



RESEARCH ARTICLE

# The stomatal characteristics of monocots and dicots at different altitudes

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## Abstract

This study investigates the differences in stomatal characteristics between plants growing in lowland and highland altitudes, focusing on parameters such as stomatal type, distribution, number, density and index. A quantitative descriptive approach was employed, with sample collection conducted through purposive sampling. A total of 144 leaves were collected from 8 species, including both dicot and monocot, originating from low and high-altitude areas. The dicot species investigated were *Capsicum annuum* L., *Mangifera indica* L., *Manilkara zapota* (L.) P. Royen, and *Psidium guajava* L., while the monocot species included *Cocos nucifera* L., *Colocasia esculenta* (L.) Schott, *Curcuma longa* L., and *Oryza sativa* L. Stomatal number, density, and index were statistically analyzed using the t-test, whereas other parameters were assessed descriptively. The results revealed diverse stomatal types and distributions among the examined plant species. For instance, *P. guajava* exhibited paracytic stomata, *C. annuum* L. displayed anisocytic stomata, and *C. esculenta* had a distinct brachy-paracytic stomatal complex of, each plant manifests unique anatomical characteristics. The finding indicated that stomatal attributes, including number, density and index did not differ significantly between plants inhabiting lowland and highland environments within an altitude range of 550 m. This study highlights the influence of broader altitude variations and ecological factors influence stomatal features across diverse plant species.

## Keywords

altitude; dicot; monocot; stomatal density; stomatal index

## Introduction

Stomata are small pores flanked by 2 protective cells, found in greater abundance on young leaves compared to mature leaves and stems. They facilitate gas exchange by allowing the absorption of carbon dioxide (CO<sub>2</sub>), which is essential for photosynthesis and the release of water vapor via transpiration. This exchange allows the movement of water vapor and CO<sub>2</sub> between the plant's internal environment and the atmosphere. The uptake of CO<sub>2</sub> via stomata plays a vital role in the process of photosynthesis within plants (1, 2).

Stomata are surrounded by specialized guard cells, which differ structurally and functionally from the surrounding epidermal cells. General-

ly, stomata are located on the lower surface of the leaves; however, in some plant species, they are found on both leaf surfaces (upper and lower). For example, water lilies have stomata exclusively on the upper leaf surface, an adaptation to their aquatic environment (3).

The shape, type, and distribution of foliar stomata vary across plant species. Stomatal distribution can vary, with some species exhibiting scattered stomata, while others, such as monocots, display rows arranged in parallel (3). In monocotyledonous plants and conifers with needle-like leaves, stomata are typically elongated and organised in parallel rows along the leaf veins (4).

The difference in temperature in the lowlands and highland regions significantly influences the plant growth, development, reproduction and survival of plants in these environments. Both high and low temperatures have a profound impact on plant physiology, affecting flavonoid accumulation, nitrogen balance indices and various physiological and morphological traits (5). Temperature plays a crucial role in regulating photosynthesis, respiration, cell wall permeability, absorption of water and nutrients, transpiration, enzyme activity and protein coagulation (6).

The characteristics of stomatal types are strongly influenced by environmental factors. Numerous studies have demonstrated that conditions such as light intensity, water availability, CO<sub>2</sub> concentration and soil properties play crucial roles in determining stomatal traits. For instance, research on moso bamboo (*Phyllostachys edulis*) forests in subtropical China identified solar radiation and mean annual precipitation as primary factors influencing stomatal traits, with stomatal size playing a central role in plant adaptation to external conditions (7). Similarly, studies on *Podocarpaceae* species found that stomatal size increases under moist and shaded conditions, whereas stomatal density increases in sunny and drier conditions, further emphasizing the role of environmental factors (8).

Elevational gradients influence the availability of CO<sub>2</sub>, a critical factor to conduct photosynthesis. Plants respond to these fluctuating condition by adjusting stomatal density, either as long-term adaptations or an immediate responses to environmental changes. The consistency of stomal type and densities over millions of years suggests an evolutionary adaptation aimed at optimizing gas exchange efficiency. Understanding stomatal parameters, such as stomatal type and distribution, is crucial for studying plant evolutionary processes and their adaptation to different environmental conditions, such as land altitude.

Kepahyang Regency is located in the highland of the Bukit Barisan Mountains in Sumatera, at an altitude ranging from approximately 250 to 1600 m above sea level (ASL). In contrast, Bengkulu city is located in a lowland area with a relatively flat contour. The coastal area of Bengkulu has an altitude ranging from 0 to 10 m ASL, while the eastern part of the city lies at an evaluation of 25 to 50 m ASL.

The comparative analysis of stomatal characteristics is significant in plant physiology, as it provides insights

into how plants adapt to varying altitudes to optimize water use and enhance photosynthetic efficiency. Stomata play a crucial role in regulating gas exchange and water loss, both of which are vital for plant survival and productivity. By examining stomatal characteristics across different plant species at varying altitude, a better understanding can be gained of the adaptive and physiological mechanisms that contribute to the development of climate-resilient crops and advancements in agricultural practices.

Environmental conditions significantly impact plant development, and plants growing at different altitudes exhibit a range of adaptive features that enable them to survive and thrive under varying environmental conditions. These adaptations include structural (9, 10), physiological (11), and biochemical (12) adaptations that allow plants to cope with altitude-related environmental changes. In terms of leaf characteristics and stomatal distribution, previous studies showed that plant leaves respond differentially to varying ecological conditions, including changes in elevation (13–15). However, information regarding stomatal characteristics in plants grown at an altitude range of approximately 600 m is still unclear.

Therefore, this study aimed to examine the stomatal characteristics of dicots and monocot species growing in the lowland of Bengkulu city and the highlands of Kepahyang Regency, Sumatera, Indonesia.

