Comparative Leaf anatomy of some species of Habenaria Wild (Orchidaceae).

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Abstract

The present study analyzed the characteristics in leaf of Terrestrial Orchidaceae member i.e different species of Habenaria Wild (H. plantaginea Lind1., H. ovalifolia. Wight., H. multicaudata Sedgw. , H. crinifera Lind1., H. heyneana. Lindl., H. elwesii Hook.f., H. longicorniculata J.Graham. , H. pelorioides Par. & Rchb.f. (Odisha cleistantha S.Mishra).). Anatomical characters are important in identification and ecological adaptations. The anatomical characters have serving as a tool for the genus's systematic. The plants was collected at different forest types from Shivamogga district. Materials was fixed in FAA solution. Transverse sections were obtained by freehand sections, stained with 1% safranin. Leaf anatomical organization of Habenaria species (Stomata ,epidermis, mesophyll, vascular bundles, hypodermis.) was observed. There was a distinct variation in the anatomical characters of the leaf. Detailed leaf anatomical characters can be used to distinguish species from others. Leaf anatomical characters with structural adaptation helps plants to survive in different vegetation. The implication of this study is the importance of leaf anatomical features to support species identification and to increase understanding of orchid biology which are important in orchid conservation.

INTRODUCTION

Orchidaceae with 25,000 -30,000 species shows a high level of specialization and a great capacity for adaptation to a different environments, which have contributed to the morphological and physiological, vegetative organization, that vary between species (Dressler, 1993;Frander *et .al* 2017; Udupa ,2011). Orchidaceae is one of the largest family among monocots. Orchidaceae is a second largest flowering plant family of herbaceous, perennials that includes terrestrial ,saprophytic, lithophytic, and epiphytic species (Pridgeon*et.al* 1993). Orchids are associated with an endophytic symbiotic fungus, mainly in their roots, terrestrial orchids mainly grows in floor of the forest and grassland.

Habenaria Wild is an orchid genus of about 600 species widely distributed throughout the tropical, subtropical and temperate regions of the world. This genus of terrestrial orchids is one of the genera having largest number of species. In India, it is represented by 17 species in Western Himalaya (Jalal and Jayanthi, 2015). In*Habenaria* Wild. Prominent paired tuberoids with a small third tuberoid is also seen (Pande *et .al* . 2010) in this species flowers are highly attractive. The species can be easily identified when in bloom, but the vegetative characteristic (number and size of tubers and leaves, stem height) overlap in many of these. These structural and physiological variations permit plants to survive and reproduce in a variety of environmental conditions and contribute to protection against stress and herbivore damage (Frander *et .al* 2017.) Particularly in the anatomy of groups are of a descriptive nature, and have focused upon the search for similarities or differences that contribute to the taxonomic determination.

MATERIALS AND METHODS

STUDY AREA

Shimogga district is a part of malnad region of Karnataka and is also known as the ' Gateway to Malnad ', There is a seven taluks: Soraba, Sagara, Hosanagara, Shimogga, Shikaripura, Thirthalli & Bhadravathi, district situated between the latitudes $13^0 27^1$ and 14^0 39^1 N and between the longitudes 74^0 $38^1$and 76^0 04^1 at mean altitude of 640 meters above sea level . Peak Kodachadri hill is at an altitude of 1343 meters above sea level is the highest point in the district. Shimogga district is rich in diversity of flora and fauna. District covers evergreen, semi-evergreen, deciduous, scrub forest, and grasslands .(figure. 1.)

METHODOLOGY

The present study was undertaken in Shimogga district during the year 2019-2020. The Terrestrial Orchids of eight different *Habenaria*species , were collected at different regions from Shivamogga district at different vegetation types includes evergreen , deciduous and open forest type.(Table 1.) *Habenaria* species, were identified with the help of available manuals and floras (Gamble ,1935; Rao , 1998). To evaluate the leaf anatomy, samples were fixed in FAA 50% for 48 hours and then transferred to Ethanol 50% (Johansen ,1940). Histological slides were prepared by freehand section. Each one was sectioned in the midline, free hand sections were obtained using a razor blade, Transverse sections were subjected to staining with 1% safranin (Kraus and Arduin, 1997). Leaf sections were analyzed in light microscope. In leaf section number of layers in epidermis, hypodermis, mesophyll and vascular bundles, were observed and noted. The epidermis of leaves were peeled from the fresh leaves to observe structure of the stomata. The most important aspects were recorded with digital camera attached to Olympus microscope.

