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Research Article

Stomata Profile Comparisons in Abaxial and Adaxial Zones of *Dendrobium aphyllum* and *Arachnis flos-aeris* Leaves

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Abstract

Stomata activity in orchids is related to orchids' adaptation process to their environment. This study aimed to investigate the stomata profile, comprising of anatomical structure and density of the two orchids species, *Dendrobium aphyllum,* and *Arachnis flos-aeris*. Data was collected by printing the leaves' surface using glue and observed at 09.00 am and 09.00 pm, then analyzed using Image raster 3.0 software and MS. Excel. The stomata of *D. aphyllum* and *A. flos-aeris* were characterized by anomocytic type. Results showed that the highest stomata density was 1177.78 um^2, found on the abaxial part of *D. aphyllum*. The adaxial leaves of both species were classified as low or no stomata. These two orchid species were epiphyte yet grow in different habitats. Arachnis flos-aeris exposed to direct sunlight tends to reduce transpiration rate, resulting in fewer stomata than *D. aphyllum* less exposed to sunlight.

Keywords: abaxial, adaxial, density, orchids, stomata.

1. Introduction

Orchidaceae adapt to environmental parameters in several ways. Orchid leaves that receive different light intensities, for example, will have different anatomy in their stomata (Zhang et al., 2018). Stomata are organs on the surface of leaves, in the form of holes and companion cells that can open and close for transpiration and photosynthesis processes. Stomata have the main task of being a route for water and gas uptake and excess transpiration (Zwieniecki et al., 2016). The arrangement of water and gas uptake from inside to outside the leaves or vice versa is different for each plant. The process of opening and closing these stomata is related to environmental conditions. When environmental conditions are unfavorable, exposure frequency will occur less frequently to avoid events such as excess transpiration (Matthews & Lawson, 2019). Crassulacean Acid Metabolism (CAM) plants such as orchids generally open their stomata from late afternoon or evening. It absorbed water and CO_2 for storage and reused in the morning in the Calvin cycle to produce organic substances such as carbohydrates (Lee, 2010; Males & Grif, 2017).

Stomata can also be used for plant identification studies because of its unique characteristics in each type of plant, including orchids (Aono et al., 2019). The identification of this plant can be made by observing and measuring the stomata's morphology, starting from its size, shape, and distribution in a leaf (Dharma & Maryani, 2018). Leaf stomata are among the most widely used characters because they have different properties for each type of plant. Diverse stomata reflect the adaptation of organs to their environment (Rindyastuti et al., 2018). Plants that are still in the same family or genus could have different stomata. Differences were ranging in size, shape, arrangement, even the position of the stomata that are found in leaves. The stomata can be found on both sides of the leaf (adaxial and abaxial) or only on one side (Hong et al., 2018). These are the leaf adaptation, according to its environment. The position of the stomata on both sides is generally found in plants growing with high light exposure. Meanwhile, stomata found on one side are generally found in plants that grow in certain areas, such as arid places or aquatic plants. The position of the stomata varies according to environmental conditions, which is very useful in keeping the leaves from excess transpiration (Matthews & Lawson, 2019: Hong et al., 2018)

Each type of orchid has different stomata that can be used to differentiate orchids between taxa. Indonesia has high orchid biodiversity, where around 5,000 of the 25,000 species of orchids in the world can be found in Indonesia (Fardhani et al., 2015). These orchids are generally divided into two major groups: epiphytic orchids, which live on substrates such as plant stems, and terrestrial, which grow on soil substrates. One of the community's orchids commonly cultivated is the *Dendrobium* genus orchid, for example, *D. Aphyllum* (Darmawati et al., 2018). *Dendrobium* is classified as an epiphytic orchid, with a fairly high diversity and distribution in the orchid family. Orchids other than *Dendrobium* are *A. flos-aeris* classified as epiphytic orchids but can grow in soil or semi-terrestrial (Agustini et al., 2016). In Indonesia, this orchid is called the scorpion orchid. It is widely cultivated because it is easy to care for, has beautiful flowers, and can be cut orchids.

