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ANATOMICAL STUDY OF LIMESTONE ZINGIBERACEAE SPECIES IN JELI DISTRICT, KELANTAN

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Zingiberaceae is the largest family in order Zingiberales and commonly known as aromatic rhizomatous herbs. Few earlier studies recorded the successful adaptation of wild gingers in limestone area. The preliminary study was carried out to investigate the difference in the anatomical characters between limestone and non-limestone ginger species. A total of four wild ginger species from limestone and non-limestone area were selected and the leaf surfaces, pseudostem, rhizome and root were used in this study. Qualitative study revealed different concentration of vascular bundle in vegetative parts between limestone and non-limestone species meanwhile quantitative study exhibited the differences in sizes of veins on leaf epidermis. The anatomy changes in the studied samples were mainly influenced by the differences of nutrients concentration in limestone and non-limestone areas due to their adaptations on different habitats.

Keywords: Zingiberaceae, leaf anatomy, vascular bundle, limestone, Kelantan

INTRODUCTION

Environmental component is essential to establish diversity of habitats which allows plant growth depending on their ecological processes (Barth 2021). Limestone is made up of mineral calcite which primarily composed of calcium carbonate ($CaCO_3$) called sedimentary rock (King 2005). Limestone soil is also known as calcareous soil as it contains $CaCO_3$ and lime accumulation (Taalab et al. 2019). Plants growing in this type of soil ecology usually lack phosphorus and potassium with high pH value of above 7 and may exceed 9 due to the high concentration of $CaCO_3$. (Taalab et al. 2019). Compared to Peninsular Malaysia, the Malaysia region on Borneo has the most abundant limestone forest area with about 130 species of limestone flora (Aimi-Syazana et al. 2017). In East Coast of Peninsular Malaysia, Kelantan remains as one of the understudied state for its limestone flora and thus more research needed to be done not only on its plant diversity and distribution of wild gingers but also the adaptation methods of these plant species to thrive in different ecology and its impact on the plant anatomy (Appalasamy et al. 2020a, Appalasamy et al. 2022a, Appalasamy et al. 2022b). Many studies reported on the distribution of wild gingers in Peninsular Malaysia (Rahman et al. 2008, Ismail et al. 2019, Appalasamy et al. 2019, Izlamira et al. 2020). In addition, wild ginger species can be found growing and adapting well to both limestone area and non-limestone area (Gobilik et al. 2003, Aimi-Syazana et al. 2017, Kiew et al. 2019, Appalasamy & Arumugam 2020, Appalasamy et al. 2020b, Appalasamy et al. 2022a, Appalasamy et al. 2022b, Arumugam et al. 2022).

Plant anatomy is a pertinent branch of study in the field of plant systematics, palaeobotany, evolutionary biology, physiology, ecology and developmental genetics science (Rudall 2007). Solereder and Meyer (1930) carried out the first anatomy analysis of family Zingiberaceae then followed by Tomlinson in 1956 who performed a thorough analysis on anatomy of Zingiberaceae and proposed to elucidate the anatomy variation between intergeneric and interspecific (Tomlinson 1956). Up till now, alterations on interspecific anatomical characters have been established for *Alpinia* (Hussin et al. 2000, Talip et al. 2005), *Zingiber* (Hussin & Ibrahim 1989), *Boesenbergia* and *Kaempferia* (Hussin et al. 2001) and *Curcuma* (Nonkratok et al. 2012). The aim of this study was to provide preliminary study data on the differences of anatomical structure in limestone ginger species compared to non-limestone ginger species.

