Florea: Jurnal Biologi dan Pembelajarannya
Vol. 11, No. 2, November 2024, pp. 40~46
ISSN 2355-6102; e-ISSN 2502-0404 DOI: 10.25273/florea.v11i2.21297

Characteristics of Stomata and Leaf Thickness in Several Liliales Plants

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Article Info

Article history:

Received October 15, 2024
Revised November 26, 2024
Accepted November 30, 2024

Keywords:

Leaf thickness
Liliales
Stomata characteristic

ABSTRACT

This study examine the stomatal characteristics and leaf thickness from six species of the Liliales plants: *Gloriosa superba, Sansevieria trifasciata, Aloe vera, Cordyline terminalis, Pleomele angustifolia,* and *Allium fistulosum*. Stomatal density, type, and distribution were measured to reveal their relationship with leaf morphology. The results showed that *Gloriosa superba* have thin leaves with stomatal density of 148.72/mm² and an index of 0.295. *Sansevieria trifasciata*, with its thick and fleshy leaves, has a lower stomatal density of 15.39/mm² and an index of 0.031. *Aloe vera*, another species with thick leaves, has a stomatal density of 38.47/mm² and an index of 0.067. In contrast, *Cordyline terminalis and Pleomele angustifolia*, which have thin leaves, exhibit higher stomatal densities of 192.31/mm² and 128.21/mm², respectively. Their stomatal indices are 0.092 for *Cordyline terminalis* and 0.163 for *Pleomele angustifolia*. *Allium fistulosum* also have thin leaves, with a stomatal density of 100/mm² and a higher stomatal index of 0.390. The study revealed that species with thicker leaves tend to have lower stomatal densities, a feature that helps reduce water loss in arid condition. These findings contribute to a deeper understanding of plant adaptation mechanisms and have implications for improving water-use efficiency in agriculture and conservation efforts.

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1. INTRODUCTION

Stomata are small, movable pores found on the surface of most aerial parts of higher plants, especially leaves. Stomata facilitate gas exchange, including the uptake of carbon dioxide (CO_2) required for photosynthesis and the release of oxygen (O_2) and water vapor [1]. The main function of stomata is to regulate the internal environment of the plant by controlling the rate of gas diffusion based on the concentration gradient and the resistance to diffusion through the stomata [2]. Stomata play an important role in controlling the entry of CO_2 for photosynthesis and the exit of O_2. This regulation is important for maintaining the overall carbon status and growth of the plant [1], [2]. Stomata also play an important role in transpiration, which is the process by which water vapor is lost from plants to the atmosphere. This process is important for nutrient transport and temperature regulation within plant tissues [3]. Stomatal behavior is influenced by various environmental factors such as light, CO2 concentration, humidity, and temperature. These factors affect the size of the stomatal opening, thus affecting the rate of photosynthesis and transpiration [2].

Stomatal characteristics, including their density, size, and distribution on the leaf surface, vary widely between plant species and can even be affected by environmental conditions within the same species [4], [5]. Stomatal density is the number of stomata per unit leaf surface area, and it can directly affect a plant's efficiency in gas exchange and water regulation. Similarly, the size and distribution of stomata affect how efficiently a plant can take up CO_2 for photosynthesis while minimizing water loss[6], [7]. These stomatal traits are important indicators of how plants adapt to their habitat, especially in response to stressors such as drought, high temperatures, and variable light conditions [8], [9].

The order Liliales plants include a large group of flowering monocotyledonous plants. Its members are widely distributed and show significant diversity in growth form, habitat preferences, and leaf morphology. Liliales plants are characterized by their linear or strap-like leaves, which can vary in width, length, and overall shape depending on the species [10]. This variation in leaf morphology is often accompanied by different ecological strategies and physiological responses to environmental factors. Despite their shared traits, Liliales plants exhibit a variety of stomatal characteristics that likely contribute to their adaptability across ecological niches [8], [11].

Variation in leaf morphology within Liliales raises the question of whether leaf shape is related to specific stomatal characteristics. For example, narrow and elongated leaves may have different stomatal densities and distributions compared to wider leaves due to differences in surface area and the need for efficient water use. Understanding the relationship between leaf shape and stomatal properties is essential for exploring how Liliales plants balance their physiological processes, such as photosynthesis and transpiration, under varying environmental conditions. Understanding the relationship between stomatal characteristics and leaf shape in Liliales plants is not only relevant to basic plant physiology but also to broader ecological and environmental research.

