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IDENTIFICATION OF *Dendrobium* Sw. BASED ON STOMATA ANATOMICAL CHARACTERS IN LIWA BOTANICAL GARDEN

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Short Communication

ABSTRACT

Orchids are ornamental plants in the superior collection of Liwa Botanical Garden, West Lampung. One of the largest types of orchids is the members of the genus *Dendrobium*. However, until now, many collections have not been identified. This study aims to determine the identification of *Dendrobium* based on the anatomical characters of leaf stomata in Liwa Botanical Garden. Four *Dendrobium* accessions were collected, namely CAT140, CAT 144, CAT 274, and IR015. The results of the observation of nine anatomical characters on the upper and lower surfaces of the leaves indicate that the leaf organs have high variations. Accessions IR015, CAT 140, and CAT 274 have stomata only on the lower surface, while CAT 144 has stomata on the upper and lower surfaces. This difference is due to the position of the leaves attached to the stem. CAT 144 forming an angle of 45°C, while the others open horizontally. In case of IR015, CAT 140, and CAT 274, the upper surface of the leaves is only composed of epidermis which is composed of cells which are irregular and pentagonal in shape. The highest number was in CAT 274 because the epidermal cells was smaller than IR015 and CAT 144. The types of stomata in all types were the same, namely tetracytic in the form of stomata surrounded by 4 neighboring cells. The stomata are kidney-shaped and the epidermal cells is irregular, pentagonal, and hexagonal in shape. The results of this study are expected to provide basic information in identifying natural orchids and conservation efforts in Liwa Botanical Garden.

Keywords: *Orchid identification; anatomical identification; stomata; dendrobium; liwa botanical garden.*

INTRODUCTION

Orchids are ornamental plants in the superior collection of Liwa Botanical Gardens, West Lampung. One of the largest epiphytic taxa in the family Orchidaceae is *Dendrobium* Sw. This orchid has a total of 1500 species that are very widespread throughout the world, from Japan, China, India, the Malacca Peninsula, Indonesia, Papua, to Australia. This type of orchid that has the beauty of various shapes, sizes, and colors of flowers. *Dendrobium* flowers can last more than 30 days with up to 20 flowers per stalk [1-5]. Copy that, these orchids are adaptable so they're easy to care for. *Dendrobium* growth will be optimal in locations less than 400 m above sea level, although maintenance in areas more than 400 m above sea level can still grow and flower [6-10]. *Dendrobium* used phylogenetically independent contrasts to test the relationships among traits, and between traits and the environment [11].

Lampung is one of the places on the island of Sumatra that has a flora conservation area, which is located in the Liwa Botanical Garden, West Lampung Regency. Liwa Botanical Garden has many unidentified orchid species, given the high increase in exploitation for economic reasons, this can threaten the existence of natural orchid plants which cause loss of their natural habitat and natural damage resulting in extinction of existing flora species. Moreover, orchids have high economic value because of the beauty of their various flower forms. This makes the reason people can just hunt the existing natural orchids. For this reason, the Liwa Botanical Garden is expected to ensure the preservation of natural orchid species that can be used sustainably. However, until now there were several types of natural orchids that it was not certain what species of natural orchids were in the Liwa Botanical Garden [12,13,2,14,15,10].

Given the importance of maintaining and preserving orchids in the region, it is necessary to follow up. One way to do this is to identify the types of natural orchids, especially the *Dendrobium* species, which have a high diversity compared to other orchid species. The results of identification will be addressed based on the morphological characters of the leaves and the anatomical structure of the leaf stomata. The results of the identification of leaf morphological characters have been previously published separately. This is important to do considering that the morphological and anatomical characters are one of the approaches that play an important role in the taxonomy and systematic basis of plants [2,16,11,17].

This study complements previous research [2] on determining variations in morphological characters, phenetic relationships, and to identify *Dendrobium* collections based on leaf morphological characters in the Liwa Botanical Garden. Five accessions of *Dendrobium* were collected, namely CAT140, CAT 144, CAT 274, and IR015. Observation of 11 morphological characters of leaves showed that leaf had high variations. The phenetic relationship based on the Gower similarity value and the UPGMA method shows that the *Dendrobium* in the Liwa Botanical Garden can be classified into 2 main groups formed with a similarity index value of 0.813. Based on Principle Component analysis values, it is known that the characters that have a large influence on grouping are the ratio of leaf length and width, leaf cross section, and leaf arrangement. The phenetic dendrogram topology is supported by the morphological character classification [2].

