaxial epidermal cells were distinctly elongated and rectangular, whereas on adaxial surface, epidermal cells were short and polygonal. Largest epidermal cells were found in alpine species, *Herminium lanceum* whereas smallest were observed in temperate species, *Habenaria ensifolia* (Table 2) Fig. 6 shows adaxial epidermal cells with cuticular striations, in Herminium lanceum. Presence of relatively larger epidermal cells in high altitude growing taxa, than those in low altitude ones was also earlier reported. Costal and intercostal regions were not distinguishable in all the species under genus *Habenaria*, however these were well differentiated in *Herminium* spp.

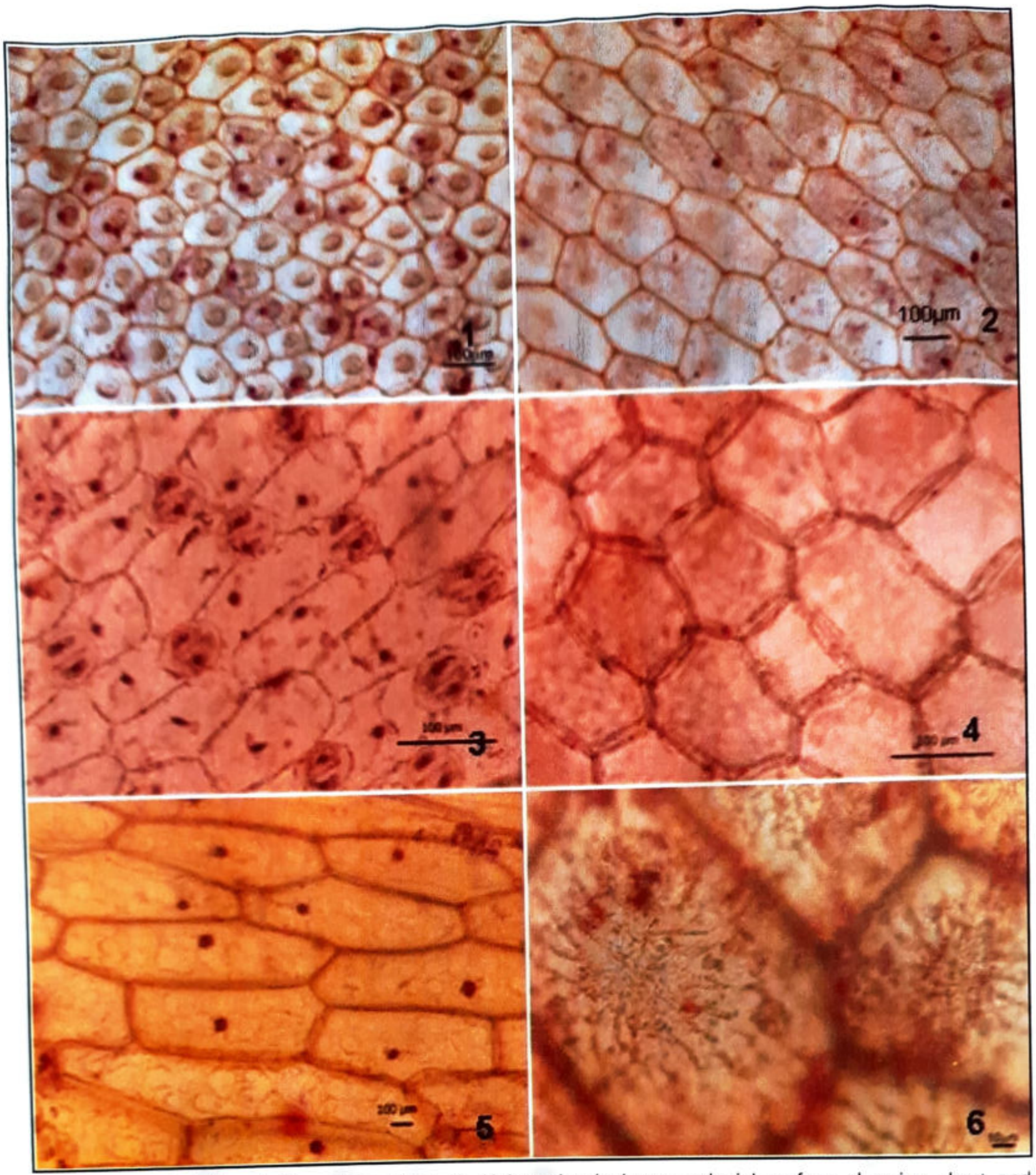
Stomatal Complex

Presently, hypostomatic leaves and anomocytic stomata were observed in all the species studied. These observations are is in accord with earlier findings made by several authors (Inamdar, 1968; Kaushik, I983; Samuel and Bhat, 1994; Singh, 1981; Stern, 1997a, and Williams, 1979). However, occasional tetracytic stomatal apparatus was also presently recorded in some species (*Habenaria clavigera*, and *H. ensifolia*) (Fig.7). Apparently, presence of hypostomatic leaves and anomocytic stomata may be considered as a characterstic features of Habenariinae. However, leaves were reported to be amphistomatic in *Habenaria digitata* (Das and Paria, 1992) and *H. goodyeroides* (Singh, 1981) and occasional presence of tetracytic stomata was reported earlier in a few species belonging to this subtribe as well (Stern, 1997a). Stomatal apparatus was usually surrounded by 4-5 cells. Highest stomatal frequency (46.6) and index (50) were found in *Herminium monophyllum* and the lowest were found in *Habenaria edgeworthii* (frequency- 14.51, index-18.4) (Table 3). Stomatal frequency was earlier correlated with different anatomical and ecological conditions by different authors. Goh *et al.* (1977) observed distinct negative correlation between leaf succulence and frequency of stomata whereas a positive correlation of stomatal density with light intensity was reported by Cyge (1930), Rasmussen, (1987),and Vij *et al.*(1991).

