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Stomatal and epidermal characteristics of Zingiberaceae in Serang District, Banten, Indonesia

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Abstract. *Windarsih G, Riastiwi I, Dewi AP. Yuriyah S. 2022. Stomatal and epidermal characteristics of Zingiberaceae in Serang District, Banten, Indonesia. Biodiversitas 23: 5373-5386.* The stomatal and epidermal characteristics can be used as additional evidence in plant taxonomy activity. The aim of the study was to identify the stomatal and epidermal characteristics of Zingiberaceae in Serang District, Banten, Indonesia. The samples used were 11 species of Zingiberaceae collected from Serang District. The stomatal and epidermal collection was carried out using the replica technique. Based on the results, all species has brachyparatetracytic type of stomata, on both adaxial and abaxial surfaces of the leaf. The densities of stomata of all species observed on abaxial were higher than adaxial surface of the leaf. The stomatal density on adaxial surface of leaf between 1.71-4.43 stomata per unit leaf area of 171,602 μm^2, while on abaxial 10.46-42.22 stomata per unit leaf area. The stomatal index on adaxial surface is between 1.34-6.97%, while on abaxial 7.54-30.25%. The length of guard cells on adaxial surface is between 30.30-47.85 µm, while on abaxial 29.86-50.68 µm. The width of guard cells on adaxial surface between 18.67-31.73 µm, while on abaxial 19.97-31.95 µm. The adaxial epidermal cells have a column, polygonal, or rectangular shapes, while the abaxial has poligonal or rectangular-shaped epidermal cells. The epidermal cells that are over leaf veins have polygonal or rectangular-shaped cells.

Keywords: Epidermal cell, guard cell, stomatal density, stomatal index, Zingiberaceae

INTRODUCTION

The gingers family, Zingiberaceae, is included in the monocotyledon group and the order of Zingiberales (Zahara 2020). Zingiberaceae consists of about 53 genera and 1,500 species around the world (Furmuly and Azemi 2020). The distribution of Zingiberaceae spread widely in tropical and subtropical areas of the world, such as Asia (Zahara 2020; Furmuly and Azemi 2020), China (Zhao et al. 2022), Thailand (Kajornjit et al. 2018), Malaysia (Salasiah and Meekiong 2018), Indonesia (Windarsih et al. 2021), India (Pemba and Sharangi 2017), West Africa, South America (Kaliyadasa and Samarasinghe 2019), Brunei (Zaini et al. 2014), Singapore, Philippines (Zahara 2020), Sri Lanka (Karunarathne et al. 2021), Japan (Elvira et al. 2022), Vietnam (Leong et al. 2016) and Australia (Setiawan et al. 2021).

Zingiberaceae is a rhizomatous plant, annual or perennial herb (Kaliyadasa and Samarasinghe 2019), it has a fibrous root system, rhizomes grow creeping horizontally in the soil, roots usually emerging from the surface of rhizomes. Zingiberaceae has a single leaf, symmetrical leaf blade, leaves are arranged alternatively in two opposite rows, leaf sheaths wrap around the true stem to form a pseudo-stem (Windarsih et al. 2021). Zingiberaceae is an economically important family for many utilization, such as traditional medicine, spice, ornamental plants, cosmetics, dye (Furmuly and Azemi 2020), food (Zahara 2020; Kaliyadasa and Samarasinghe 2019), aromatic agent, and insecticide (Zahara 2020). The rhizome of Zingiberaceae is widely utilized as a medicinal plant due to the presence of bioactive compounds (Zahara 2020) with pharmacological activities, such as anti-inflammatory (Sikha et al. 2015), antimicrobial, hypocholesterolemic (Shafreen et al. 2018), antirheumatic (Abdel-Lateef et al. 2016), antiviral (Pant et al. 2013), antifibrotic, antihepatotoxic, antidiabetic, antinociceptive, anticancerous (Li et al. 2014), gastroprotective properties (Kaliyadasa and Samarasinghe 2019), and antidiarrheal (Furmuly and Azemi 2020).

In most plants, stomata can be found on both adaxial and abaxial surfaces of the leaf. In some species, i.e. trees, stomata can be found on the abaxial surface of the leaf, while in aquatic plants, stomata can be found on the adaxial surface of the leaf. Stoma consists of a pore, paired guard cells, and subsidiary cells, forming the stomatal complex. The subsidiary cells are commonly called also as neighbor cells and surround guard cells. The presence of leaf cuticles on the leaf surface is impermeable to water and CO_2, but stomata pores function to regulate the exchange of CO_2 and H_2O between the inside of the leaf and the external environment (Lawson 2008). The photosynthesis process depends on the opening and closing of stomata, which is regulated by guard cells (Zahara 2020), while subsidiary cells play a role in the mechanical movements of guard cells. In most species, the guard cells contain chloroplasts that have an important role in the photosynthesis process. (Lawson 2008).

The leaf anatomy features were considered to be used as additional evidence in plant taxonomy activity. The variation of epidermal shape and characteristics of guard cells and subsidiary cells of stomata can be useful evidence for the identification of plant species (Rahayu et al. 2012). Nine species of Zingiberaceae are widely cultivated in Serang District, Banten, include in ginger, galangal, East Indian galangal, yellow turmeric, black turmeric, javanese cardamom, lempuyang wangi, Chinese keys, and javanese turmeric, that are generally utilized by the local society for the rhizome part as biopharmaceutical plants (BPS-Statistics of Banten Province 2021). Because of the importance of those plants' utilization in that region, it is necessary to determine the characters that can be used to help the identification process of Zingiberaceae. Twelve species of Zingiberaceae were successfully investigated from Serang District by Windarsih et al. (2021) for identification based on morphological characters, included in *Wurfbainia compacta*, *Alpinia purpurata*, *Curcuma xanthorrhiza*, *C. longa*, *C. heyneana*, *C. mangga*, *C. aeruginosa*, *Kaempferia rotunda*, *Etlingera elatior*, *Zingiber zerumbet*, *Z. officinale*, and *Z. cassumunar*. But, the information on stomatal and epidermal characteristics of Zingiberaceae is still limited. The study of stomatal characteristics of Zingiberaceae had been conducted by Setiawan et al. (2021) revealed that the size of the stomata, the density of the stomata, and the stomatal index could be used to differentiate the species among the members of *Alpinia* genus. Therefore, the aim of the study was to identify the stomatal and epidermal characteristics of Zingiberaceae in Serang District, Banten, Indonesia.

MATERIALS AND METHODS

Samples collection

The plant materials used as samples were 11 species collected from Serang District, Banten, Indonesia consisting of Pabuaran, Gunungsari, and Ciomas sub-districts (Figure 1) in January-August 2022. The eleven species included red galangal, black turmeric, *temu giring*, yellow turmeric, mango ginger, Javanese turmeric, torch ginger, *kunyit putih*, ginger, *bangle*, and Javanese cardamom (Table 1).

Procedures

Slide preparation

The preparation of slides was carried out using a replica technique (Setiawati et al. 2018) by smearing the nail polish on both the adaxial and abaxial surfaces of the leaf. The leaf was collected from a perfectly opened leaf on the fourth blade from the top of the shoot from three plants per species with one leaf per plant. After the nail polish dried (about ±5 min), each surface of the leaf was peeled off using transparent tape. Each epidermal layer was taken place on a glass slide, 3 repetitions per leaf. The stomata sampling was conducted at 09.00-10.00 a.m. and the weather was sunny to obtain opened stomata.

