28-38 J. Nat. Hist. Mus. Vol. 30, 2016-18

Taxonomic significance of stomatal complex in fifteen species of *Dendrobium* Swartz (Orchidaceae) of Nepal

Baba Maiya Pradhan[1]✉ and Devendra M. Bajracharya[2]

[1]Department of Botany, Patan Multiple Campus, Tribhuvan University, Nepal
[2]Amrit Science Campus, Tribhuvan University, Nepal
baba.m.pradhan@gmail.com

ABSTRACT

Dendrobium Swartz is one of the largest and most problematic genus of the tribe Dendrobieae of the family Orchidaceae. Nepal harbors about thirty-one species of *Dendrobium*. Micromorphological characters like the stomatal complex in fifteen species of *Dendrobium* Swartz (Orchidaceae) of Nepal were studied for understanding the taxonomic significance of stomatal complex within the genus. For the study of stomatal complex of leaf, Carpenter's method was followed. Stomatal index and stomatal frequency were calculated using formula given by Salisbury. The terminologies used for the stomatal complex type were followed those of Patel. In all species the leaf surface was found hypostamic. Tetracytic (a-tetra-monocyclic, b-tetra-monocyclic and c-tetra-monocyclic) and eupara twi-monocyclic with hexa-monocyclic types of stomata were recorded in the genus. Tetracytic type was found in fourteen species and eupara twi-monocyclic with hexa-monocyclic was found in only one species. The elliptical shape of stomata was found in more species than the circular shape. The epidermal cells of leaf were found rectangular, square, polygonal to irregular. The distribution of stomata on leaf surface, type of stomata and shape of epidermal cells were found to be taxonomically significant. These characters could be used as distinguishing characters to delimit the species in the genus.

Keywords: anatomical key, *Dendrobium*, epidermal cells, micromorphological character, stomatal variation

INTRODUCTION

The family Orchidaceae is widely distributed in the tropical and subtropical regions of the world and is regarded as one of the largest, most diverse and distinctive families of flowering plants (Burns-Balogh & Funk, 1986; Dressler, 1981, 1993). *Dendrobium* Swartz is a dominant genus of the tribe Dendrobieae of the family Orchidaceae. The genus was established by Olof Swartz (1799) based on *Dendrobium moniliforme* (L.) Sw. as the type species of the genus. The genus *Dendrobium* Swartz consists of about 900 species, widely distributed in China, Japan, India, Malaysia, Indonesia to New Guinea, Australia, New Zealand and the Pacific islands (Seidenfaden, 1985; Pearce & Cribb, 2002). In Nepal they are distributed from tropical to alpine regions. Hara *et al.* (1978) and Press *et al.* (2000) recorded 26 species of *Dendrobium* from Nepal. However, Rokaya *et al.* (2013) enumerated 31 species of *Dendrobium* from Nepal Himalaya. Species of *Dendrobium* are mostly epiphytes with some

are lithophytes. The distinguishing characters of the genus *Dendrobium* are presence of cane-like stem with pseudobulb covered with or without sheathing leaf bases, distichous or duplicate leaves, spiral flowers with basally jointed lips with prominent spurs formed by the column foot and naked pollinia.

Micromorphological study on leaf epidermal surface had been widely used in taxonomic and phylogenetic studies as they can provide valuable information (Patel, 1979; Stace, 1980; Davis, 1997). The notable work on stomatal patterns in some angiosperms had studied by Stebbins & Khush (1961), and Croxdale (2000). Leaf epidermal anatomy particularly of stomata was reported to provide valuable taxonomic and systematic evidence in both living and fossil plants (Wilkinson, 1979; Kong, 2001). In Orchidaceae, several micromorphological studies on stomatal types based on their structures have been done by Kaushik (1983) and Khasim & Rao (1990). Singh & Singh (1974) studied the organization of stomatal complex in family Orchidaceae. Kaushik (1983) and Rasmussen (1987) studied the ecological significance of dermal characters. Yukawa *et al.* (1992) reported the existence of two types of stomatal shape in the genus *Dendrobium* and its systematic significance. Arditti (1992) suggested that the shape of subsidiary cells had been used to distinguish the stomatal complex of Orchidaceae into several types. Vij *et al.* (1991) also examined epidermal feature of Indian orchids for taxonomic and ecological implications. Where Himalayan species are concerned, among the twelve species of the genus *Oberonia*, eight types of stomata had classified according to the arrangement of subsidiary cells (Shakya, 1999). In case of Himalayan genus *Eria* seven types of stomata complex has been studied (Bajracharya, 2005).The stomatal types had been reported to be significant in establishing the various ranks and clarifying the interrelationship among the different taxa and also resolving many taxonomic problems (Baranova, 1992).