This study aims to examine the stomatal characteristics of different Liliales species and investigate the relationship between these characteristics and leaf morphology. By analyzing the density, size, and distribution of stomata in leaves with different shapes, we seek to determine whether certain leaf shapes are associated with certain stomatal properties. This study will focus on a selection of Liliales species with diverse leaf shapes, providing a comprehensive analysis of the potential relationship between stomatal characteristics and leaf morphology. The findings of this study may provide insight into how plants within the Liliales adapt to different environmental conditions through variations in leaf morphology and stomatal function. This knowledge could be applied in areas such as agriculture, where optimizing plant water use is critical in the face of climate change and water scarcity. Furthermore, this study could contribute to conservation efforts by highlighting adaptive traits that allow certain species to thrive in the environment.

2. METHOD

2.1 Types and Samples of Research

This is a descriptive study of plants from Liliales. The study consists of six species, *Gloriosa superba, Sansevieria trifasciata, Aloe vera, Cordyline terminalis, Pleomele angustifolia, and Allium fistulosum.* The sample plants were collected from Teluk Segara District, Bengkulu City.

2.2 Determination and preparation for stomata observation

Stomata observations were carried out on the middle part of the 3rd or 4th leaf from the upper tip of the branch or stem of the plant, which had fully developed [12]. Stomata observations were carried out using the replica method on the abaxial part of the leaf because the main surface of the stomata in this plant is located as shown in the preliminary study. Fresh leaf samples were washed and cleaned to remove dirt and leaf hairs or trichomes. The lower surface of the leaf sample was coated with cellulose acetate or nail polish and then waited for about 10 minutes to dry. The dried sample was evenly coated with clear tape. Carefully remove the tape, then stick it on a glass object, smooth it out, and label it with the name of the plant species.

Observation of the number of stomata per field of view using a microscope with a magnification of 400 times. Observations were made on three leaves collected for each plant. Three plants were used for each species studied in this study. Thus, nine leaves were collected from three plants belonging to one plant species, so the total number of leaves collected from six species of Liliales plants was 54 leaves.

2.3 Stomatal distribution, type, density, and index

The types and distribution of stomata were observed under a light microscope. The distribution patterns of stomata are divided into three groups, uniform, random, and aggregate distribution [13]. Detection of stomata distribution patterns and calculation of the number of stomata using micrographs with a magnification of 400x, filtered, and determined using ImageJ version 1.54i (National Institute of Health, USA). Stomatal density (SD) is the number of stomata in square mm of leaf area, which is recorded by observing the abaxial leaf traces under a microscope and counting the number of stomata in the field of view at a magnification of 400x. The number of epidermal cells and stomata were counted on the same slide prepared for SD observation. The stomatal index (SI) is determined by dividing the number of stomata by the number of stomata plus epidermal cells multiplied by 100. The formula is SI (%) = (S/S+E) x 100, where SI is the stomatal index, S is the number of stomata, and E is the number of epidermal cells, calculated in a microscopic field of view at 400x magnification [12]. The unit area of the field of view at 400x magnification is equivalent to 0.1372 mm^2 and the diameter of the field of view is 0.418 mm.

2.4 Data analysis

The relationship between parameters of the number of stomata, such as density, and index to leaf shape was analyzed descriptively.

3. RESULTS AND DISCUSSION

3.1 Types and Distribution of Stomata in Some Liliales Plants

Figure 1 shows the leaf shapes of the six Liliales plant species in this study.

