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Anatomical Comparison of Two *Grammatophyllum* spp. (Orchidaceae) Species and Their Specific Ecological Adaptation

S Ishmah[1], D Metusala[2*], Nisyawati[1] and J Supriatna[1]

[1]Department of Biology, FMIPA Universitas Indonesia, Kampus UI Depok, Depok 16424, Indonesia
[2] Purwodadi Botanic Garden, Indonesian Institute of Sciences, Jl. Raya Surabaya-Malang km.65, Pasuruan 67163, East Java, Indonesia, ORCID ID 0000-000-0483-1883

[1]sharfinaia@ui.ac.id, [2*]metusala.destario@gmail.com, [1]nsywt@yahoo.com, [1]jatna@conservation.or.id

Abstract. The genus *Grammatophyllum* (Orchidaceae) has two sections with a very different habitus. The *Grammatophyllum* from section "*Grammatophyllum*" has a long cylindrical pseudobulb with linear leaves, while the *Grammatophyllum* section "Gabertia" has a shorter pseudobulb with a larger diameter and broader lanceolate shaped leaves. The study is aimed to compare the anatomical characters of leaf and root between G. *speciosum* (representation of "Pattonia" section) and G. *scriptum* (representation of "Gabertia" section). Leaf and root sections were obtained using the mini-microtome with liquid preservation method in 10 replications. Data were analyzed statistically using a t-test at 5% significance. The results showed significant differences between the two species in the leaf's primary vascular bundle area, velamen area, number of velamen layers, root's cortex area, and stele area. Future research with more organs and parameters being explored and experimental research regarding its anatomical response to drought is suggested.

1. Introduction

Genus *Grammatophyllum* is a genus from the Cymbidieae tribe naturally occurring in Indonesia, containing two sections and 13 species. The genus *Grammatophyllum* is both valuable as an ornamental and medicinal plant. Research about microanatomy of genus *Grammatophyllum* is still scarcely done. Previous related research about the anatomy of the Cymbidiae tribe was done by Yukawa & Stern [1], of which the genus *Grammatophyllum* is one of the topics covered and described. Other research covering the genus from when was conducted-either about its physiology or only mentioned as a part of species inventory. This research aims to find the anatomical differences of significant value between the root and leaf of *Grammatophyllum scriptum* and *Grammatophyllum speciosum* and connect them with the environmental adaptations of both species. This research is also conducted to contribute to the available data of *Grammaptophyllum* anatomy

Orchidaceae is the second largest plant family comprising around 900 genera and 30,000 species. The orchid leaf is simple and has parallel venation as most monocots. Some orchids have a specialized structure called pseudobulb; an enlarged, bulbous structure at the base of an orchid's leaf. The function of pseudobulb is predicted to be food and water storage, as its tissue is rich in starch. The pseudobulb tissue has few chlorophylls that can function as a photosynthetic organ, especially if the leaves are shed. Pseudobulb in orchids is also linked to epiphytic lifestyle, as it is harder to obtain water and nutrition in that life form [2][3]. Pseudobulb can arise from a single node (heteroblastic) or multiple internodes (homoblastic) and has a variety of shapes that can help in identification [2,4].

The orchid root is more anatomically adapted and distinct than the leaf or stem [5]; while the root varies in thickness, it is never thin and fibrous [4]. Orchid has a dominant or only secondary root system, which is divided into terrestrial and aerial roots. Terrestrial root functions as an anchor to substrate and nutrient uptake, while the aerial root is exposed to air and aids in collecting moisture and climbing [2]. The tip of the aerial root of the orchid could contain chlorophyll and have a photosynthetic function. Orchid root has a specialized structure outside exodermis called velamen, which comprises multiple epidermal cells which range from 1–20 layers thick [5,6]. Velamen is an adaptation suited for an epiphytic lifestyle, but it is found that many terrestrial orchids possess velamen [3,4].

Genus *Grammatophyllum* belongs to the tribe Cymbidieae, comprising two sections and 13 species [7]. The members of the *Grammatophyllum* genus can have terrestrial, epiphytic, lithophytic, or saprophytic lifestyles [5], while the genus is described by Groom [8] as essentially epiphytic. The distribution of *Grammatophyllum* ranges from South East Asia, New Guinea, and Oceania.