## Materials and Methods

### Plant materials

The plant specimens used in this study included *C. annuum* L. (chili pepper), *M. indica* L. (mango), *M. zapota* (L.) P. Royen (sapodilla), and *P. guajava* L. (guava) as representatives of dicotyledonous plants. Monocotyledonous plants studied were *C. nucifera* L. (coconut), *C. esculenta* (L.) Schott (taro), *C. longa* L. (turmeric), and *O. sativa* L. (rice). All specimens, both dicots or monocots were procured from both lowland and highland altitude locations.

### Research location and sampling method

#### Research sampling

This research employed a quantitative descriptive approach, with sample collection conducted using a purposive sampling technique. The parameters assessed included various stomatal characteristics, such as stomatal type, distribution, number, density (SD) and index (SI), offering a comprehensive analysis of the stomatal features under consideration.

### Determination and preparation for stomata observation

Stomatal observation were conducted on the middle part of the 3<sup>rd</sup> or 4<sup>th</sup> fully developed leaves from the upper end of the plant branch or stem (16). Stomatal observations were performed using the replica method on the abaxial (lower) leaf surface, which was identified as the primary stomatal region based on preliminary studies.

Fresh leaf samples were washed and cleaned to remove dirt and leaf hairs (trichomes). A thin layer of clear nail polish was applied to the lower surface of the leaves and left to dry for approximately 10 min. Once dried, trans-

parent adhesive tape was evenly placed over the coated surface. The tape was then carefully peeled off and affixed to a glass slide, smoothed and labeled with the corresponding plant species name. Stomatal characteristics, including distribution, type, density, and index, were observed using light microscopy at 400x magnification. Observations were conducted on 3 leaves from each plant, with 3 plants sampled per species. As a result, 9 leaves were collected for each plant species, totaling 144 leaves from 8 species at both lowland and highland altitudes.

#### Stomatal distribution, type, density, and index

Stomatal types and distribution were observed under a microscope. Stomatal distribution patterns were classified into 3 groups: uniform, random, and aggregated (17). The pattern of stomatal distribution, along with the calculation of stomata number and density, was determined using image analysing software (ImageJ version 1.54i, National Institute of Health, USA).

Stomatal density (SD) was defined as the number of stomata in mm square area of the leaf surfaces. The stomatal index (SI) was determined using formula:

$$SI (\%) = (S/S+E) \times 100$$

Where SI is the stomatal index, S is the number of stomata, and E is the number of epidermal cells. These parameters

were counted from photo micrograph taken under a microscopy at 400x magnification (16).

#### Data analysis

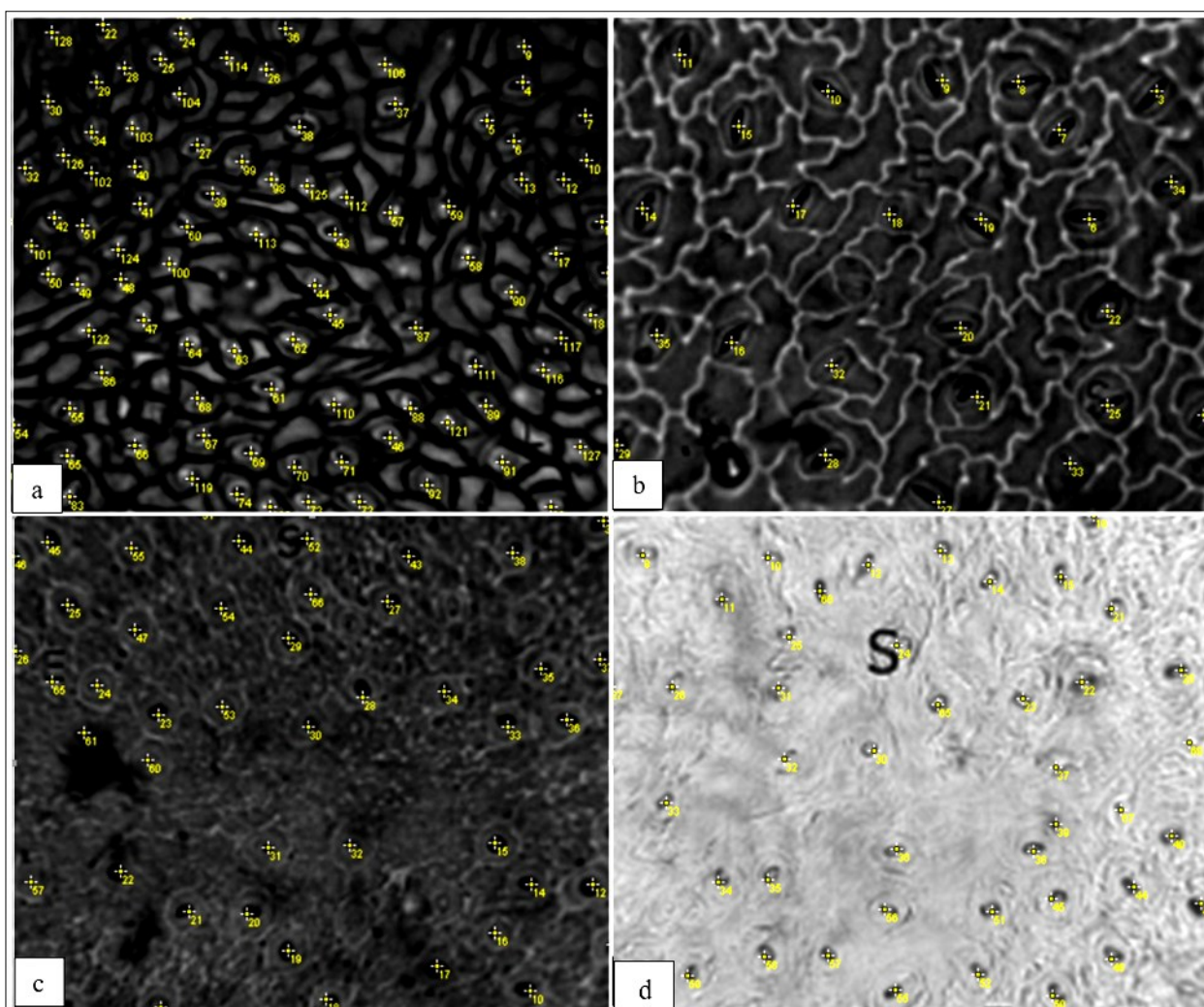
The stomatal number, density, and index of plants growing in low-land and highland habitats were statistically analysed using the t-test. Apart from those parameters, the type and distribution of stomata were analysed descriptively.