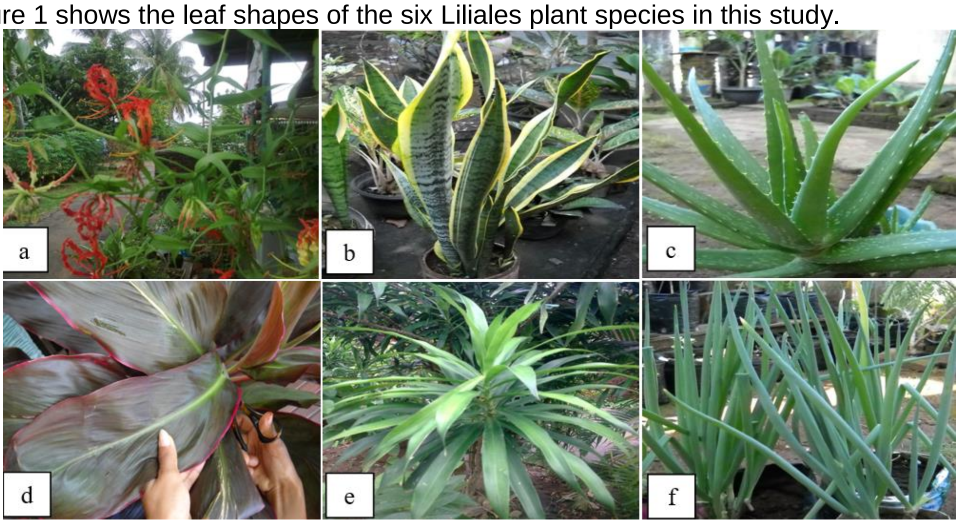

Figure 1. Liliales plants have a diversity of leaf forms among the six species observed during this research. a. *Gloriosa superba; b. Sansevieria trifasciata; c. Aloe vera; d. Cordyline terminalis; e. Pleomele angustifolia; e. Allium fistulosum.*

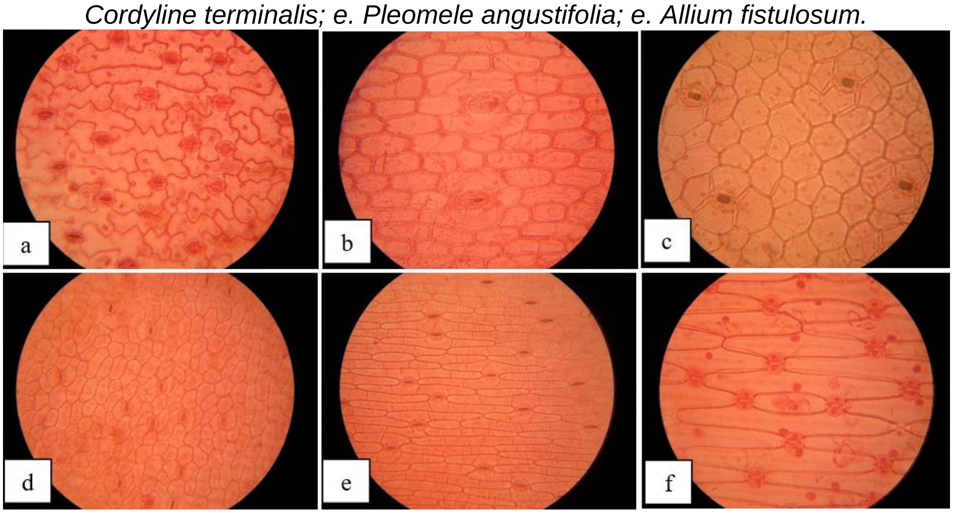

Figure 1. Variation of stomata type and distribution of Liliales leaves. a. *Gloriosa superba; b. Sansevieria trifasciata; c. Aloe vera; d. Cordyline terminalis; e. Pleomele angustifolia; e. Allium fistulosum.*

Table 1. Type and distribution of stomata in stomatal density in six Liliales species.

No	Species Name	Stomata Types	Distribution of Stomata
1.	*Gloriosa superba*	Anomocytic	Line up in a row
2.	*Sansevieria trifasciata*	Parasitic	Line up in a row
3.	*Aloe Vera*	Actinocytic	Scattered randomly
4.	*Cordyline terminalis*	Anomocytic	Scattered randomly
5.	*Pleomele angustifolia*	Parasitic	Line up in a row
6.	*Allium fistulosum*	Parasitic	Line up in a row

The results of the study presented in Table 1 show that Liliales plants have various types of stomata. *Gloriosa superba* has stomata whose distribution is parallel with the anomocytic type. The morphological characteristics of the leaves are parallel leaf veins, thin and soft, smooth surface with flat edges, pointed leaf tips, leaf length 8-25 cm, width 1-4 cm, oval, green. *Sansevieria trifasciata, Pleomele angustifolia* and *Allium fistulosum* have stomata whose distribution is parallel with the parasitic type. *Sansevieria trifasciata* has hard, very thick and fleshy leaves, parallel veins, succulent, smooth leaf surface with flat edges, leaves tapering to the tip with a leaf length of 50-75 cm with a combination of green and yellow leaf colors. *Pleomele angustifolia* has thin leaves with parallel veins, smooth leaf surface with flat edges, and elongated. *Allium fistulosum* has thin and soft fleshy leaves, parallel leaf veins, pointed tips, leaf base hugs the stem, leaf length ± 30 cm with a width of ± 5 cm, green leaves. *Aloe vera* has stomata whose distribution is randomly distributed with an actinocytic type. The morphological characteristics of the leaves are parallel leaf veins, thick fleshy, with a green and cool color, and slimy, smooth leaf surface with prickly edges, pointed tips, leaf length 15-37 cm and width 2-6 cm. *Cordyline terminalis* has stomata whose distribution is randomly distributed with anomocytic type. The morphological characteristics of the leaves are pinnate leaf veins, thin, smooth leaf surface with flat edges, elongated, pointed leaf base and tip, leaf length 20-60 cm leaf width 10-13 cm, reddish green leaf color.