The genus *Grammatophyllum* has a special characteristic in which the secondary root defies geotropic growth to form a basket-like structure that collects organic materials; this is done as an adaptation for epiphytic life in which nutrients are scarcer [2,8]. Pseudobulb shape in *Grammatophyllum* can be ovoid for the section Gabertia or cylindrical for the section Pattonia [9]. The leaf of *Grammatophyllum* is simple, described as smooth with no hair, has cuticule, and has tetracytic stomata, which sometimes appear adaxially [1].

The species *Grammatophyllum speciosum* has a distribution range from Cambodia, Laos, Sumatra, Java, Borneo and the Philippines. Its conservation status is currently not evaluated by IUCN, but it is listed as appendix II on CITES. The species G. *scriptum* is notable as the largest orchid in the world, with huge and long-lived inflorescence. This orchid is said to naturally grow in terrestrial and epiphytic life forms [8].

The species *Grammatophyllum scriptum* has a distribution range from Malesia to the Southwest Pacific. The conservation status of G. *scriptum* is currently not evaluated by IUCN, but it is listed as appendix II on CITES. The species G. *scriptum* is valued as an ornamental plant with showy inflorescence, with floret's pattern resembling a letter from which its species name derived. In its native range, such as the Moluccas, G. *scriptum* is also a medicinal plant [10]. The orchid is an epiphytic orchid that can also grow terrestrially.

2. Material and Methods

This research was done in 2018, spanning around a year from sample collection, preservation to writing. The sample of *Grammatophyllum scriptum* was obtained from the drier coastal lowland of Jambi, while the *Grammatophyllum speciosum* was obtained from a wet rainforest in Central Sulawesi, Indonesia. Both plants were brought and habituated for two months in Dikara Orchid Nursery, Depok. The leaf and root morphology still reflect its adaptation to environmental conditions in their natural habitats.

Samples used for observation were obtained from both plants' mature leaf and aerial roots; every sample was taken in ten repetitions. Leaves were observed in the transversal and paradermal sections. A transversal section was obtained by cutting the leaf in a 1x1 cm area, including its costae. The pieces were then held in place with a cassava pith and cut transversely with a hand microtome. The paradermal section of the leaf was done with the scraping method by a razor blade. A transversal section of the root was obtained by selecting straight parts of the roots and cut transversely with a hand microtome.

The sample preservation method followed the protocol by Metusala [11]. Each sample was dehydrated gradually with ethanol 50% for 5 minutes, ethanol 70% for 1 minute, and ethanol 96% for 1 minute to dissolve the chlorophyll content. Samples were then transferred to a tube containing preservation liquid composed of safranin dye with ethanol 70% + glycerine (1:1).

The preserved sample was then prepared and observed with a light microscope LEICA. Measurement of the obtained images was done with the software ImageJ. Obtained data were processed with unpaired t-test at 5% significance to determine which characteristics have significant difference.

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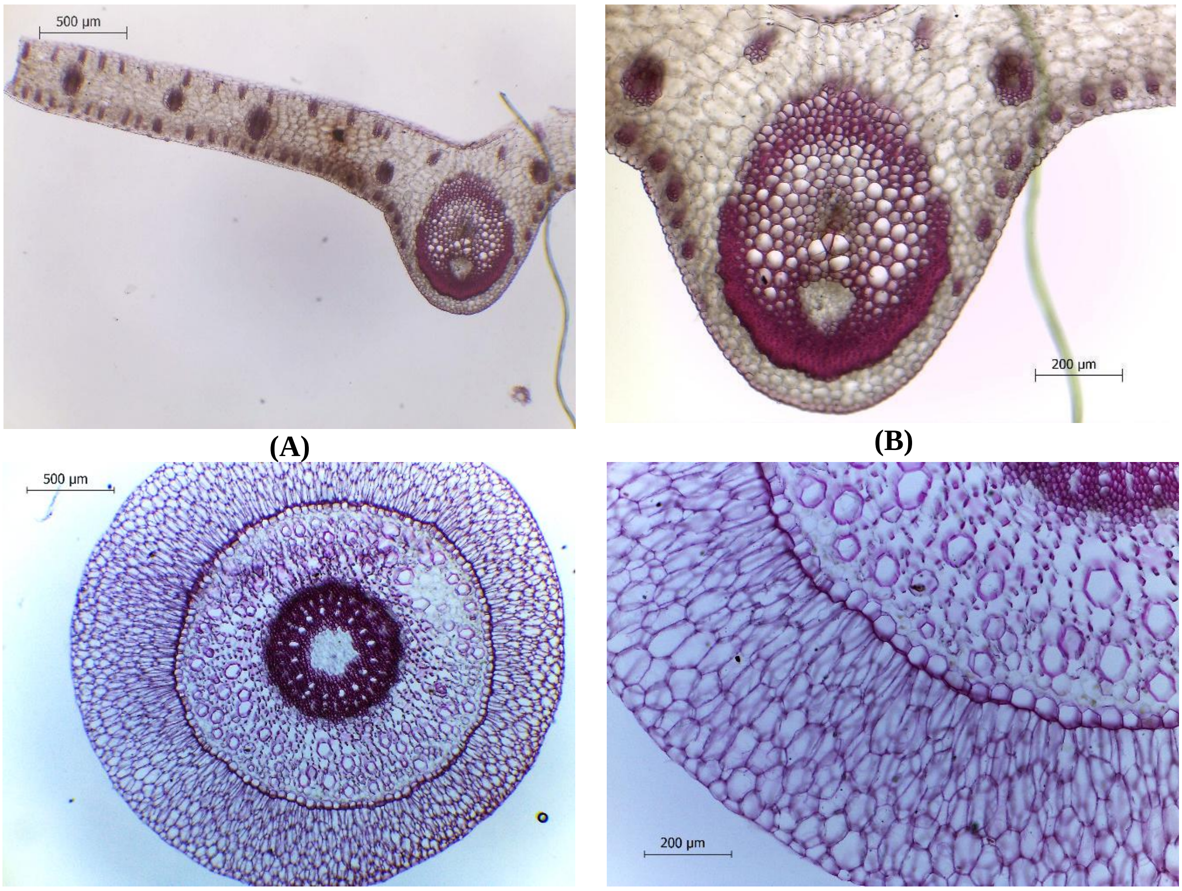
3. Result and Discussion

