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Epiphytic ferns and orchids adaptation mechanism based on stomatal structure and chlorophyll content in Urban Forest of Jember University, Indonesia

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Abstract

Epiphytic orchids and ferns in urban forests simulating a lowland tropical forest ecology were studied. This study aimed to provide information about the epiphytes' adaptation to living in a dynamic microclimate of the tree canopy. Five epiphytes from the two taxa (orchids and ferns) were observed in their stomatal structure and chlorophyll contents. The stomatal type, size, and density were analysed with microscopic observation. The chlorophyll content was measured using a spectrophotometer. The orchids developed different mechanisms for water conservation compared to the ferns. Orchids develop smaller sizes and denser stomata to support photosynthesis rate as they had water absorbers and storage organs. On the other hand, ferns tend to avoid drought stress by developing fewer numbers and sparse stomata. The higher chlorophyll content in ferns was for photosynthesis efficiency purposes.

Keywords: chlorophyll, epiphytes, ferns, orchids, stomata

Introduction

The presence of epiphyte domination on the surface of tree trunks and branches is a unique characteristic of tropical forests. Those epiphytes developed survival strategies to high exposure to sunlight, humidity, and a stable temperature range (Petter et al., 2016; Woods et al., 2015). The epiphytes live with non-parasitic on the host trees (Bhatt et al., 2015). In established urban forests, trees and epiphytes contributed to the absorbed air particle pollution and influenced atmospheric quality (Tan et al., 2022). Epiphytes play a role in shaping complex habitats and forms, such as nutrient cycling, water retention, and microclimate protection. The vegetation of epiphytic ferns and orchids was reported as habitats for several plants and animals that increased the species diversity and ecosystem balance (Seidl et al., 2020).

The largest epiphytic taxa in tropical Asia were orchids and ferns (Pittermann et al., 2013; Wang et al., 2016). Both taxa occupy three trunks rapidly as they produce a high number of reproductive structures. The ferns produce spores, while orchids produce light seeds that both are dispersed by wind and germinate on the bark (Silvera et al., 2016). The orchids commonly have a long juvenile period, low growth rate and photosynthesis performance (Zhang et al., 2018). Orchids differ from ferns in environmental adaptation by having succulent leaves with thicker cuticles, smaller size and higher density of stomata. In contrast, ferns have thinner leaves, lower water content and water use efficiency (Hietz et al., 2022), reduced stomatal density, short stipes (Campany et al., 2021), hair and scale protector, and humus collection structure (Nitta et al., 2020). The stomata contribute to the photosynthesis balance with their pore as the water vapor and carbon dioxide uptake organ (Cheng and Raissing, 2023). The stomatal types refers to the shape and arrangement of the stomatal subsidiary cells (Rudall and Knowles, 2013). The recent publication purpose the roles of two stomatal types among the grasses and succulent (Cheng and Raissing, 2023).

Some tree species in the urban forest of Jember University have been occupied by ferns and orchids epiphytes. The species diversity of epiphytes ferns and their potential for medicinal purposes were reported (Komaria, 2015; Rahmawati, 2018). Altogether trees and ferns report With the increase of CO_2 in the atmosphere of the campus area, the vegetation might be producing higher biomass that potentially damages the host tree (Silvera et al., 2016). The ecological contribution and vegetation values of the epiphytes in the urban forest are still less studied. Since the potential alternation of three canopies in the urban forest is at a higher risk than the natural forest (Groffman et al., 2017). The information about the adaptation of the epiphytes in this dynamic environment would be beneficial for the long-term epiphytes and the host tree conservation. Therefore, it is crucial to figure out the adaptation mechanism of the epiphytes in the campus area for the vegetation conservation and management strategies. This research was conducted to determine the structure of stomata and adaptation mechanism as indicated by the chlorophyll content of epiphytic orchids and ferns in the urban forest of the Jember University Campus area.

Materials and Methods

Material

The leaves sample of five epiphytes, i.e., *Asplenium nidus* L., *Microsorum punctatum* (L.) Copel., *Dendrobium linearifolium* Teijsm. & Binn., *Liparis resupinata* Ridl., and *Vanda tricolor* Lindl. Were collected from the urban forest in the University of Jember area, Jember district, East Java Province, Indonesia. It is situated at tropical lowland area and is surrounded by Mount Argopuro on the west side and Mount Raung on the east side. A total of 9.905 tree stands were reported from 80 species (Qomah, 2015).