This study aims to determine the variation of *Dendrobium* identification based on the anatomical characters of leaf stomata in Liwa Botanical Garden. The results of this study are expected to provide basic information in identifying natural orchids and conservation efforts in Liwa Botanical Garden.

METHODOLOGY

Sample Collection

The sample collection was carried out on *Dendrobium* leaves in Liwa Botanical Garden. Overall, the sample accessions were natural orchids, native to flora from Lampung. All samples were tabulated and documented with photographs.

Identification of Stomata Anatomical Characters

The research stage of leaf stomata identification was carried out by direct observation using a miscroscope shortly after sampling in the field. The anatomical characters of leaf stomata identified include the average number of stomata, average length of stomata, average stomata width, average number of epidermal cells, average length of epidermal cells, average width of epidermal cells, stomata index, stomata type, shape of epidermal cells on the upper and lower surfaces of the leaves [18].

RESULTS AND DISCUSSION

Sample Collection

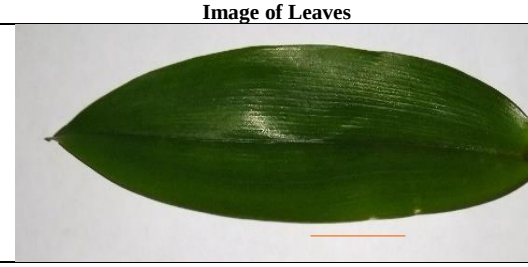
The sample collection stage was carried out in December 2020 - February 2021 at the orchid green house in the Liwa Botanical Garden. *Dendrobium* samples were selected based on unidentified orchid data. The collection results obtained 4 accessions of *Dendrobium* samples with sample codes CAT140, CAT 144, CAT 274, and IR015 (Table 1 and Table 2). Overall, the sample accessions were natural orchids, native to flora from Lampung.

Table 1. List of *Dendrobium* sample accessions in Liwa Botanical Garden

No. Accession	Species	Origin Location
CAT274	*Dendrobium* sp.	Taman Nasional Bukit Barisan Selatan
CAT144	*Dendrobium* sp.	Hutan Seminung
CAT140	*Dendrobium* sp.	Taman Nasional Bukit Barisan Selatan
IR015	*Dendrobium* sp.	Taman Nasional Bukit Barisan Selatan

Table 2. Documentation of *Dendrobium* sample accession leaf in Liwa Botanical Garden, Bar = 1 cm

No. Accession	Image of Leaves
CAT 274	
CAT 144	
CAT 140	

No. Accession	Image of Leaves
IR 015	

Identification of Leaf Stomata Anatomical Characters

The epidermis is a system of cells, varying in structure and function that protects the primary plant body. The leaf epidermis is the outermost layer of cells, generally only one thick layer. In the leaf epidermis, there are usually epidermal derivatives in the form of stomas (plural: stomata). A stoma is a gap in the epidermis that is bounded by two special epidermal cells, namely the covering cell that functions to widen or narrow the gap. The stoma is surrounded by cells that can be the same or different from other epidermal cells called neighboring cells [12,18,4,11]

In this study, observations were made on 4 types of *Dendrobium*, namely *Dendrobium* IR015, *Dendrobium* CAT 140, *Dendrobium* CAT 144, and *Dendrobium* 274. Observations were made on the upper and lower surfaces of leaves (Table 3).