Dendrobium is one of the largest and most problematic genera with respect to its intrageneric classification and relationships to other taxa in the family orchidaceae. The large number of species and diversity of gross morphology are the most prominent reasons for the complexity of *Dendrobium* taxonomy and the confusion over its intrageneric relationships. Under such circumstances orchid systematics needs a thorough revision using parameters other than gross morphology to clarify the intrageneric relationships among *Dendrobium* species. So in the present study the preliminary attempts were made on the study of distribution of stomata on leaf surface, stomatal types and epidermal cell types to clarify the intrageneric relationship among these taxa.

MATERIALS AND METHODS

In present study 15 species of *Dendrobium* Swartz viz. *D. amoenum* Wall. ex Lindl., *D. anceps* Sw., *D. aphyllum* (Roxb.) C.E.C. Fisch., *D. bicameratum* Lindl., *D. chrysanthum* Wall. ex Lindl., *D. crepidatum* Lindl. & Paxton, *D. densiflorum* Lindl., *D. denudans* D. Don., *D. eriiflorum* Griff., *D. formosum* Roxb. ex Lindl., *D. heterocarpum* Wall. ex Lindl., *D. longicornu* Lindl., *D. moniliforme* (L.) Sw., *D. moschatum* (Buch.-Ham.) Sw., *D. transparens* Wall.ex Lindl. were studied. The study was based on the live specimens. Field visits were carried out in different parts of Nepal to collect the live specimens. Live specimens were identified by studying protologues, type specimens and authentic herbarium specimens deposited at KATH, TUCH, CAL, BM, K, P and TI. Protologues and images of type specimens have been downloaded

from different websites including www.botanicus.org, www.biodiversitylibrary.org, www.plants.jstor.org, www.kew.org and www.nybg.org. Update nomenclature of *Dendrobium* species have been acquired from websites www.kew.org.wcsp/monocots and www.theplantlist.org.

The study of stomata was done on the fresh leaves of *Dendrobium* species collected from various localities of Nepal Himalaya. For the study of stomata, the method described by Carpenter (2005) with some modification was followed. Stomatal index and stomatal frequencies were calculated using the formula given by Salisbury (1928). The terminologies used for stomatal complex types were followed those of Patel (1979).

RESULTS AND DISCUSSION

The stomatal complex of 15 species of *Dendrobium* had examined with light microscope. The species of *Dendrobium* were found hypostamic leaf surfaces (i.e., stomata occurring on the abaxial surface only). The stomata were largely of tetracytic type (a, b and c-tetra-monocyclic) whereas eupara twi-monocyclic with hexa-monocyclic rarely present in some species. The stomata possess four to five subsidiary cells.

The stomatal distribution on the leaf surface, stomatal size with guard cells, stomatal pore size, stomatal frequency, stomatal index, shape of stomata, type of stomata, shape and type of epidermal cells on the abaxial surface of leaves was found to be variable in different species of *Dendrobium* (table 1).