The gap in the research, there is not many studies regarding the comparison of the stomata profile on the abaxial and adaxial sides of orchids. The purpose of this study is to the comparison of stomatal profiles was observed in two types of orchids, namely *Dendrobium aphyllum* and *Arachnis flos-aeris*.

2. Material and Method

Samples of curtain orchid (*Dendrobium aphyllum*) and scorpion orchid (*Arachnis flos-aeris*) were collected in Pogung Kidul, Sleman, Special Region of Yogyakarta (110° 22′31.5″ West Longitude, 7° 45′47.6″ South Latitude) at an altitude of 113 m asl. Weather conditions and temperature at the observation field were sunny weather with a temperature range of 22-33ºC. This experiment was carried out directly in the field to make stomata molds, and then the stomata molds were collected and continued by anatomical observation in the laboratory. The leaves used to make stomata molds are characterized by third and fourth leaf parts from the shoot, green, and healthy. Stomata mold is made directly on the leaves that are still attached to the orchid.

Furthermore, stomata prints were observed under a microscope. In this study, one individual plant was used for each orchid species. Then three replications were carried out, with three leaves for each individual was selected to make stomata prints. The anatomical preparation method for stomata on leaves follows (Haryanti, 2010). Stomata molds on the leaf surface are made with the help of glue and tape. Before mold was made, upper (adaxial) and lower (abaxial) surfaces of the leaves are first cleaned with 70% technical alcohol to remove dust; debris, microbes, and another foreign substance. Leaf stomata molds of both orchid species were made at 09.00 am and 09.00 pm with a time difference of 12 hours to know the differences in stomata activity in the morning and evening. Leaves are smeared with glue evenly and left for ± 10 minutes to dry for mold preparations. Glue mold that has been dry, then affixed with tape and gently flattened to get a stomata mold.

In Comparison, the tape was slowly removed to lift the stomata mold from the leaf surface. Molds were then attached to the object-glass and flattened. Sample collection was labeled and observed stomatal profiles, including stomata anatomical structure, number of open and closed stomata, and stomata density. It was observed with a light microscope and opti-lab with a magnification of 40 × 40 with an average area of 45,000 μm^2. In this study, an observation area of 45,000 μm^2 was used because the distance between stomata on the orchid leaves is quite far, and the number of stomata is relatively low. This area was used to obtain a broad and representative description of the distribution of stomata. Data obtained were then analyzed with the help of Image raster 3.0 and Microsoft Excel 2010.

3. Results and Discussion

3.1. Results

Based on the results of this research on both types of orchids, the type of stomata, the density of the stomata, and the number of open and closed stomata at two collection times on each side (adaxial and abaxial) are below.

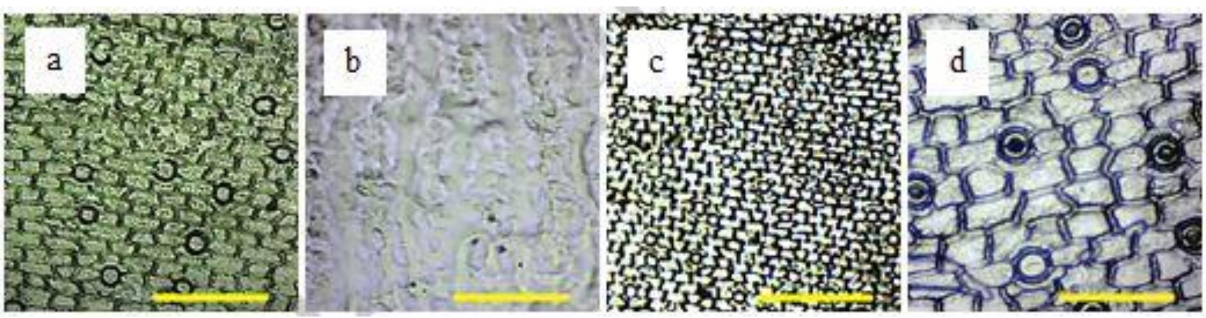

Figure 1. Anatomical profile of the stomata in *Arachnis flos-aeris*. (a) Adaxial leaves collected at 09.00 am, (b) Adaxial leaves collected at 09.00 pm, (c) Abaxial leaves collected at 09.00 am, (d) Abaxial leaves collected at 09.00 pm. Bar: 100 µm