### Results

#### The distribution of stomata in several dicot and monocot plants

Fig. 1 illustrates the distribution pattern of stomata in the dicot plants examine in this study. *P. guajava* L. possesses a higher stomatal count compared to *M. indica* L., *C. annuum* L., *M. zapota* (L.) P. Royen, and all monocotyledonous species analysed.

The stomata in *M. indica* and *C. annuum* are uniformly distributed across the abaxial surface of the leaf. *C. annuum* possesses the smallest stomata, which are distributed randomly. Meanwhile, *M. zapota* features small stomata with a distinct distribution pattern, appearing randomly in some areas while exhibiting uniformly in others (Fig. 1d).



**Fig.1.** Distribution of stomata in dicotyledonous plants. **a.** *Psidium guajava*, **b.** *Mangifera indica* L., **c.** *Capsicum annuum*, **d.** *Manilkara zapota*. Microscope magnification is 400x. Determination of stomatal number used ImageJ version 1.54i software (National Institute of Health, USA).

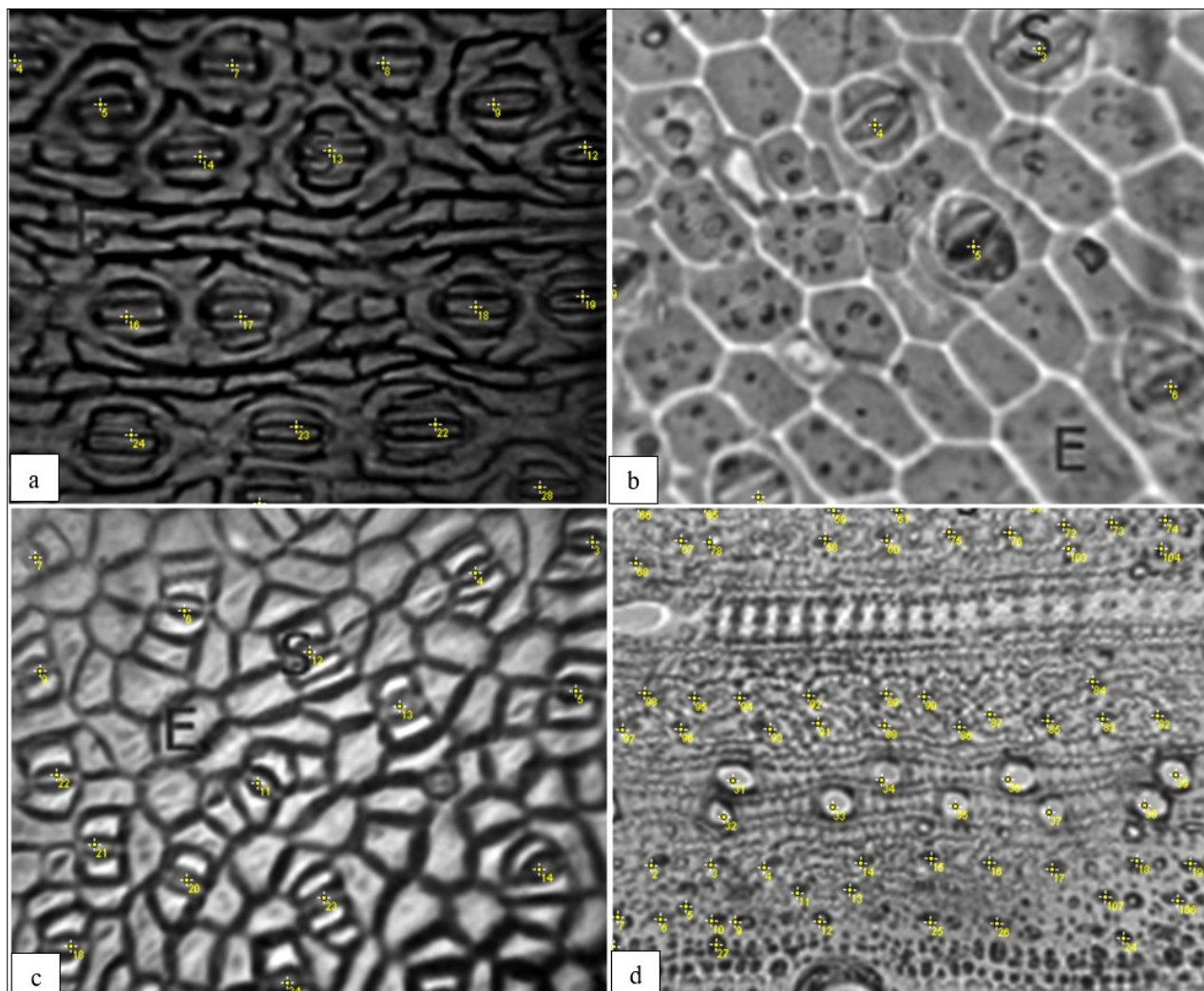


Fig. 2 shows the spatial arrangement of stomata in the monocotyledonous plants analysed in this study. *C. nucifera* L., *C. longa*, and *C. esculenta* have large stomata, in contrast to the small stomata observed in *O. sativa*.