The epidermal cells on abaxial surface are rectangular to polygonal and regular to irregular in shape. In most of species like *D. amoenum, D. anceps, D. aphyllum, D. eriiflorum, D. formosum, D. heterocarpum, D. longicornu, D. moniliforme, D. moschatum* and *D. transparens* the epidermal cells were found to be rectangular to polygonal where as in species like *D. bicameratum, D. chrysanthum, D. crepidatum, D. densiflorum* and *D. denudans* the epidermal cells were found to be squarish, pentagonal, hexagonal to polygonal and irregular size in some species with anticlinal wall varying from straight-sided to curvilinear. The epidermal cells were mostly parenchymatous (thin-walled or thick-walled) in species like *D. amoenum, D. anceps, D. aphyllum, D. bicameratum, D. chrysanthum,*

TABLE 1. Micromorphological characters of leaf surface in species of *Dendrobium.*

(Abbreviations used : Rect.= Rectangular, poly. = polygonal, Squa. = Squarish, Paren. = Parenchymatous, Scleren. = Sclerenchymatous)

Taxa	Leaf surface	Stomatal size with guard cells (LxB) µm	Stomatal pore size (LxB) µm	Stomatal frequency mm²	Stomatal index %	Shape of stomata	Type of stomata	Shape of epidermal cells	Type of epidermal cells
D. amoenum	Abaxial	29.97 x 23.31	13.32 x 4.99	76.92	5.81	Elliptic	b-tetra-monocyclic	Rect. to poly.	Paren.
D. anceps	Abaxial	29.97 x 25.30	13.32 x 6.66	46.15	3.75	Elliptic	eupara twi-monocyclic +hexa-monocyclic	Rect. to poly.	Paren.
D. aphyllum	Abaxial	33.30 x 23.31	16.65 x 9.99	46.15	7.31	Elliptic	a-tetra-monocyclic	Rect. to poly.	Paren.
D. bicameratum	Abaxial	33.30 x 29.97	23.31 x 9.99	76.92	7.04	Elliptic	a-tetra-monocyclic	Squa. to poly.	Paren.
D. chrysanthum	Abaxial	33.30 x 23.31	16.65 x 6.66	76.92	5.68	Circular	a-tetra-monocyclic	Squa. to poly.	Paren.
D. crepidatum	Abaxial	32.63 x 26.64	15.32 x 7.32	130.77	13.04	Elliptic	b-tetra-monocyclic	Squa. to poly.	Paren.
D. densiflorum	Abaxial	33.30 x 26.64	26.64 x 13.32	76.92	6.76	Elliptic	a-tetra-monocyclic	Squa. to poly.	Scleren.
D. denudans	Abaxial	39.96 x 26.64	26.64 x 9.99	92.3	10.71	Elliptic	a-tetra-monocyclic	Squa. to poly.	Paren.
D. eriiflorum	Abaxial	38.62 x 25.97	20.64 x 10.65	123.07	10.66	Elliptic	c-tetra-monocyclic	Rect. to poly.	Paren.
D. formosum	Abaxial	45.96 x 41.96	24.64 x 6.66	76.92	12.5	Circular	c-tetra-monocyclic	Rect. to poly.	Scleren.
D. heterocarpum	Abaxial	38.30 x 33.30	16.65 x 10.00	92.3	13.04	Circular	a-tetra-monocyclic	Rect. to poly.	Paren.
D. longicornu	Abaxial	29.97 x 23.31	13.32 x 4.99	123.07	10.53	Elliptic	a-tetra-monocyclic	Rect. to poly.	Paren.
D. moniliforme	Abaxial	38.30 x 34.00	16.65 x 6.66	138.5	12.5	Circular	a-tetra-monocyclic	Rect. to poly.	Paren.
D. moschatum	Abaxial	27.97 x 26.64	16.65 x 9.99	76.92	5.0	Circular	a-tetra-monocyclic	Rect. to poly.	Paren.
D. transparens	Abaxial	33.30 x 27.31	17.98 x 6.66	138.46	10.71	Elliptic	b-tetra-monocyclic	Rect. to poly.	Paren.

D. crepidatum, D. denudans, D. eriiflorum, D. heterocarpum, D. longicornu, D. moniliforme, D. moschatum and *D. transparens* and in remaining species like *D. densiflorum* and *D. formosum* the epidermal cells were sclerenchymatous type.