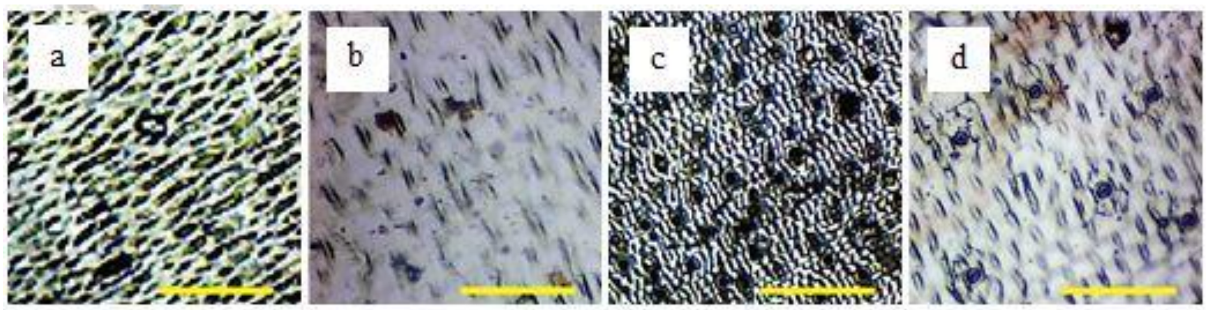

Figure 2. Anatomical profile of the stomata in *Dendrobium aphyllum*. (a) Adaxial leaves collected at 09.00 am, (b) Adaxial leaves collected at 09.00 pm, (c) Abaxial leaves collected at 09.00 am, (d) Abaxial leaves collected at 09.00 pm. Bar : 100 μm

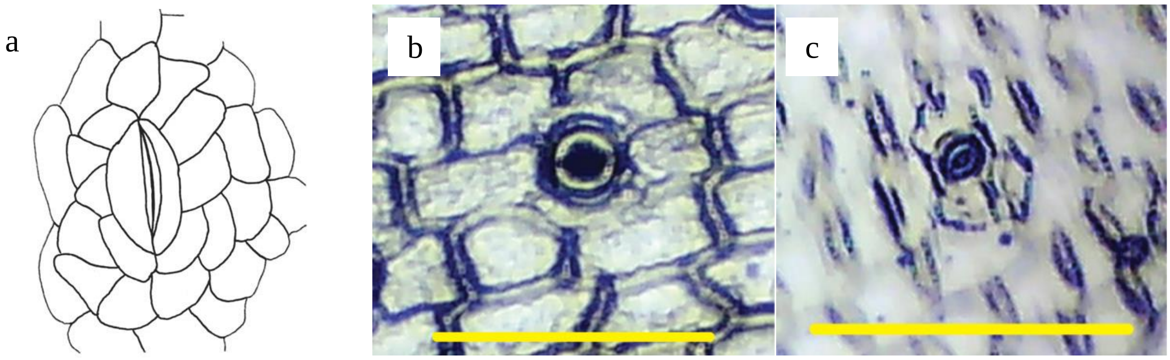

Figure 3. Anatomical structure of stomata. (a) Sketch of anatomical structure from anomocytic type stomata (b) Anatomical structure of stomata on the adaxial and abaxial surfaces from *Arachnis flos-aeris* leaves, (c) Anatomical structure of stomata on the adaxial and abaxial surfaces from *Dendrobium aphyllum* leaves. Bar: 100 μm

The stomata of *Arachnis flos-aeris* leaves (Figure 1) have an anomocytic type where the stomata are accompanied by several neighboring cells that resemble surrounding epidermal cells (Figure 3). The stomata are amphistomatic found on both sides (adaxial and abaxial). Closed stomata can be found on both sides at different hours. Open stomata also can be found on both sides, at different hours. Stomata of *Dendrobium aphyllum* leaves (Figure 2) have an anomocytic type, with the stomata accompanied by several neighboring cells that resemble the surrounding epidermal cells (Figure 3). The stomata are amphistomatic found on both sides (adaxial and abaxial). Closed stomata were not found on either side at different hours. Open stomata can be found on both sides at different hours. Stomata in *A. flos-aeris* have a larger size than the stomata size of *D. aphyllum*. *A. flos-aeris* has a stomatal diameter of ± 30.3 μm, while *D. aphyllum* has a stomatal diameter of ± 25 μm (Figure 3).