Measurements of parameters were taken by calculating distances between two points with a straight line in ImageJ. The area measurements of root parameters were done using the calculation from the circle's circumference as some root samples were too big to be measured manually using ImageJ. The stomatal density and velamen layer numbers were counted manually using ImageJ. The data obtained from each repetition was then averaged and put into the t-test.

The total root area of *Grammatophyllum scriptum* (figure 1 C and D, table 1) was averagely measured at 7,437,367.599 μm^2, with root cortex area 3,985,361.117 μm^2, and root stele area of 993,887.089 μm^2. The root of G. *scriptum* has thicker velamen with several cell layers ranging from 5–8 cells thick, while it was stated as 4–6 cells thick in previous research by Yukawa & Stern [1]. The velamen layers are more numerous than *Grammatophyllum speciosum* and have a significantly larger area of 4,140,413.257 μm^2. The thin velamen wall does not absorb the dye much, indicating little lignin and suberin content. The exodermis cells are hexagonal-shaped and have no thickening. The root has a larger cortex area compared to smaller stele. There was also observed mycorrhizal infection in some repetitions.

The leaf of *Grammatophyllum scriptum* observed has an average thickness of 402.077 μm, which does not significantly differ from *Grammatophyllum speciosum*. The mesophyll cells are rectangular-shaped, with vascular bundles spread between the abaxial and adaxial sides. Some sclerenchymatous fiber cells near the abaxial and adaxial surface, with lesser density than G. *speciosum*. The abaxial stomatal density measures 46/mm^2, while stomata were found on an adaxial surface with density of 32/mm^2.



(A) (B) (C) (D)

Figure 1. (A) leaf cross section of *Grammatophyllum scriptum* 4X10 magnification LM (B) leaf cross section of G. *scriptum* 10X10 magnification LM (C) root cross section of G. *scriptum* 4X10 magnification LM (D) root cross section of G. *scriptum* 4X10 magnification LM

