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LEAF MICROMORPHOLOGY IN GENUS *Alpinia* (ZINGIBERACEAE)

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Abstract: Leaf epidermal characteristics are essential for phylogenetic and taxonomic studies of many plants. Among the family *Zingiberaceae*, *Alpinia* is recorded as the largest genus. This study aimed to identify the leaf micromorphological characteristics of the genus *Alpinia* that can be used in species identification as supportive data in classification and also to determine the taxonomic value of their micromorphological characteristics. In fact, the genus *Alpinia* has been studied less in Malaysia and requires more supporting data for species identification. Hence, a study has been conducted on eight species of *Alpinia,* which are *Alpinia assimilis, A. javanica, A. ligulata, A. malaccensis, A. mutica*, *A. pahangensis, A. petiolata,* and *A. rafflesiana.* Characters such as epidermal cell shape, trichomes, and stomatal type and distribution were observed. The result from this study suggests that all the species studied are amphistomatic, which means the stomata are present in both the abaxial and adaxial surfaces of the leaf. All the stomas in the species studied are tetracytic. However, trichomes on the leaf surface can only be discovered in *A. assimilis, A. malaccensis*, *A. rafflesiana,* and *A. pahangensis.* Notably, all the trichomes possessed simple and unicellular types. In conclusion, studies on leaf micromorphological in genus *Alpinia* have taxonomic significance and can be used in species identification and classification, especially at the species level.

Keywords: Epidermal cells, light microscope, morphology, stomata, trichomes.

Introduction

Zingiberaceae, commonly known as ginger, is the largest of the eight available families classified under the order Zingiberales (Kress *et al*., 2002; Zahara, 2020). About 53 genera and 1,500 species are classified globally as *Zingiberaceae* (Furmuly & Azemi, 2020). *Zingiberaceae* spread widely in tropical and subtropical areas of the world (Zaini *et al*., 2014; Kajornjit *et al*., 2018; Salasiah & Meekiong, 2018; Furmuly & Azemi, 2020; Zahara, 2020; Windarsih *et al*., 2021; Zhao *et al*., 2022) and this pantropical herb generally distributed from lowland to hill forests (Larsen *et al*., 1999). Furthermore, the gingers family is abundant in lowland tropical rainforests of the Malesian region (Mohamad & Kalu, 2018). In addition, about 200 ginger species from the family *Zingiberaceae* have been recorded in Peninsular Malaysia (Appalasamy *et al*., 2019).

The genus *Alpinia* Robx. is the largest and most widespread genus in the *Zingiberaceae* and comprises approximately 230 species distributed across tropical South Asia to Australia (Kress *et al*., 2005), with 160 species reported in the Malesian region (Larsen, 1996). Most of the peninsular Malaysian species are wild. They are distributed from the lowlands to hill forests, except for a few species that can be discovered in montane forests, such as *A. petiolata* and *A. murdochii* (Talip *et al*., 2005). Note that *Alpinia* is the only genus in the tribe Alpinieae that bears terminal inflorescences on the leafy shoot, i.e., *A. galanga* and *A. mutica,* and mostly have long petioles (Smith, 1985; Kress *et al*., 2002).

The leaf anatomy features were considered to be used as additional evidence in plant phylogeny taxonomy activity. The variation of epidermal shape and characteristics of guard

cells and subsidiary cells of stomata, surface ornamentation, and trichome type can be useful evidence for identifying plant species (Rahayu *et al*., 2012; Zhao *et al*., 2022). According to Barthlott (1990), the micromorphology of cuticular wax is beneficial in taxa delineation at several taxonomic levels within flowering plants. Notably, variations between *Alpinia* species in floral micromorphology (Normalawati *et al*., 1997), pollen morphology (Liang, 1989), and leaf anatomy (Hussin *et al*., 2000; Talip *et al*., 2005) have been reported. A recent study by Setiawan *et al*. (2021) revealed that the size of the stomata, the density of the stomata, and the stomatal index could be utilised to differentiate the species among the members of the *Alpinia* genus.

Many taxonomists have difficulties identifying and classifying species in Alpinia due to morphological similarities, especially without the presence of inflorescents and fruits. To date, anatomical evidence via leaf micromorphological characteristics can be used as supportive data in the identification and classification of plant species. Therefore, this study aimed to explore the possibility of using the stomata and epidermal characteristics for identification in the absence of inflorescence and provide useful additional data for species identification and classification in the genus *Alpinia*.

