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A Study on Stomatal Complex and Phenology of Certain Epiphytic Orchids of Western Ghatts of Karnataka

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A B S T R A C T

The study indicated genotypic differences with respect to habitat, inflorescence, floral position, phenology, orientation, texture, leaf surface and stomatal complex of eleven species of epiphytic orchids belonging to three genera Viz., *Aeridis, Bulbophyllus and Dendrobium* of Western ghats of Karnataka. *A ringens, A.maculosum, B.neilgeriensis* and *D.ovalifolium* have shown receme type of inflorescence. *B.fischerii D.nutantifolium D.cripidatum D.macrostychum* and *D.heterocarpum* cyme type inflorescence whereas *B. fimbriatum* has shown umbellate and *D. herbaceum* have shown umbellate terminal raceme type of inflorescence. The floral position varied from base of leaf sheath, base of the bulb, from the nodes and node of the pseudo stem. Flower phenology indicated different climatic periods like pre monsoon, monsoon and even summer indicating influence of climatic factors. The leaf surface was hypostomatic in all the species except *B.Neilgeriensis* where it was Amphistomatic. The type of stomata was Anomocytic in *A.ringens, A.maculosum, B.fischerii, D.ovalifolium* and *D.heterocarpum.* Anisocytic in *B.neilgeriensis D.cripidatum* and paracytic in *B.fimbriatum D.herbaceum, D.Nutantifolium* and *D.macrostychum*. The Species *Dendrobium macrostychum* has shown least stomatal index and frequency followed by *B.neilgeriensis*. This could be due to strong drought tolerant mechanism by which these species are capable of curtailing transpiration water loss as these species generally found in harsh climatic conditions like rocks and dry bark of trees and also open conditions.

Keywords: Orchids, *Aeridis maculosam,* Niluguli, Stomata, Frequency

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CONTENTS

1. Introduction

Orchidaceae is a unique family among plant groups; they constitute strange, yet interesting Characteristics in almost all aspects. *Orchidaceae* stands apart from the rest of the plant families, maintaining intriguing individuality and all its own. The various morphological, physiological and genetic peculiarities, so liberally displayed by this group of

plants have stimulated fields of research so vast and varied that Orchidology today has developed into one of the most dynamic branch of Botany (Abraham and Vatsala, 1981). Orchidaceae is represented by 25000-35000 species belonging to 600-800 genera (Arditti, 1979). In India this family is represented by about 152 genera and 1300 species (Rao, 1979). Krishnaswamy *et al.,* (2004a) reported 203 species and 59 genera of orchids in Karnataka state.

Plant tissues just like animal tissues were composed of specialized cells to perform specific functions. Plants have an outer skin like epidermis which is protective in function. A single elliptical shaped pore present on plant epidermis is called a stoma. (Eames and Mac Daniels 1947) Usually gaseous exchange takes place between intercellular spaces of the sub epidermal cells and the atmosphere through stomata. Leaf stomata are the principal means of gas exchange in vascular plants. Stomata are small pores, typically on the undersides of leaves which are opened or closed under the control of a pair of kidney-shaped cells called guard cells. When open stomata allow CO_2 to enter the leaf for Photosynthetic process. The stomata also allow water and free oxygen to escape. In addition to opening and closing the stomata plants may exert control over their gas exchange rates by varying stomata density in leaves based on climatic conditions and periods. Plants also regulate transpiration water loss by stomatal behavior. These structures are also involved in loss of water in the form of vapour from the leaf surface (Meinzer *et al*., 1997). Normally stomata are abundant in the lower epidermis of the leaf. The wall near the opening is thickened in some species of the plants and the epidermal cells around the guard cells are distinct from the other cells are referred as subsidiary cells. Based on type of subsidiary cells stomata are classified as Anomocytic, Anisocytic, Paracytic, and Diacytic. (Rubina abid *et.al* (2007)).