MATERIALS AND METHOD

Plants materials

Whole flowering plant consisted of 2–3 individuals (Figure 1) for each of four wild ginger species such as *Alpinia javanica*, *Etlingera punicea*, *Globba patens*, *Globba leucantha* were collected at Jeli District in Kelantan from January 2020 to May 2020 at different locations which were classified into limestone in Gua Setir and non-limestone area in Lata Janggut (Table 1). The fertile samples were identified using the flower identification keys and literature related to ginger (Larsen et al. 1999). Collected fresh specimens were brought to the Biochemical and Microbiology Laboratory, Faculty of Earth Science, Universiti Malaysia Kelantan Jeli Campus for further analysis. Voucher specimens were also collected and deposited at Earth Science Museum in Universiti Malaysia Kelantan for future reference.

Light microscopy

The leaves, root, rhizome and pseudostem of selected limestone and non-limestone dwelling wild ginger specimens were collected in the field locations. For leaf atomical studies, the epidermal of leaves were scrapped using a blade between its midrib and margin meanwhile root, rhizome and pseudostem were sectioned at the middle part by using sliding microtome at thickness of 6 to 10 µm. The specimens were then stained using methylene blue or iodine and were rinsed with dehydration series of ethyl alcohol to discard excess water in the sample. The samples then were mounted on slides using Canada Balsam and covered with cover slip. The slides were dried on the slide dryer. The well-mounted slides were examined using polarized light microscope in Biochemical and Microbiology Laboratory at Faculty of Earth Science, UMK. The photographs of the leaf structure were taken with aid of a microscopic camera.

RESULTS AND DISCUSSION

A total of four species belongs to the family Zingiberaceae from limestone and non-limestone areas were collected (Figure 1 & Table 1). An anatomical analysis was conducted on the collected ginger species where leaf, root, rhizome and pseudostem parts were used for anatomy study.

Anatomy of leaves

Epidermal cells

The epidermal cells in leaves for both limestone and non-limestone gingers were studied. The epidermal cells are the outer layer on leaves and generally consists of straight anticlinal walls on both abaxial and adaxial surfaces. The epidermal cells observed were rectangular or hexagonal in shape.

Stomata

Based on visual observation, the stomata could be found on both adaxial and abaxial surfaces which was also known as amphistomatic. The stomata were more concentrated on the abaxial surfaces compared to adaxial surfaces (Figure 2). The stomata in all studied species were in tetracytic form whereby each stoma was surrounded by four subsidiary cells.

Vein

Leaf veins composed of vascular tissue which was made up by xylem and phloem in tube-shaped structures. Based on the observation of leaf surfaces of *Etlingera punicea*, veins in limestone species indicated slightly bigger size compared to non-limestone species due to higher concentration of nutrients in limestone areas (Figure 3).

Transverse section of pseudostem

The pseudostem is the part of plant whereby the leaf sheaths overlapped tightly to form the false stem as the leaves emerge one after

Figure 1 The inflorescence of identified ginger species in Kelantan (a) *Alpinia javanica,* (b) *Etlingera punicea,* (c) *Globba leucantha* and (d) *Globba patens*

Table 1 Fertile ginger species collected at limestone and non-limestone area in Kelantan, Malaysia

No	Species	Location	Habitat
1.	*Alpinia javanica*	Gua Setir, Jeli Lata Janggut, Jeli	Limestone Non-limestone
2.	*Etlingera punicea*	Gua Setir, Jeli Lata Janggut, Jeli	Limestone Non-limestone
3.	*Globba patens*	Gua Setir, Jeli Ginger & Herbs Nursery, Institute of Food Security and Sustainable Agriculture	Limestone Non-limestone
4.	*Globba leucantha*	Gua Setir, Jeli Ginger & Herbs Nursery, Institute of Food Security and Sustainable Agriculture	Limestone Non-limestone