Liliales, like other monocots, have different types of stomata. These types include anomocytic, parasitic, and tetracytic, to name a few. The parasitic type is considered primitive and is the common ancestor of the other types of stomata, which evolved from it. This diversity is not only a result of evolutionary adaptation, but also plays a role in the taxonomic classification [14]. Anomocytic types of stomata are characterized by the absence of subsidiary cells surrounding the guard cells. This is one of the types of stomata observed in Liliales, as noted in the broader context of monocots [15]. Parasitic stomata have a pair of daughter cells flanking the guard cells. Although more common in Magnoliopsida, parasitic stomata are also found in some monocots, including Liliales [14], [16]. Anisocytic stomata, usually having three daughter cells, anisocytic stomata are less common in Liliales but are part of the diversity observed in monocots [14].

The diversity of stomatal types in the Liliales reflects evolutionary adaptations and genetic regulation that allow these plants to thrive in a variety of environments. Stomata, which are essential for gas exchange and water regulation, shows significant variability in size, density, and structure across species within Liliales, contributing to physiological functions and taxonomic classification. This diversity is shaped by evolutionary processes, genetic factors, and environmental pressures, highlighting the adaptability of these plants [17].

Morphological variability in *Lilium rosthornii*, stomatal length and width are affected by ploidy level, indicating an adaptive mechanism to increase water consumption efficiency. Plant leaf structure, including significant leaf thickness and defensive adaptations, indicates resilience to environmental stress [18]. Similarly in *Allium tuberosum* , stomatal size and chloroplast number increase with ploidy level, although stomatal density is not directly correlated with ploidy, indicating a complex adaptive response to polyploidization [19].

The distribution of stomata in the Liliales is a complex topic that involves understanding the diversity of stomatal structures and their distribution across species. Stomatal distribution can vary greatly depending on the position on the leaf. For example, in monocotyledonous plants such as *Chlorophytum comosum*, the upper part of the plant shows a higher distribution of stomata compared to the middle and lower parts [20]. In a study of Lilium species, *L. carniolicum* was found to have hypostomatic leaves, i.e. stomata only on the lower surface, while *L. bosniacum* showed a transitional type towards amphistomatic leaves, i.e. stomata on both surfaces [21].

The development of stomatal types in Liliales is influenced by phylogenetic relationships and developmental pathways. The presence of different stomatal types can be associated with evolutionary adaptations [14], [15]. Environmental and Morphological Influences The distribution of stomatal is also influenced by environmental conditions and plant morphological characteristics. For example, the length of epidermal cells can affect the likelihood of stomatal occurrence [17].

Liliales, like other monocots, have a variety of stomata types, including those with multiple daughter cells. This diversity reflects the adaptation strategies of these plants to different environments [22]. Variation in stomata types and their distribution can be used as taxonomic characters to distinguish between species and understand phylogenetic relationships within Liliales [22]. Although stomatal distribution in Liliales is influenced by a variety of factors, it is important to note that these patterns are not unique to this order. Similar variations are observed across plant orders, indicating complex interactions between genetic, environmental, and evolutionary factors that shape stomatal distribution. Understanding these patterns in Liliales may provide insights into broader evolutionary strategies in monocots.

3.2 Stomatal Density, Stomatal Index, and Leaf Thickness

Stomatal density in plants, including those belonging to Liliales, is an important factor affecting gas exchange and water regulation. Although specific studies on Liliales are limited, insights can be gained from broader studies on stomatal density across plant groups. These findings contribute to a comprehensive understanding of stomatal density, its regulation, and its implications for plant physiology and adaptation. Table 2 shows the stomatal density, stomatal index, and leaf thickness morphology of 6 species of Liliales.

Table 2. Stomatal density, stomatal index, and leaf thickness morphology of 6 Liliales species.