Table 2. Leaf number, texture, orientation, and epidermal characters.

| Species | Number | Orientation | Texture | Epidermal Cell | | | | | Adaxial vs Abaxial cell size | T and /or H |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Range of L (µm) x Range of W ( µm) | | Shape | | Wall pattern | | |
| | | | | Adaxial | Abaxial | Adaxial | Abaxial | | | |
| *Habenaria clavigera* (Lindl.) Dandy | many | S | NFl | 125.3- 140.4 x 95.2- 130.4 | 130.3-150.2 x 70.3-100.4 | penta-hexagonal | polygonal | non-sinuous | 1.25 | - |
| *H. commelinifolia* (Roxb.) Wall. ex Lindl. | 4-5 | S | NFl | 153-204.2 x 102.3- 153 | 110.2- 155.4 x 75- 85.5 | polygonal | rectangular-polygonal | non-sinuous | 2.14 | - |
| *H. edgeworthii* Hook. f. ex Collet | 4- 6 | S | NFl | 170.3- 187.8x119.4- 153.4 | 153.4-187.8 x 119.3-136.6 | polygonal-rectangular | Polygonal-rectangular | non-sinuous | 1.12 | - |
| *H. ensifolia* Lindl. | 4-5 | S | NFl | 90-125.5 x 80.4- 85 | 80.4- 92.5 x 75- 85.4 | hexagonal | polygonal | non-<br>-hexagonal sinuous | 1.29 | - |
| *H. intermedia* D. Don | 3-5 | S | NFl | 145.2- 155.8 x 95- 101.4 | 140.2-150 x 87.4-93 | hexagonal-polygonal | hexagonal-polygonal | non-sinuous | 1.13 | - |
| *H. latilabris* (Lindl.) Hook. f. | 3-4 | S | NFl | 75-180.8x70.4-135.2-140.4 | 130.3- 147.8 x 70.4- 93 | polygonal-hexagonal | polygonal-rectangular | non-sinuous | 1.19 | - |
| *H. pectinata* (J. E. Sm.) D.Don | 3-6 | S | NFl | 120-125.4 x 110.4- 125 | 110.4- 120.8 x 75- 80.5 | hexagonal | polygonal-hexagonal | non-sinuous | 1.61 | - |
| *Herminium lanceum* (Thun. ex Sw.) Vujik | 2-3 | S | NFl | 120.5- 144.4 x 100.2-120 | 60.5-76.5x 30.2 - 36.5 | polygonal-rectangular | polygonal-rectangular | non-sinuous | 6.38 | - |
| *H. monophyllum* (D. Don) P. F. Hunt & Summerh. | 1 | S | F | 136-272 x 187- 221 | 172.5-180 x 55-60 | pentagonal-hexagonal | columnar-polygonal | non-sinuous | 4.11 | - |
| *H. monorchis* (L.) R.Br. | 2-3 | S | F | 357.2- 510.4 x 119.2-238.6 | 170.2-374.5 x 68.2 - 85.6 | elongated, rectangular | elongated, rectangular | non-sinuous | 3.71 | - |

Ad-Adaxial, Ab-Abaxial, F-Foliar, H-Handle cells, L- Length, NFl-Nearly Fleshy, S-Spreading, T-Trichome, V-Vertical, W- Width.



Figs. 1-6. Leaf epidermis in surface view. *1, Habenaria clavigera* : adaxial surface showing short and polygonal cells; 2, *H. edgeworthii* : adaxial surface showing elongated polygonal cells; 3-4, *H. commelinifolia* : 3, abaxial surface showing elongated rectangular to polygonal cells and globose to oval stomata; 4, adaxial surface showing polygonal cells with thicker walls than abaxial one; 5, *Herminium monorchis* : adaxial surface showing thick walled polygonal and rectangular cells having prominent pit fields; *6, H. lanceum* : adaxial epidermal cells showing cuticular striations.

Presently, no such correlation was observed, though stomatal frequency was slightly higher in species (*Herminium monophyllum,* and *H. monorchis*) having less succulent leaves. It is interesting to note that these two species were also collected from exposed conditions. Shape of stomatal apparatus varies from nearly globose (*e.g. H. ensifolia*; Fig. 7); globose to oval (*e.g., H. commelinifolia;* Fig. 3); oval (*e.g., Habenaria clavigera, H. edgeworthii, H. intermedia, H. latilabris,* and *H. pectinata;* Fig. 8); to oval to oblong (*e.g., Herminium lanceum, H. monophyllum,* and *H. monorchis;* Figs. 9,10). Figures 11 and 12 shows T.S. of a part of lamina, showing, superficial stomata in *Herminium monorchis* and raised stomata with pointed outer ledges forming a pore between them, in *Herminium lanceum.* Presence of circular to nearly circular stomatal apparatuses were earlier reported in some species (*e.g. Bonatea steudneri*; *Cynorkis fastigiata, Habenaria leonensis, H. plantaginea, Stenoglottis fimbriata,*) belonging to this sub-tribe (Stern,1997a). The greatest length/width differences occur in *Habenaria clavigera* (about 75mm). Trichomes

Table 3. Stomatal characters.