Stomatal analysis

The stomatal type was analysed by anatomical observation of the healthy and mature leaves sample at the middle area of the blade. A drop of clear nail polish was applied to the abaxial and adaxial leaves' surfaces to make a leaf surface imprint. After left for drying, plastic tape was attached to the nail print to attach and peel them off. The tape then was mounted on a microscope slide and analysed using a Nikon Eclipse L100LED MVR microscope with 40x magnification. The stomatal type definition was according Rudall et al. (2013). A total of five positions in the same area size (0.3 mm^2) of the four biological replicates measurements were conducted for stomata density analysis per individual (Clemens et al., 2022).

Chlorophyll analysis

The contents of chlorophyll a and b were determined according to Wang et al. (2022). Leaves sample of 0.1 gram were chopped with a razor blade dan suspended in 10 ml ethanol 96% for 3 days in dark condition. Afterwards, 100 µl suspension was measured with a spectrophotometer in optical density of 665 and 649 and alcohol 96 % as the blank control. The contents of chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll were respectively calculated according to Wellburn & Lichtenthaler (1984) with the following formulae:

$$\text{Chl a} = 13.95 \times OD665 - 6.88 \times OD649$$
$$\text{Chl b} = 24.96 \times OD649 - 7.32 \times OD665$$
$$\text{Chl(a+b)} = 18.08 \times OD649 + 6.63 \times OD665$$

Statistical analysis

Data were analysed with R version 4.1.2 for Windows (R Foundation for Statistical Computing). Statistical data visualization was using ggplot2 (Wickham, et. al. 2016). Significant differences were analized with the non-parametric test (Kruskal Wallis) and the posthoc with Duncan's used Package 'agricolae' (de Mendiburu & de Mendiburu, 2019).

Results and Discussion

Stomatal type dan density

The picture of the stomata among five epiphytes were presented in Figure 1. A total of four stomatal types were identified. They are anomocytic, anisocytic, cyclocytic, and paracytic (Fig. 1a). The two orchid species, *V. tricolor* and *L. resupinata*, have a similar type of anomocytic stomata, while *D. linearifolium* has a paracytic stomatal type. On the other hand, the two ferns have different stomatal types, i.e., *A. nidus* has a cyclocytic type but the *M. punctatum* has an anisocytic type. The stomatal type is categorised by the shape and formation of the subsidiary cell. The anomocytic type is characterized by the absence of subsidiary cells. The anisocytic type has three subsidiary cells with another one having a smaller size. The cyclocytic stomata have four subsidiary cells arranged in a ring formation. The paracytic stomata have two subsidiary cells that are arranged in parallel to the axis of guard cells (Rudall et al., 2013). The orchids family were reported to have an anomocytic and paracytic stomatal type (Rudall et al., 2017), whereas the paracytic is the most observed stomatal type among Angiospermae (Rundall, 2023). On the other hand, ferns were reported to have all types of stomata (Van Cotthem, 1970). The subsidiary cells support the guard cell movement for the opening and closure of the stomata. The absence of the subsidiary cells reduced the stomata's sensitivity to light (Harrison, et al. 2020). The *V. tricolor* and *L. resupinata* might be less sensitive to light fluctuation and their anomocytic stomata were better adaptive for the denser canopy of big trees inside the natural forest. The rest of the three stomatal types had several subsidiary cells that might better adaptive in light fluctuating environments.