*C. nucifera* exhibits a uniform distribution of stomata, aligned parallel to the leaf blade. In contrast, *C. longa* and *C. esculenta* display a random distribution of stomata. *O. sativa* is characterized by the presence of small stomata

functions, particularly in photosynthesis. *M. indica* typically feature paracytic stomata with lateral subsidiary cells. However, other types, like anomocytic and anisocytic stomata, have also reported (18).

*C. annuum* exhibits anisocytic stomata. However, a previous study reported the presence of tetracytic stomata on its leaves, with anisocytic stomata predominantly on both leaf surfaces (19). Stomata in *C. annuum* are mainly



**Fig. 2.** Distribution of stomata in monocotyledonous plants. **a.** *Cocos nucifera* L., **b.** *Curcuma longa*, **c.** *Colocasia esculenta*, **d.** *Oryza sativa*. Microscope magnification is 400x. Determination of stomatal number used ImageJ version 1.54i software (National Institute of Health, USA).

that are arranged in parallel aggregation in which stomata are distributed in a regular manner at several areas parallel along the leaf axis.

#### **The types of stomata in several dicots and monocots plants grown in lowland and highland habitats**

Fig. 3 illustrates the stomata (S) and epidermal (E) cells in four dicotyledonous species, while Fig. 4 presents the stomata of 4 monocotyledonous plants, both observed in the lowlands and highland environments. The dicotyledonous plants examined in this study include guava, mango, chili, and sapodilla, which were collected from both lowland and highland habitats.

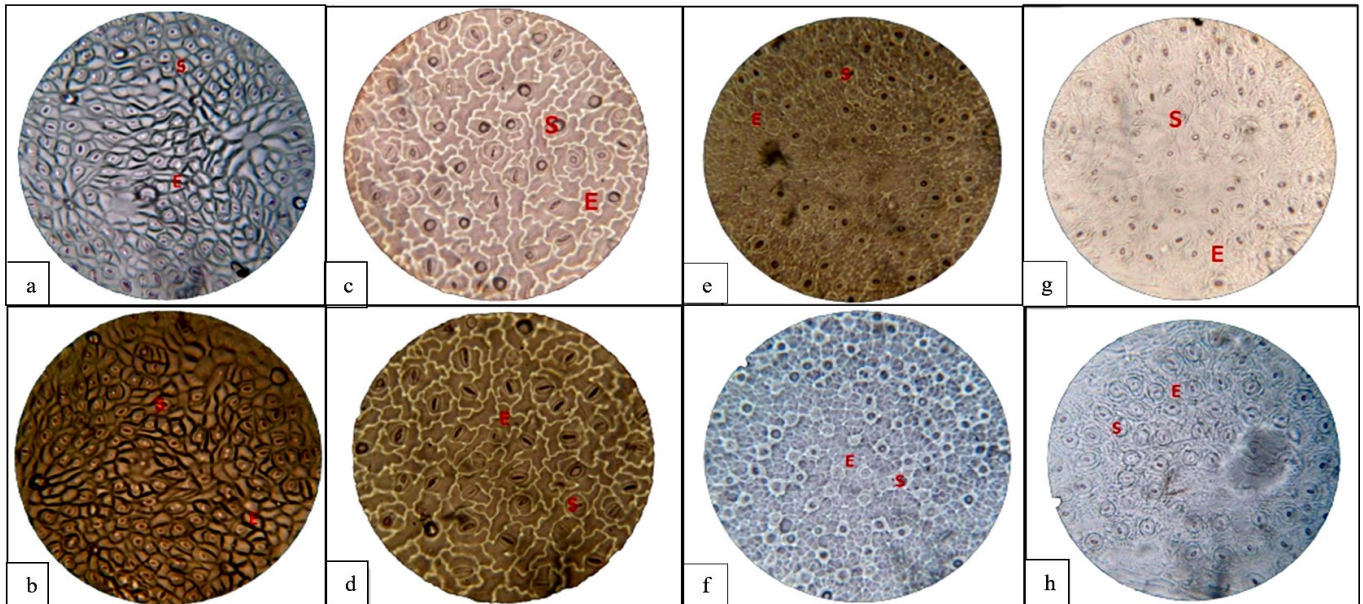
*P. guajava*, commonly known as guava, exhibits paracytic stomata on the abaxial surface of its leaves. These stomata are evenly distributed across the leaf surface (Fig. 1 and 2), contributing to the plant's physiological

located on the abaxial surface, with variations in trichome presence across different varieties. On the other hand, *M. zapota* exhibits a paracytic stomata on its abaxial leaf surface.