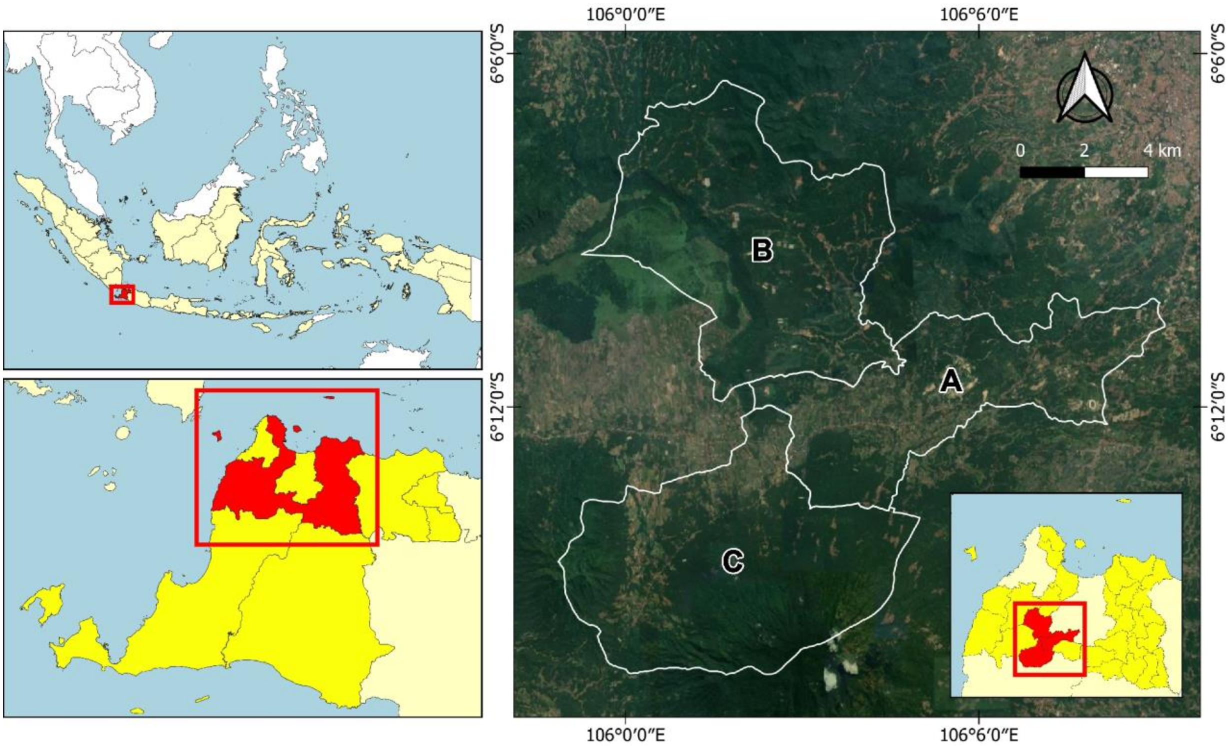


Figure 1. Sampling location of Zingiberaceae in Serang District, Banten, Indonesia. A. Pabuaran, B. Gunungsari, C. Ciomas sub-districts

Table 1. The eleven species of Zingiberaceae collected from Serang District, Indonesia for stomatal and epidermal characteristics observation

Species	Scientific name	Origin
Red galangal	*Alpinia purpurata* (Vieill.) K. Schum.	Pabuaran sub-district
Black turmeric	*Curcuma aeruginosa* Roxb.	Pabuaran sub-district
Temu giring	*Curcuma heyneana* Valeton & Zijp.	Pabuaran sub-district
Yellow turmeric	*Curcuma longa* L.	Pabuaran sub-district
Mango ginger	*Curcuma mangga* Val.	Pabuaran sub-district
Javanese turmeric	*Curcuma zanthorrhiza* Roxb.	Pabuaran sub-district
Torch ginger	*Etlingera elatior* (Jack) R.M.Sm.	Ciomas sub-district
Kunyit putih	*Kaempferia rotunda* L.	Pabuaran sub-district
Ginger	*Zingiber officinale* Roscoe	Gunungsari sub-district
Bangle	*Zingiber purpureum* Roscoe	Pabuaran sub-district
Javanese cardamom	*Wurfbainia compacta* (Sol. ex Maton) Skornick. & A.D.Poulsen	Ciomas sub-district

Observation of stomatal characteristics

The stomatal characteristics observed included the type of stomata, length and width of guard cells, density of stomata, and stomatal index on both the adaxial and abaxial surfaces of the leaf. The stomatal density and stomatal index were observed on the 9-unit leaf area per leaf, each unit leaf area was observed with a measure of 478 µm x 359 µm. Ten stomata were randomly observed on each unit leaf area to measure the size of guard cells and identify the type of stomata. The observation of guard cells was conducted at the opened stomata. The observation of stomatal characteristics was conducted under a microscope with a total magnification of 100x. The identification of stomatal type was conducted according to Willmer & Fricker (2012), Setiawan et al. (2021), and Dilcher (1974).

Observation of epidermal cells

The determination of cell shape and measurement of epidermal cell size was conducted on 9 unit leaf areas (478 µm x 359 µm) per leaf, with 20 epidermal cells per unit leaf area, on both adaxial and abaxial surfaces of the leaf. These characters were observed under microscopy with a total magnification of 100x.

Data analysis

Data analysis was conducted using a descriptive comparative method with qualitative and quantitative approaches. The stomatal density and stomatal index were determined based on the formulas below (Paul et al. 2017).

$$\text{stomatal density} = \frac{\text{number of stomata}}{\text{unit leaf area}}$$

$$\text{stomatal index} = \frac{\text{number of stomata}}{\text{number of stomata} + \text{number of epidermal cells}} \times 100\%$$

Note: unit leaf area = 478 µm x 359 µm = 171602 µm^2

The correlation analysis of stomatal and epidermal characteristics was carried out using SPSS 15.00, while the determination of mean and standard deviation was conducted using Microsoft Office Excel 2007.

RESULTS AND DISCUSSION

Results

Based on the results obtained in this study, there were 11 species observed for stomatal and epidermal characteristics, included in a type of stomata, stomatal density, stomatal index, length and width of guard cell, and epidermal cell shape and size. From all species observed, the stomata can be found on both adaxial and abaxial surfaces of the leaf.

Type of stomata

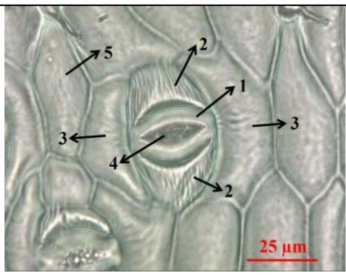
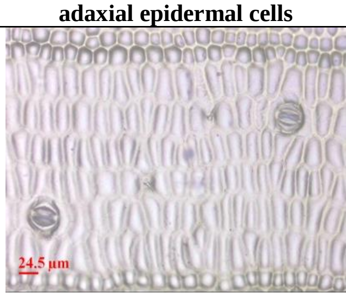
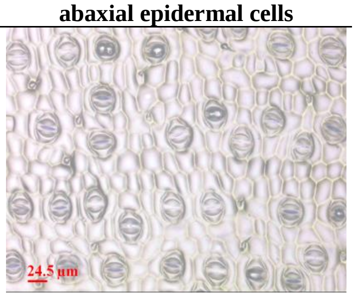
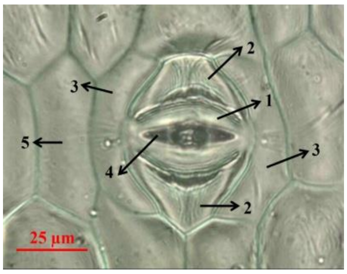
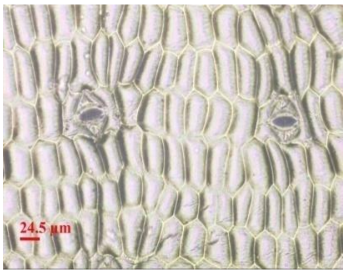
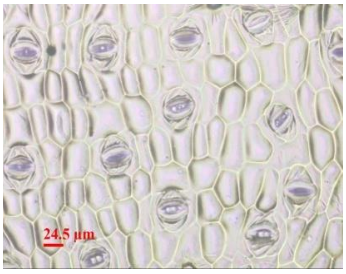
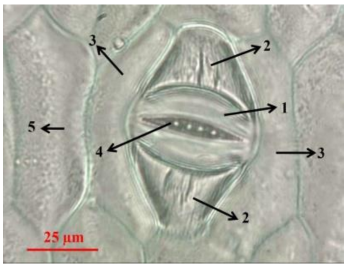
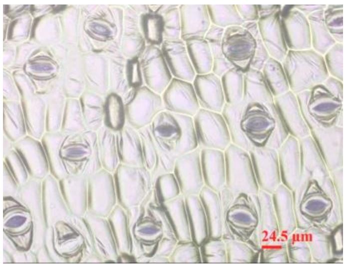
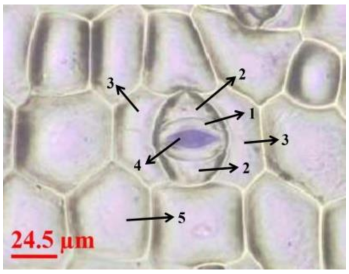
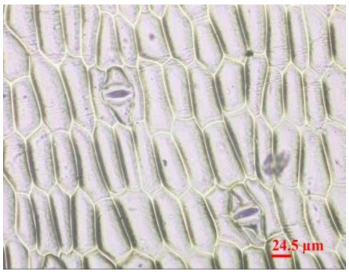
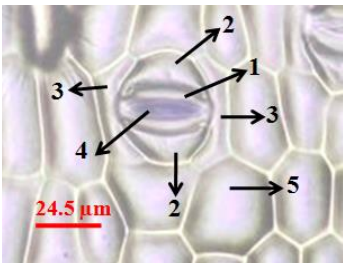
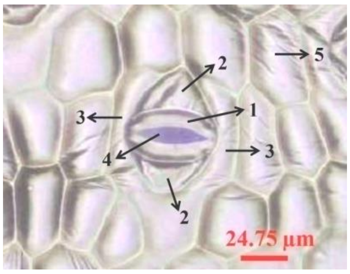
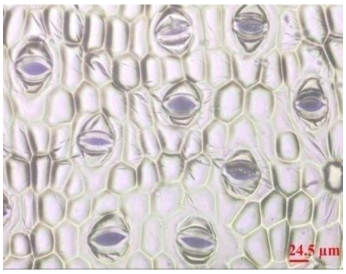
All species have the tetracytic type of stomata, which are specifically called as brachyparatetracytic type (Dilcher 1974). This type of stomata was found on both the adaxial and abaxial surfaces of the leaf. The brachyparatetracytic stomata type has a structure in which one stoma is surrounded by four subsidiary cells, consisting of two lateral subsidiary cells are parallel to the guard cells, whereas two polar (terminal) subsidiary cells are perpendicular to the guard cells as well as bordered wide to the guard cell and lateral subsidiary cells (Dilcher 1974).