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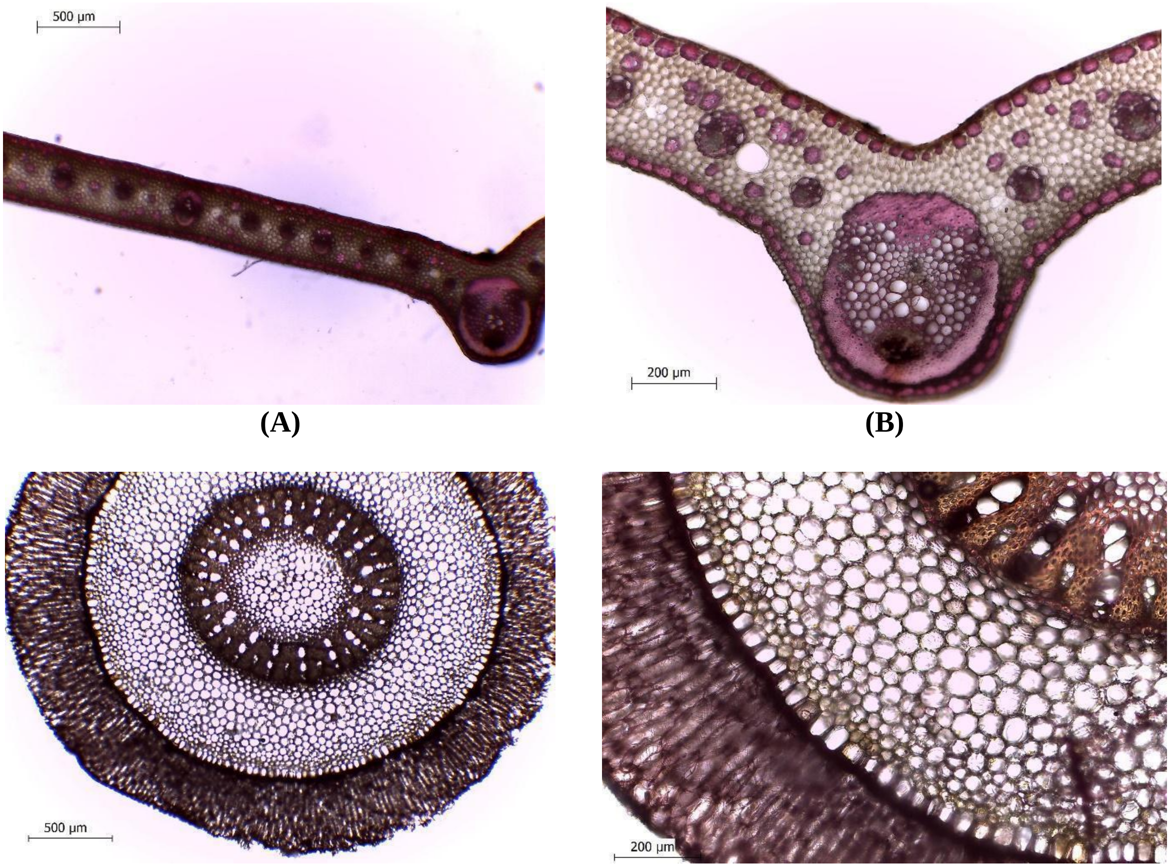

(A) (B)

(C) (D)

Figure 2. (A) leaf cross section of *Grammatophyllum speciosum* 4X10 magnification LM (B) leaf cross section of G. *speciosum* 10X10 magnification LM (C) root cross section of G. *speciosum* 4X10 magnification LM (D) root cross section of G. *speciosum* 4X10 magnification LM

The total root area of *Grammatophyllum speciosum* (figure 2 C-D, table 1) was averagely measured 6,616,727.956 μm^2, with root cortex area of 199,9617.672 μm^2, and root stele area of 221,081.683 μm^2. The root of G. *speciosum* has fewer velamen layers of 2–5 cells thicker than 2–4 layers found in previous research of Yukawa & Stern [1]. The velamen layers are less than *Grammatophyllum scriptum* and have a significantly smaller area of 2,689,448.419 μm^2. The velament cells themselves have thicker walls and absorb the safranin dye, indicating lignin and suberin content. The exodermis cells are hexagonal-shaped with a thickened wall bordering the velament. A smaller cortex area in contrast with an enormous stele was observed. In G. *speciosum* root was also observed mycorrhizal infection in some repetitions.

The leaf of *Grammatophyllum speciosum* observed has average thickness of 337.922 µm (Image 1 D), which does not significantly differ from *Grammatophyllum scriptum*. Mesophyll cells' shape was more circular and isodiametric than G. *Scriptum*, with vascular bundles spread between the abaxial and adaxial sides. There were tight rows of sclerenchymatous fiber cells near the abaxial and adaxial surface, indicated by safranin absorption. Adaxial cuticle thickness in G. *speciosum* measured 2.796 µm thick. The abaxial stomata density measures 44/ mm^2, while no stomata were found on the adaxial surface.