Table 1. Stomata density from *Arachnis flos-aeris* and *Dendrobium aphyllum*

Time	Species	Surface Side	Stomata		Total of Stomata	Stomata Density (ind/ µm²)
			Open	Close		
09.00 am	*Arachnis flos-aeris*	Adaxial	10	4	14	311.11
		Abaxial	22	0	22	488.89
	Dendrobium aphyllum	Adaxial	11	0	11	244.44
		Abaxial	53	0	53	1177.78
09.00 pm	*Arachnis flos-aeris*	Adaxial	10	0	10	222.22
		Abaxial	5	2	7	155.56
	Dendrobium aphyllum	Adaxial	7	0	7	155.56
		Abaxial	7	0	7	155.56

The observed leaf stomata of *A. flos-aeris* collected at 09.00 am for the adaxial side were 14, with ten open stomata and four closed stomata. The abaxial side totals 22 stomata were all open. The adaxial side's stromata density was 311.11 ind/µm^{2,} and the abaxial side was 488.89 ind/µm^2 (Table 1). There were ten stomata collected at 09.00 pm for an adaxial side. The abaxial side is 7, with 5 is open stomata and two closed stomata. The density of adaxial stomata was 222.22 ind/µm^{2,} and the abaxial side was 155.56 ind/µm^2. Stomata of *D. aphyllum* leaves were observed and collected at 09.00 am for the adaxial side totaling 11 open stomata. Abaxial side totals 53 open stomata. The density of adaxial stomata was 244.44 ind/µm^{2,} and the abaxial side was 1177.78 ind/µm^2. Stomata collected at 09.00 pm for both adaxial and abaxial sides were seven open stomata. Stomata density of both sides was 155.56 ind/µm^2.

3.2. Discussion

One of the prominent characters of *Dendrobium aphyllum* is elongated pseudobulb to store water. The pseudobulb structure is quite hard or dense, with the flowering of the subterminal type. Flower parts, such as sepals and petals, have a striped pattern of purple gradation. Orchids other than *Dendrobium* are *Arachnis flos-aeris* classified as epiphytic orchids but can be cultivated in the soil (Agustini et al., 2016). Scorpion orchids (*A. flos-aeris*) character are stems that are quite thick, hard, and long leaves. The leaves of this orchid are thick and green, coupled with gray roots that can grow randomly, far from the stem. This is what makes this orchid can be cultivated by cuttings and planted in the ground even though it is generally epiphytic. This orchid has yellow flowers with brownish spots.

One of the characters that can be used in the identification process of orchids is the anatomical character, the stomata on the leaves (Rindyastuti et al., 2018). Stomata are part of the leaves in the epidermal layer that play a role in gas exchange for respiration and transpiration (Baillie & Fleming, 2020). Stomata have several types that are different in each plant. Stomata in orchids can appear in the lower epidermal layer but can also be at the top due to the adaptation of each type (Fan et al., 2014; Rindyastuti et al., 2018). Stomata consist of a porous or slit accompanied by a pair of curved guard cells and several neighboring cells. These neighboring cells in each species have various forms. Some resemble surrounding epidermal cells or different (Matthews & Lawson, 2019). The stomata's location in orchids generally follows the path of the leaf repetition where, if the leaf bones are parallel, the stomata location will also be parallel. Stomata position in orchids also varies according to the side of the stomata found in leaves. The hypostomatic type is the position of the stomata in the lower epidermis. The stomata's position on the leaves' underside is related to the adaptation of orchids, where the stomata help the leaves store water and reduce excess transpiration by reducing the stomata on the top of the leaves (Garvita & Wawangningrum, 2020). Other types of stomata that can be found include anomocytic, anisocytosis, paracytic, and diacytic based on the pattern of neighboring cells around the stomata (Nunes et al., 2020). Stomata are generally rounded when they open. This opening is caused by the closing cell that changes shape to become curved because it absorbs water and other substances (Raissig et al., 2017). Stomata characters can distinguish one type from another, ranging from stomatal size, stomatal density, stomatal frequency, stomata type, neighboring cell patterns, and stomata location on the leaves surface side (Hong et al., 2018). The different stomata characters reflect how these species grow in their habitat and adapt to their environmental parameters. For example, *D. aphyllum* and A. *flos-aeris* are epiphytic orchids. *D. aphyllum* and *A. flos-aeris* are both epiphytic orchids that generally have more stomata in the abaxial part to reduce excess transpiration. However, this can change if the orchid grows in the shade of a dense tree so that sunlight does not easily reach the leaf surface. This makes the number of adaxial stomata more abundant because it's assisted by tree shade to reduce excess transpiration (Agustini et al., 2016).