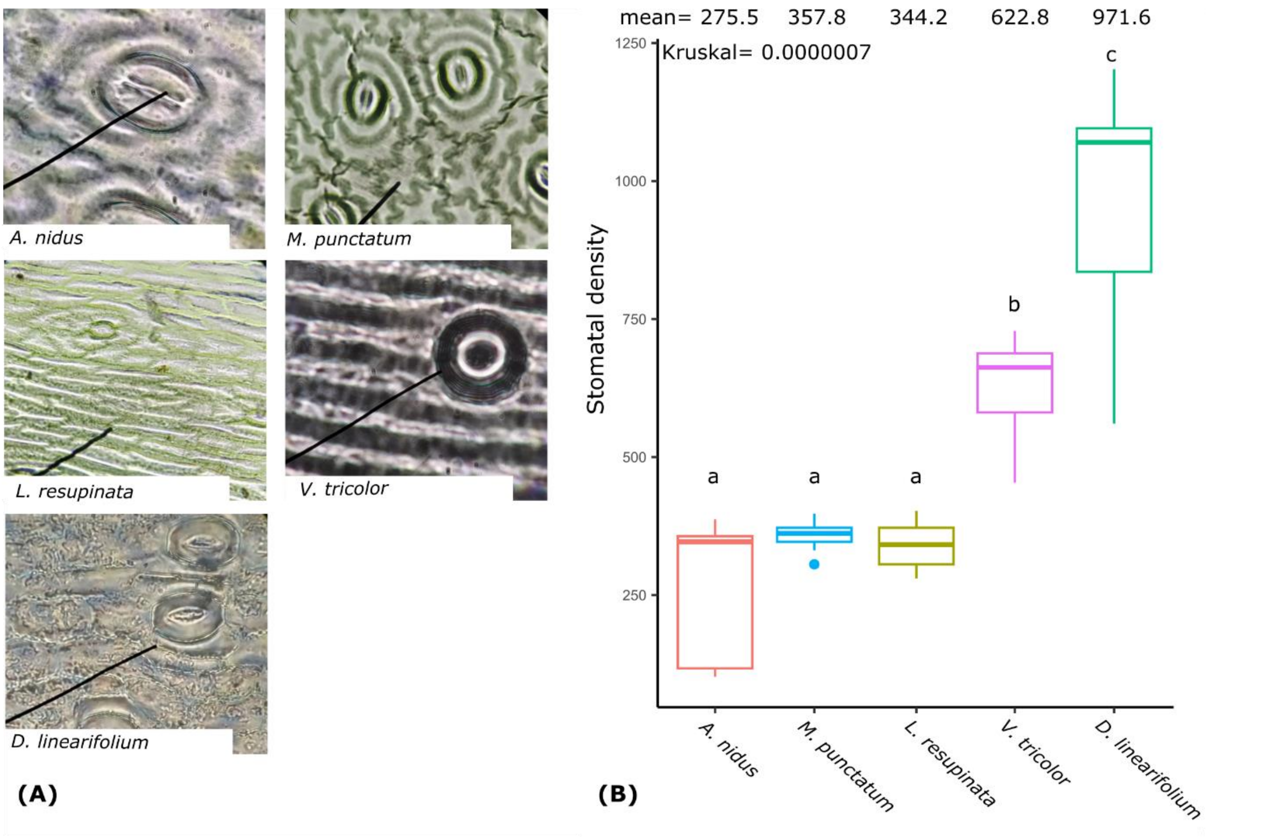

Figure 1. The Stomatal shape and density among five epiphytes: (A) The stomatal shape; (B) stomatal density

Two orchids, *V. tricolor* and *D. linearis*, had a higher stomatal density compared to three plants in this study (Fig. 1b). Duncan's test confirmed significant differences among the species, with the highest density observed in *D. linearifolium* followed by *V. tricolor*. Epiphytic ferns and orchids play an important role in the forest canopy ecology (Mendieta-Leiva et al., 2020; Shen et al., 2022). The tropical forest is the hot spot of epiphytes which are characterized by high humidity, and frequent precipitation, but the fast canopy dries out required adaptation for the water limitation. Ferns develop different strategies compared to the orchid in water deficiency adaptation. The epiphytic ferns forms leave, stem, scales and another vegetative organ to avoid drought (Campany et al., 2021). On the other hand, orchids develop specific organs for drought tolerance mechanisms, i.e., they have velamen on the root for fast water uptake, succulent leaves and pseudobulb for water storage, thick leaves with a dense cuticle to reduce water loss (Zhang et al., 2018). In this study, we confirmed the anatomical structure of stomata as an adaptation strategy to water stress in three orchids and two ferns. Ferns have a lower number of stomata to reduce water loss. In contrast, orchids have a higher number of stomata to support photosynthesis. This result on orchids was related to the other study reported by Hietz et al. (2022).