| Species | Distribution | Shape | Type | Size (L µm ´ W µm ) | Pore size | Frequency (/mm²) | Index |
|---|---|---|---|---|---|---|---|
| *Habenaria clavigera* (Lindl.) Dandy | hypostomatic | oval | anomocytic | 100.2-105.4 x 25.2-30.3 | 40 x 5 | 26.79 | 25 |
| *H. commelinifolia* (Roxb.) Wall. ex Lindl. | hypostomatic | globose/ oval | anomocytic | 65.2-75.5 x 55.4-60.6 | 35 x 7.5 | 32.43 | 40 |
| *H. edgeworthii* Hook. f. ex Collet | hypostomatic | oval | anomocytic | 85.2-102.4 x 68.2-102.2 | 16.3 x 4.75 | 14.51 | 18.4 |
| *H. ensifolia* Lindl. | hypostomatic | globose | anomocytic | 80- 90.4 x 75.3-85.6 | 15 x 5 | 35.33 | 24.99 |
| *H. intermedia* D. Don | hypostomatic | oval | anomocytic | 70.2- 80.4 x 64.5-68.8 | 24.3 x 2.5 | 24.1 | 19 |
| *H. latilabris* (Lindl.) Hook. f. | hypostomatic | oval | anomocytic | 85.2- 86.4 x 74.2-75.6 | 26.3 x 4.75 | 24.51 | 17 |
| *H. pectinata* (J. E. Sm.) D.Don | hypostomatic | oval | anomocytic | 85.4-90.4 x 70.4-80.6 | 30 x 5 | 33.33 | 28.85 |
| *Herminium lanceum* (Thun. ex Sw.) Vujik | hypostomatic | oval to oblong | anomocytic | 35.2-48.4 x 30.2-35.5 | 15.2 x 3.75 | 25 | 29 |
| *H. monophyllum* (D. Don) Hunt & Summerh. | hypostomatic | oval to oblong | anomocytic | 81-72.5 x 50.5-55 | 38.75 x 5.75 | 46.6 | 50 |
| *H. monorchis* ( L.) R. Br. | hypostomatic | oblong | anomocytic | 70.2-75.4 x 55.2-65.5 | 50.2 x 9.16 | 35 | 28.5 |

ab- Abaxial surface, ad- Adaxial surface, L- Length, W- Width.

and handle cells were absent on both the leaf surfaces.

*Transverse Section (T.S.) of Leaf*

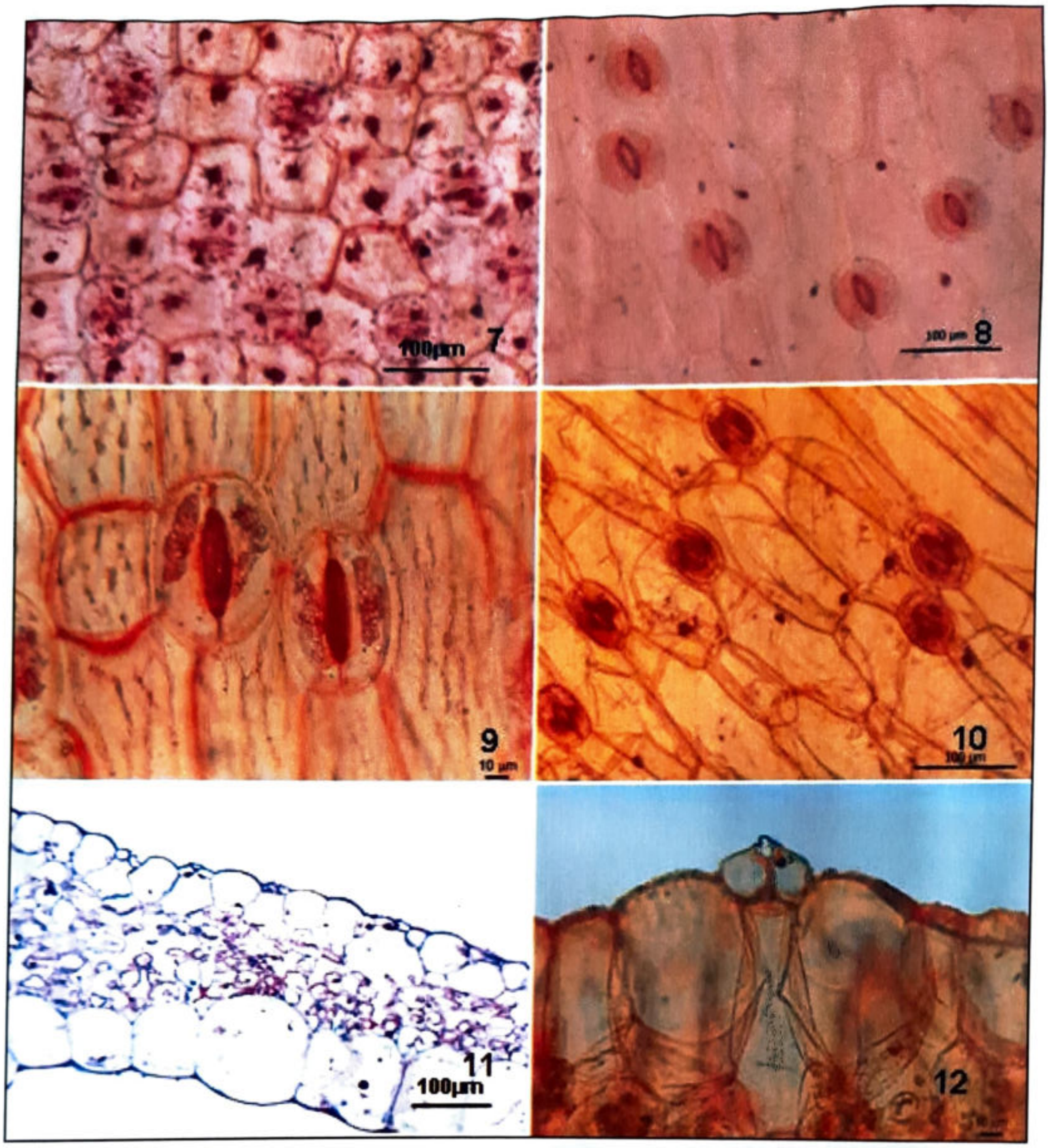
The T.S. of leaf was elongated, dorsiventrally flattened with the midrib region elongated downwards abaxially to form prominent V-shaped region (*Habenaria* spp.) (Fig. 13), or the leaf was broadly V shaped at the midrib region in T.S. as in *Herminium* spp. (Fig 14). Edges were blunt and bent downward abaxially. In some species of *Habenaria* (e.g., *H. edgeworthii*, *H. ensifolia*, *H. latilabris*, and *H. pectinata*), large laminar bundle regions were also elongated downwardly to form V-shaped region at regular intervals.