The stomata were found mostly tetracytic type (a, b and c-tetra-monocyclic). In species like *D. aphyllum* (table 1, plate-I C)*, D. bicameratum* (table 1, plate-I D)*, D. chrysanthum* (table 1, plate-I E)*, D. densiflorum* (table 1, plate-I G)*, D. denudans* (table 1, plate-I H)*, D. heterocarpum* (table 1, plate-II B)*, D. longicornu* (table 1, plate-II C), *D. moniliforme* (table 1, plate-II D) and *D. moschatum* (table 1, plate-II E) a-tetra-monocyclic type was found whereas b-tetra-monocyclic type was found in *D. amoenum* (table 1, plate-I A), *D. crepidatum* (table 1, plate-I F)*, D. transparens* (table 1, plate-II F) and c-tetra-monocyclic type was found in *D. eriiflorum* (table 1, plate-I I) *and D. formosum* (table 1, plate-II A). Eupara twi-monocyclic with hexa-monocyclic was found in *D. anceps* (table 1, plate-I B).

Shape of stomata in *Dendrobium* varied from elliptical to circular. Elliptic type of stomata had found more in number than circular type. In species like *D. amoenum* (table 1, plate-I A)*, D. anceps* (table 1, plate-I B)*, D. aphyllum* (table 1, plate-I C)*, D. bicameratum* (table 1, plate-I D)*, D. crepidatum* (table 1, plate-I F)*, D. densiflorum* (table 1, plate-I G)*, D. denudans* (table 1, plate-I H), *D. eriiflorum* (table 1, plate-I I)*, D. longicornu* (table 1, plate-II C) and *D. transparens* (table 1, plate-II F) the stomata were elliptical type whereas the species like *D. chrysanthum* (table 1, plate-I E)*, D. formosum* (table 1, plate-II A), *D. heterocarpum* (table 1, plate-II B), *D. moniliforme* (table 1, plate-II D) and *D. moschatum* (table 1, plate-II E) had circular type.

The largest stoma was found in *D. formosum* with the area of 1928.48 μm^2 and the smallest in *D. amoenum* and *D. longicornu* with the area of 698.60 μm^2. The stomatal pore size was found smallest in *D. amoenum* and *D. longicornu* ranging from 66.46 µm to largest size 354.84 µm in *D. densiflorum.* The stomatal frequency varied from 46.15 mm^2– 138.50 mm^2. The highest stomatal frequency was found in *D. moniliforme* with 138.50 mm^2 and the lowest in *D. anceps* and *D. aphyllum* with 46.15 mm^2. The stomatal index was found highest in *D. heterocarpum* with 13.04 and the lowest in *D. anceps* with 3.75.

On the basis of micromorphological characters like distribution of stomata on leaf surface, shape of epidermal cell, type of stomata, *Dendrobium* species of Nepal has been divided into different groups to delimit the taxa.

Anatomical key for *Dendrobium* species

Epidermal cell sclerenchymatous:

A. Rectangular to polygonal shaped epidermal cells, c-tetra-monocyclic with circular stomata ----------*D. formosum*

B. Square to polygonal shaped epidermal cells, a-tetra-monocyclic with elliptical stomata----------*D. densiflorum*

Epidermal cells parenchymatous:

A. Rectangular to polygonal shaped epidermal cells, a, b or c-tetra-monocyclic, eupara-twi-monocyclic with elliptical or circular stomata

- a, b or c-tetra-monocyclic, eupara-twi-monocyclic with elliptical stomata
    - a-tetra-monocyclic ----------*D. aphyllum*, *D. longicornu*
    - b-tetra-monocyclic ----------*D. amoenum*, *D. transparens*
    - c-tetra-monocyclic ----------*D. eriiflorum*
    - eupara twi-monocyclic ----------*D. anceps*
- a-tetra-monocyclic with circular stomata---------- -*D.heterocarpum*, *D. moniliforme, D. moschatum*

B. Square to polygonal shaped epidermal cells, a or b-tetra-monocyclic with elliptical or circular stomata

- a or b-tetra-monocyclic with elliptical stomata
    - a-tetra-monocyclic ----------*D. bicameratum*, *D. denudans*
    - b-tetra-monocyclic ----------*D. crepidatum*
- a-tetra-monocyclic with circular stomata ----------*D. chrysanthum*