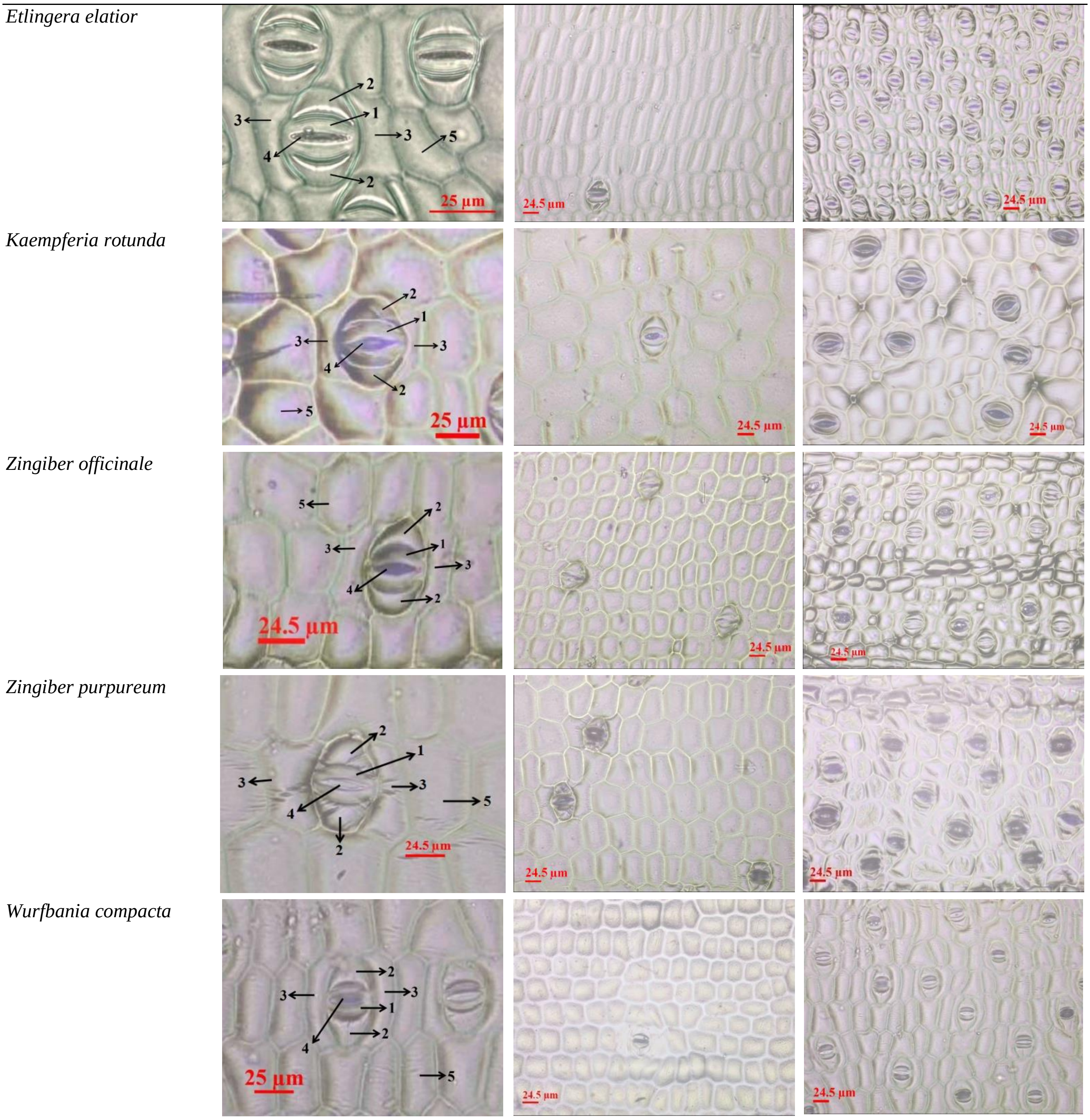
Distribution of stomata in all species was arranged irregularly and spread in intervein areas on both adaxial and abaxial surfaces of the leaf. Stomata and epidermal cells were arranged in the same rows, but more regular on adaxial than abaxial epidermis. The guard cell was kidney-shaped and arranged symmetrically on both sides of the stoma. The number of epidermal cells surrounding the stoma showed a variation and was not significantly different among 11 species observed, both on adaxial (6-13 epidermal cells) and adaxial epidermis (4-12 epidermal cells) (Table 2).

Stomatal density

The highest stomatal density on the adaxial surface of the leaf was obtained from *Z. purpureum* by 4.43±1.29 stomata per unit leaf area, while the lowest was obtained from *Kaempferia rotunda* by 1.71±0.46 stomata per unit leaf area. Meanwhile, the highest stomatal density on the abaxial surface of the leaf was obtained from *E. elatior* by 42.22±10.52 stomata per unit leaf area, while the lowest was obtained from *C. heyneana* by 10.46±1.82 stomata per unit leaf area. The stomatal density on the adaxial surface of the leaf did not show any variation among all species observed, while on the abaxial surface of the leaf, it showed a variation among 11 species which can be distinguished significantly into two groups, i.e. group I (*E. elatior*, *A. purpurata*) with the stomatal density value is more than 20, and group II (*C. aeruginosa*, *C. heyneana*, *C. longa*, *C. mangga*, *C. zanthorrhiza*, *K. rotunda*, *Z. officinale*, *Z. purpureum*, *W. compacta*) with the stomatal density value is below 20 (Tables 3 and 4).

Table 2. Stomatal characteristics of Zingiberaceae collected from Serang District, Banten, Indonesia

Species	Structure of stomata	Distribution of stomata on adaxial epidermal cells	Distribution of stomata on abaxial epidermal cells
Alpinia purpurata			
Curcuma aeruginosa			
Curcuma heyneana			
Curcuma longa			
Curcuma mangga			
Curcuma zanthorrhiza			



Note: 1. Guard cell, 2. lateral subsidiary cell, 3. polar subsidiary cell, 4. stomatal pore, 5. epidermal cell

Table 3. The stomatal characteristics on adaxial surface of the leaf in Zingiberaceae collected from Serang District, Banten, Indonesia

Species	Stomatal density	Stomatal index (%)	Length of guard cell (µm)	Width of guard cell (µm)
Alpinia purpurata	2.95±0.89a	1.34±0.40a	30.30±2.56a	18.67±1.84a
Curcuma aeruginosa	2.50±0.73a	2.74±0.83a	43.20±2.61a	26.81±2.50a
Curcuma heyneana	2.26±0.58a	2.87±0.83a	43.78±3.20a	28.55±2.84a
Curcuma longa	3.59±1.02a	4.50±1.51a	40.18±2.13a	26.33±2.22a
Curcuma mangga	3.62±1.07a	3.10±0.84a	40.68±1.77a	25.62±2.31a
Curcuma zanthorrhiza	3.40±1.19a	3.90±1.30a	47.73±2.99a	29.13±2.89a
Etlingera elatior	2.07±0.59a	1.63±0.44a	38.25±3.12a	24.48±2.30a
Kaempferia rotunda	1.71±0.46a	5.14±1.46a	**47.85±3.53a**	**31.73±2.78a**
Zingiber officinale	4.42±0.90a	3.60±0.67a	37.49±2.26a	23.30±2.40a
Zingiber purpureum	**4.43±1.29a**	**6.97±1.90a**	42.62±2.07a	23.75±2.39a
Wurfbania compacta	2.56±0.76a	2.46±0.78a	34.12±2.55a	22.91±1.86a

