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Comparative Leaf and Root Anatomy of Two *Dendrobium* Species (Orchidaceae) from Different Habitat in Relation to Their Potential Adaptation to Drought

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Abstract. *Dendrobium capra* and *Dendrobium arcuatum* are closely related in phylogeny, but they have very contrasting vegetative morphology and habitats. *D. capra* is known as a species that is well-adapted to dry lowland teak forest habitat in East Java, where most trees drop their leaves in summer, while *D. arcuatum* has adapted to mid or high land moist forest at elevation up to 800 m dpl. In order to investigate their potential adaptation to drought stress in the climate change era, we have compared and analyzed the leaf and root anatomical characteristics of both species. Transversal sections were made using hand mini microtome, dehydrated in graded alcohol series and stained with safranin 1 % and fastgreen 1 %. Leaf scraping technique has been used to prepare paradermal sections, and then dehydrated in graded alcohol series and stained with safranin 1 %. Quantitative anatomical characteristics between *D. capra* and *D. arcuatum* have been compared using a t-test. The result showed that there were significant differences on anatomical characters between both species. Compared to *D. arcuatum*, *D. capra* shows more developed anatomical features for adapting to drought and dry condition. These anatomical features were a thicker cuticle, thicker epidermis, presence of hypodermis, thicker mesophyll, broader primary vascular bundle, well developed xylem's sclerenchyma, lower stomatal density, thicker and high proportion of velamen.

Keywords: leaf, root, dendrobium, adaptation, drought

INTRODUCTION

Dendrobium is one of three largest orchid genera, as well as one of the most important in commercial ornamental flowers [1]. Due to its importance in science and horticulture, *Dendrobium* is therefore of interest not only to botanists, but also to orchid growers, ecologists and conservationists. *Dendrobium*, along with many other orchid species, is facing great threats from climate change, especially drought stress [2, 3]. Most species of *Dendrobium* are epiphytes in primary forest, less often lithophytes; and only very few are obligate terrestrials [4]. This epiphytic environment is expected to be more vulnerable to drought pressure, particularly in the dry season when the humidity is very low.

Dendrobium capra is an epiphytic *Dendrobium* species from Indonesia, which is facing a high risk of extinction in its natural habitat [5, 6, 7, 8]. The distribution of *D. capra* is restricted to the dry lowland teak forest in East Java. Their natural habitat has temperature range between 30-33 °C with the air relative humidity at 40-60 %, and the plants are regularly exposed to 100 % full sunlight in the summer period. This species was recorded growing naturally in some lowland areas, such as Bojonegoro, Madiun, and Purbolinggo, all of which are in East Java [5, 7]. In many areas where *D. capra* grows, rainfall and consequent humidity is low. Rainfall is usually seasonal so that

water is not easily retained and the plants in such areas often have to survive in some moderate to long dry periods. Another species of *Dendrobium*, called *Dendrobium arcuatum* is also an endemic species with a natural distribution restricted to East Java. This species grows in the shade of hill forests at elevations up to 800 metres. In contrast to *D. capra*, the environment where *D. arcuatum* grows usually has a high level of humidity (80-90 %) and the plants are shaded by tree canopies, resulting in low light intensity (30-50 %).

Knowledge of their adaptive strategies is essential for their conservation and continued use in the ornamental trade. However, little is known about the leaf traits in *D. capra* and *D. arcuatum*, or their adaptive strategies for adapting to drought stress in their natural habitat. In the present study, we investigated the leaf and root anatomical structures of *D. capra* and *D. arcuatum* to understand their potential adaptation to drought stress. We hypothesize that the divergence of leaf anatomical structures in *D. capra* and *D. arcuatum* reflect adaptation strategy to their habitats, and that *D. capra* should exhibit more developed features for adapting to drought and dry condition than in *D. arcuatum*.