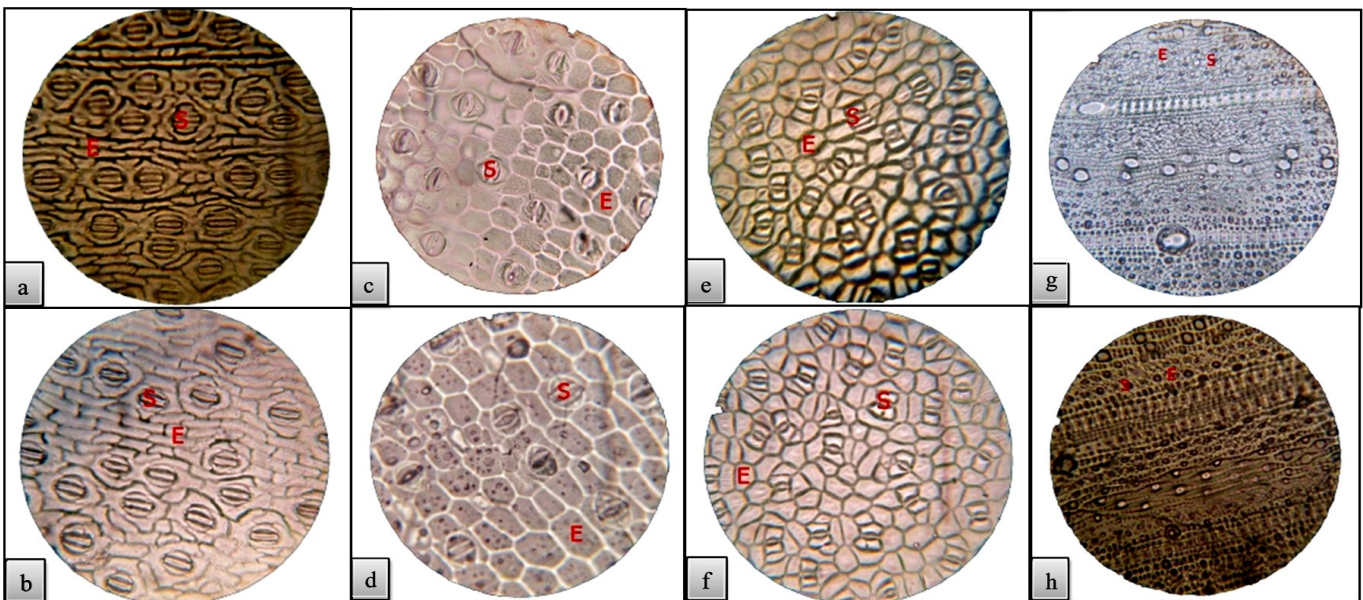
Fig. 4 shows the stomatal characteristics of monocotyledonous plants examined in this study. *C. nucifera* exhibits paracytic stomata with a complex stomatal structure. The stomatal complex is characterized by the presence of 2 guard cells and 4 subsidiary cells, 2 of which are rounded, while the other 2 are positioned laterally to the guard cells. The stomata are located on the abaxial epidermis, arranged in parallel rows, and sunken below the epidermis.

*Curcuma longa* L., commonly known as turmeric, exhibits amphistomatous leaves with varying stomata present on both the upper and lower surfaces, with variations in stomatal frequency. In this study, stomata in





**Fig. 3.** Stomata and epidermal cells in dicotyledonous plants at the lowland and highland locations. **a.** lowland guava; **b.** highland guava; **c.** lowland mango; **d.** highland mango; **e.** lowland chilies; **f.** highland chilies; **g.** lowland sapodilla; **h.** sapodilla highlands. **S** = stomata; **E** = epidermal cells. Microscope magnification is 400x.



**Fig.4.** Stomata and epidermal cells in monocot plants in the lowlands and highlands. **a.** Lowland coconut; **b.** highland coconut; **c.** lowland turmeric; **d.** highland turmeric; **e.** lowland taro; **f.** upland taro; **g.** lowland rice; **h.** highland rice. **S** = stomata; **E** = epidermal cells. Microscope magnification is 400x.

*C. longa* have tetracytic types that are uniformly distributed. Previous studies have reported the presence of pentacytic and hexacytic stomatal types on both leaf surfaces, with higher densities observed on the abaxial side (20).

*C. esculenta* (L.) Schott, commonly known as taro, also exhibits amphistomatic leaves (plant leaf that possesses stomata on both the upper and lower leaf surfaces). The stomatal complex type in *C. esculenta* (L.) Schott is brachy-paracytic, with a unique uniform distribution.

This research has shown that *O. sativa* exhibits small-sized gramineous-type stomata. The gramineous stomatal type, refer to a specific morphological structure found in grasses (Poaceae). This type stomata are typically composed of 2 dumbbell-shaped guard cells flanked by 2 lateral subsidiary cells, which facilitate rapid stomatal movements and efficient water use. This configuration is a significant evolutionary adaptation in grasses, contributing to their ecological success (21). Studies on *Oryza*

leaves grown under varying environmental conditions have revealed variations in stomatal traits, with higher abaxial stomatal density compared to the adaxial surface across species. Additionally, a correlation has been observed between stomatal traits and photosynthetic capacity (22).

#### **The stomatal number, density, and index in the leaves of some dicotyledonous and monocotyledonous plants at low and high altitudes**

Tables 1 and 2 present data on stomatal number with their comparative t-test analysis for various dicotyledonous and monocotyledonous plants grown in lowland and highland environments.

Table 1 focuses on dicotyledonous plants, such as guava, chili, mango, and sapodilla, while Table 2 examines monocotyledonous plants, such as coconut, turmeric, taro and rice. The results indicate that differences in stomatal number between plants in lowland and highland

areas are statistically insignificant for all examined species, as evidenced by p-values greater than 0.05.

For instance, among dicotyledonous plants, guava shows an average stomatal number of 148 in lowland regions compared to 153.67 in highland areas, with a t-statistic of -0.763 and a p-value of 0.525, indicating no significant difference.

plants, such as coconut, turmeric, taro, and rice. There was no significant differences between lowlands and highlands plants, with a p-value greater than 0.05. For example, guava exhibits a stomatal density of 1138.46 in the lowlands and 1182.08 in the highlands, with a t-statistic value of -0.11 and a p-value of 0.91, confirming insignificant variations.

Similarly, Table 4 shows that the stomatal index

**Table 1.** Number of stomata of several dicotyledonous plants that grow in the lowlands and highlands

No	Plant species	Stomatal number		t-statistic	p-value
		Lowland	Highland		
1	<i>Capsicum annuum</i> L.	39	43.33	-1.982	0.186
2	<i>Mangifera indica</i> L.	85	84	0.15	0.894
3	<i>Manilkara zapota</i> (L.) P. Royen	70	75.67	-0.612	0.603
4	<i>Psidium guajava</i> L.	148	153.67	-0.763	0.525

The difference in the number of stomata for each species between the lowland and the highland habitat is statistically significant if p-value < 0.05.