Table 4. The stomatal characteristics on abaxial surface of the leaf in Zingiberaceae collected from Serang District, Banten, Indonesia

Species	Stomatal density	Stomatal index (%)	Length of guard cell (µm)	Width of guard cell (µm)
Alpinia purpurata	29.33±4.95b	14.48±3.26a	29.86±2.21a	19.97±2.18a
Curcuma aeruginosa	11.22±2.14a	11.42±2.77a	46.17±2.54c	27.73±2.43a
Curcuma heyneana	10.46±1.82a	11.55±2.52a	48.07±2.85c	29.15±2.68a
Curcuma longa	15.50±1.96a	16.34±1.44a	43.48±2.32c	27.73±2.51a
Curcuma mangga	16.18±1.47a	10.82±1.14a	43.14±2.05c	24.95±2.18a
Curcuma zanthorrhiza	14.09±2.02a	13.82±2.29a	50.55±2.65c	30.00±3.10a
Etlingera elatior	**42.22±10.52b**	**30.25±3.34b**	37.00±2.11b	23.37±1.88a
Kaempferia rotunda	11.14±1.92a	15.71±2.70a	**50.68±2.68c**	**31.95±2.87a**
Zingiber officinale	13.50±1.73a	7.54±1.05a	38.31±1.97b	21.81±2.15a
Zingiber purpureum	17.05±2.55a	19.11±2.79a	45.63±2.08c	28.96±2.67a
Wurfbania compacta	11.00±1.80a	14.22±1.69a	35.26±1.54b	23.35±1.73a

Stomatal index

The highest stomatal index on the adaxial surface of the leaf was produced by *Z. purpureum* (6.97±1.90%), while the lowest was produced by *A. purpurata* (1.34±0.40%). Meanwhile, the highest stomatal index on the abaxial surface was obtained from *E. elatior* (30.25±3.34%), while the lowest was obtained from *Z. officinale* (7.54±1.05%). The stomatal index on adaxial surface of the leaf did not show any variation among all species, while the abaxial surface showed a variation among 11 species which can be divided significantly into two groups, i.e. group I (*E. elatior*) with the stomatal index value is more than 25.00% and group II (*A. purpurata*, *C. aeruginosa*, *C. heyneana*, *C. longa*, *C. mangga*, *C. zanthorrhiza*, *K. rotunda*, *Z. officinale*, *Z. purpureum*, *W. compacta*) with the stomatal index value is below 25.00% (Tables 3 and 4).

Size of guard cell

The highest length of guard cell was obtained from *K. rotunda* on both adaxial (47.85±3.53 µm) and abaxial (50.68±2.68 µm) surfaces of the leaf, while the lowest was produced by *A. purpurata* on both adaxial (30.30±2.56 µm) and abaxial (29.86±2.21 µm) surfaces of leaf. The data obtained in this study showed the same results with *C. rubrobracteata* by 30.13-77.70 µm (Chen and Xia 2010). The adaxial surface of leaf did not show any variation of guard cell length significantly among 11 species, while on abaxial surface of leaf, it showed a variation that can divide all species into three groups, i.e. group I (*A. purpurata*) with the length of guard cell is below 30.00 µm, group II (*E. elatior*, *Z. officinale*, *W. compacta*) with the length of guard cell ranges between 30.00-40.00 µm, and group III (*C. aeruginosa*, *C. heyneana*, *C. longa*, *C. mangga*, *C. zanthorrhiza*, *K. rotunda*, *Z. purpureum*) with the length of guard cell more than 40.00 µm. Meanwhile, the highest width of guard cell was obtained from *K. rotunda*, both on adaxial (31.73±2.78 µm) and abaxial (31.95±2.87 µm) surfaces of the leaf, while the lowest was produced by *A. purpurata* on both adaxial (18.67±1.84 µm) and abaxial (19.97±2.18 µm) surfaces of the leaf. Both the adaxial and abaxial surfaces of the leaf did not show any variation in the width of guard cells significantly among the 11 species observed (Table 3-4).

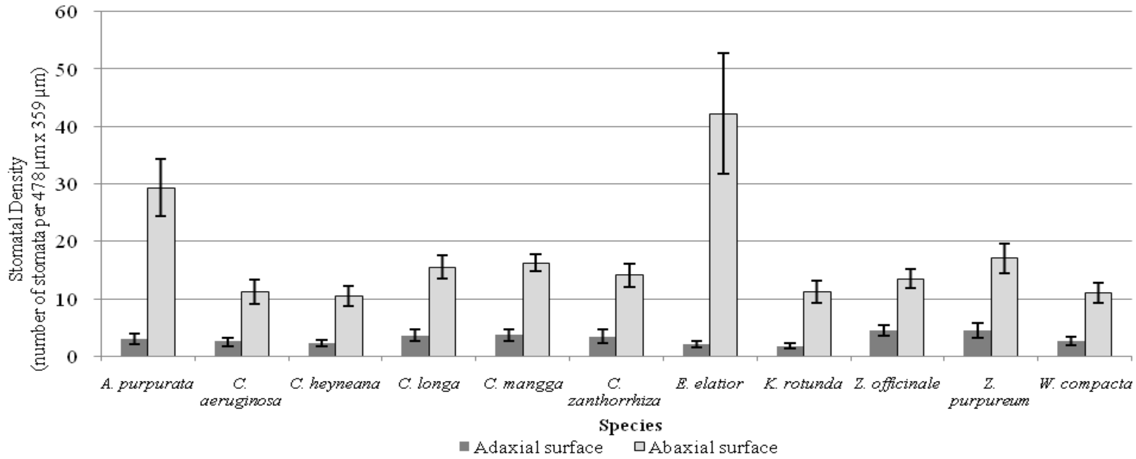


Figure 2. The stomatal density of Zingiberaceae from Serang District, Banten, Indonesia

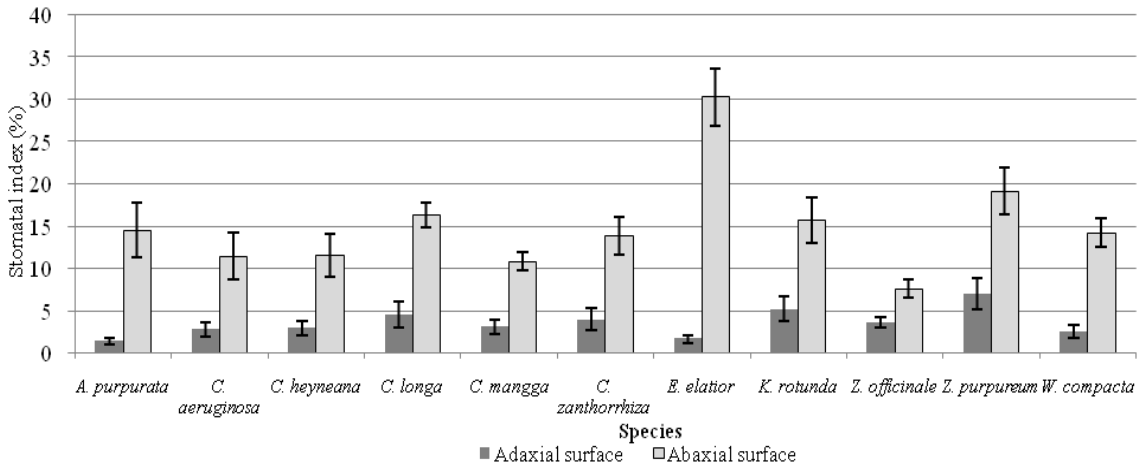


Figure 3. The stomatal index of Zingiberaceae in Serang District, Banten, Indonesia