Stomata observation uses a simple printing technique. From this technique, the leaf surface pattern is obtained starting from the stomata condition, the position of the stomata, the appearance of the epidermal layer, and so on. The observed stomata types from the two types of orchids were all anomocytic stomata (Figure 1, Figure 2). Anomocytic stomata have the characteristics of neighboring cells that accompany guard cells, which have a shape that resembles the surrounding epidermal cells. The type of stomata in *D. aphyllum* is different from the research of Arif & Ratnawati (2018), which states that *D. aphyllum* stomata are parasitic. Both species have the same type of stomata, namely anomocytic, according to the research by (Rompas, 2011). This can be caused by the two species adapting to the same environmental conditions and habitats in the Pogung area so that the adaptation process affects the anatomical structure of stomata in both species.

D. aphyllum is an epiphytic orchid that has lanceolate leaves, stomata that generally hypostomatic type. According to the observation, the stomata are amphistomatic and panerophore type or the position of the stomata found are in both sides, and also parallel to the epidermis, kidney-shaped guard cells, and have parasitic stomata where guard cells are accompanied by several neighboring cells. The results obtained are in accordance with the results of a research, according to Arif & Ratnawati (2018). *A. flos-aeris,* which is an epiphytic orchid but can live in the soil substrate, is characterized by the presence of two types of roots, namely aerial roots and terrestrial roots (Zhang et al., 2018). This orchid has stomata characteristics that classified as anomocytic type where guard cells are accompanied by neighboring cells that resemble surrounding epidermal cells (Rompas, 2011). Orchids include Crassulacean Acid Metabolism (CAM) plants so that the stomata are more closed in the morning to noon to reduce water loss but prevent the release of CO_2 (Males & Grif, 2017). Then the leaves experience the opening of the stomata for transpiration and absorption of CO_2 at night. When the stomata open, plants will absorb carbon dioxide which is then bound by the PEP carboxylase system complex to produce oxaloacetate and malate (Santelia & Lawson, 2016). CAM plants then use the results to supply CO_2 in the metabolic process.

The observations on the stomata profile of *D. aphyllum* and *A. flos-aeris* showed that the density of the stomata obtained in the abaxial part of the leaf was higher than that of the adaxial part (Table 1). These conditions are closely related to plant physiological processes to prevent excess transpiration. Sunlight generally shines on the adaxial side directly so that transpiration is faster and has a more significant effect (Zhang et al., 2016). This also makes many plants modify the epidermal layer to help leaves fight against excess transpiration in the adaxial area (Ullah et al., 2020). Modifications can be in the form of cuticle layers, trichomes, fan cells, or a higher frequency of closure of the stomata in the adaxial area (Arif & Ratnawati, 2018).