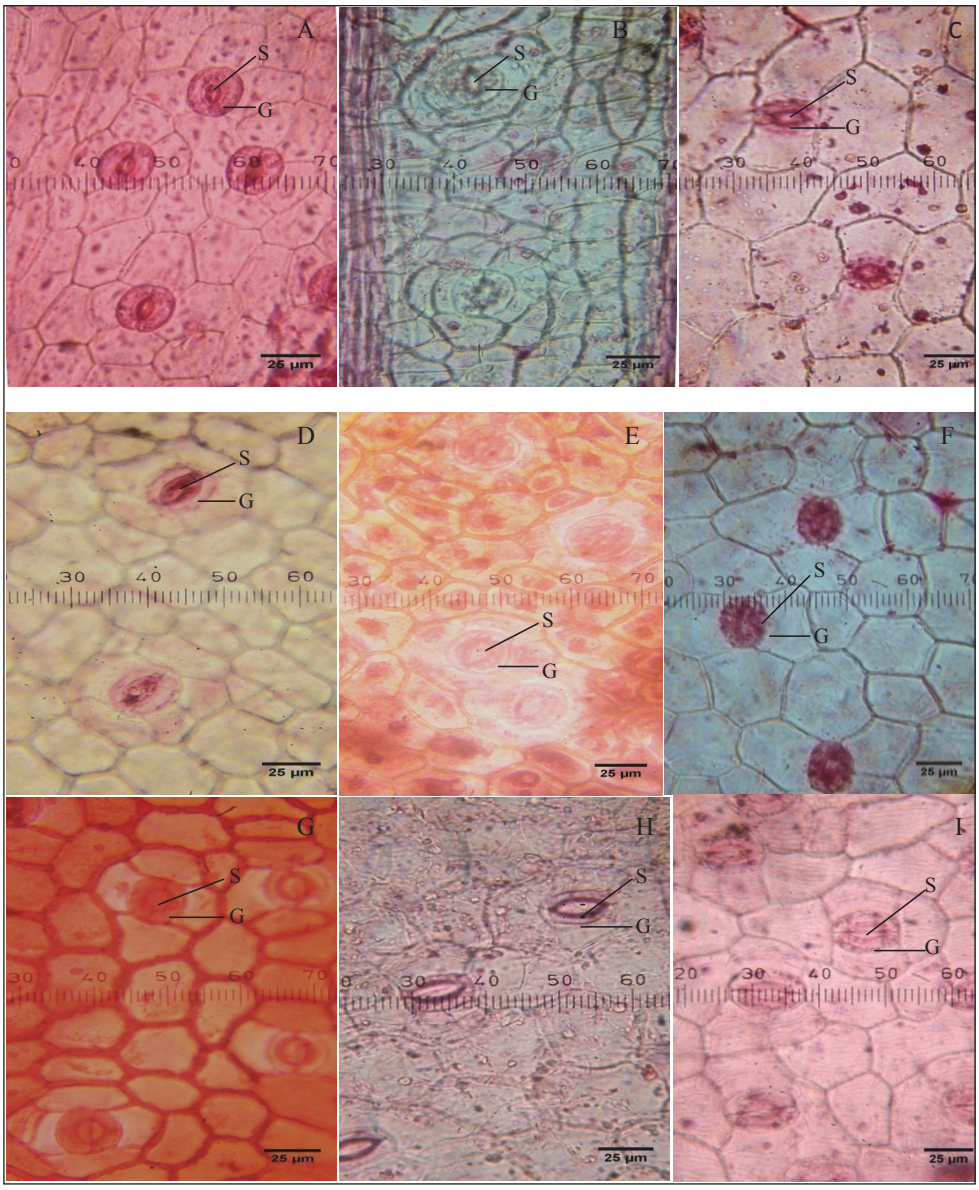

PLATE – I. Stomatal variations in different species of *Dendrobium* Swartz (Abaxial surface of leaf): A. *D. amoenum* (b-tetra-monocyclic); B. *D. anceps* (eupara twi-monocyclic); C. *D. aphyllum* (a-tetra-monocyclic); D. *D. bicameratum* (a-tetra-monocyclic), E. *D. chrysanthum* (a-tetra-monocyclic); F. *D. crepidatum* (b-tetra-monocyclic); G. *D. densiflorum* (a-tetra-monocyclic); H. *D. denudans* (a-tetra-monocyclic). I. *D. eriiflorum* (c-tetra-monocyclic) (Abbreviations used on photos: S = Stoma; G = Guard cell).