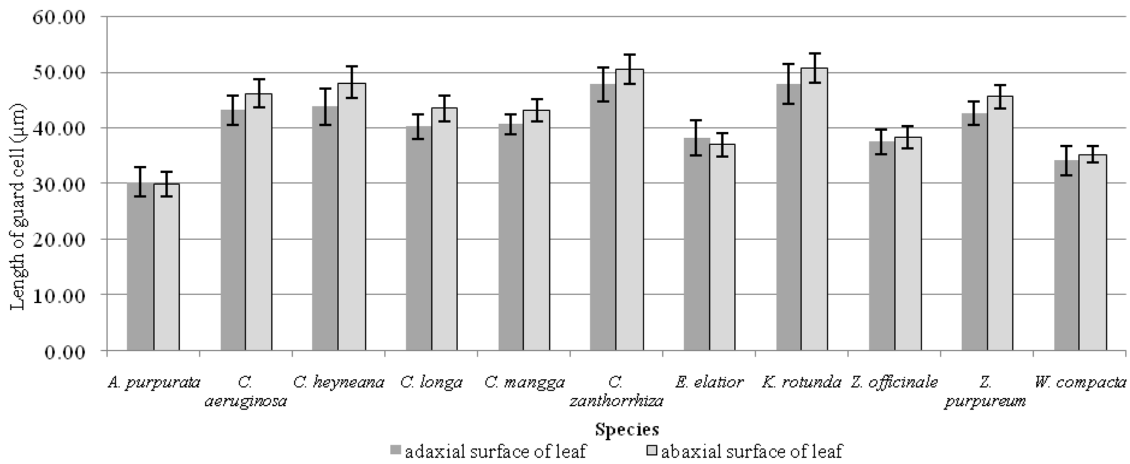


Figure 4. The length of stomatal guard cell in Zingiberaceae collected from Serang District, Banten, Indonesia

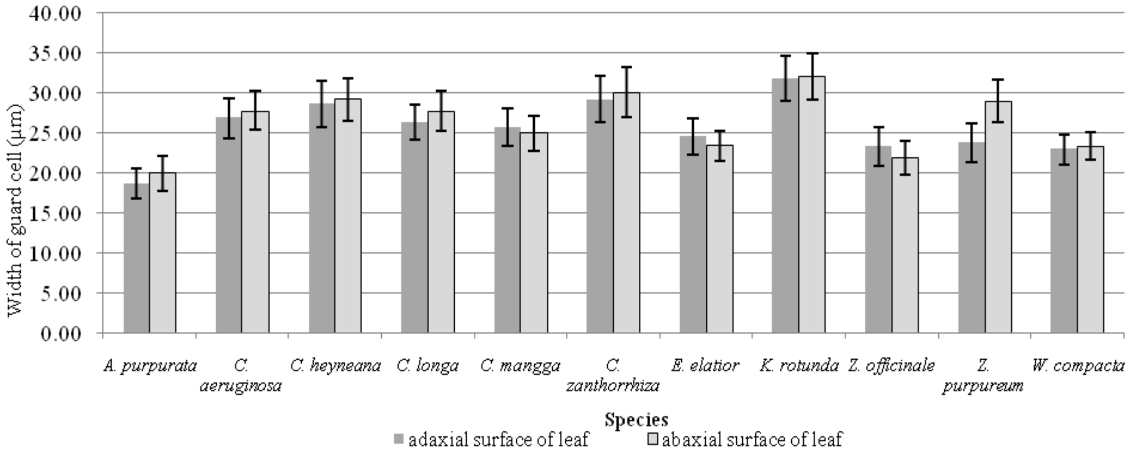


Figure 5. The width of guard cell in Zingiberaceae collected from Serang District, Banten, Indonesia

Epidermal cells

Overall, all species have epidermal cells that consist of relatively unspecialized ground cells interspersed with specialized cells type, such as stomata, on both the adaxial and abaxial surfaces of the leaf. The polygonal or rectangular-shaped epidermal cells are over leaf veins arranged horizontally and regularly. Otherwise, stomata are located between leaf veins. The shape of adaxial epidermal cells can be divided into two classes, i.e. (1) column in *A. purpurata*, *C. aeruginosa*, *C. heyneana*, *C. longa*, *C. mangga*, *C. zanthorrhiza*, and *E. elatior*, and (2) 5, 6, 7, or 8-sided polygonal or rectangular shapes in *K. rotunda*, *Z. officinale*, *Z. purpureum*, and *W. compacta*. Meanwhile on the abaxial surface of the leaf, all species have 5, 6, 7, or 8-sided polygonal or rectangular-shaped epidermal cells. The anticlinal walls of epidermal cells, on both the adaxial and abaxial surfaces of the leaf, in all species observed are straight (Table 5 and 84-5). The type of anticlinal walls is constant at the species level (Rahayu et al. 2012).

Kaempferia rotunda has the biggest epidermal cells on both the adaxial surface of the leaf with a length of 87.06±11.88 µm and a width of 68.90±9.01 µm, and the abaxial surface of the leaf with a length of 64.83±11.22 µm and a width of 45.54±7.93 µm. There was no significant difference in the length of epidermal cells among all species, both on the adaxial and abaxial surfaces of the leaf. But, the adaxial surface of the leaf showed a variation in the width of epidermal cells that can distinguish all species observed into two groups, i.e. group I (*K. rotunda*) with the width of epidermal cell value above 60.00 µm and group II (*A. purpurata*, *C. aeruginosa*, *C. heyneana*, *C. longa*, *C. mangga*, *C. zanthorrhiza*, *E. elatior*, *Z. officinale*, *Z. purpureum*, *W. compacta*) with the width of epidermal cell value is below 60.00 µm (Table 6).

Table 5. Epidermal cell shape on the leaf surface of Zingiberaceae collected from Serang District, Banten, Indonesia

Species	Surface of leaf	Epidermal cells
Alpinia purpurata	Adaxial	Column, arranged regularly
	Abaxial	Polygonal or rectangular, arranged irregularly
Curcuma aeruginosa	Adaxial	Column, arranged regularly
	Abaxial	Polygonal or rectangular, arranged irregularly
Curcuma heyneana	Adaxial	Column, arranged regularly
	Abaxial	Polygonal or rectangular, arranged irregularly
Curcuma longa	Adaxial	Column, arranged regularly
	Abaxial	Polygonal or rectangular, arranged irregularly
Curcuma mangga	Adaxial	Column, arranged regularly
	Abaxial	Polygonal or rectangular, arranged irregularly
Curcuma zanthorrhiza	Adaxial	Column, arranged regularly
	Abaxial	Polygonal or rectangular, arranged irregularly
Etlingera elatior	Adaxial	Column, arranged regularly
	Abaxial	Polygonal or rectangular, arranged irregularly
Kaempferia rotunda	Adaxial	Polygonal or rectangular, arranged regularly
	Abaxial	Polygonal or rectangular, arranged irregularly
Zingiber officinale	Adaxial	Polygonal or rectangular, arranged regularly
	Abaxial	Polygonal or rectangular, arranged irregularly
Zingiber purpureum	Adaxial	Polygonal or rectangular, arranged regularly
	Abaxial	Polygonal or rectangular, arranged irregularly
Wurfbainia compacta	Adaxial	Polygonal or rectangular, arranged regularly
	Abaxial	Polygonal or rectangular, arranged irregularly

Table 6. Epidermal cell size on adaxial surface of the leaf in Zingiberaceae collected from Serang District, Banten, Indonesia

Species	Adaxial surface of leaf		Abaxial surface of leaf	
	Length of epidermal cells (µm)	Width of epidermal cells (µm)	Length of epidermal cells (µm)	Width of epidermal cells (µm)
Alpinia purpurata	50.32±5.91a	27.56±4.39a	34.80±4.89a	24.91±3.93a
Curcuma aeruginosa	73.87±10.41a	30.79±5.09a	53.47±7.99a	35.72±5.57a
Curcuma heyneana	74.61±12.24a	35.87±6.05a	55.15±8.34a	37.50±5.46a
Curcuma longa	72.36±11.97a	35.85±7.09a	52.64±13.59a	39.01±5.63a
Curcuma mangga	68.92±10.00a	27.15±3.92a	46.08±6.26a	28.44±3.83a
Curcuma zanthorrhiza	76.29±9.68a	30.80±5.29a	51.19±6.87a	35.92±5.26a
Etlingera elatior	57.05±10.42a	30.29±4.96a	40.23±9.50a	26.06±5.97a
Kaempferia rotunda	**87.06±11.88a**	**68.90±9.01b**	**64.83±11.22a**	**45.54±7.93a**
Zingiber officinale	48.83±7.48a	37.23±5.45a	41.38±7.49a	27.77±4.44a
Zingiber purpureum	63.80±7.52a	49.69±5.97a	49.74±7.15a	36.67±4.77a
Wurfbainia compacta	54.00±7.15a	38.43±5.58a	60.90±10.08a	40.21±6.53a