Table 3. Anatomical characters on the upper and lower surfaces of leaves

No.	Parameter	IR 015	CAT 140	CAT 144	CAT 274
1.	**Leaf Upper Surface**				
	Average number of stomata	-	-	2,80	-
	Average length of stomata (µm)	-	-	2,45	-
	Average stomata width (µm)	-	-	2,17	-
	Average number of epidermal cell	29	41	45	42,8
	Average epidermal cell length (µm)	5,22	5,49	4,31	4,64
	Average epidermal cell (µm)	4,22	2,79	2,65	2,88
	Stomata index (%)	-	-	5,86	-
	Stomata type	-	-	Tetracytic (1 stomata surrounded by 4 neighboring cells)	-
	Epidermal cell shape	Irregular, pentagonal, hexagonal	Irregular, pentagonal	Irregular, quadrilateral, pentagonal	Irregular, quadrilateral, pentagonal
2.	**Leaf Lower Surface**				
	Average number of stomata	5,2	2,50	2,50	3,4
	Average length of stomata (µm)	2,75	2,78	2,33	2,57
	Average stomata width (µm)	1,96	2,45	2,07	1,82
	Average number of epidermal cells	32,2	30,75	43,50	42,2
	Average of epidermal cell length (µm)	3,54	5,13	4,42	3,74
	Average of epidermal cell width (µm)	2,72	3,03	3,06	2,45
	Stomata index (%)	13,90	7,52	5,43	7,46
	Stomata type	Tetracytic (1 stomata surrounded by 4 neighboring cells)	Tetracytic (1 stomata surrounded by 4 neighboring cells)	Tetracytic (1 stomata surrounded by 4 neighboring cells)	Tetracytic (1 stomata surrounded by 4 neighboring cells)
	Epidermis shape	Irregular, pentagonal, hexagonal	Irregular, pentagonal	Irregular, quadrilateral, pentagonal	Irregular, quadrilateral, pentagonal

Based on Table 3, it is known that the types of IR015, CAT 140, and CAT 274 have stomata only on the lower surface, while CAT 144 has stomata on the upper and lower surfaces. This difference is due to the position of the leaves attached to the stem. CAT 144 forming an angle of 45^{o}C, while the others open horizontally. This causes the top and bottom sides to be the same. The anatomical characters on CAT 144 on the upper and lower surfaces of the leaves, such as the number of stomata, length of stomata, width of stomata, number of epidermal cell, length of epidermal cell, width of epidermal cell, index of stomata are not much different. Stomata are kidney-shaped, and belong to the tetracytic type, namely in the form of stomata surrounded by 4 neighboring cells. In IR015, CAT 140, and CAT 274 the upper surface of the leaves is composed only of the epidermal cell which is mostly irregular and pentagonal in shape. The highest number was in CAT 274 because the epidermis was smaller than IR015 and CAT 144.

On the lower leaf surface, it is known that the number of stomata at IR015 is the highest and the number of epidermal cell is the least, so that the stomata index is the largest. The stomata index looks the largest because the stomata index shows the number of stomata divided by the number of stomata plus the number of epidermal cell and multiplied by 100%. The types of stomata in all types are the same, namely tetracytic in the form of stomata surrounded by 4 neighboring cells. The stomata are kidney-shaped and the epidermal cell is irregular, pentagonal, and hexagonal in shape. The overall cell shape of the epidermis and leaf stomata in each accession is shown in Figs. 1,2,3,4.

The model of *Dendrobium* leaf is an important epiphytic taxon. The majority of leaves were shaped by the environment rather than evolutionary constraints. To maintain water balance and improve water use efficiency, leaf and root traits showed close coordination in *Dendrobium*. The traits related to water uptake and conservation might play an important role in helping Dendrobium species to adapt to cold and dry conditions at high elevations [14].