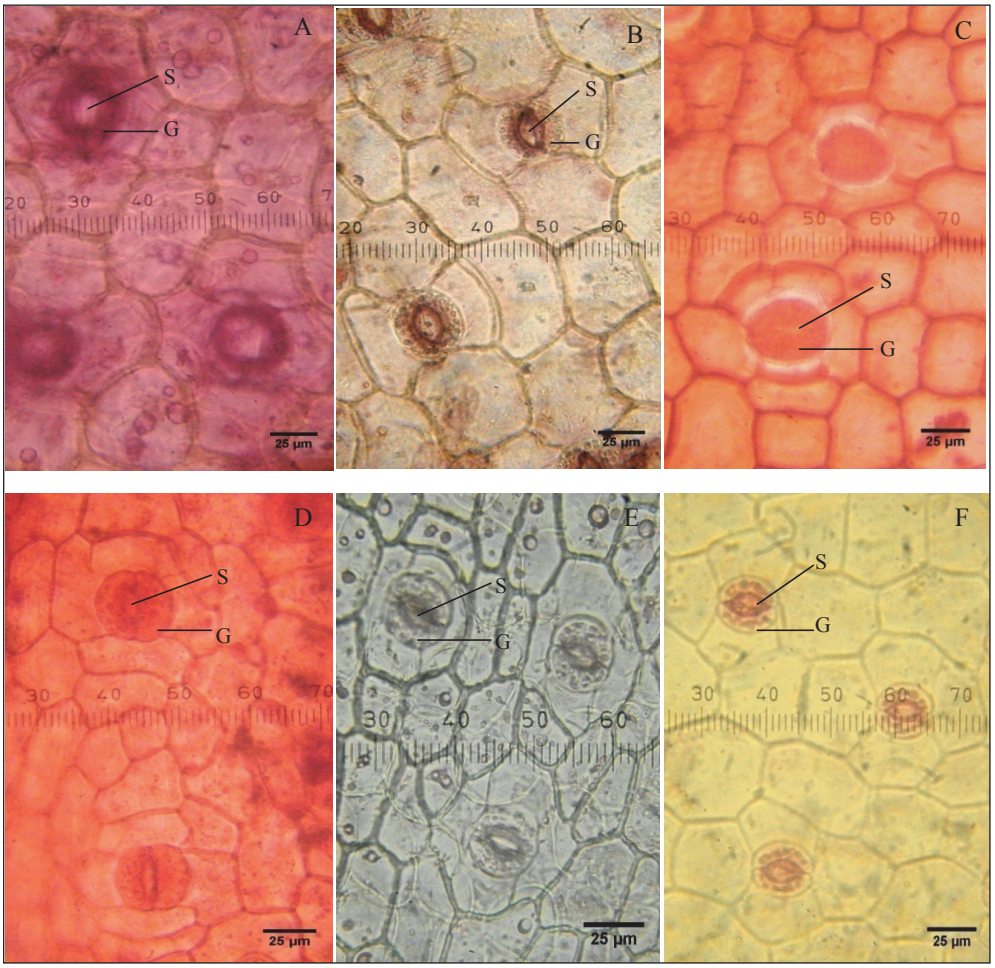

PLATE II. Stomatal variations in different species of *Dendrobium* Swartz (Abaxial surface of leaf): A. *D. formosum* (c-tetra-monocyclic); B. *D. heterocarpum* (a-tetra-monocyclic); C. *D. longicornu* (a-tetra-monocyclic); D. *D. moniliforme* (a-tetra-monocyclic); E. *D. moschatum* (a-tetra-monocyclic); F. *D. transparens* (b-tetra-monocyclic). (Abbreviations used on photos: S = Stoma; G = Guard cell).