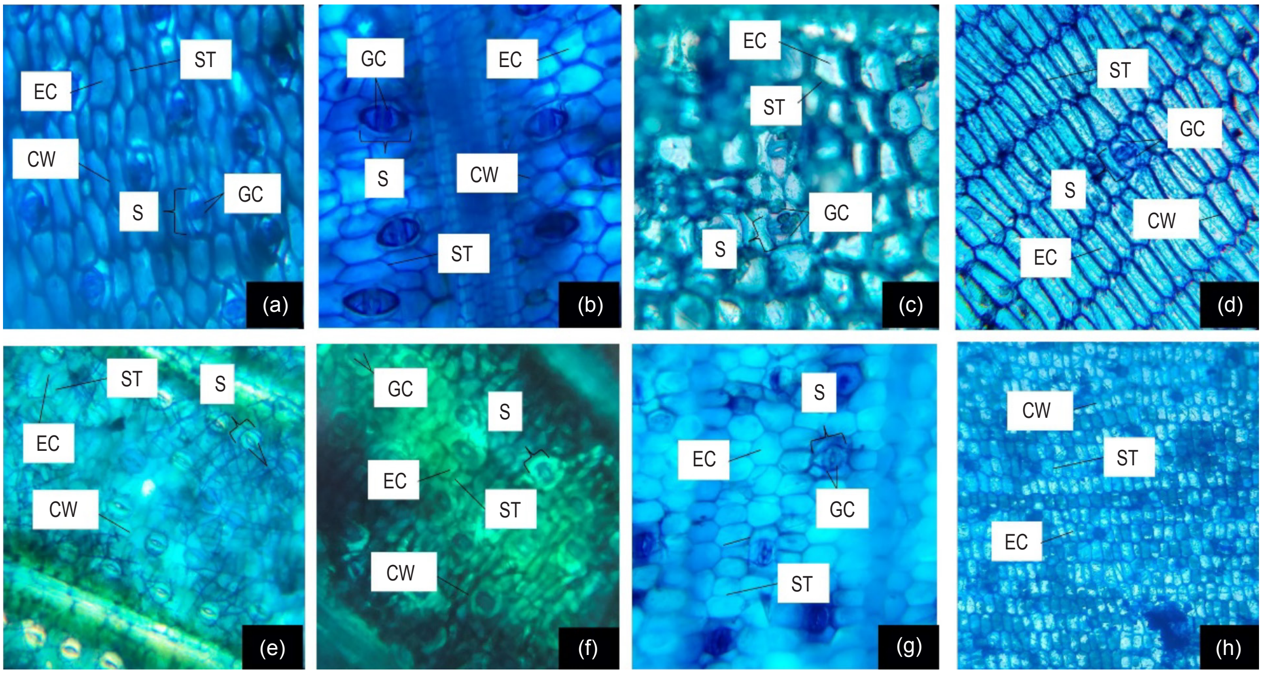

Figure 2 Leaf epidermis of studied species (a) non-limestone *Alpinia javanica*, (b) limestone *A. javanica*, (c) non-limestone *Globba patens*, (d) limestone *G. patens*, (e) non-limestone *Etlingera punicea*, (f) limestone *E. punicea*, (g) non-limestone *Globba leucantha* and (h) limestone *G. leucantha* Magnification = 4 ×100, microscope type = light microscope; CW = cell wall, EC = epidermal cell, GC = guard cell, S = stomata, ST = straight anticlinal walls