Materials and Methods

Sample Collection

Fresh leave samples of eight species of *Alpinia* (*Alpinia assimilis*, *A. javanica*, *A. malaccensis*, *A. mutica*, *A. rafflesiana*, *A. pahangensis*, *A. petiolata* and *A. ligulata*) were used in this study. Fresh specimens were obtained from areas such as Simpang Pulai, Bukit Fraser, and Taman Botani Putrajaya. Leaf specimens were fixed in a mixture of 70% ethanol and acetic acid (3:1) for preservation.

Epidermal Peeling

Epidermal peels were prepared by scraping both the abaxial and adaxial surfaces of the leaf until a thin transparent layer was achieved (Hussin *et al*., 2000; Talip *et al*., 2005). The epidermal layers were washed with distilled water stained in safranin. All slides were mounted in Euparal after dehydration, and images were then captured using a light microscope and examined.

Observation of Stomata and Trichome Characteristics

The identification of stomata and trichome types was conducted according to Talip *et al.* (2019) and Amirul-Aiman *et al*. (2017).

Results and Discussion

Based on the results obtained in this study, eight species of *Alpinia* were observed for epidermis cell shapes in abaxial and adaxial, stomatal type, and the presence of trichomes in the genus *Alpinia*. From all species observed, the stomata can be discovered in both the abaxial and adaxial surfaces of the leaf, known as an amphistomatic arrangement. Meanwhile, the trichomes can only be discovered in a few species (Table 1).

Epidermal Cell

The anticlinal walls are straight in cells of both adaxial and abaxial surfaces. The epidermal cells for both adaxial and abaxial surfaces are elongated-hexagonal or more or less polygonal in shape. In this case, a polygonal shape refers to a two-dimensional shape with straight lines consisting of rectangular, pentagonal, hexagonal, heptagonal, and octagonal shapes. Note that different species present different epidermal cell sizes. Figures 1 (a) to (f) illustrate the leaf epidermis of several specimens examined.

Table 1: Leaf epidermal characters of specimens examined

Species	Stomata		Trichome		Epidermis cell shape	
	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial
Alpinia assimilis	Tetracytic	Tetracytic	Simple & unicellular	Simple & unicellular	Elongated-hexagonal	Elongated-hexagonal
Alpinia javanica	Tetracytic	Tetracytic	-	-	Elongated-hexagonal	Elongated-hexagonal
Alpinia malaccensis	Tetracytic	Tetracytic	Simple & unicellular	Simple & unicellular	Polygonal	Polygonal
Alpinia mutica	Tetracytic	Tetracytic	-	-	Polygonal	Polygonal
Alpinia rafflesiana	Tetracytic	Tetracytic	Simple & unicellular	Simple & unicellular	Polygonal	Polygonal
Alpinia ligulata	Tetracytic	Tetracytic	-	-	Polygonal	Polygonal
Alpinia petiolata	Tetracytic	Tetracytic	-	-	Elongated-hexagonal	Elongated-hexagonal
Alpinia pahangensis	Tetracytic	Tetracytic	Simple & unicellular	Simple & unicellular	Elongated-hexagonal	Elongated-hexagonal

Stomata

Generally, the stomata are amphistomatic but are always more abundant on the abaxial than the adaxial surface. The stomata are randomly distributed in *A. javanica, A. rafflesiana, A. petiolata,* and *A. pahangensis*. Meanwhile, in *A. assimilis, A. malaccensis, A. mutica,* and *A. ligulata*, stomata were distributed in rows near veins, with fewer stomata randomly distributed in between. The sizes of the stomata are different in all species yet constant on both surfaces. The stomata of all species studied are of tetracytic form, which refers to guard cells, and are surrounded by four subsidiary cells (two polar and two laterals). Figures 1 (a) and (b) display the types of stomata on the abaxial epidermis in the species examined.

Trichomes

Simple and unicellular trichome types were discovered in the four species studied. The trichomes were scattered on the leaf surface, especially the abaxial epidermis. *Alpinia javanica, A. mutica, A. petiolata,* and *A. ligulata* did not exhibit any presence of trichomes on either surface. Figures 1 (e) and (f) present the type of trichomes in the selected species examined.