The present study revealed the stomatal complex of *Dendrobium* which showed variation in types of epidermal cells, shape, size and types of stomata, stomatal frequency and stomatal index.

All species of *Dendrobium* showed hypostomatic leaf surface. The leaves are found hypostamic condition in most of orchids (Avadhani *et al.,* 1982). Stomata were found only on the abaxial surface (hypostomatic). This is because these plants are adapted to aerial habitats to minimize

water loss through stomata (Stebbins & Khush, 1961). The epidermal cells on the abaxial surface varied from rectangular, squarish, pentagonal, hexagonal to polygonal, irregular with thick or thin walled parenchymatous cells and some species with sclerenchymatous cells. The anticlinal walls of epidermal cell varied from straight-sided to curvilinear.

The shape of stomata varied from elliptical to circular. Elliptical stomata were found in most species of *Dendrobium* whereas few species had circular stomata. Various modifications of stomatal shape such as elliptical, circular, transversely elliptical and angular are known to exist within Orchidaceae (Rasmussen, 1987). All modifications except the angular shape were found in *Dendrobium* (Yukawa *et al.,* 1992). Two types of stomatal shape (Stoma I and Stoma II) were reported in *Dendrobium* (Yukawa *et al.,* 1992). Stoma I is elliptical with a slit-like opening whereas Stoma II is circular with a round to spindle-shaped opening. Similarly two types of stomatal shape i.e. elliptical and circular were found in our study. The size of stomata showed a wide variation from 698.60 μm^2 to 1928.48 μm^2. The largest stoma was found in *D. formosum* and the smallest in *D. amoenum* and *D. longicornu*.

Tetracytic type of stomata had been reported in many monocotyledons (Metcalfe, 1961). Mostly three types of stomata paracytic, tetracytic and anomocytric were reported in mostly in monocots (Cheadle, 1953; Stebbins & Khush, 1961). Stebbins & Khush (1961) reported that anomocytic type is limited only to the orders closely related to Liliales. Patel (1979) distinguished five types of stomata i.e. tetracytic (a-tetra-monocyclic, b-tetra-monocyclic and c-tetra-monocyclic), twicytic, perihaplocytic, anisocytic and hexacytic and each type with four to six subsidiary cells. Rasmussen (1987) distinguished four types of stomata such as tetracytic, anisocytic, cyclocytic and floating stomatal complexes. In the present study tetracytic type (a-tetra-monocyclic, b-tetra-monocyclic and c-tetra-monocyclic) was found in most species of *Dendrobium* whereas eupara twi-monocyclic with hexa-monocyclic types was found in one species (*D. anceps*). In the stomatal type the monocyclic means the guard cells of the stomata are surrounded by a single cycle of subsdiary cells (Patel, 1979).

The stomatal frequency ranged from 46.15 mm^2-138.50 mm^2. The highest stomatal frequency was found in *D. moniliforme* and lowest in *D. anceps* and *D. aphyllum*. 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). Similarly in species from marshy habitat, the highest stomatal frequency was recorded (Ziegenspeck, 1936). The stomatal index ranged from 3.75 to 13.04. The highest stomatal index was found in *D. heterocarpum* and lowest in *D. anceps.* Similar variation in stomatal index was reported in orchid species (Saadu *et al.,* 2009). The variation in stomatal index could be on account of variation in sunlight intensity (Santosh *et al.,* 2015). A direct correlation between light intensity and stomatal index was observed in orchids (Rasmussen, 1987).

These studies clearly indicated that the micromorphological characters such as distribution of stomata on leaf surface, type of stomata and shape of epidermal cells were found to be taxonomically significant. This parameter will be helpful in solving the existing problems in *Dendrobium* species.

AKNOWLEDGEMENTS

Authors are thankful to National Herbarium and Plant Laboratories, Godawari, Nepal (KATH) and Tribhuvan University Central Herbarium, Kirtipur, Nepal (TUCH) for their cooperation and allowing me to study the herbarium specimens housed over there. My sincere thanks go to Patan Multiple Campus and Amrit Campus for providing the laboratories facilities.

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