**Table 2.** Number of stomata of several monocotyledonous plants that grow in the lowlands and highlands

No	Plant species	Stomatal number		t-statistic	p-value
		Lowland	Highland		
1	<i>Cocos nucifera</i> L.	28	22.67	1.576	0.256
2	<i>Colocasia esculenta</i> (L.) Schott	29	25	2	0.184
3	<i>Curcuma longa</i>	15	13	1.732	0.225
4	<i>Oryza sativa</i>	60.3	63.33	-0.283	0.804

The difference in the number of stomata for each species between the lowland and the highland habitat is statistically significant if p-value < 0.05.

Similarly, among monocotyledonous plants, chili shows a stomatal count of 39 in lowlands regions and 43.33 in the highlands areas, with a t-statistic = -1.982 and a p-value of 0.186, further confirming the absence of significant variations.

Table 3 and Table 4 present data on stomatal density and stomatal index for various dicot and monocot plants growing in both lowlands and highlands areas.

Table 3 presents data on stomatal density, measured as the number of stomata per mm<sup>2</sup> of leaf area, while Table 4 presents the stomatal index, calculated as the ratio of the number of stomata to the total number of epidermal cells.

The t-test analysis in Table 3 shows that stomatal density in dicotyledonous plants, such as guava, chili, mango and sapodilla, as well as monocotyledonous

does not exhibit statistically significant difference between lowland and highland conditions across all analysed species. For example, the guava stomata index in the lowlands is 0.42, whereas in the highlands, it is 0.41, with a t-statistic value of 0.06 and a p-value of 0.95, further indicating the absence of altitude-induced effects.

These results indicate that altitudinal differences do not have a significant effect on the density or stomata index of the plants studied. However, further research may be needed to understand other factors that may influence stomatal density and index, such as microclimatic conditions and genetic factors.

## Discussion

Stomata play a critical role as essential structures located on the surfaces of plants, enabling the process of gas exchange. Their development and classifications of stomata

**Table 3.** Stomata density in several dicotyledonous and monocotyledonous plants in the lowlands and highlands

Plant type	Plant species	Stomata density (in mm <sup>-2</sup> )		t-statistic	p-value
		Lowland	Highland		
Dicotyledonous	<i>Capsicum annuum</i> L.	300	333.31	-0.87	0.42
	<i>Mangifera indica</i> L.	653.85	646.15	0.11	0.92
	<i>Manilkara zapota</i> (L.) P. Royen	538.46	582.08	-0.58	0.58
	<i>Psidium guajava</i> L.	1138.46	1182.08	-0.11	0.91
Monocotyledonous	<i>Cocos nucifera</i> L.	215.38	174.38	1.05	0.35
	<i>Colocasia esculenta</i> (L.) Schott	223.08	192.31	0.7	0.52
	<i>Curcuma longa</i> L.	115.38	100	0.73	0.5
	<i>Oryza sativa</i> L.	463.85	487.15	-0.48	0.64

The difference of stomatal density for each species between the lowland and the highland habitat is statistically significant if p-value < 0.05.



**Table 4.** Stomatal index in several dicotyledonous and monocotyledonous plants in the lowlands and highlands

Plant type	Plant species	Stomatal index		t-Statistic	p-value
		Lowland	Highland		
Dicotyledonous	<i>Psidium guajava</i> L.	0.42	0.41	0.06	0.95
	<i>Capsicum annuum</i> L.	0.23	0.26	-1	0.38
	<i>Mangifera indica</i> L.	0.29	0.29	0	1
	<i>Manilkara zapota</i> (L.) P. Royen	0.28	0.28	0	1
Monocotyledonous	<i>Cocos nucifera</i> L.	0.24	0.14	1.45	0.22
	<i>Colocasia esculenta</i> (L.) Schott	0.31	0.31	0	1
	<i>Curcuma longa</i> L.	0.13	0.13	0	1
	<i>Oryza sativa</i> L.	0.32	0.32	0	1

The difference of stomatal index for each species between the lowland and the highland habitat is statistically significant if p-value < 0.05.

vary substantially across species. Comprised of a pair of guard cells, stomata regulate their opening and closing, a process crucial for water vapor emission and CO<sub>2</sub> intake.

Stomatal types differ widely among species. In this study, the observed types include paracytic (*P. guajava*, *M. indica*, *M. zapota*), anisocytic (*C. annuum*), tetracytic (*C. longa*), paracytic complex (*C. nucifera*), complex brachy-paracytic (*C. esculenta*), and gramineous (*O. sativa*). Notably, the types of stomata of the plants in this study are consistent between the same species growing in both lowland and highland locations (Fig. 2 and 3).

The development of stomata involves asymmetric divisions of meristemoids, which are stomatal-lineage stem cells regulated by key transcription factors (23,24). Stomatal characteristics are closely interconnected with photosynthetic processes and water efficiency (7). They play a vital role in the evolution of vascular plants by maintaining hydration and enabling adaptation to terrestrial habitats (25). Recent progress in comprehending the molecular mechanisms and environmental responses of stomatal development have provided valuable knowledge on the ecological importance of various stomata types stomata across plant lineages (26).

Table 1-4 presents the stomatal number, density, and index of dicotyledonous plants (guava, chili, mango, and sapodilla) and monocotyledonous (coconut, turmeric, taro, and rice) grown at low (11-18 m above sea level) and high (545 – 560 m above sea level) altitudes. The findings reveal no significant differences in these parameters between lowland and highland populations, regardless of plant type. These results align with recent studies suggesting that stomatal density can be influenced by environmental factors but may not always show significant altitudinal variation.

Previous study highlight that stomatal density is influenced by multiple factors, including light availability, humidity, and CO<sub>2</sub> levels, which may overshadow the effects of altitude alone (27). Similarly, a study observed that stomatal density can adapt to different environmental conditions, the response is often species-specific and not solely dependent on altitude (28).