Cuticle

Cuticle was present on both the leaf surfaces; it was thicker on adaxial surface than on abaxial one. It was usually thin and smooth in most species, however it was striated and rugulose in *Habenaria commelinifolia*, *H. ensifolia*, and *Herminium lanceum* (Fig. 6). Stern (1997a) observed general presence of smooth cuticle in Habinariinae; however he also reported rugulose cuticle in few species namely *Habenaria leonensis*, *H. monorrhiza*, and all *Stenoglottis* spp. Presence of striated cuticular layer was also reported earlier in *Habenaria pectinata*, *Herminium gramineum*, and *H. lanceum* (Kaushik, 1983). According to Barthlott and Ziegler (1981), surface sculpturing increase the surface area and hence the reflectability of incoming radiation, which further results in an increased energy exchange with the surrounding air, and surface roughness may cause turbulency in laminar air flow and thereby increasing the thermodynamic exchange. According to Cyge (1930), the extent of development of cuticle in the leaves depends upon their degree of exposure (to sun); vertically oriented leaves which are similarly exposed on both adaxial and abaxial sides tend to exhibit uniform development of cuticle than spreading leaves, which have their only upper side exposed to sun light. Moreover, in spreading leaves, adaxial laminar surface is usually subjected to more exposure to sun light and hence resulting in more desiccation than abaxial ones. Presently, in transverse section, the adaxial epidermal cells were found to be enlarged and modified to different extent, which may act as water storage cells, indicating thereby that presence of a thick coating of cuticle in these species is probably an adaptive feature to minimize the water loss due to transpiration and/or guttation.