MATERIALS AND METHODS

Ten individuals of *D. capra* and *D. arcuatum* were collected from their natural habitat in East Java, Indonesia. As the environmental requirements of the two species are different, they were cultivated at two places with different growing conditions. The growing conditions of *D. capra* were 80-100 % of full sunlight and *D. arcuatum* were 25% of sunlight. All individuals were sampled for leaf anatomical observations. The middle parts of mature leaves were cut off to make cross-section samples. Cross-sections have been made by hand mini microtome and were dehydrated in graded alcohol series. The sections were stained with safranin 1 % and fastgreen 1 %. The samples were examined and photographed under a light microscope LEICA DM500 (Leica Inc., Bensheim, Germany). The thickness of cuticles, epidermis, hypodermis, mesophyll, and leaf lamina, as well as primary vascular bundle area were measured with ImageJ software

The adaxial and abaxial epidermis of middle mature leaf parts were peeled from fresh leaves and photographed under a light microscope. Digital images were manually analyzed with ImageJ. For leaf histological observations, leaves from ten different individuals were examined for each species, and more than ten images per leaf were analyzed. Comparison between *D. capra* and *D. arcuatum* were tested by independent sample t test.

RESULTS AND DISCUSSION

Cuticle layers in the leaves of *D. capra*, both sides adaxial and abaxial, were thicker and heavily cuticularised than in *D. arcuata* leaves (Fig. 1). Different with *D. capra* leaves, cuticles were not obvious on the surface of *D. arcuatum* leaves. Thicker cuticles found on both sides of the *D. capra* leaves surface suggest a better defence against drought pressure than those in *D. arcuatum*. Thicker cuticles are better for preventing water transpiration from the surface of leaves. A thicker cuticle is the anatomical trait of xeric conditions, and this may be an adaptations of xeric plants [9]. Structural cuticles defenses is one of the evolutionary forms observed in plants experiencing environmental stress [10]. The major function of the cuticle is to prevent water loss from the leaf interior during surface transpiration [11, 12] and can help increase water-use efficiency, especially when water availability is reduced [13]. The cuticles in evergreen plants tend to have a lower permeability than those of deciduous species, reflecting the adaptation in conserving water during dry periods [14]. A thick cuticle is the most reliable trait for drought resistance in four clones of tea [15].

Dendrobium capra showed thicker epidermis cells that were somewhat thickened at the outer wall. Therefore, water loss through transpiration in *D. capra* should be minimised by the thicker upper and lower epidermis, since they are providing more protection against desiccation for the below leaf tissues, especially mesophyll [16, 17, 18].

The primary vascular bundle in *D. capra* was significantly larger than in *D. arcuatum*, due to a larger xylem area, phloem area, and xylem's sclerenchyma (Table 1). The area of phloem sclerenchyma in *D. capra* was not significantly different with *D. arcuatum*. The percentage of xylem and area compared to vascular bundle area in *D. capra* was also significantly different with *D. arcuatum*. The same result was also found in the percentage of phloem area compared to vascular bundle area, which were significantly larger in *D. capra* than in *D. arcuatum*.

Broader vascular bundles, protected with well-developed sclerenchyma cells as showed in *D. capra*, may provide better defense against extreme drought conditions. Primary vascular bundles have a role as the main path in water transport system heading to the leaves, and assimilates from leaves to the other organs. Therefore, primary vascular bundles are a vital component in water distribution to the leaves' mesophyll. Broader xylem sclerenchyma

area in *D. capra* showed a well-developed vascular system, supporting water transportation optimally to the leaf. One of the phloem/xylem sclerenchyma's functions is to provide mechanical protection to the vascular bundles, especially when leaves experienced moderate shrinkage due to desiccation.