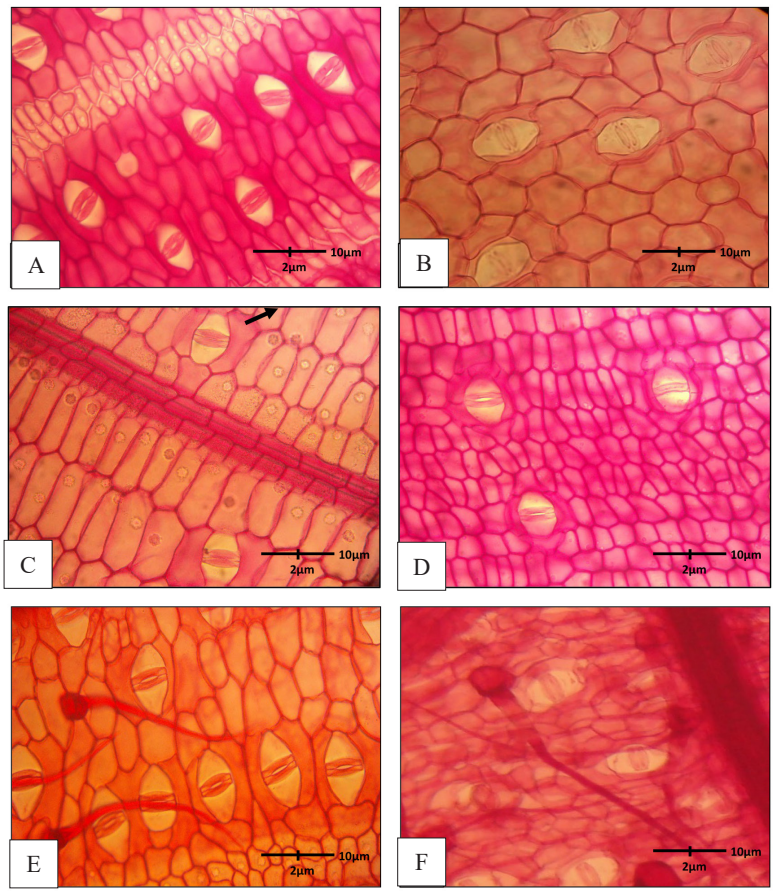

Figure 1: Leaf epidermis of studied specimens. (a) Tetracytic stomata on abaxial surface of *Alpinia asimilis*, (b) abaxial epidermis showing stomata of tetracytic form in *A. petiolate,* (c) the adaxial epidermis of *A. javanica* with crystals in each cell (arrow), (d) the adaxial epidermis of *A. mutica,* (e), and (f) trichomes of simple, unicellular and long in *A. malaccensis* and *A. pahangensis* Scale bar = 20 µm

Our results reveal that the epidermal cells of *Alpinia* species are very similar in shape, i.e., elongated-hexagonal or polygonal, and findings were consistent with the reported characteristics of *Alpinia* (Tomlinson, 1956; Hussin *et al*., 2000; Talip *et al*., 2005; Jayasree, 2007; Salasiah & Meekiong, 2018) which was classified under *Zingiberaceae* Tomlinson (1956, 1969). The straight epidermal anticlinal walls and tetracytic type of stomata were indeed characteristics of *Alpinia* (Tomlinson, 1959; Talip *et al*., 2005). However, some *Alpinia* exhibits tetracytic-type stomata, as indicated by Setiawan *et al*. (2020). For this ginger group (*Zingiberaceae*), the leaf surface trichomes were simple and unicellular. Yet, the leaves of *A. ligulata* and *A. petiolata* did not have trichomes, and this finding is consistent with the early descriptions published by Talip *et al*. (2005). Overall, the leaves of *Alpinia* could be differentiated using a comparison between stomata structures and the shape and form of its trichome. An additional feature described as crystal-like formation in epidermal cells was the characteristics of *A. javanica*. Overall, ginger plants appear similar and can only be distinguished if they bear flowers. However, this study provided new information on identifying *Alpina* using stomata

and trichome characteristics. Therefore, the use of microscopic features may provide useful support to distinguish look-alike plants that were classified in the same taxonomic group.

Conclusion

As in other genera of *Zingiberaceae*, the epidermal cells of *Alpinia* are elongated-hexagonal or polygonal, and tetracytic stomata are distributed on both surfaces. The results from this research suggested that the foliar epidermal features of the species were almost similar. Nevertheless, some of these characteristics can be useful in identifying and classifying the studied taxa. The study also suggests the presence of crystals, one crystal body per cell, on the adaxial surface of *A. javanica*. In some species, the crystal can be found above veins (*A. asimilis*). This study also delineated variations in the traits of the examined species, providing distinguishing features for their differentiation. In conclusion, findings in this study demonstrated the significance of micromorphological epidermal characters, offering valuable systematic and taxonomic information as well as providing additional data for species identification.

Conflict of Interest

All authors declared that they have no conflicts of interest.

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