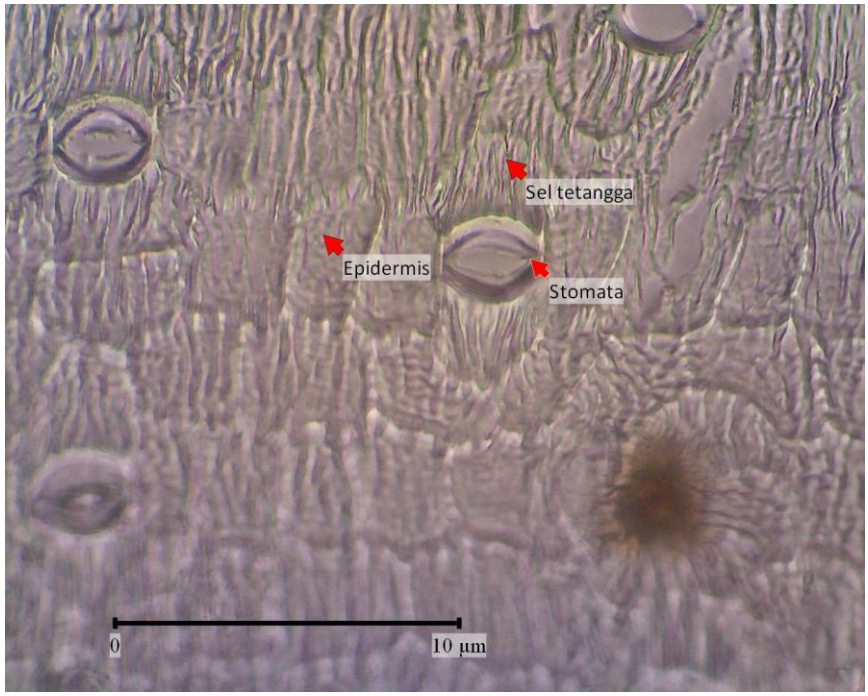


Fig. 1. The shape of the epidermal cells and leaf stomata IR015 (400x)

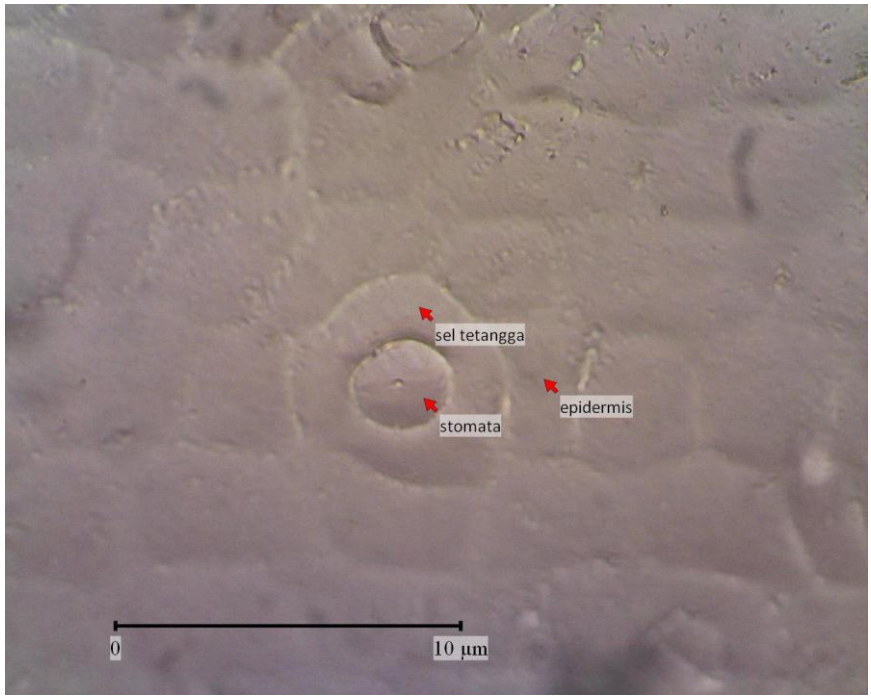


Fig. 2. The shape of the epidermal cells and leaf stomata CAT 140 (400x)