The size and the number of stomata vary from species to species. The number of stomata per unit area is sometime reflective of the environment in which plants are growing. The developmental patterns of stomatal complex shows absolute consistency in the majority of the plant families(VanCothen1970).Stomatal size, shape and orientation pattern of the stomatal guard cells with other epidermal cells is of immense taxonomic importance (Tomlison1974 ,Stace 1984).Studies on structure ,distribution and taxonomic importance of stomata are still in progress in different parts of the world (Leela&Rao,1996:Sahin1998,yousufzai,1999). In the present study attempts were made to understand phenology and stomatal complex of certain epiphytic orchids of Western ghats of Karnataka.

2. Materials and methods

The present investigation was carried out using eleven species of epiphytic orchids belonging to three genera Viz., *Aeridis maculosam, A. Ringens, Bulbophyllum fimbriatum, B.fischerii B.neilgeriensis, Dendrobium cripidatum, D.herbaceum, D.heterocarpum D.macrostychyuim, D.nutantifolium, D.ovalifolium. The* Orchids are collected from the different regions of the western ghats of chikmagalore District of Karnataka. Types of Inflorescence, floral position and flower phenology are recorded.

In the present study stomatal complex was studied by peeling method of using mature leaves. The dermal characters were studied using the the methods suggested for grass by Metcaffe (1960) and for orchids Williams (1975). A portion of each macerated cuticle was taken for microscopic studies. It was stained in 1% aqueous solution of saffranin for about 3 -5 min. Excess stain was rinsed off with clean water. The stained cuticle was mounted in DPX. Observations were made on the microscope to determine stomatal complex types, stomatal frequencies, stomatal size and stomatal index. The size of the guard cell as well as aperture was measured under light microscope with micrometer and slides are observed in Trinocular Microscope. The photography was taken in camera under 45 x magnifications.

Using ten fields of view at 40X objective as quadrants the numbers of subsidiary cells per stoma were noted to determine the types of stomatal complex present in each specimen. Frequency of each stomatal complex type was expressed as % occurrence of each stomatal complex type based on all occurrences of stomatal complex types (Carr and Carr, (1990)); Obiremi and Oladele, (2001). Terminologies used for stomatal complex types were followed those of Dilcher (1974), Metcalfe and Chalk (1988), and Saadu *et al* (2009)

Stomatal index (SI) was determined as: SI = S/E+S X 100Where: SI = Stomatal index, S = Number of stomatal per square millimeter, E = Number of ordinary epidermal cells per square millimeter. The mean stomatal size or area was determined by measuring length and breadth using a micrometer of a sample of ten stomata using eye-piece micrometer.

3. Result and Discussion

Present studies on phenological characteristics in eleven species of epiphytic orchids yielded interesting results. The eleven species of epiphytic orchids in different localities indicated different type of inflorescence like raceme, umbellate, cyme and terminal raceme (Table 1). .*A ringens, A.maculosum, B.neilgeriensis* and *D.ovalifolium* have shown receme type of inflorescence. *B.fischerii D.nutantifolium D.cripidatum D.macrostychum* and *D.heterocarpum* have shown cyme type inflorescence. Whereas *B. fimbriatum* has shown umbellate and *D. herbaceum* have shown umbellate and terminal raceme type of inflorescence. The floral position also varied from base of leaf sheath, base of the bulb, from the nodes and node of the pseudo stem. Flower phenology also indicated different climatic periods like pre monsoon, monsoon and even summer in different orchids indicating influence of climatic factors (Table 1).

The eleven orchid species studied were found to be epiphytic. However there is a genotypic difference between

the species with respect to orientation, texture and also leaf surface. The species *A.ringens* and *D.heterocarpum* has shown V type of orientation (Table 2). *A.maculosam*, S type of orientation, *B.fimbriatum, B.fischerii*, and *B.neilgeriensis* P type of orientation of other four species of *Dendrobium* D type of orientation. The texture also varied between the species. *A.ringens, A.maculosam, B.fimbriatum, B.fischerrii* and *B.neilgeriensis* have shown F type of texture where as *D.herbacium, , D.cripidatum D.ovalifolium* and *D.macrostychum* have shown M type of texture, *D.nutantifolium* MF type of texture. The species *D.heterocarpum* showed LS type of texture. The leaf surface was hypostomatic in all the species except B.Neilgeriensis where it was Amphistomatic.