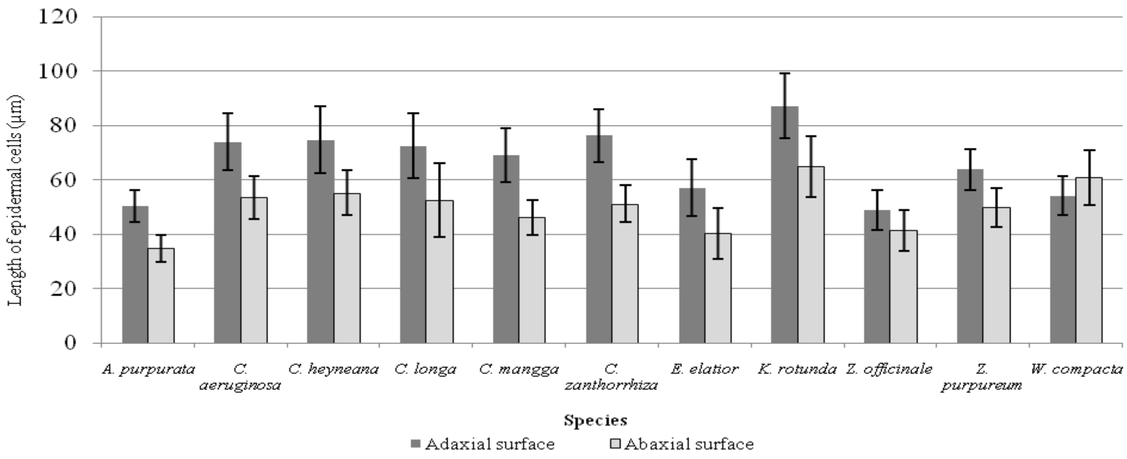


Figure 6. The length of epidermal cells in Zingiberaceae collected from Serang District, Banten, Indonesia

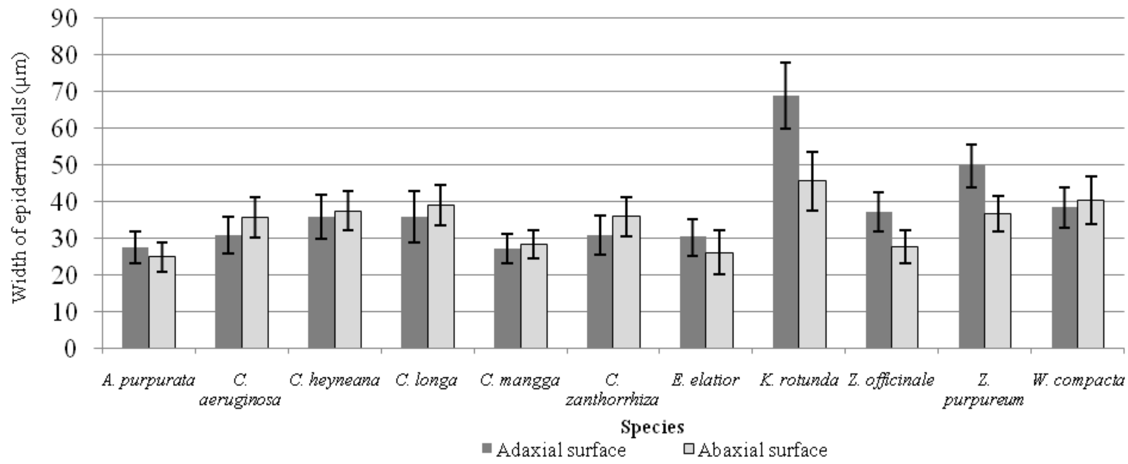


Figure 7. The width of epidermal cells in Zingiberaceae collected from Serang District, Banten, Indonesia

Meanwhile, the shape of epidermal cells that are over leaf vein on the adaxial surface of the leaf can be divided into two classes, i.e. (i) polygonal or rectangular in *A. purpurata*, and (ii) rectangular in *C. aeruginosa*, *C. heyneana*, *C. longa*, *C. mangga*, *C. zanthorrhiza*, *E. elatior*, *K. rotunda*, *Z. officinale*, *Z. purpureum*, and *W. compacta*. The abaxial epidermal cells over leaf veins can also be divided into two classes, i.e. (i) polygonal or rectangular in *A. purpurata*, *C. aeruginosa*, *C. heyneana*, *C. longa*, *C. mangga*, *C. zanthorrhiza*, and *E. elatior*, and (ii) rectangular in *K. rotunda*, *Z. officinale*, *Z. purpureum*, and *W. compacta*. *K. rotunda* has the biggest epidermal cells that are over leaf vein, both on the adaxial surface with a length of 78.79±16.18 µm and a width of 26.70±3.93 µm, and the abaxial surface with a length of 73.92±13.84 µm and a width of 31.73±4.19 µm. The length of epidermal cells that are over leaf vein, on both the adaxial and abaxial surfaces of the leaf, showed a variation that can significantly separate *A. purpurata* from all of the others, while width of adaxial epidermal cells can significantly separate *K. rotunda* from all of the others (Tables 7 and 8).

Correlation analysis of characteristic stomatal parameters

The result of Pearson correlation analysis showed that the stomatal index on abaxial leaf surfaces in 11 species observed was significantly positively correlated with stomatal density ($P<0.05$). The stomatal density was significantly positively correlated with the length of the guard cell of *A. purpurata* and *C. aeruginosa* and the width of the guard cell of *C. heyneana* and *C. longa*, but the stomatal density was significantly negatively correlated with the length of the guard cell of *K. rotunda* and the width of guard cell of *Z. purpureum*. The stomatal density on abaxial surface in the genus *Curcuma* was significantly negatively correlated with the width of epidermal cell ($P<0.05$), as well as in genus *Zingiber* the stomatal density on adaxial surface was significantly negatively correlated with the width of epidermal cell ($P<0.01$).

Table 7. Epidermal cells of leaf epidermis that are over leaf vein in Zingiberaceae collected from Serang District, Banten, Indonesia

Species	Adaxial surface of leaf			Abaxial surface of leaf		
	Epidermal cell shape	**Length of epidermal cells (µm)**	**Width of epidermal cells (µm)**	**Epidermal cell shape**	**Length of epidermal cells (µm)**	**Width of epidermal cells (µm)**
Alpinia purpurata	Polygonal or rectangular	20.08±2.83a	16.78±2.36a	Polygonal or rectangular	18.99±2.54a	16.46±2.12a
Curcuma aeruginosa	Rectangular	41.42±14.00b	17.88±2.29a	Polygonal or rectangular	39.63±4.65b	31.59±3.96a
Curcuma heyneana	Rectangular	54.54±11.13b	15.80±2.13a	Polygonal or rectangular	35.70±6.35b	31.38±4.63a
Curcuma longa	Rectangular	44.82±8.82b	16.40±2.41a	Polygonal or rectangular	33.87±4.24b	29.10±4.24a
Curcuma mangga	Rectangular	31.70±4.27b	13.77±1.93a	Polygonal or rectangular	27.30±3.82b	21.51±3.32a
Curcuma zanthorrhiza	Rectangular	48.46±8.18b	17.66±2.26a	Polygonal or rectangular	31.63±3.41b	27.59±3.73a
Etlingera elatior	Rectangular	31.41±4.73b	14.96±2.25a	Polygonal or rectangular	32.05±6.96b	21.90±3.14a
Kaempferia rotunda	Rectangular	**78.79±16.18b**	**26.70±3.93b**	Rectangular	**73.92±13.84b**	**31.73±4.19a**
Zingiber officinale	Rectangular	44.36±9.07b	14.76±2.61a	Rectangular	60.21±15.59b	17.78±2.77a
Zingiber purpureum	Rectangular	56.00±11.16b	19.33±2.15a	Rectangular	71.96±17.56b	17.83±2.05a
Wurfbainia compacta	Rectangular	50.89±10.44b	14.95±2.06a	Rectangular	60.48±14.02b	20.05±2.09a