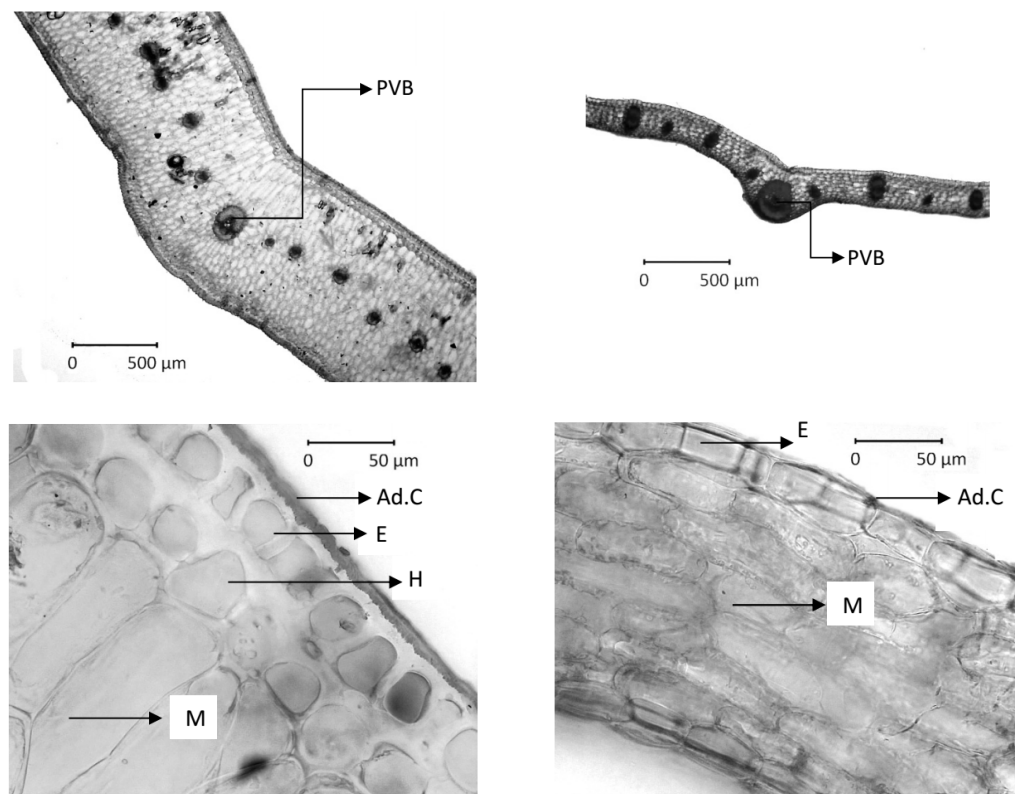


FIGURE 1. Leaf cross section of *Dendrobium capra* and *Dendrobium arcuatum*. (PVB- primary vascular bundle; Ad.C-adaxial cuticles; E-epidermis; H-hypodermis; M-Mesophyll).

TABLE 1. Leaf anatomical comparison between *Dendrobium capra* and *Dendrobium arcuatum*

No	Parameters	<i>Dendrobium capra</i>	<i>Dendrobium arcuatum</i>	P Values
1	Adaxial cuticle thickness (μm)	7.893 a	1.118 b	***
2	Abaxial cuticle thickness (μm)	5.855 a	0.694 b	***
3	Adaxial epidermis thickness (μm)	49.850 a	26.799 b	***
4	Abaxial epidermis thickness (μm)	37.742 a	17.171 b	***
5	Adaxial hypodermis thickness (μm)	50.974	absent	-
6	Abaxial hypodermis thickness (μm)	42.473	absent	-
7	Leaf lamina thickness (μm)	1023.152 a	186.514 b	***
8	Mesophyll thickness (μm)	827.525 a	137.024 b	***
9	Primary vascular bundle (PVB) area (μm ²)	35253.615 a	15977.239 b	***
10	Xylem area on PVB (μm ²)	7863.076 a	4445.720 b	***
11	Phloem area on PVB (μm ²)	1384.692 a	958.746 b	**
12	Xylem sclerenchyma area on PVB (μm ²)	13529.133 a	4232.923 b	***
13	Phloem sclerenchyma area on PVB (μm ²)	7092.365 a	5670.568 a	NS
14	Stomatal density per 1 mm ² (abaxial)	31.838 b	51.010 a	***

NS not significant; * significant at P < 0.05 %; ** significant at P < 0.01 %; *** significant at P < 0.001 %

TABLE 2. Root anatomical comparison between *Dendrobium capra* and *Dendrobium arcuatum* (cross-section).