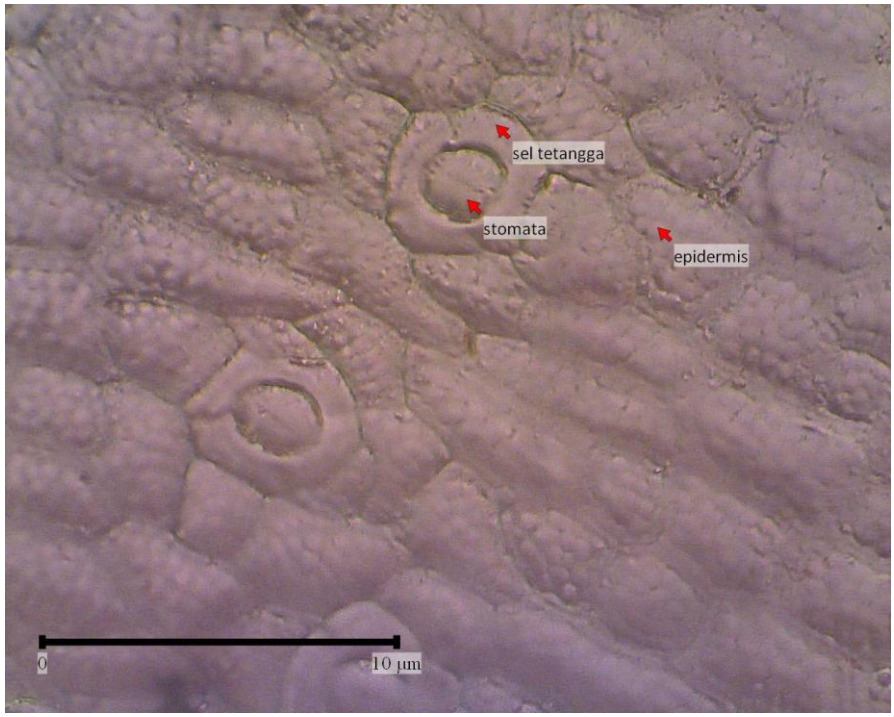


Fig. 3. The shape of the epidermal cells and leaf stomata CAT 144 (400x)

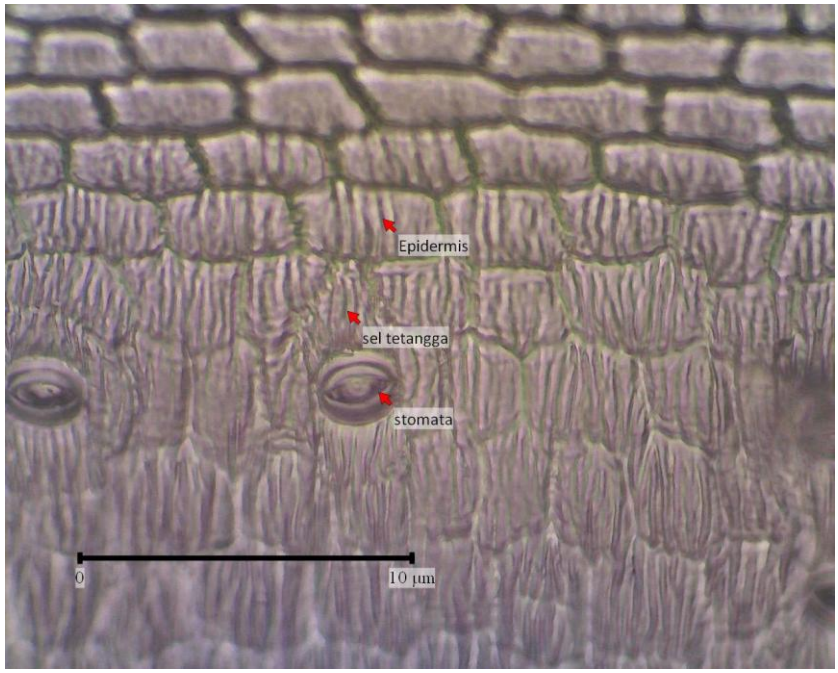


Fig. 4. The shape of the epidermal cells and leaf stomata CAT 274 (400x)

CONCLUSION

The observation of nine anatomical characters on the upper and lower surfaces of the leaves indicate that the leaf organs have high variations. The types of stomata in all types were the same, namely tetracytic in the form of stomata surrounded by 4 neighboring cells. The stomata are kidney-shaped and the epidermal cell is irregular, pentagonal, and hexagonal in shape.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mahfut. Indonesia Darurat Konservasi: Sudah Amankah Kebun Raya Kita? [Indonesia Conservation Emergency: Are Our Botanical Gardens Safe?]. Prosiding Seminar Nasional Biodiversitas Indonesia. UIN Alauddin Makassar; 2019.
DOI: https://doi.org/10.24252/psb.v5i1.11847
2. Mahfut Handayani TT, Wahyuningsih S, Sukimin. Identification of *Dendrobium* (Orchidaceae) in Liwa Botanical Garden Based on Leaf Morphological Characters. Journal of Tropical Biodiversity and Biotechnology. 2021;6(1):1-6.
DOI: https://doi.org/10.22146/jtbb.59423
3. Mahfut Indrianto A, Somowiyarjo S, Daryono BS. Molecular phylogeny of orchids mycorrhiza isolated from native tropical orchids in Indonesia. Malaysian Journal of Microbiology. 2020;16(1):68-72.
DOI: http://dx.doi.org/10.21161/mjm.190425
4. Medeiros JS, Burns JH, Nicholson J, Rogers L, Valverde-Barrantes O. Decoupled leaf and root carbon economics is a key component in the ecological diversity and evolutionary divergence of deciduous and evergreen lineages of Genus *Rhododendron*. American Journal of Botany. 2017;104(6):803-816.
DOI: 10.3732/ajb.1700051
5. Munawaroh E, Yuzammi Solihah SM, Suhendar. Koleksi Kebun Raya Liwa, Lampung Tumbuhan Berpotensi sebagai Tanaman Hias [Liwa Botanical Garden