The results of Stomata revealed presence three major types of stomata such as paracytic, anomocytic,and anisocytic type. The type of Stoma was Anomocytic in *A.ringens, A.maculosum, B.fischerii, D.ovalifolium* and *D.heterocarpum.* Anisocytic in *B.neilgeriensis D.cripidatum* and paracytic in *B.fimbriatum D.herbaceum, D.Nutantifolium* and *D.macrostychum*. Earlier these type of stomata reported in number of monocots (Cheadle, 1953; Stebbins and khush, 1961). Stebins and khush (1961) suggested that anomocytic type of stomata is limited only to the orders closely related to liliales. Most of the plant species shows hypostomatic condition. Very few species shows amphistomatic condition. This is because these plants are adopted for aerial habitats in order to avoid water loss through stomata.

The two important processes viz., photosynthesis and transpiration are influenced by stomatal frequency (Imamdar *et al*.,1991).The stomatal frequency in adaxial surface has been found higher than abaxial surface (Col and Dobrenz,1971). All the leaves of selected orchids are leathery in texture. Earlier Dressler and Dodson (1960) noted a strong association of leathery leaves with epiphytic habit and suggested their independent origin from the membranous ones along several evolutionary lines in orchids. The Fig.3 shows patterns of stomata of different orchids studied.

Stomatal Size

The results of stomatal size indicated genotypic difference between different orchids studied in this programme the size of the stomata varied from a minimum of 562.18 to the maximum of 2531.91 maximum stomatal size was observed in *B.neilgeriensis* followed by *A.maculosam, A.ringens* and *B.fimbriatum* lowest stomatal size was observed in *D.machrostychum* followed by *D.herbaceum* and *D.ovalifolium* (Table 2). It is interesting to note that the the *Dendrobium sps* have shown comparatively smaller size of stomata comparing to *Aeridis* and *bulbophyllum* genera.Similar variations in stomatal size was reported in tropical tuber species by Saadu *et al* (2009).

Stomatal Index

In the present Investigation wide variation with respect to stomatal index (SI) was observed between the orchids studied. The ranged from 4.34 to 19.58 (Figure 1a). The maximum SI was observed in *D.nutantifolium* (19.58) followed by *B. fimbriatum* (18.79) and *D.cripidatum* (14.44). The minimum stomatal index was recorded in *D.macrostychum* (4.34) and *B.neilgeriensis* (8.36). The species of genus *Aeridis* showed SI of 9.76 and 9.26. In the genus *Dendrobium herbaceum, D.cripidatum, D.ovalifolium* the SI values are 11.01, 14.44 and 13.77 respectively. Similar variation in stomatal index was reported in orchid species (Saadu *et al* 2009). Interestingly a higher stomatal index is observed in *D.nutantifolium* and lowest in *D.macrostychum* could be on account of variations in sun light intensity. Because *D.nutantifolium* and *B.fimbriatum* grow in more sun light intensity areas compared to *D.*macrostychum. Such observations were also reported earlier by Vij.*et.al*., (1991). A direct positive correlation between light intensity and stomatal index was observed in orchids Rasmussen, (1987).

Stomatal Frequency

The observations on stomatal frequency (SF) indicated wide variations among the orchids studied. The Sf ranged from a minimum of 1308.4 to a maximum of 11831.8 (Fig.1b). Among the eleven orchids species the highest frequency was found in *D.nutantifolium* (11831.8) followed by *B.fimbriatum* (9539.38), *D.heterocarpum*(9266.8), *D.cripidatum* (8994.27), *D.ovalifolium* (7904.05) and least frequency is recorded in *B.fischerii (1308.4)*. The reduced stomatal frequency was distinctly related with the extent of leaf succulence and more the succulence lesser the frequency of stomata (Goh *et al*., 1977). On the other hand similarly higher stomatal frequency in the species from marshy habitats was reported (Ziegenspeck, 1936). It is interesting to note that the species showing higher SF also have shown higher SI in the present study. These species were found inhabited in open and well lighted situations. Similar reports between light intensity and stomatal index were available (Rasmussen, 1981). Correlation and regression analysis was also carried out to find out the relationship between stomatal index, stomatal frequency and stomatal size of the orchids studied. No significant relationship was observed between stomatal size, stomatal index and stomatal frequency. However a strong ($p<0.01$) positive relationship ($r^2=0.762$) was noticed between stomatal index and stomatal frequency in Orchids (Fig.2).