Based on the unpaired t-test (Table 1 and 2), there are significant differences between Grammatophyllum scriptum and Grammatophyllum speciosum in the leaf vascular bundle area. For determining the value differences between the two species are statistically significant, an unpaired t-test with 5% significance was used. A p-value below 0.05 indicates a significant difference between the two species. There are significant differences in the root cortex area, stele area, velamen layers number and velamen area. Orchids observed have a thicker cuticle and velament layer than orchids described in the previous research by Yukawa & Stern [1]. The other characteristics of root and leaf observed are primarily similar to the previous description [1], which used garden-grown Grammatophyllum samples from unknown countries of origin. Whether between the samples or previous research, differences in anatomy might reflect the plant's lifestyle, environmental conditions, and genetic factors.

Table 1. Root Anatomy

Parameter	*Grammatophyllum scriptum*	*Grammatophyllum speciosum*	P Value
Root area (μm^2)	7,437,367.599	6,616,727.956	NS
Velamen area (μm^2)	4,140,413.257	2,689,448.419	*
Root cortex area (μm^2)	3,985,361.117	1,999,617.672	*
Root stele area (μm^2)	993,887.089	221,081.683	*
Average velamen layers	6.5	3.7	*

* Significant difference at p value 0.05

Table 2. Leaf anatomy

Parameter	*Grammatophyllum scriptum*	*Grammatophyllum speciosum*	P Value
Leaf thickness (μm)	402.077	337.922	NS
Primary vascular bundle area (μm^2)	257,121.753	208,937.206	*
Abaxial stomata/ mm^2	46	44	
Abaxial stomata/ mm^2	32	0	–
Adaxial cuticle thickness (μm)	3.21	2.796	NS

* Significant difference at p value 0.05

More velamen layers might be attributed to the larger root area and-adaptation for dry conditions and intense sun exposure [12]. In this case, the latter is more likely, as there is no significant difference in the root area of *Grammatophyllum scriptum* and *Grammatophyllum speciosum* observed. Velamen function as sponge-like structures that retain water and nutrients temporarily, which can serve as reservoirs for a limited time. Velamen also serves as mechanical protection, provides protection of cortex from ultraviolet rays [12,13], and one of its proposed functions is a defense against pathogens [4]. Velamen protects the root from UV- B radiation damage and protects photosynthetic root pigment by scattering incoming light [2,14]. The thickness of the velamen cell wall may be a feature that supports its function to retain water, preventing cellular dehydration collapse, and mechanical protection [12].

The orchids observed have significantly different values of cortex and stele area. Smaller cortex areas in monocotyledons such as maize (*Zea mays*) are attributed to drought tolerance. The reduced cortex area is thought to reduce metabolic cost, increase water acquisition, and water permeability to stele [15]. Joca et al. [16] research about root anatomy and nutrient uptake of 18 orchid taxa shows that roots with bigger relative cortex size have more passage cells and protoxylem, which facilitates more water transport. In *Grammatophyllum scriptum*, a large cortex area compared to stele was observed, while in *Grammatophyllum speciosum,* the cortex is smaller than stele. The smaller stele is thought of as a drought-resistant mechanism as it reduces water transport capacity, as seen in *Astragalus gombiformis* [17]. This is consistent with the native habitat of G. *scriptum*, which is drier than one of G. *speciosum*, that it could be linked as its anatomical adaptation.

A significant difference is observed in the leaf primary vascular bundle area. The primary vascular bundle is composed of xylem and phloem, which mainly transports water and nutrients from and to the leaf. However, based on the research by Boughaleb et al. [17], plants given drought treatment showed a significant decrease in vascular bundle area.

Adaxial cuticle is directly exposed to the sun. The thicker adaxial cuticle may be an adaptation to conserve water mainly seen in epiphytic orchids, as it serves as a barrier from transpiration when the stomata is closed [18]. Adaxial cuticle layer also shields from and protect tissues below it rom intense radiation [19–21].

Stomata in orchids are usually restricted to the abaxial surface of the leaf [22]. Stomata is a main place for gas exchange, more stomata means more transpiration and gas exchange to facilitate photosynthesis. The adaxial stomata observed in *Grammatophyllum scriptum* might be a feature which helps its leaf to cool off in a hot environment. Increase in the number of stomata is also observed as an adaptation to intense sunlight, for cooling function and photosynthetic capacity [20] as it lowers stomatal resistance [23].