Recent research has indicated that the spatial distri-

bution of stomata can exhibit notable fluctuations because of environmental factors such as light exposure, concentration of carbon dioxide and the presence of water. However, this variability does not consistently show a strong connection with elevation. For example, a study on barley plants revealed that increased light exposure enhanced stomatal spatial distribution. Additionally, lower carbon dioxide levels contributed to a higher stomatal density, although the disparities between standard and increased levels of carbon dioxide were not statistically significant (29).

Similarly, in tea plants, stomatal density exhibited a significant positive correlation with photosynthetic rate and water-use efficiency, indicating its potential for genetic improvement through breeding strategies (30). In oak species, notable variations in stomatal density were noted among different species and populations, with a strong inverse relationship between stomatal density and leaf length. This indicates that local adaptations play a more significant role than altitude-specific patterns (31).

Research on *Brachypodium distachyon* has underscored the variability of stomatal characteristics within the same species in response to seasonal fluctuations, highlighting the importance of considering long-term environmental influences (32). Moreover, an investigation into a multitude of plant species across different elevations in Changbai Mountain found that the stomatal density varied among plant functional types, including trees, shrubs and herbs. However, the correlation between stomatal attributes and elevation was not straight forward, as distinct trends were noted in trees compared to shrubs and herbs (33).

Additionally, research on ferns and flowering plants suggested that external environmental factors have a great influence on stomatal density than stomatal dimensions, which is largely genetically determined (34). Overall, while environmental factors such as light availability and water conditions significantly impact stomatal density, the relationship with altitude remains complex. It is often mediated by other ecological and genetic factors, leading to variable patterns across different species and functional types (32,34,35).

Conversely, other studies have reported significant

variations in stomatal density with altitude. Stomatal density tends to increase at high altitude, which can enhance water-use efficiency and photosynthesis under the fluctuating conditions typical of high-altitude environments. For instance, research conducted on the Qinghai-Tibetan Plateau demonstrated a significant increase in stomatal density in high-altitude plants compared to lowland species, suggesting an adaptive response to the harsher climatic conditions (36,37).

This adaptation is further supported by evidence that higher stomatal density facilitates faster photosynthetic induction and increased biomass production under fluctuating light conditions, as observed in *Arabidopsis thaliana* lines. Additionally, the relationship between stomatal density and leaf vein density is crucial for maintaining water balance, with a positive correlation observed across various species and altitudes, particularly in shrubs and herbs (38). However, the impact of altitude on stomatal density is not uniform across all plant species, indicating a complex interplay between genetic and environmental factors. For example, apple trees exhibit variations in stomatal morphology and density under different altitudinal conditions, with stress conditions at higher altitudes leading to smaller epidermal cells and altered stomatal characteristics (38,39).

Furthermore, the intrinsic water-use efficiency of trees has been shown to increase with altitude, by enhanced photosynthesis at tree lines and reduced stomatal conductance at lower elevations. This highlights the role of moisture conditions and vapor pressure deficit in regulating stomatal behaviour (40). These findings underscore the multifaceted nature of plant responses to altitude, involving physiological, anatomical, and genetic adaptations to optimize water use and photosynthesis in diverse environmental condition.

At certain altitude, stomatal density significantly influences transpiration rates and photosynthesis in some plant species. As altitude increases, the partial pressure of atmospheric gases, including CO<sub>2</sub>, decreases, prompting plants to adapt by modifying their stomatal characteristics. For instance, high-altitude species like *Arabis alpina* exhibit increased stomatal density to optimize CO<sub>2</sub> uptake during brief favourable weather conditions, unlike low-altitude species such as *A. thaliana*, which do not show this adaptation (41). Similarly, *Hordeum vulgare* and *Pisum sativum* cultivars in the Himalayas exhibit increased stomatal density and conductance at higher altitudes, facilitating sufficient CO<sub>2</sub> influx and efficient leaf cooling through transpiration (36).

Additionally, plants such as *Epilobium amurense* and *Potentilla fulgens* in the Yulong Mountains adapt by increasing leaf thickness and stomatal density, enhancing photosynthetic efficiency and internal water retention. However, this adaptation is not uniform across all species; for example, Qinghai spruce exhibits increased stomatal density up to an optimal altitude of 3000 m, beyond which it declines due to other environmental factors, such as temperature and rainfall variability (38). The lack of signifi-

cant differences in stomatal density and stomatal index between plants at lowland and highland sites in this study may be attributed to the limited altitude range (10 – 600 m ASL), which may not be sufficient to induce notable changes in stomatal properties.

The phenotypic plasticity observed in trees along altitudinal gradients, such as in *Fagus sylvatica* and *Quercus petraea*, further underscores the complex interplay between genetic and environmental factors in determining stomatal traits (42). Moreover, the unique environmental conditions at high altitudes, including low temperatures and high UV radiation, necessitate specific photosynthetic adaptations, such as increased carboxylation capacity and CO<sub>2</sub> drawdown (43,44). These adaptations are crucial for maintaining gas exchange and water-use efficiency, as seen in *Adenophora lobophylla* and *Phacelia secunda*, which adjust their stomatal conductance and photosynthetic rates in response to varying altitudinal conditions (45,46).

Overall, the interplay of atmospheric pressure, temperature, and other environmental factors at high altitudes drives significant changes in stomatal density, influencing transpiration and photosynthesis in diverse plant species.

The impact of altitude on stomatal density varies significantly across species and environmental conditions, indicating that altitude does not universally affect stomatal density. For instance, in *A. alpina*, stomatal density increased with altitude due to the decreased partial pressure of CO<sub>2</sub>, while *A. thaliana* showed no significant change in stomatal density under similar conditions (41). Similarly, a study of 105 angiosperm species across tropical and subtropical montane forests that stomatal density was independent of altitude (47). However, in *Qinghai spruce*, stomatal density increased with altitude up to 3000 m but decreased beyond this point, suggesting that the optimal growth altitude for this plant is reached at 3000 m above sea level (43).