A. nidus is a C3 fern that is adaptative to water stress by altering transcriptomic factors, especially in the regulation of lateral root formation (Zhang et al., 2018). These ferns were found widespread on *Samea saman* upper canopy cooccurrence with the *M. punctatum* on the lower canopy position. Both ferns have simple shapes, big sizes, and coriaceous leaves. This leaves texture advantages for water scarcity, while, the large size of the leaves supports the light exposure. The stomatal type of cyclocytic and shade condition in *A. nidus* might influence the density of the stomata across the abaxial leaves, as the variant data of the stomatal density was wider in our box plot (Fig. 1b). Further study will be useful to compare the density of stomata in this species across the different light intensity habitat, even though it was found a homogenous data of the stomatal density from the paracytic stomatal type in *M. punctatum*. For the long-established epiphytic ferns in the urban forest of Jember University, *the M. punctatum* and *A. nidus* might develop fewer stomata due to the higher CO_2 through the urban forest. The study of Harrison et al. (2020) implied that the escalation of CO_2 reduced the stomatal density.

Epiphytic orchids in this study have two distinctive leaves. The succulent leaves of *V. tricolor* and *L. resupinata* had a different number of stomata, while the coriaceous compound leaves of *D. linearifolium* had the highest stomata number. The lowest number of stomata in *L. resupinata* among other orchids might be related to the total leaves number per individual, as only a total of three leaves per individual. This species had been reported to occupy a wide range of habitats with a stable morphological characteristics (Tetsana et al., 2014). Overall, Angiospermae has a higher stomatal density compared to the older taxa of Pteridophytes which might be related to the evolutionary adaptation to CO_2 as reported by Haworth et al. (2023).

Chlorophyll content

It was found that ferns had more chlorophyll content than orchids (Table 1). The chlorophyll content of thick leaves orchids was lower compared to the thin leaves' epiphytes. Orchids *D. linearifolium* had more than double chlorophyll a, b, and total than the *V. tricolor* and *L. resupinata*. *M. puncatatum* had the highest chlorophyll than other epiphytes in this study.

Table 1. Chlorophyll content of five epiphytes in urban forest

Plant group	Species	Chlorophyll		
		a	b	total
Orchids	*V. tricolor*	1.92^e	2.19^d	4.11^e
	L. resupinata	3.37^d	1.79^e	5.17^d
	D. linearifolium	8.59^b	4.65^c	13.3^b
Ferns	*A. nidus*	6.62^c	6.25^b	12.9^c
	M. punctatum	10.3^a	10.3^a	20.7^a

Different in letter a, b, c, d, e indicate statistical significant difference (Duncan, $p<0.05$)

Chlorophyll is the main chloroplast component for photosynthesis. Drought stress can reduce the chlorophyll content by chlorophyll degradation (Anjum et al., 2026). Such a higher content of chlorophylls in ferns compared to the orchids was an adaptive mechanism to survive in severe stress conditions. With a higher number of chlorophylls, the water deficit effect might be less suffering the chlorophyll contained in the leaves. Therefore *A. nidus* is still able to survive under three weeks of water stress with stable chlorophyll content as reported by Suhaimi & Cicuzza (2020). *M. punctatum* with the highest chlorophyll content might be more adaptive to drought stress than other studied epiphytes.

Orchids were reported to have long-shaped and thick leaves with lower photosynthetic capacity per leaf area compared to ferns (Cardelús and Mack 2010). The thin-leaf orchid, *D. linearifolium*, that have more chlorophyll than the other two succulent orchids might be due to the water content inside the leaves. Having succulent leaves is a strategy to survive through water deficit. It has a high amount of *hydrenchyma*, an enlarged spongy structure to store water in succulent leaves. The thick leaf was also related to the photosynthesis type of the Calvin cycle, i.e., C3, C4, and CAM. The epiphytes with limitation of water condition majority follow CAM or C3 type, whereas other commercial crops such as sugarcane and corn which can combat photorespiration follow C4 (Wang et al., 2008). The succulent was associated with δ13C values in the CAM range, although thin leaves orchids were also reported to have CAM pathways (Silvera et al., 2016).

The two ways adaptation strategy of ferns and orchid to survive as an epiphyte in this study were indicated by the stomatal density and chlorophyll contents. Ferns have a lower number of stomata to avoid water loss and protect photosynthesis with a higher amount of chlorophyll. On the other hand, orchids which were evolutionary and more adapt to the current condition related to the lower CO_2 in the atmosphere, developed morphological structure and physiology mechanisms to avoid water stress. Better water storage in orchids than in ferns might be more efficient for supporting a lower amount of chlorophyll in performing CAM. Further study by evaluation of chlorophyll content along rainy seasons compared to the dry season might be useful to explain the short-term adaptation mechanism of both taxa related to the humidity and water flow in the urban forest of Jember University.

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