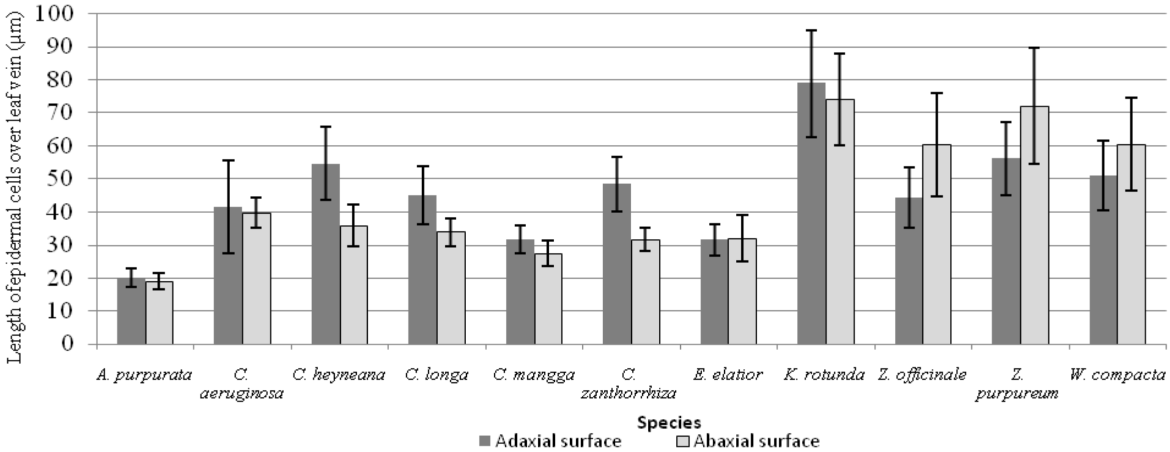


Figure 8. The length of epidermal cells that are over leaf vein in Zingiberaceae collected from Serang District, Banten, Indonesia

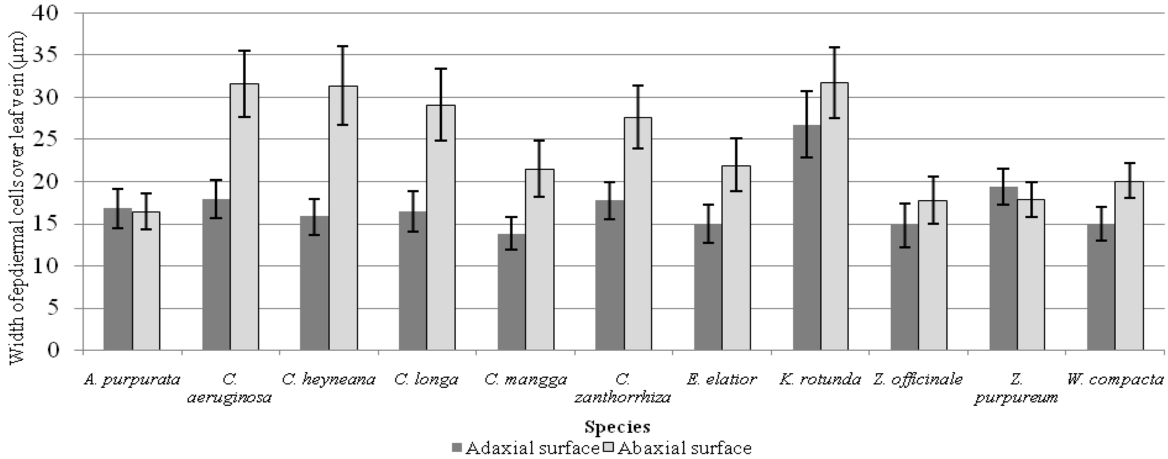
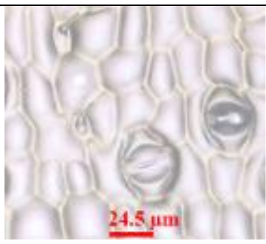
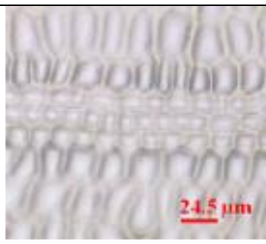
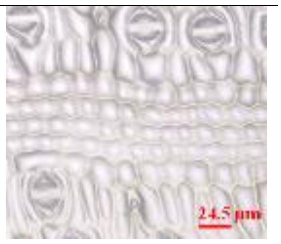
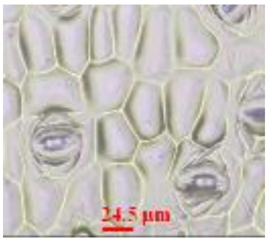
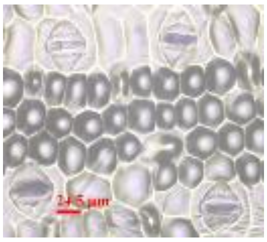
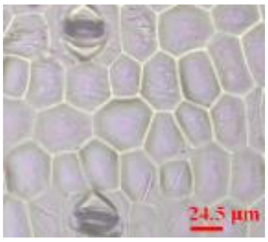
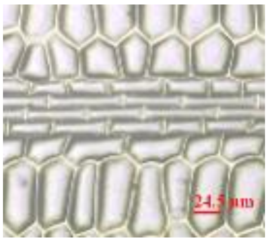
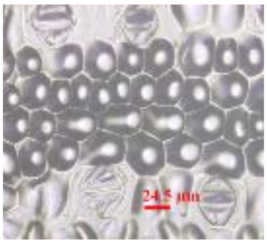
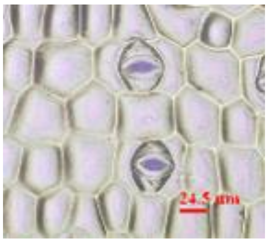
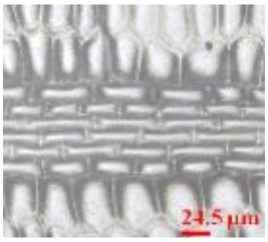
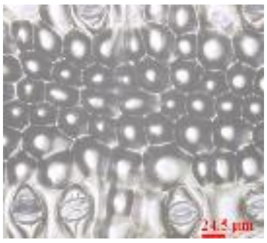
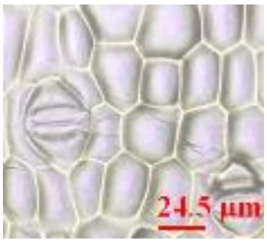
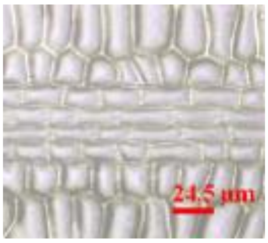
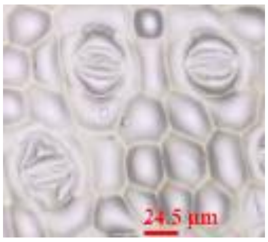
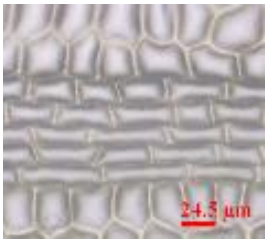
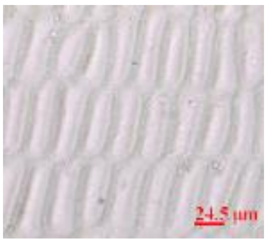
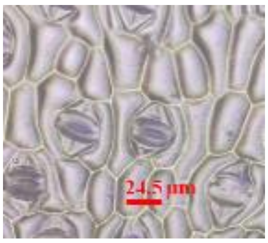
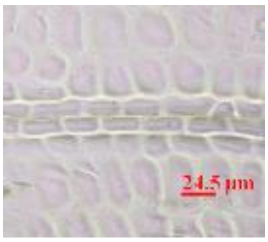
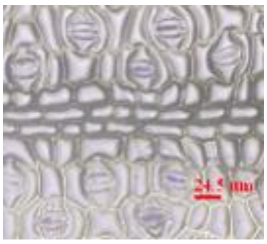
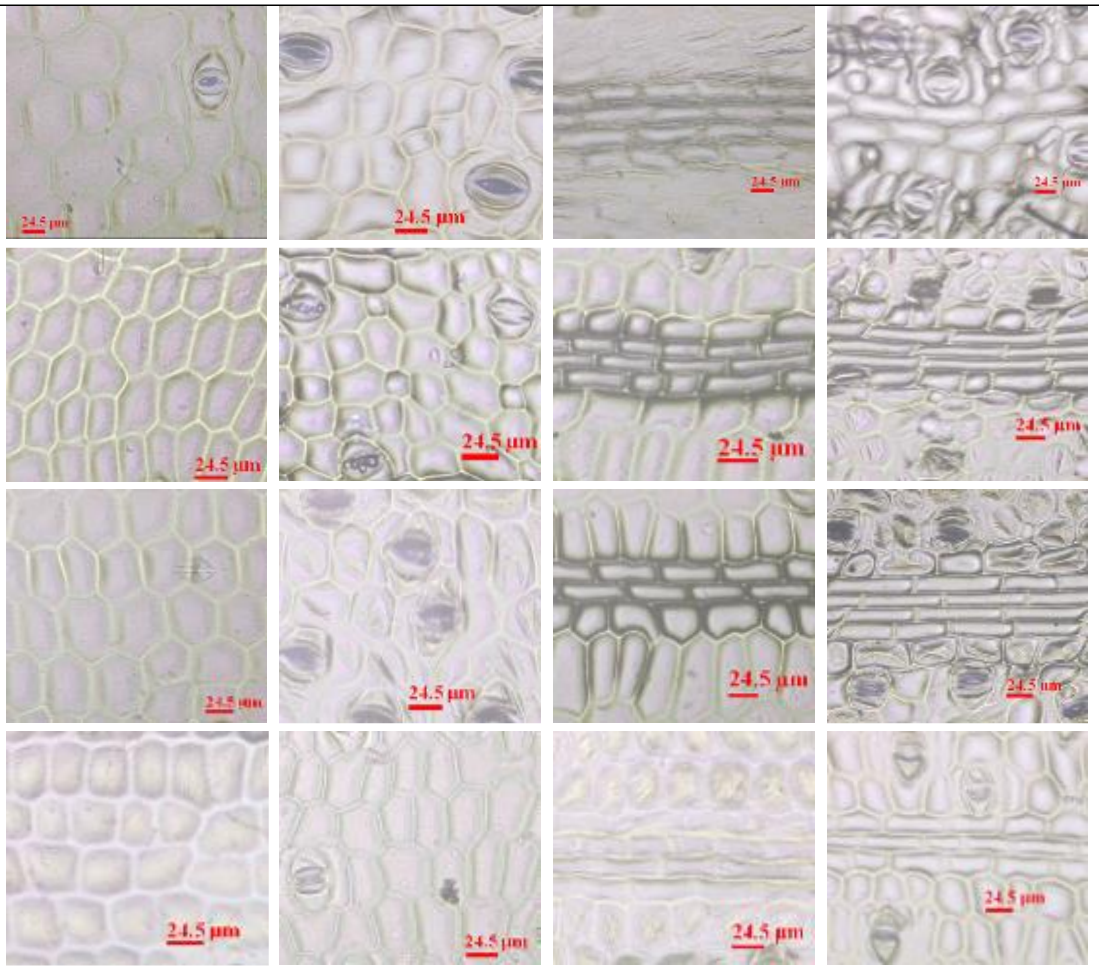