Collection, Lampung Potential Plants as Ornamental Plants]. LIPI Press. Jakarta; 2017.
6. Chang W, Zhang SB, Li SY, Hu H. Ecophysiological Significance of Leaf Traits in *Cypripedium* and *Paphiopedilum*. Physiologia Plantarum. 2011;141(1):30-9.
DOI: 10.1111/j.1399-3054.2010.01418.x
7. Rammitsu K, Yagame T, Yamashita Y, Yukawa T, Isshiki S, Ogura-Tsujita Y. A Leafless Epiphytic Orchid, *Taeniophyllum glandulosum* Blume (Orchidaceae), is Specifically Associated with the Ceratobasidiaceae Family of Basidiomycetous Fungi. Mycorrhiza. 2019; 29(2):159-166.
DOI: 10.1007/s00572-019-00881-7
8. Rosanti D, Widianjaya RR. Morfologi Orchidaceae di Kebun Raya Liwa Kabupaten Lampung Barat Provinsi Lampung [Orchidaceae Morphology in Liwa Botanical Gardens, West Lampung Regency, Lampung Province]. Sainmatika: Jurnal Ilmiah Matematika dan Ilmu Pengetahuan Alam. 2018;15(2): 84-89.
DOI: http://dx.doi.org/10.31851/sainmatika.v15i2.2371
9. Solihah SM. Koleksi, Status, dan Potensi Anggrek di Kebun Raya Liwa [Collection, Status, and Potential of Orchids in Liwa Botanical Gardens]. Warta Kebun Raya. 2015;13(1):14-23.
10. Zotz G, Schickenberg N, Albach D. The velamen radicum is common among terrestrial monocotyledons. Annals of Botany. 2017;120(5):625-632.
DOI: 10.1093/aob/mcx097
11. Qi Y, Huang JL, Zhang SB. Correlated Evolution of Leaf and Root Anatomic Traits in *Dendrobium* (Orchidaceae). AoB Plants. 2020;12(4):1-13.
DOI: 10.1093/aobpla/plaa034
12. Arif A, Ratnawati. Hubungan kekerabatan anggrek dendrobium berdasarkan karakteristik morfologis dan anatomis daun [Dendrobium Orchid Relationship Based on Morphological and Anatomical Characteristics of Leaves]. Jurnal Prodi Biologi. 2018;7(4):213–222.
13. Guan ZJ, Zhang SB, Guan KY, Li SY, Hu H. Leaf anatomical structures of *Paphiopedilum* and *Cypripedium* and their adaptive significance. Journal of Plant Research. 2011;124(2):289-98.
DOI: 10.1007/s10265-010-0372-z
14. Yang SJ, Sun M, Yang QY, Ma RY, Zhang JL, Zhang SB. Two Strategies by Epiphytic Orchids For Maintaining Water Balance: Thick Cuticles in Leaves and Water Storage in Pseudobulbs. AoB Plants. 2016;3(8).
DOI: 10.1093/aobpla/plw046
15. Zhang FP, Feng JQ, Huang JL, Huang W, Fu XW, Hu H, Zhang SB. Floral Longevity of *Paphiopedilum* and *Cypripedium* is Associated With Floral Morphology. Frontiers in Plant Science. 2021;12:637236.
DOI: 10.3389/fpls.2021.637236
16. Zhang S, Yang Y, Li J, Qin J, Zhang W, Huang W, Hu H. Physiological Diversity of Orchids. Plant Diversity. 2018;40(4):196-208.
DOI: 10.1016/j.pld.2018.06.003
17. Sun M, Feng CH, Liu ZY, Tian K. Evolutionary Correlation of Water-Related Traits Between Different Structures of *Dendrobium* Plants. Botanical Studies. 2020;61(1):1-16.
DOI: 10.1186/s40529-020-00292-4
18. Esau K. Anatomy of Seed Plants. John Wiley & Sons, Inc. Canada; 1977.