In contrast to the adaxial side, the stomata's density on the abaxial side of the leaf is higher. This is related to plants' physiological adaptation process, where sunlight generally does not shine on the abaxial side leaves directly. This event makes transpiration that occurs in the abaxial part, not too large. Therefore, according to Harrison et al. (2020), Soares et al. (2008), the density of stomata in the abaxial part is higher, which can help plants in the respiration process. The stomata density of *D. aphyllum* was higher than that of *A. flos-aeris*. The different density of stomata is one of the adaptation steps for orchids to their habitat. If epiphytic plants grow in places with more shade, sun exposure will decrease so that transpiration does not occur seriously. If there is little shade, then transpiration can be extreme. This is what makes the stomata density of *D. aphyllum* higher than *A. flos-aeris* because *D. aphyllum* grows in places with more shade so that transpiration does not go extreme, and the stomata can maximize the CO_2 uptake process (Agustini et al., 2016).

Stomata activity in this study was carried out by observing the number of closed and open stomata in the morning and evening associated with the CAM metabolism process in orchids. The number of open stomata at 09.00 am a relatively high number when compared to the number of open stomata at 09.00 pm (Table 1). The number of open stomata was mostly found in the observation of *D. aphyllum* leaf stomata at 09.00 am. It is inversely proportional to *A. flos-aeris,* which has more closed stomata at the same time. Stomata that are widely exposed can be associated with the place where orchids grow under shade. Orchids generally grow in the shade of other plants so that they are not exposed to direct sunlight, and the intensity of light received is not too high

(Puspitaningtyas, 2018). Crassulacean Acid Metabolism (CAM) plants such as orchids perform photosynthesis in the morning to evening by absorbing sunlight and combining CO_2 absorbed at night to be broken down into carbohydrates in the Calvin cycle (Sma-Air & Ritchie, 2020). In the morning, the number of open stomata in *D. aphyllum* was higher than that in *A. flos-aeris*. This is related to *D. aphyllum,* which grows epiphytes under the shade so that the light intensity received is less and the activity of the stomata is higher, which is indicated by a higher number of open stomata.

In contrast to *A.flos-aeris,* which grows semi-terrestrial, not under the shade, the light intensity obtained is higher. Stomata activity is lower to avoid excess transpiration, and the number of open stomata is less. The higher number of closed stomata of *A.flos-aeris* can also be attributed to the orchid's adaptation to survive in dry environments. Plants that are dry resistant generally have stomata that are easily closed, so that when observing the stomata in closed conditions (Clauw et al., 2015) Then the number of open stomata at 09.00 pm was less than at 09.00 am even though orchids, including CAM plants, generally open stomata at night to uptake CO_2. The results showed different results where the stomata that were open at night were less. This can be caused by different water availability during the morning and during the day. More stomata that open in the morning can occur because the water content in the morning is higher so that the guard cells can open. Stomata open due to the movement of guard cells or cover which, under pressure after absorbing water. When the water pressure is adequate, the guard cells will expand and open the stomata. If the availability of water is sufficient, the stomata can open (Anosheh et al., 2016).

The higher stomata density in the abaxial part of the leaf can indicate how the stomata condition is in that area. The higher density in the abaxial part is adapted to the inner leaf, reducing water loss during CO_2 uptake during transpiration. However, in a dry environment in both the adaxial and abaxial parts, the density of stomata required by plants is not much. This is because the higher the stomata density, the higher the water loss (Bertolino et al., 2019; Rindyastuti & Hapsari, 2017). Then the stomata of dicot plants are generally scattered, in contrast to monocot plants such as orchids, which have a parallel pattern to the epidermis arrangement and are located in a row (Haryanti, 2010; Rudall et al., 2017). According to research by Rindyastuti & Hapsari (2017), the density of stomata that is owned is directly proportional to the transpiration that occurs. The higher the stomata density, the more stomata found in one study area. If the plant has many stomata, the transpiration will be faster, and the water that will come out will also be more. Therefore, plants that live in dry areas have a density of stomata that is not too high to reduce water loss during transpiration. The results showed that the abaxial stomata density was generally higher, but the spacing between the stomata was uneven and tended to be dense. The abaxial side's density is also denser than the adaxial side because more stomata are found on the abaxial side.

Conclusion

This present study concludes that the abaxial stomata of *D. aphyllum* and *A. flos-aeris* differ from adaxial in their response to sunlight. *A. flos-aeris* has a lower activity of *stomata* than *D. aphyllum* because it grows with greater direct exposure to sunlight.

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