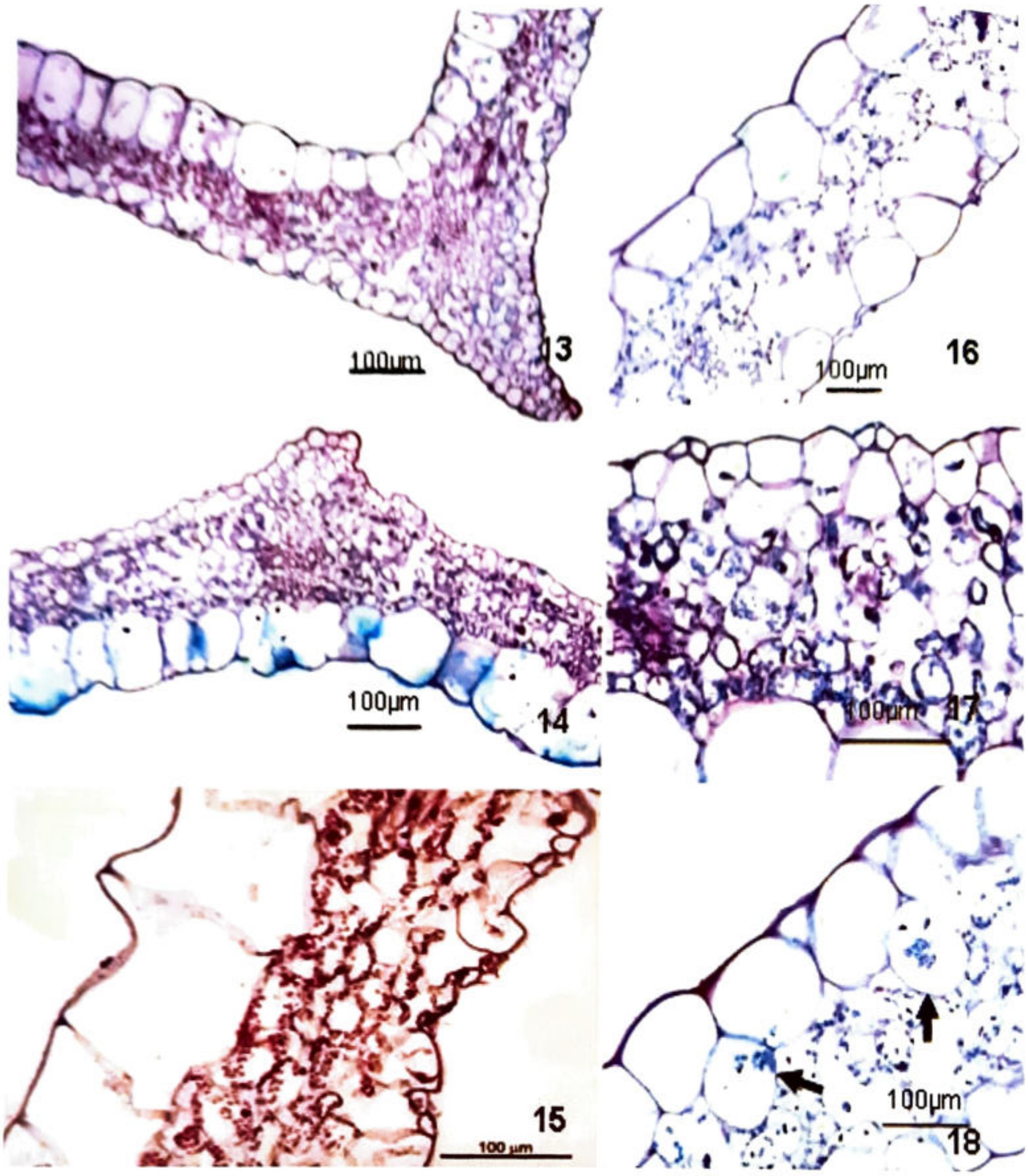
Epidermis

Epidermal cells were generally slightly rounded on both the surfaces in *Habenaria clavigera*, *H. ensifolia*, *H. intermedia*, and *H. pectinata*. Outer epidermal cell walls were thicker than other walls. In *Habenaria edgeworthii*, *Herminium monophyllum*, and *H. monorchis*, the outer margin of the abaxial cells was prominently rounded to dome shaped (Figs. 13,14), but it was usually flat to slightly rounded on adaxial surface and outer walls of some of the adaxial cells were inwardly compressed to form shallow grooves, resulting into their uneven surface. Some darkly stained epidermal cells were occasionally found in some species (*Habenaria edgeworthii*) (Fig. 23). Prominent epidermal papillae were also found to be present on laminar edges in some of the presently studied species (*Habenaria edgeworthii* and *H. monorchis*) (Figs. 23, 24). Presence of dome shaped epidermal cells was reported earlier in some orchid species , namely *Bonatea steudneri*, *H. rhodocheila*, *Stenoglottis fimbriata*, *S. longifolia*, and *S. zambesiaca* belonging to subtribe Habenarrinae (Stern, 1997a). Presence of papillose laminar epidermal cells has also been noticed in some other forest-floor dwelling orchid species (Atwood and Williams,1979). According to Samuel and Bhat (1994), the papillose (dome-shaped) cells present on the adaxial foliar epidermis of *Stenoglottis fimbriata* represented water vesicles. However, Stern (1997b) indicated their importance in light gathering as these are more apparent in forest floor plants growing under diminished light. Vogelmann *et al.*, (1996) described the function of these papillose epidermal cells as to gather or focus light. Juniper and Jefferee (1983) also considered these structures as a device to scatter light. Presently, papillose epidermal cells were also more apparent in species inhabiting semi exposed forest floors where natural light is less; presence of these cells, however, in sun exposed *Herminium monorchis* may also indicate its role in reducing the rate of transpiration as suggested earlier by Pridgeon (1982). Adaxial cells were thin walled, elongated, hyaline and generally larger anticlinally than abaxial cells ranging from 1.1 times in *Habenaria edgeworthii* to 2.14 times in *H. commelinifolia*. In genus *Herminium*, this condition was exaggerated so much that the dimensions of the upper epidermis account for almost about half of the laminar thickness. In *Herminium monorchis* and *H. monophyllum*, these were 3.71 and 4.10 times larger than abaxial cells. However, in *H. lanceum*, this condition was exaggerated to 6.38 times (Table 2) (Figs. 13-15). A similar condition has earlier been reported in *Holothrix* spp. (Stern, 1997a). These larger epidermal cells were usually thin walled, hyaline, and may function as water storage cells. Mobius (1887) first time described the vertically elongated epidermal cells, as water storage cells, in conduplicate taxa. A very thin wall coupled with plumpness and yielding



Figs. 7-12. 7-10: Leaf epidermis in surface view: 7, *Habenaria ensifolia* : abaxial surface showing globose stomatal apparatus with anomocytic to occasionally tetracytic configuration, and paired stomata; 8, *H. intermedia* : abaxial surface showing oval and anomocytic stomata; 9, *Herminium lanceum* : abaxial surface showing paired and oblong stomata, 10, *H. monorchis* : abaxial surface showing oblong stomata and occasional presence and paired stomata; 11, *H. monorchis* : T.S. of a part of lamina showing superficial stomata; 12, *H. lanceum* : T.S. of a part of lamina showing raised stomata with pointed outer ledges forming a pore between them.

quality of the actual tissue provides some evidence of their potential water storing capability. Mohana Rao and Khasim (1987) also observed some thin walled and hyaline water storage cells on adaxial epidermis in *Habenaria edgeworthii*, collected from Darjeeling and Sikkim hills (Eastern Himalaya). Vij *et al.,* (1991) suggested that the epidermal features in orchidaceae are appreciably affected by the environmental factors. Presently, stomata were superficial (*Habenaria clavigera*, *H. edgeworthii*, *H. ensifolia*, *H. pectinata*, and *Herminium monophyllum*; Figs. 11,15,16) to somewhat raised (*Herminium lanceum*, and *H. monorchis*; Fig. 12). Superficial to somewhat raised stomata were earlier observed in *Cynorkis fastigiata*, *H. odontopetala*, and *H. rhodocheila* (Stern, 1997a). Substomatal chambers were small, generally not much exceeding in size than adjacent epidermal cells (*Habenaria clavigera*, *Herminium lanceum*, *H. monophyllum* and *H. monorchis*; Fig 17); or moderately sized, almost double sized or more than adjacent epidermal cells (*Habenaria edgeworthii*, *H. ensifolia*, *H. intermedia*, *H. pectinata*; Fig. 16). Amongst the