Table 1: Locality and Phenology of Presently investigated orchids

S.NO	Name of the orchid	Locality	Inflorescence	Floral position	Flower phenology
1	*Aeridis ringens*	Nagalapura,Koppa	Raceme	Base of leaf sheath	April-June
2	*A.maculosum*	Kotte gudda,Koppa	Raceme	Base of leaf sheath	April-June
3	*Bulbophyllum fimbriatum*	Near Sethallyangiri	Umbellate	Base of the bulb	March-May
4	*B.fischerii*	Near Basarikatte	Cyme	Base of the bulb	August-Dec
5	*B.neilgeriensis*	Niluguli,Koppa	Raceme	Base of the bulb	July-Sept

6	*Dendrobium herbaceum*	Addagadde,Sringeri	Terminal raceme	From the node	March-April
7	*D.nutantifolium*	Near Seethallyangiri	Cyme	From the node	April-Nov
8	*D.cripidatum*	Near Kammaradi	Cyme	From the node	March-June
9	*D.ovalifolium*	Near Basarikatte	Raceme	From the node	March-April
10	*D.macrostychum*	Kaimara,N R Pura	Cyme	Node of pseudo stem	May-Dec
11	*D.heterocarpum*	Meruthigudda	Cyme	From the node	Dec-Feb

Table 2: Habit, orientation, texture, leaf surface, type of stomata, size of stomata, stomatal index and Frequency of the orchids studied.

S.N	Name of Orchid	Habit	Orientation	Texture	Leaf surface	Type of stomata	Size of stomata	Stomatal index	Stomatal frequency
1	*A.ringens*	E	V	F	Hypostomatic	Anomocytic	2045.06	9.77	6813.84
2	*A.maculosum*	E	S	F	Hypostomatic	Anomocytic	2370.07	9.26	6268.73
3	*B.fimbriatum*	E	P	F	Hypostomatic	Paracytic	1985.14	18.79	9539.38
4	*B.fischerii*	E	P	F	Hypostomatic	Anomocytic	1398.92	9.04	1308.4
5	*B.neilgeriensis*	E	P	F	Amphistomatic	Anisocytic	2531.91	8.36	3815.74
6	*D.herbaceum*	E	D	M	Hypostomatic	Paracytic	871.3	11.01	6813.84
7	*D.nutantifolium*	E	D	MF	Hypostomatic	Paracytic	1652.32	19.58	11831.80
8	*D.cripidatum*	E	D	M	Hypostomatic	Anisocytic	1337.01	14.44	8994.27
9	*D.ovalifolium*	E	D	M	Hypostomatic	Anomocytic	963.88	13.77	7904.05
10	*D.macrostychum*	E	D	M	Hypostomatic	Paracytic	562.18	4.34	1907.86
11	*D.heterocarpum*	E	V	LS	Hypostomatic	Anomocytic	1289.48	12.24	9266.80

E=Epiphytic; S=Spreading;
V= Vertical; P= Pseudo bulb; D=Drooping;
F=Fleshy; M= Membranous;
MF= Membranous Fleshy; LS=Soft leathery

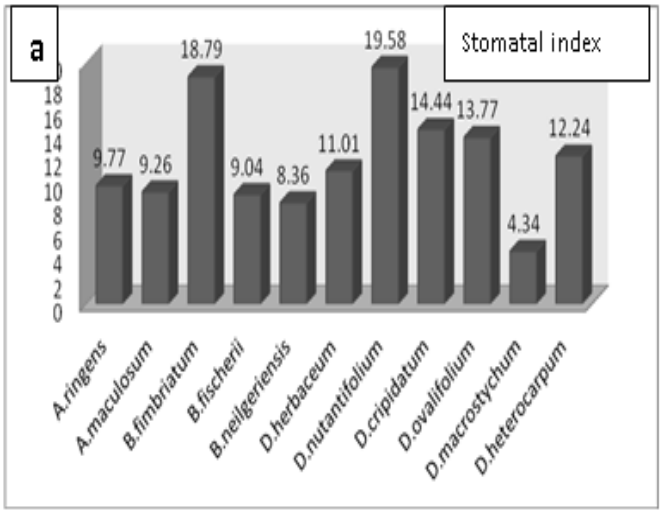

Figure 1 a: Stomatal index and frequency of different orchids studied.