4. Conclusion

There are significant differences between *Grammatophyllum scriptum* and *Grammatophyllum speciosum* in leaf vascular bundle area, root cortex area, root stele area, velamen layers number, and velamen area. Differences may be contributed by many factors such as life form, adaptations to environmental conditions such as drought, and genetic factors or inherent differences between species. In future research, more anatomical parameters such as ŧ stomata, and the areas of individual xylem and phloem vessels could be added. More organs such as pseudobulb could be explored. Experimental research regarding the two speciesè anatomical response to environmental stressors such as drought is also suggested.

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References

[1] Yukawa T and Stern W L 2002 Comparative vegetative anatomy and systematics of *Cymbidium* (Cymbidieae: Orchidaceae) *Bot. J. Linn. Soc.* **138**

[2] Hew C S and Yong J W 2004 *The Physiology of Tropical Orchids in Relation to the Industry* (Singapore: World Scientific)

[3] Lüttge U 2012 *Vascular Plants as Epiphytes: Evolution and Ecophysiology* (Berlin: Springer-Verlag)

[4] Dressler R L 1993 *Phylogeny and Classification of the Orchid Family* (Cambridge: Cambridge University Press)

[5] Stern W L, Gregory M and Cutler D F 2014 *Anatomy of the Monocotyledons* (New York: Oxford University Press)

[6] Evert R F 2006 *Esau's Plant Anatomy: Meristems, Cells, and Tissues of the Plant Body: Their Structure, Function, and Development* (New York: John Wiley & Sons)

[7] WCSP 2018 World Checklist of Monocotyledon available at http://wcsp.science.kew.org/

[8] Groom P 1893 Botanical Notes, No 4 –On The Velamen Of Orchids *Ann. Bot.* ***7*** 143-51

[9] Teoh E S 2005 *Orchids of Asia* (Singapore: Marshall Cavendish)

[10] Teoh E S 2016 *Medicinal Orchids of Asia* (Switzerland: Springer)

[11] Metusala D 2017 An alternative simple method for preparing and preserving cross-section of leaves and roots in herbaceous plants: Case study in Orchidaceae *AIP Conf. Proc.* **1862** *030113*

[12] Moreira A S and Isaias R M 2008 Comparative anatomy of the absorption roots of terrestrial and epiphytic orchids *Braz. Arch. Biol. Technol.* **51** 83-93

[13] Metusala D, Supriatna J N and Sopandie D 2017 Comparative leaf and root anatomy of two *Dendrobium* species (Orchidaceae) from different habitats in relation to their potential adaptation to drought *AIP Conf. Proc.* **1862** 030118

[14] Chomicki G, Bidel L P, Ming F, Coiro M, Zhang X, Wang Y and Renner S S 2014 The velamen protects photosynthetic orchid roots against UV-B damage, and a large dated phylogeny implies multiple gains and losses of this function during the Cenozoic *New Phytol.* ***205*** 1330-41

[15] Chimungu J G, Brown K M and Lynch J P 2014 Reduced root cortical cell file number improves drought tolerance in maize *Plant Physiol.* **166** 1943-55

[16] Joca T A C, Oliveira D, Zotz G, Winkler U and Moreira A S 2017 The velamen of epiphytic orchids: Variation in structure and correlations with nutrient absorption *Flora* **230** 66-74

[17] Boughalleb F, Abdellaoui R, Ben-Brahim N and Neffati M 2014 Anatomical adaptations of Astragalus gombiformis Pomel under drought stress *Cent. Eur. J. Biol.* **9** 1215-25
[18] Yang S et al. 2016 Two strategies by epiphytic orchids for maintaining water balance: Thick cuticles in leaves and water storage in pseudobulbs *AoB Plants **8** plw046*
[19] Esau K 1977 *Anatomy of Seed Plants* (New York: John Wiley & Sons)
[20] Dickison W C 2000 *Integrative Plant Anatomy* (San Diego: Academic Press)
[21] Pfündel E E, Agati G and Cerovic Z G 2006 *Optical Properties of Plant Surfaces in Biology of the Plant Cuticle* (Oxford: Blackwell)
[22] Stancato G C, Mazzoni-Viveiros S C and Luchi A E 1999 Stomatal characteristics in different habitat forms of Brazilian species of Epidendrum (Orchidaceae) *Nord. J. Bot*. **19** 271-5
[23] Wild A and Wolf G 1980 The Effect of Different Light Intensities on the Frequency and Size of Stomata, the Size of Cells, the Number, Size and Chlorophyll Content of Chloroplasts in the Mesophyll and the Guard Cells during the Ontogeny of Primary Leaves of *Sinapis alba Zeitschrift für Pflanzenphysiologie* **97** 325-42