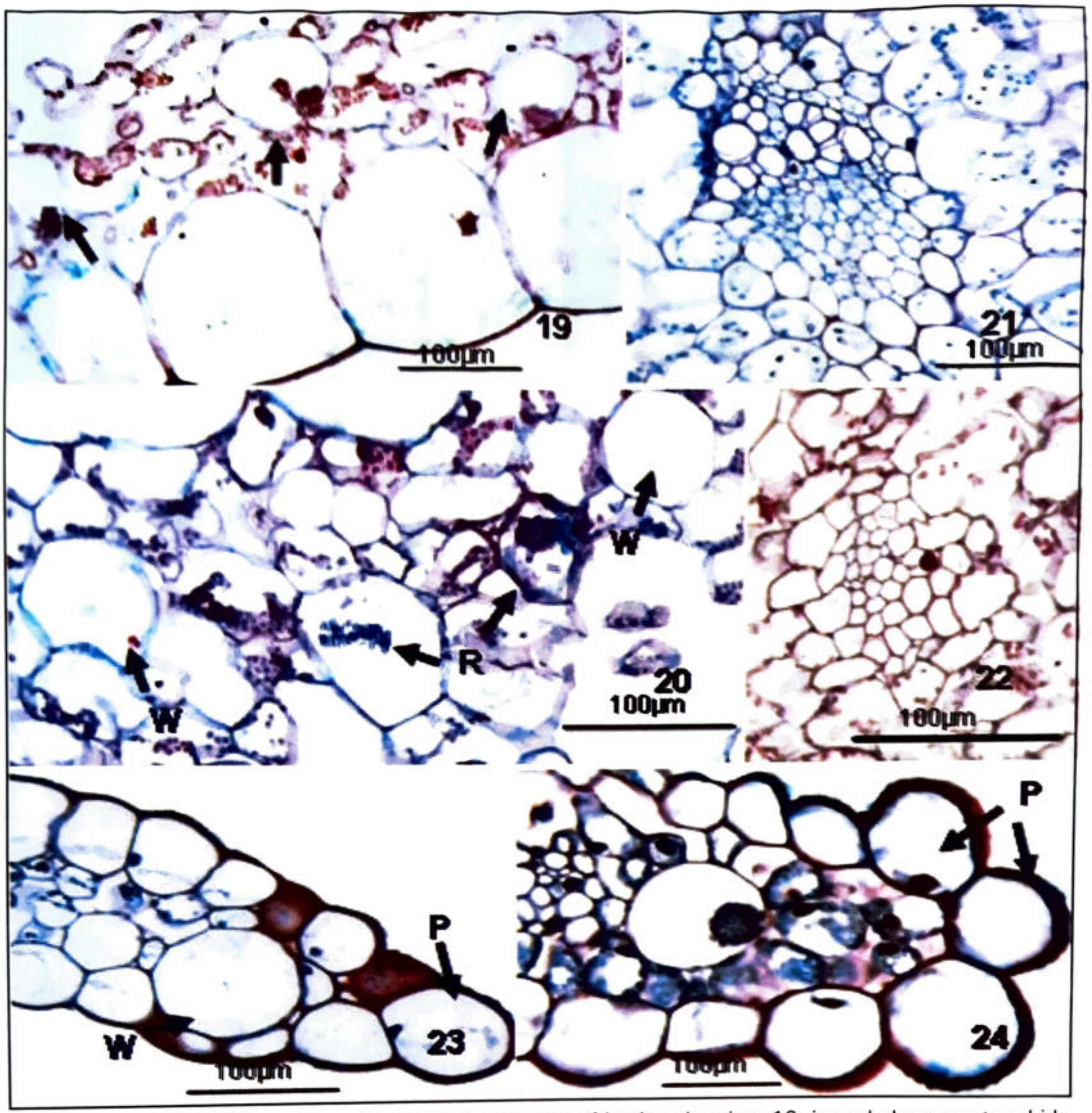


Figs. 13-18. Transverse section (T.S.) of a part of lamina showing V shaped midrib region, enlarged adaxial epidermal cells than abaxial one, and undifferentiated mesophyll with air spaces in: 13*Habenaria ensifolia;* 14, *Herminium monorchis* :15, *Herminium monophyllum* T.S. of a part of lamina showing 4.10 times larger adaxial epidermal cells than abaxial one, large and pointed ouer ledges of guard cells; 16, *Habenaria edgeworthii* : T.S. of a part of lamina showing undifferentiated mesophyll with large air spaces and moderate sized substomatal cavities; 17, *Herminium monorchis* :T.S. of a part of lamina showing small substomatal cavities; 18, *H. edgeworthii* : T.S. of a part of lamina showing irregularly present raphide idioblasts (arrow) below epidermis.

xeromorphic traits, encountered in presently studied orchid species, were the well developed outer cuticular ledges of stomata. Inner cuticular ledges were often absent and weakly developed. Presently outer guard cell ledges were large and blunt in *Habenaria clavigera*, and *H. intermedia*; large and pointed in *Herminium monophyllum* (Fig 15); and moderate and pointed in *Habenaria edgeworthii*, *H. pectinata*, and *Herminium lanceum*. Large distinct and pointed outer guard cell ledges were earlier reported in this sub-tribe (Kaushik, 1983). The outer ledges were curved leaving a small pore between them (*Herminium* spp.; Fig 12). According to Rusmussen (1987), sometimes in orchids, the inner wall of the outer chamber bulges out from the two sides, thus narrowing the middle of the cavity into a slit between an upper and lower space. Small to moderately sized substomatal chambers and outer stomatal ledges were earlier reported in members of sub-tribe Habenariinae (Stern, 1997a).