Figure 9. The width of epidermal cells that are over leaf vein in Zingiberaceae collected from Serang District, Banten, Indonesia

Table 8. The epidermal cells of Zingiberaceae collected from Serang District, Banten, Indonesia

Species	Epidermal cells are located between veins		Epidermal cells over leaf vein	
	Adaxial surface	**Abaxial surface**	**Adaxial surface**	**Abaxial surface**
Alpinia purpurata				
Curcuma aeruginosa				
Curcuma heyneana				
Curcuma longa				
Curcuma mangga				
Curcuma zanthorrhiza				
Etlingera elatior				



Discussion

Eleven species observed have the tetracytic type of stomata, specifically brachyparatetracytic type. The tetracytic type had already been observed in a previous study by Setiawan et al. (2020) which found the tetracytic type of stomata in *Alpinia* genus. Zhao et al. (2022) also found that the stomatal type, both on the adaxial and abaxial leaf epidermis, in all *Zingiber* species was the tetracytic type with four subsidiary cells around the stoma, one on each side and one at each pole. However, the result of this study is not according to the result obtained by Zahara (2020) in *Z. officinale* and *E. elatior* which have paracytic stomatal type, while *C. longa* has the stomata type of diacitic by Ferrari (2020).

In all species, stomata occur on adaxial and abaxial leaf epidermis, but stomata are always more abundant on abaxial than adaxial epidermis. All species had a density of stomata on the abaxial surface of the leaf that was higher than on the adaxial surface of the leaf. This result is according to the result obtained by Zahara (2019) revealed that the densities of stomata of *Z. officinale* and *E. elatior* on the abaxial surface of the leaf are more than on the adaxial surface of the leaf. It was presumably due to the presence of the cuticle and the light exposure directly on the upper surface of the leaf which may cause the stomatal damage (Papuangan et al. 2014). Meanwhile, the abaxial surface of the leaf in *E. leatior* showed the closest distance between stomata. The distance between stomata that are too close can inhibit evaporation from other stomata (Sungkar et al. 2017).

The stomatal density on abaxial surface of the leaf significantly separated *E. elatior* and *A. purpurata* from others, while the stomata index on abaxial surface of the leaf just separated *E. elatior* from others. The length of the guard cell on abaxial surface of the leaf divided all species into three groups. The width of adaxial epidermal cells, both between leaf veins and over leaf vein, separated *K. rotunda* from others. Meanwhile, the length of epidermal cells that are over leaf vein, both on adaxial and abaxial surfaces of the leaf, separated *A. purpurata* from others. The results of this study showed that stomatal and epidermal characteristics are not consistent as distinguishing character for Zingiberaceae taxon. However, Hong (2018) reported significant differences in stomatal density, area, as well as width and length of stoma in nine *A. montana* provenances. The characteristic stomatal variation is affected by genetic and environmental factors, such as temperature, air humidity, radiation, CO_2 content in the atmosphere, and humidity and nutrient content in the soil. The genetic variation is associated with the growth environment factors (Hong et al. 2018). Besides that, the stomata are also associated with many physiological functions in plants (Mishra 1997), such as photosynthesis, respiration, and transpiration (Hong et al. 2018).

The stomatal density and size affected directly the transpiration and photosynthesis rate (Hong et al. 2018). Higher stomatal density causes a faster photosynthetic process, so higher stomatal density is able to enhance biomass production in plants (Sakoda et al. 2020). A high stomatal density can help increase conductance and photosynthetic gas exchange before the final senescence of mature leaves (Sabina and Sameena 2022). Stomatal density could be influenced by environmental factors, such as water vapor and CO_2 levels. A high concentration of CO_2 can cause the plant to optimize the absorption of CO_2. To prevent water loss during the photosynthetic process, the plant responds by reducing the number of stomata and stomatal pores to be smaller (Royer 2001). Plant adaptation to salinity and drought can be increased with high density and small stomata size so that the regulation of water transport and plant transpiration will be more efficient (El-Kady et al. 2021). Plants with lower stomatal density and smaller stomatal size have the ability to maintain a high rate of gas exchange under high-temperature stress by opening stomatal pores (Caine et al. 2019).

The mechanism of stomatal opening and closure is affected by turgor pressure in guard cells. Turgor changes of guard cells regulate stomatal movement. Malate has an important role in controlling the turgor pressure within the guard cell for stomatal opening. Otherwise, the presence of abscisic acid reduces the concentration of malate in guard cells causing stomatal closure (Dong et al. 2018). The stomatal aperture is a limiting factor for plant growth. The stomatal opening induces CO_2 uptake for photosynthesis and transpiration, which increases nutrient absorption by roots. The enhancement of stomatal opening increases the photosynthetic activity and growth in plants (Toh et al. 2018). Hong et al. (2018) reported stomatal density is significantly negatively correlated with the long-axis and short-axis length of stomata. The result of this study was consistent with Hong et al. (2018) showed that the stomatal density was significantly negatively correlated with both guard cell length and width.

In some previous studies, the stomatal index was not significantly different between the parental and the offspring (Martin and Stimart 2005) or different ploidy levels (Mishra 1997) in one species. In this study, it was found that the stomatal index on the abaxial surface of the leaf can significantly differentiate *E. elatior* from other species of Zingiberaceae. Another study by Segev et al. (2015) also found that the stomatal index was different between the species from different genera and families. The stomatal index is correlated to the net rate of photosynthesis (Segev et al. 2015). Plants with a lower stomatal index cause a lower net rate of photosynthesis, vice versa (Segev et al. 2015). Unfortunately, in this study, the rate of photosynthesis was not measured a relation between the stomatal index and the rate of photosynthesis could not be observed. However, the results may be assumed that *E. elatior*, which has a higher stomatal index may have a higher net rate of photosynthesis among other species examined.

There are a number of variations found in the epidermis between species, such as differences in cell size and type on both abaxial and adaxial surfaces of leaf. In this study, it was obtained that differences in cell size and type of epidermal cells can be used to distinguish the species. One previous study from Zhao et al. (2022) was also mentioning similar results to our study on *Zingiber* species. The epidermal cells on both the adaxial and abaxial epidermis were found to be hexagonal or polygonal, with non-sinuous anticlinal walls arranged parallel to leaf veins. The result of this study showed the width of epidermal cells was significantly negatively correlated with stomatal density in genus *Curcuma* and *Zingiber*. Many factors affect the characteristic stomatal parameters, so the stability of stomatal characteristics can be one of the important factors in the selection of a plant with considering a better ability of stomatal environmental.

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