RESULT AND DISCUSSION

The results of this study showed leaf anatomical features of*Habenaria* Wild Species in Shimogga district, comparable data among the species supports species identification and to helps to understand their structures adaption to different habitat in a district. The leaf anatomical structures showed organizations of components forming leaves, consisted of stomata, cuticle, epidermis, mesophyll and vascular bundles, hypodermis in cells are particular in orchid species.

1.*Habenaria heyneana* Lindl**.** : Leaf transverse section of*H* . *heyneana* showed tetracyclic type of stomata, cuticle was thick and smooth, The epidermic was uniseriate with elongated shaped cells, hypodermis was not clear. Mesophyll were homogeneous consisted of 10-12 thin walled , round-shaped ,parenchymatic cell layers, filled with chlorophylls (chlorenchyms) vascular bundles consisted of xylem and phloem, arranged collaterally. There were 4 large and 4-6 small arches arranged in a row with parenchyma cells bundle sheath surrounded the vascular bundles, scherenchyma cells in vascular bundle were not observed (figure 2. A , B. figure 4. I)

2. *Habenaria Plantaginea* Lind1**.** : *H.Plantaginea* anatomical characters showed that stomata was cyclocytic, cuticle was relatively thick and smooth. The epidermis was a single layer composed of elongated shaped cells . mesophyll was 13 -15 layers, consisted of elongated parenchymatous cells, chlorophylls were present in the Parenchymatous cells of mesophyll. vascular bundles were arranged within parenchymatous cells of mesophyll layer. Vascular bundles consisted of 7-9 Arches of xylem and phloem. Bundles sheath was thin walled surrounds the vascular bundles (Figure 2. F , G,H. Figure 4. A)

3. *Habenaria longicorniculata* J.Graham**.** : *H. longicorniculata* anemocytic and tetracytic stomata configuration, cuticle layer was thick and smooth, epidermic was uniseriate composed of polygonal shaped cells. Hypodermis was not observed. Mesophyll was 14-15 layers of homogenous polygonal shaped parenchymatous cells. Vascular bundles consisted of xylem and phloem arranged collaterally. Sclerenchyma cells were not associated with vascular bundles, bundle sheath cells were thin . there were 8-9 arches of xylem and phloem arranged in a row embedded within parenchymatous cells of mesophyll. (figure 2 I, G Figure .4 B)

4. *Habenaria elwessi* Hook.f : *H. elwessi* had anemocytic stomata configuration and cuticle was thick and smooth, Epidermis is had one layer made up of elongated shaped cells . Hypodermis was absent or not clear. Mesophyll was Homogenous with 15-16 layers consisting of polygonal shaped parenchymatous cells.

Chlorophylls were present in mesophyll cells. Vascular bundles consisted of xylem and phloem arranged collaterally. There were 9-10 xylem and phloem arches arranged in rows embedded in parenchymatous cells. Vascular bundles are surrounded with thin bundle sheath. (Figure 3 , A, B, C figure 4. J).

5. *Habenaria crinifera* Lind1 : *H. crinifera* had tetracyclic stomata configuration with thick and smooth cuticle. Epidermis is single layered with elongated cells. Hypodermis was not clearly distinguishable. Mesophyll layer is made with Homogenous parenchymatous cells. Mesophyll cells stores starch grains and water. There is a large water storage cells were present. Mesophyll cells with 12-13 layers of parenchymatous cells, they were polygonal in shape. Vascular bundles with 10-11 xylem and phloem arches arranged in a rows embedded within parenchymatous cells. (Figure 3. D, E figure 4. H)

6. *Habenaria ovalifolia* Wight.: *H. ovalifolia* had tetracyclic stomata configuration with thick cuticle layer. The epidermis was uniseriate with polygonal shaped cells. Hypodermis is not clearly seen. Mesophyll was 14-15 layers with homogenous polygonal shaped parenchyma cells. Vascular bundles consisted of xylem and phloem arranged collaterally. Sclerenchyma cells not associated with vascular bundles. Bundle sheath was indistinct. There were 5-6 arches of vascular bundle arranged in a number of rows embedded within parenchymatous cells of mesophyll. (figure 3. F, G figure 4.F, G).