No	Parameters	<i>Dendrobium capra</i>	<i>Dendrobium arcuatum</i>	P Values
1	Cross sectioned area (μm^2)	1506533.811 a	1126514.394 a	NS
2	Velamen thickness (μm)	334.390 a	193.432 b	***
3	Percentage of velamen (%)	69.008 a	47.484 b	***
4	Percentage of cortex area (%)	17.558 b	28.802 a	***
5	Percentage of stele and endodermis area (%)	6.244 b	15.385 a	***

NS not significant; * significant at $P < 0.05$ %; ** significant at $P < 0.01$ %; *** significant at $P < 0.001$ %

The leaves's lamina of *D. capra* were thicker and more succulent than those of *D. arcuatum* (Table 1). This was mainly caused by the thicker epidermal cells, the presence of hipodermal cells, and the thicker mesophyll layers of *D. capra*. There were a layer of hipodermal cells arranged adaxially and abaxially in *D. capra*, but there were no hipodermal cells in *D. arcuatum* leaves. The lamina thickness of *D. capra* leaves was thicker than that of *D. arcuatum*, which was mainly due to the thicker epidermis cells, presence of hipodermal cells, and thicker mesophyll. Thicker mesophyll containing high density of thin walled parenchyma cells may provide higher capacity in water storage. The capacity of parenchyma tissue in mesophyll to hold moisture or water is essential in providing water supply for the photosynthetic process, especially in dry conditions when external humidity is very low.

The stomatal density of *D. capra* was lower than those of *D. arcuatum* (Table 1) Stomata has the function of controlling the exchange of gases, and most importantly water vapour and CO₂, between the interior of the leaf and the atmosphere [19]. Stomatal density is closely associated with plant transpiration [20]. Plants with lower stomatal density tend to have lower transpiration rates and are usually able to tolerate a more arid environment than plants with higher stomatal density [21]. The lower stomatal density in *D. capra* may allow this species to adapt to more dry environment.

Cross sectioned areas of these two species were not different, suggesting that *D. capra* has a root diameter of relatively the same size as *D. arcuatum*. However, the root's velamen layers in *D. capra* were significantly thicker with a higher percentage than in *D. arcuatum* (Table 2), indicating that velamen tissue in *D. capra* was more developed as an adaptation of the root to face dry environment and high sunlight intensity. Velamen has its function related to water and nutrition absorption, and protecting the cortex from ultraviolet exposure [22, 23]. Moreover, a thicker velamen layer indicates that the roots are capable of absorbing larger amounts of water when it is available, especially in the rainy season. Many African orchid species growing in dry habitats have developed more velamen layers [24].

On the other hand, proportion of cortex and stele in *D. capra* was significantly lower than *D. arcuatum* (Table 2). This reduced cortical tissue improves drought tolerance by reducing the metabolic cost, greater water acquisition and providing a shorter way for water to reach stele [25]. Smaller stele are expected as one of the adaptation strategies to limit the water transport capacity when experiencing extreme drought condition. This adaptation strategy to decrease the stele size was also observed in *Astragalus gombiformis* when treated with drought stress treatment for 30 days [26]. Based on the higher cortex and stele area in *D. arcuatum*, it can be assumed that this species has developed water storage function in root tissues as a strategy for adapting to the moderate dry epiphytic environment with fluctuating humidity. Meanwhile, *D. capra* has developed more absorption and protection function in their roots.

Overall, the results confirmed the hypothesis that the leaf anatomical structure of *D. capra* showed many xeromorphic features linked to reducing water loss and water-use efficiency, which contribute to growth and survival in dry habitats. The divergence in leaf anatomical structures between *D. capra* and *D. arcuatum* reflects adaptations to their growing environments. This study provides evidence of the divergent evolution of congeneric orchids under natural selection and also giving a new approaches to the conservation and cultivation for these two endemic orchid species.

CONCLUSIONS

The leaf and root of *Dendrobium capra* exhibit more developed anatomical features for adapting to drought and dry conditions than in *Dendrobium arcuatum*. Those anatomical features were: thicker cuticle, thicker epidermis, presence of hypodermis, thicker mesophyll, broader primary vascular bundle, well developed xylem's

sclerenchyma, lower stomatal density, thicker and high proportion of velamen. The divergence in leaf anatomical structures between *D. capra* and *D. arcuatum* reflects adaptations to their growing environments.

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