No	Species Name	Stomatal Density (in mm^2)	Stomatal Index	Leaf Thickness
1.	*Gloriosa superba*	148.72	0.295	Thin
2.	*Sansevieria trifasciata*	15.39	0.031	Thick and fleshy
3.	*Aloe Vera*	38.47	0.067	Thick and fleshy
4.	*Cordyline terminalis*	192.31	0.092	Thin
5.	*Pleomele angustifolia*	128.21	0.163	Thin
6.	*Allium fistulosum*	100.00	0.390	Thin

Plants such as *Sansevieria trifasciata* and *Aloe vera* have thicker leaves that are described as “thick-fleshy”. These leaves are typically adapted to conserve water, especially in arid or semi-arid environments. These species exhibit low stomatal densities. *Sansevieria trifasciata* has a stomatal density of 15.39/mm^2, and *Aloe vera* has a stomatal density of 38.47/mm^2. Lower stomatal densities may help reduce water loss through transpiration, which is especially important in drought conditions. The thick-fleshy nature of these leaves allows them to store water, so they are less dependent on frequent gas exchange (for photosynthesis and transpiration), thus requiring fewer stomata. Plants such as *Gloriosa superba, Cordyline terminalis, Pleomele angustifolia,* and *Allium fistulosum* have thinner leaves. These species tend to have higher stomatal densities. For example, *Cordyline terminalis* has 192.31/mm^2, and *Allium fistulosum* has 100.00/mm^2.

Stomatal index is the proportion of stomata relative to total epidermal cells which follows a somewhat similar trend. *Sansevieria trifasciata* (thick leaves) have the lowest stomatal index (0.031), while *Allium fistulosum* (thin leaves) has the highest stomatal index (0.390). This further strengthens the idea that thicker leaves, which require less gas exchange, will have fewer stomata compared to thinner leaves.

Thick and fleshy-leaved plants, such as *Sansevieria trifasciata* and *Aloe vera* in this study showed fewer stomata due to adaptations that optimize water retention and minimize water loss in dry environments. This adaptation is essential for survival in water-stressed conditions and involves complex interactions between morphological and physiological traits. The surface-to-volume (S/V) ratio is an important factor in the adaptation of thick and fleshy-leaved succulent plants. A lower S/V ratio, achieved through increased leaf thickness, reduces the surface area available for transpiration, thereby conserving water. Succulents have evolved to store water in their thick leaves, allowing them to survive prolonged drought. This water storage ability is essential for maintaining cellular function during the dry season [22].

Stomatal density is inversely related to leaf thickness in many succulent plants. Fewer stomata reduce water loss through transpiration, which is a significant advantage in dry environments [23]. Regulation of stomatal density and aperture is a key mechanism for optimizing water use efficiency. In agave plants, stomatal aperture is primarily influenced by temperature, with stomata closing during the hottest part of the day to minimize water loss [3]. Succulents with thick, fleshy leaves often utilize Crassulacean Acid Metabolism (CAM) photosynthesis, which allows them to open their stomata at night when temperatures are cooler and humidity is higher, reducing water loss while still allowing CO_2 uptake. [24]. This adaptation is important to maintain photosynthetic activity while minimizing water loss, a vital balance for survival in dry conditions.

The evolution of succulent traits, including reduced stomatal density, is a response to the selective pressures of dry environments. These traits are part of a broader strategy to maintain hydration and ensure survival during long periods without water [25]. The coordination of leaf and stem traits in succulent plants such as agave reflects a complex strategy to regulate water loss and tolerate drought. This includes not only reduced stomatal density but also other morphological adaptations such as increased leaf mass per area. Although reduced stomatal number is a major adaptation for water conservation in succulents, it is important to note that this trait may also limit the plant's ability to absorb CO_2, potentially affecting photosynthetic efficiency. However, compensation is generally beneficial in dry environments where water conservation is paramount. Furthermore, the evolution of these traits is not solely driven by environmental factors but also involves genetic and developmental processes that influence stomatal development and distribution [26]. Understanding these complex interactions is critical to predicting how plants may adapt to climate change and to developing crops that are more resistant to drought conditions.

4. CONCLUSION

The results showed variations in stomatal types (such as anomocytic, parasitic, and actinocytic) and their distribution patterns (scattered or parallel) in the six Liliales species in this study. The study highlighted the relationship between leaf thickness and stomatal density, where species with thicker leaves, such as *Sansevieria trifasciata* and *Aloe vera*, showed lower stomatal density, an adaptation that helps reduce water loss in dry conditions. In contrast, species with thinner leaves, such as *Cordyline terminalis*, had higher stomatal density to facilitate more efficient gas exchange in water-stressed environments. These findings suggest that variation in stomatal characteristics is key to the adaptive strategies of Liliales plants, allowing them to thrive in diverse environments. This research can contribute to improved agriculture, especially in water management, and offer insights into conservation efforts in the context of climate change.

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