7. *Habenaria multicaudata* Sedgw. : *H.multicaudata* leaves has cyclocytic stomata configuration. cuticle was thick ,epidermis was a single layer with elongated cells. Hypodermis cells were not clear. Mesophyll was 13-14 layers made with homogenous , rounded to elongated shaped parenchyma cells. Chlorophylls were abundantly present in mesophyll cells. Vascular bundles were arranged with 4-5 arches. sclerenchyma cells were not associated with bundle sheath cells. Bundle sheath cells are thin cleanly visible. (figure 2 D, E figure 4.D).

8. *Habenaria pelorioides* Par. & Rchb.f. (*Odisha cleistantha* S.Mishra) : Leaf transverse section of *H. peloriodes* showed the stomata with anemocytic and tetracyclic configuration. Cuticle was thick and smooth in nature. The epidermis was uniseriate with elongated shaped cells. Hypodermis was absent or not clear. Mesophyll was homogenous consisted of 14-15 layers with thin walled parenchyma cells. Vascular bundles consisted of xylem and phloem arranged collaterally. There were 4-5 Arches arranged in a rows embedded with parenchyma cells. Sclerenchyma cells were not associated with vascular bundles. Thin walled bundle sheath surrounded the vascular bundle. (figure 3. H. I. figure 4. C,E).

Discussion

The leaf outline was 'V' shaped in all *Habenaria* species. In all species studied, the cuticle was smooth on leaf surface. The cuticle thickness was greatest in all studied species. The cuticle is a thick, hydrophobic membrane composed of a polymer matrix (cutin) and associated with solvent soluble lipids (cuticular waxes) (Rasmussen, 1987); Angela*et. al* 2015). Plant cuticle plays an important role in the interaction of plant with environment, helps to reduce water transpiration (Fahn 1982; Rindyastuiti *et .al* 2018).*Habenaria* species has a variation in cuticle thickness, the thick cuticle is an ecological adaption to reduce transpiration (Moreira*et. al* 2013).

Epidermis is the outer cell layer of a leaf, act as a barrier between the leaves and environment it is also important in maintaining the external stimulus (Dietz and Hartung 1996). Epidermis is important to protect mesophyll and vascular bundles from higher solar radiation. Thick epidermis cell helps to reduce water transpiration.

Large epidermal cell in many species of orchids serve as water storage (Guan *et .al* 2011). Size of the epidermal cell is significantly different among species. Larger epidermal cells support the species to be more adaptive to the warmer environment . epidermal cell has various shape among species such as polygonal, isodiametric rectangular and elongated. (Aybake *et.al* .2010).

The hypodermis is a structure beneath the epidermis this structure can be present or absent in orchid species (Pridgeon 1982; stern 1997; Rindyastuti *et.al* 2018). Along with epidermis, hypodermis protect the

mesophyll cells & vascular bundles. In the present study hypodermis was absent or not clear in the *Habenaria* species.

The mesophyll is important structure for leaves that is filled with photosynthesis component (chlorophyll) to assimilate nutrients. The number of mesophyll layers in the present study varies. The thicker leaves supports the leaves succulence. The succulence level of leaves related to the parenchymal capacity of mesophylls, to provide water supply for photosynthetic process and leaves cells, turgor mainly in the dry environment (Hsiao 1973; Lack and Evans 2001; Metusala *et.al*2017). In the present study *Habenaria elwessi* Hook.f has more mesophyll layers than the other *Habenaria* species. In present study *Habenaria* species shows mesophyll layer is made with homogenous parenchymatic cells.

The vascular bundle is a transport system containing xylem and phloem that are important in the water and nutrient transport (Lack and Evans 2001; Fahn 1982).

The vascular bundles arrangement in the mesophyll of *Habenaria*species has aligned in rows in the center of mesophyll. Vascular bundles arrangement in a single row was reported in *Habenaria cornuta* ,*H . holothrix, H . monorrhiza, H. occidentalis, H. odontoptala, H. snowtenii, H. vaginatum.* (Stern 1997) in present study sclerenchyma cells were not associated with xylem and phloem. Bundle sheath consisted of cells surrounding the vascular bundle. Bundle sheath cells were made with thin walled parenchymatic cells. Fibre bundles, spiral thickening, raphide bundles were absent or not clearly visible.