### Hypodermis

Although distinct hypodermal layer was absent in most of the species, large hyaline raphide idioblasts were found irregularly scattered below epidermis on both



Figs. 19-24. 19-20, *Habenaria pectinata :* T.S. of a part of lamina showing: 19, irregularly present raphide idioblasts (arrow) below epidermis and in mesophyll; 20, presence of both hyalibe water storage cells (W) and raphide idioblasts (R) in mesophyll; 21, *H. edgeworthii :* T.S. of a part of lamina showing midrib bundle surrounded by distinct parenchymatous bundle sheath, xylem present in two patches and pholoem intermingled with parenchymatous cells; 22, *H. clavigera :* T.S. of a part of lamina showing midrib bundle surrounded by indistinct parenchymatous bundle sheath, distinct xylem and intermingled phloem; 23, *H. edgeworthii,* T.S. of a part of lamina showing occasionally present darkly stained epidermal cells, enlarged epidermal cell forming epidermal papilla and large thin walled water storage cell (W); 24, *Herminium monorchis :* T.S. of a part of lamina showing epidermal papillae present at laminar edge.

the laminar surfaces in *Habenaria edgeworthii, H. latilabris* and *H. pectinata* (Figs. 18,19). Kaushik (1983) noted the presence of some large hyaline water storage cells below both epidermal layers in *H. pectinata* (Fig. 19). We did not find any similar kind of hypodermal water-storage cells described by Kaushik (1983), we suspect these to be transverse sections of crystalliferous idioblasts, which were mentioned above. However, Stern (1997a) reported throughout absence of hypodermis in sub-tribe Habenariinae. To conclude, though a typical hypodermis is usually absent in Habenariinae, in some species, large hyaline raphide idioblasts are irregularly scattered below epidermis.

### Mesophyll

Mesophyll was parenchymatous, undifferentiated, thin-walled, 3-11 cells wide, and mostly 4-8 cells thick (Figs. 13, 14). Though, Stern (1997a) mentioned the mesophyll in Habenariinae as chlorenchymatous, present findings of parenchymatous mesophyll is in conformity with Kaushik (1983). However, we also noticed irregular presence of some thick walled cells in it. Mesophyll cells were oval and elliptical to rounded, sometimes polygonal or irregular and rich in chloroplasts. Intercellular spaces were usually abundant except in *Habenaria clavigera* and *Herminium monophyllum*, where they were small. Crystalliferous raphide idioblasts were scattered in mesophyll; these were globose and sub-globose, in transverse section. Presence of abundant intercellular spaces and raphide idioblasts was earlier reported in almost all the species studied under this sub-tribe (Kaushik, 1983; Stern, 1997a). The lacunae characteristic of leaves, stems, and roots of subaquatic *H. repens* were assumed to be storage areas for oxygen evolved during photosynthesis and conveyed around the plant to serve in respiration of submerged portions (Stern, 1997a). It is worthwhile to mention here that presence of abundant intercellular spaces was reported to be positively related with $C_3$ function (Nelson and Sage, 2008). Some of the mesophyll cells were larger in size, hyaline and isodiametric and may serve as water storage cells (Figs. 20, 23). Stern (1997a) did not see the isodiametric to polygonal water-storage cells in Habenariinae, earlier described by Kaushik (1983), in some taxa belonging to this sub-family and he (Stern, 1997a) argued these to be transverse sections of crystalliferous idioblasts; however, we noticed presence of both raphide idioblasts and water storage cells in most of the presently studied species (Fig. 20).

Presently water storage cells were more frequent in some habenarias (*Habenaria edgeworthii*, and *H. pectinata*) and almost absent in *Herminium* spp. It is interesting to note that in *Herminium* spp., the adaxial epidermal cells were highly enlarged ; these are probably meant for conserving significant amount of water and may compensate for less number of mesophyll water storage cells. Though Mohana Rao and Kashim (1987) observed some thin walled and hyaline water storage cells on adaxial epidermis in *Habenaria edgeworthii*, collected from Darjeeling and Sikkim hills (Eastern Himalaya), Vij *et al.* (1991) could not observe such cells in individuals collected from Shimla hills (Western Himalaya) suggesting thereby that the epidermal features in Orchidaceae are appreciably affected by the environmental factors. Our findings also indicate some role of environmental factors in depicting orchid leaf dermal and anatomical features.

## Vascular Bundle

Collateral vascular bundles (Figs. 21, 22) were present in a single series across the leaf blade. Larger midrib vascular bundle was flanked on either side by relatively smaller laminar bundles. Usually laminar bundles were of nearly same size (*Habenaria clavigera*, *H ensifolia*, *H. intermedia*, *Herminium lanceum*, *H. monophyllum* and *H. monorchis*); however in *Habenaria edgeworthii*, and *H. pectinata* midrib bundle was flanked either side by one or two relatively larger and few smaller laminar bundles. In all the presently studied species, vascular bundles were surrounded by parenchymatous bundle sheath. Bundle sheath cells were thin-walled containing chloroplasts (Figs. 21, 22). Similar cells were observed earlier in members of Habenariinae and Orchidinae (Stern, 1997a,b). Presently, these were not well distinguishable in *Habenaria clavigera*, *H. intermedia*, *Herminium lanceum* and *H. monophyllum* (Fig. 22). In most of the presently studied species, xylem and phloem were usually well distinguishable in midrib bundles but indistinct in small laminar bundles; though more than one distinct xylem patches were found in *Habenaria edgeworthii, H. ensifolia*, and *H. pectinata*, the phloem was intermingled with parenchymatous cells (Fig. 21). Presence of two distinct xylem patches and intermingled phloem with parenchymatous cells was also reported earlier in *Habenaria pectinata* (Kaushik, 1983). Fibre cap and stigmata were absent in all the species as reported earlier in this sub-tribe (Stern, 1997a).

In addition to the fibre strands associated with the vascular bundles, some extra-vascular strands, the commissural bundles were also observed in *Habenaria ensifolia* and *H. pectinata.* According to Esau (1965, 1977), their occurrence was reported to be closely related with the stiffness of the leaf thereby imparting mechanical strength by reducing the injurious effect of wilting during stress conditions. However, Stern (1997b) suggested that the presence of the cross anastomoses of veinlets indicates a transitional state between the monocotyledonous and dicotyledonous conditions. The commissural bundles besides providing mechanical strength to leaf, probably help in lateral conduction of water.