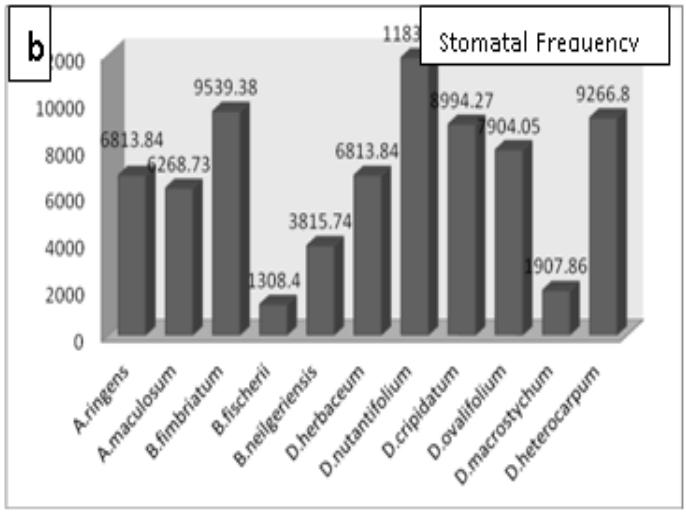

Figure 1 b: Stomatal index and frequency of different orchids studied.

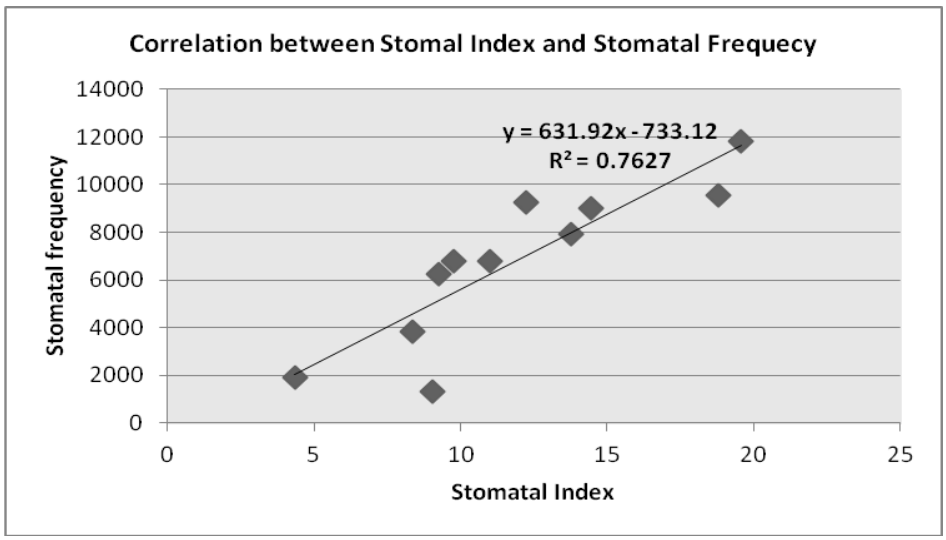

Figure 2: Correlation between Stomatal Index and Stomatal Frequency in Orchids

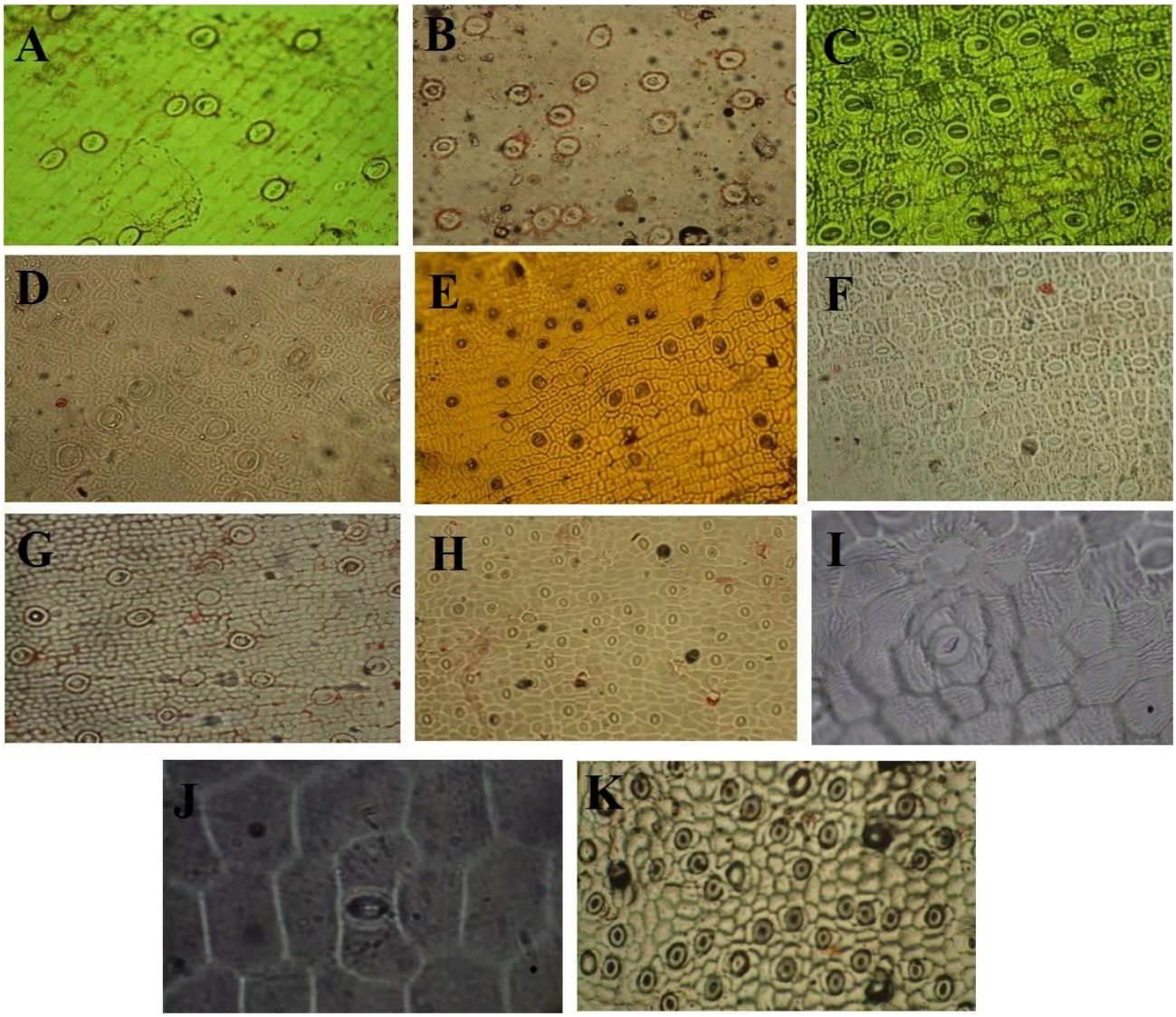

Figure 3: Stomatal structures in different epiphytic orchids of Western Ghats. **a)** *Aeridis ringens* **b)** *Aeridis maculosam* **c)** *Bulbophylum fimbriatum* **d)** *Bulbophylum neelgherense* **e)** *Bulbophylum fischerii* **f)** *Dendrobium herbaceum* **g)** *Dendrobium nutantifolium* **h)** *Dendrobium cripidatum* **i)** *Dendrobium ovalifolium* **j)** *Dendrobium macrostychyum* **k)** *Dendrobium heterocarpum*.

4. Conclusion

The Species *Dendrobium macrostychum* has shown least stomatal index and frequency followed by *B.neilgeriensis*. This could be due to strong drought tolerant mechanism by which these species are capable of curtaining transpiration water loss as these species generally found in harsh climatic conditions like rocks and dry bark of trees and also open conditions. These findings clearly indicated that the difference in stomatal complex in orchid species could be due to environmental conditions besides genetic factors.

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