In contrast, a study on *Ficus septica* showed a strong linear increase in stomatal density with elevation (48). Additionally, research on *Thalictrum alpinum* and *Kobresia humilis* indicated that environmental factors significantly influenced stomatal density in the former, while genetic factors played a more influential role in the latter (49). Other studies have shown mixed results, demonstrating varying plant responses to differential altitude. For example, in Changbai Mountain, stomatal density decreased with elevation in trees but exhibited a non-linear pattern in shrubs and herbs, initially increasing before declining (37). Furthermore, in the White Mountains, no clear elevational trends in stomatal density were observed across four different species (50).

These findings suggest that the relationship between altitude and stomatal density is complex and species-specific, influenced by a combination of genetic and environmental factors, including CO<sub>2</sub> partial pressure, light intensity, and other climatic variables (51). Therefore, while altitude can impact stomatal density, the extent and



direction of this effect are not uniform across all plant species and environments.

The absence of significant differences in the current data may be attributed to the specific plant species studied, which may possess inherent adaptive mechanisms that maintain consistent stomatal densities across different altitudes. Local environmental conditions, such as microclimate variations and soil properties, might also play a crucial role in influencing stomatal development, potentially more so than altitude alone. The presented data show no significant differences in stomatal counts between lowland and highland environments for the examined plants. However, this outcome may not be universally applicable. Future research should include a broader range of species and consider various environmental factors to better understand the complex interactions governing stomatal development in different altitudinal settings.

SI defined as the ratio of the number of stomata to the total number of stomata and epidermal cells, is considered relatively constant under different environmental conditions and leaf ages, making it a robust diagnostic characteristic for a given genotype or species (52). However, stomatal density, which refers to the number of stomata per unit area, is more variable and sensitive to environmental changes, including atmospheric CO<sub>2</sub> concentration, temperature, humidity and soil water content (53).

The large stomatal size, number and density provide an advantage to plants in absorbing atmospheric pollutants, especially carbon monoxide. In this study, monocotyledonous plants (except *O. sativa*) possess larger stomatal sizes than dicotyledonous plants (Fig. 1-3). Both SI and SD can be inversely related to CO<sub>2</sub> concentration, with higher CO<sub>2</sub> levels generally leading to lower values of both parameters (54). However, some research suggests that SI and SD may not always respond predictably to rising CO<sub>2</sub> levels in natural conditions, indicating a potential decoupling under certain conditions.

Additionally, the relationship between SD and SS is inherently negative and non-linear due to geometric constraints, which also affects the overall stomatal area and gas exchange efficiency (16).

In contrast, some studies have reported that the SI exhibits varied responses to altitude across different plant species and environments. Research suggests that the SI generally increases with altitude, primarily due to the decrease in CO<sub>2</sub> partial pressure, which necessitates a higher number of stomata to ensure adequate CO<sub>2</sub> uptake for photosynthesis (43).

For instance, in *A. thaliana*, most ecotypes demonstrated an increased SI under CO<sub>2</sub> enrichment, reflecting an adaptation to the lower CO<sub>2</sub> availability at higher altitudes (48). Similarly, *Quercus kelloggii* and *Nothofagus solandri* var. *cliffortioides* exhibited increased SI and SD with elevation, suggesting a physiological adaptation to counteract the limited photosynthetic potential resulting from reduced CO<sub>2</sub> partial pressure (55).

However, this response can be species-specific and influenced by additional environmental factors. For exam-

ple, in the northern Alps, the stomatal pore area index (SPI), which is closely related to SI, exhibited species-specific variations along elevational gradients, with some species displaying positive correlations between SPI, photosynthetic rates and leaf nitrogen content (51).

The variation in SI with altitude is also evident in *A. alpina*, where stomatal density increased with altitude, influencing stomatal conductance and carbon assimilation rates. Furthermore, in Changbai Mountain, China, trees exhibited a decrease in stomatal density and an increase in stomatal length with elevation, while shrubs and herbs displayed a more complex pattern, in which stomatal density initially increased until reaching 2008 m ASL, after which it declined (50).

Overall, while the general trend suggests an increase in stomatal index with altitude, species-specific responses can vary significantly and are influenced by a combination of environmental factors and plant functional types (37).

## Conclusion

This research demonstrates that the distribution, density, and index of stomata between dicotyledonous or monocotyledonous plants cultivated in habitat with altitude differences of approximately 600 m are not significantly different. This finding suggests that factors such as light exposure, moisture levels, and CO<sub>2</sub> concentrations may have a more pronounced impact on stomatal traits than altitude alone within this range.

Despite variations in stomatal morphology across plant species, the adjustment of stomata to environmental conditions appears to be complex and species-specific, with responses not always directly correlated with altitudinal changes. The study highlights the necessity of considering a broader range of environmental factors when analysing stomatal adaptations, as their responses are not uniform but instead highly dependent on specific ecological conditions and plant functional types.

These findings provide valuable insights into the diversity and ecological significance of stomatal variations, contributing to a deeper understanding of plant development and adaptation across different environmental conditions.

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## Authors' contributions

RH carried out the research design, field sampling, morphology anatomy analysis, data analysis, and drafted the manuscript. TS carried out statistical analysis and drafted manuscript. MN participated in the drafted manuscript.

and design coordination. NN participated in the research design and coordination. All authors read and approved the final manuscript.

## Compliance with Ethical Standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

**Ethical issues:** None

## Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, authors used Chatsonic in order to collect more up to date references and its highlights that are relevant to some parts of the discussion. After using this tool, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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