Present studies indicate a considerable uniformity, in leaf anatomy between subtribes Habenariinae and Orchiidinae. Similar observations were earlier made by Stern(1997a); according to him, in both the cases, stomata are anomocytic and often almost circular, superficial, with small to moderately sized substomatal chambers; mesophyll homogeneous; collateral vascular bundles occur in a single series across the lamina; bundle sheath cells are thin-walled and contain chloroplasts; foliar sclerenchyma and stigmata absent. Earlier, Luer (1972, 1975) had separated *Platanthera* (Orchidinae) from *Habenaria* ( Habenariinae). Stern (1997), however, considering the uniformity in anatomical characters of Habenariinae and Orchidinae, had voiced in favour of uniting this genus with '*Habenaria* group'.

## References

Atwood., J.T. and N.H. Williams.1979. Surface feature of the adaxial epidermis in conduplicate leaved cypripedioideae (Orchidaceae). *Bot. J. Linn. Soc.,* **78**:141-56.

Barthlott, W. and B. Ziegler. 1981. Mikromorphologie der Samenschalen als systematisch Merkmal bei Orchideen. *Berichte der Deutsche Botanische Gesellschaft,* **94**: 267-73.

Cyge, T. 1930. Etudes anatomiques et ecologiques sur les feuilles des Orchidees indigenes. *Exit·. Mem. Acad. Polon. Sci. Lett. Ser.B,* **12**:16-32.

Das, S. N. and N. Paria. 1992. Stomatal structure of some Indian orchids with reference to taxonomy. *Bangladesh J. Bot.,* **21**(1):65-72.

Dressler, R. L. 1993. *Phylogeny and Classification of the Orchid Family.* Cambridge University Press, Cambridge.

Esau, K.1965. *Plant Anatomy.* John Wiley and Sons, New York, USA.

Esau, K. 1977. *Anatomy of Seed Plants.* John Wiley and Sons, New York, USA.

Goh, C. J., P. N. Avadhani, C. S. Loh, C. Manegraff, and J. Arditti. 1977. Diurnal stomatal and acidity rhythms in orchid leaves. *New Phytol.,* **78**:365-72.

Inamdar, J. A. 1968. Stomatal ontogeny in *Habenaria marginata* Coleb. *Curr. Sci.,* **37**:24-25.

Johansen, D.A. 1940. Plant microtechnique. Mc Graw Hill Book Company, New York, USA.

Juniper, B. E. and C. E. Jefferee. 1983. *Plant Surfaces.* Edward Arnold, London.

Kaushik, P. 1983. *Ecological and Anatomical Marvels of the Himalayan Orchids.* Today and Tomorrow's Printers and Publishers, New Delhi, India.

Luer C.A. 1972. *The Native Orchids of Florida.* The New York Botanical Garden. New York. USA

Luer C. A. 1975. *The Native Orchids of The United States and Canada Excluding Florida.* The New York Botanical Garden. New York, USA.

Mobius, M. 1887. Uber den anatomischen bau der orchideen blatter and dessen bedeutung fur das system dieser familia. *Jahrb. Wiss. Bot.,* **18**:530-607.

Mohana Rao, P. R. and S. M. Khasim. 1987. Evolutionary trends in growth habit and vegetative anatomy of Indian orchids. *J. Orchid Soc. India,* **1**:57-70.

Nelson, A. E. and R. F. Sage. 2008. Functional constraints of CAM leaf anatomy: Tight cell packing is associated with increased CAM function across a gradient of CAM expresson. *J. Exp. Bot.,* **59**(7):1841-50.

Pridgeon, A. M. 1982. Diagnostic anatomical characters in Pleuro-thallidinae (Orchidaceae). *Amer. J. Bot.,* **69**:921-38.

Rasmussen, H. 1981. The diversity of stomatal development in Orchidaceae: subfamily Orchidoideae. *Bot. J. Linn. Soc.,* **82**:381-93.

Rasmussen, H. 1987. Orchid stomata structure, differentiation, function and phylogeny. *In: Orchid Biology, Reviews, and Perspectives* Vol. IV (ed. J. Arditti) pp. 105-38. Cornell University Press, Ithaca, New York, USA.

Samuel J. and R. B. Bhat. 1994. Epidermal structure, organographic distribution and ontogeny of stomata in vegetative and floral organs of *Stenoglottis fimbriata* (Orchidaceae). *South African journal of Botany,* **60**:113-17.

Singh, H. 1981. Development and organisation of stomata in Orchidaceae. *Acta. Bot. Indica,* **9**:94-100.

Stern, W. L. 1997a. Vegetative anatomy of subtribe Habenariinae(Orchidaceae). *Bot. J. Linn. Soc.,***125**:211-27.

Stern, W. L. 1997b.Vegetative anatomy of subtribe Orchidinae. *Bot. J. Linn. Soc.,***124**:121-36.

Vij, S. P., P. S. Kaushal, and P. Kaur. 1991. Observations on leaf epidermal features in some Indian orchids: Taxonomic and ecological implications. *J. Orchid Soc. India,* **5**(1,2):13-53.

Vogelmann T. C., J. F. Bornman, and D. J. Yates. 1996. Focusing of light by leaf epidermal cells. *Physiol. Plant.,* **98**:43-56.

Williams, N.H. 1975. Stomatal development in *Ludisia discolor* (Orchidaceae): Mesoperigenous subsidiary cells in the monocotyledons. *Taxon,* **24**:281-88.

Williams, N. H. 1979. Subsidiary cells in Orchidaceae: Their general distribution with special reference to development in Oncidieae. *Bot. J. Linn. Soc.,* **78**:41-66.