Conclusion

All the species studied possessed both morphological and anatomical features which are most suitable to xeromorphic condition, species are efficient in water use and can adopt to very well even under water stress condition. This study implied the importance of anatomical characters in species identification. Taxonomic knowledge and species identification are important in the conservation, Therefore, these*Habenaria* species need more attention in conservation than the more adaptive species ,for their future survival.

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Table 1. Ecological traits of *Habenaia* species collected in Shimogga district.

Sl.no	SPECIES	SOURCE	FLOWERING PERIOD	VEGETATION TYPE
		locality		
1.	*Habenaria longicorniculata* **J.Graham.**	Shimogga, hosanagara, Sharavati valley	August- October	evergreen ,semi-evergreen grasslands
2.	*Habenaria elwesii* **Hook.f**	Soraba, Sagara kodachadri hills	July- October	semi-evergreen forest ,grasslands, forest edges ,
3.	*Habenaria heyneana.* **Lindl.**	Shikaripura, Sagara	August- October	Hills, evergreen to semi evergreen forest.
4.	Habenaria crinifera **Lind1**.	Thirtahalli, Sagara, sharavati valley	July- September	Grasslands, evergreen to semi evergreen forest ,hills

Sl.no	SPECIES	SOURCE	FLOWERING PERIOD	VEGETATION TYPE
5.	Habenaria plantaginea **Lind1.**	Sagara Bhadravati, soraba, shikaripura	July- September	Semi evergreen ,Deciduous forest scrub forest
6.	Habenaria ovalifolia. **Wight.**	Hosanagara,, Sagara	August- October	Semi evergreen , scrub forest
7.	Habenaria multicaudata **Sedgw.**	Hosanagara, Thirtahalli,	July- September	Evergreen forest
8.	*Habenaria pelorioides* **Par. & Rchb.f. (*Odisha cleistantha* S.Mishra)**	Sagara	September-December	Evergreen to semi evergreen forest

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Fig.1. Map shows Shimogga district is the study area

Figure 2. Transverse section of leaf **A.***Habenaria heyneana* Lindl **B.** Mesophyll in *H. heyneana* **C.** Vascular region *of H. heyneana* **D** .*T.S of Habenaria multicaudata* Sedgw. **E.** Vascular region *H. multicaudata* **F** *T.S of Habenaria plantaginea* Lind1. **G.** Vascular region of *H. plantaginea.* **H .** Mesophyll in *H. plantaginea.***I** *T.S of Habenaria longicorniculata* J. Graham. J. Epidermal region in *H. longicorniculata.* C-cuticle, UE-upper epidermis, M-mesophyll, PVB-primary vascular bundles. X-xylem, PH - phloem, WSC -water storage cells.

Figure 3. Transverse section of leaf **A.***Habenaria elwessi* Hook.f **B** .Mesophyll in *H. elwessi* **C.** Vascular region *of H. elwessi* D. *T.S of Habenaria crinifera* Lind1**. E** Vascular region *of H. crinifera* **F.** T.S of *Habenaria ovalifolia* Wight**G** . Vascular region *of H. ovalifolia* **H** . T.S of*Habenaria pelorioides* Par. & Rchb. f. **I.** Vascular region *of Habenaria pelorioide.* C-cuticle, UE-upper epidermis, M-mesophyll, PVB-primary vascular bundles. X-xylem, PH-phloem, WSC

Figure 4 . **A** . Cyclocytic stomata in *Habenaria plantaginea* Lind1. **B** . Tetracyclic Stomata *Habenaria longicorniculata* J. Graham**. C** . Tetracyclic Stomata*Habenaria pelorioides* Par. & Rchb. f. **D** . Cyclocytic stomata Habenaria multicaudata Sedgw **E** . Anomocytic stomata in*Habenaria pelorioides* Par. & Rchb. f. F, **G** . Tetracyclic Stomata *Habenaria ovalifolia* Wight. **H** . Tetracyclic Stomata *Habenaria crinifera* Lind1 **I** . Tetracyclic Stomata in *Habenaria heyneana* Lindl **J** . Anomocytic stomata*Habenaria elwessi* Hook.f. **.**

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