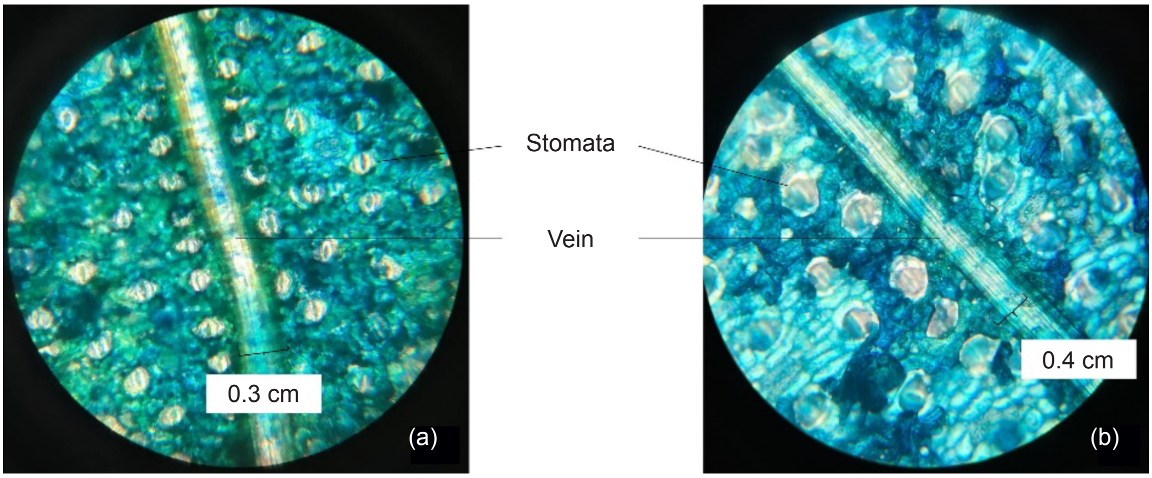

Figure 3 Leaf epidermis of studied species (a) non-limestone *Etlingera punicea* and (b) limestone *E. punicea* Magnification = 4 × 100, microscope type = light microscope

the other (Ortiz et al. 1995). The transverse section of the pseudostem (Figure 4) indicated that the arrangement of vascular bundle was distinguishable between limestone dwelling gingers and non-limestone gingers.

Based on visual observation, phloem in every specimen could be seen clearly by staining, therefore able to differentiate between the phloem and xylem. In addition, xylem could be visualized as tiny hole as it acted as pathway for water source transportation throughout the plant (Figure 4). In limestone gingers *G. patens* and *G. leucantha*, the phloem was well stained and expected to be larger than for non-limestone gingers *G. patens* and *G. leucantha*. In addition, the arrangement of vascular bundle was oriented in a proper O-shaped in non-limestone gingers as compared to limestone gingers.

Transverse section of rhizome

In family Zingiberaceae, sessile rhizome was known for producing aroma, containing secretory cells and producing essential oil (Roy et al. 2013). Rhizome also acted as food storage for plant. A pioneer study was done on rhizomes of *Curcuma* species in 1997 and *Zingiber officinale* in 1998 (Uma & Muthukumar 2014). The transverse section of rhizome (Figure 5) indicated that the density and concentration of vascular bundle was distinguishable between limestone dwelling gingers and non-limestone gingers.

Based on visual observation, the phloem in every specimen could be seen clearly as it well stained and therefore able to differentiate between phloem and xylem (Figure 5). Moreover, tiny hole in specimen shown was xylem as it was responsible to transport water to the cells throughout the plant. In limestone ginger *A. javanica*, phloem was stained well and expected to be larger than in non-limestone ginger *A. javanica*. Generally, the vascular bundle in limestone dwelling gingers was dense and close to each other. In *G. patens*, vascular bundle showed yellow colour after the staining process and the vascular bundle was compacted more in the pith for limestone *G. patens*. In addition, limestone *G. patens* indicated O-shaped endodermis thickening meanwhile non-limestone *G. patens* and non-limestone *G. leucantha* showed U-shaped endodermis thickening.

Transverse section of root

There were insufficient morphological and anatomical studies for roots compared to other vegetative parts because it was located hidden in the ground (Waisel et al. 2002). The roots also indicated not much differences in morphology that led to complication in plant identification (Rewald et al. 2012). However, roots were always used for studies in nutrient cycling, plant genetics and tissue growth patterns (Lux & Rost 2012). The roots also played an important role for plant development by supplying nutrients and for support purposes (Smith & Smet 2012).

In Zingiberaceae, roots could develop from the rhizome or sessile tubers whereby both demonstrated difference structure and functions (Uma & Muthukumar 2014). In tribe Globbae, the rhizomes produced adventitious roots from

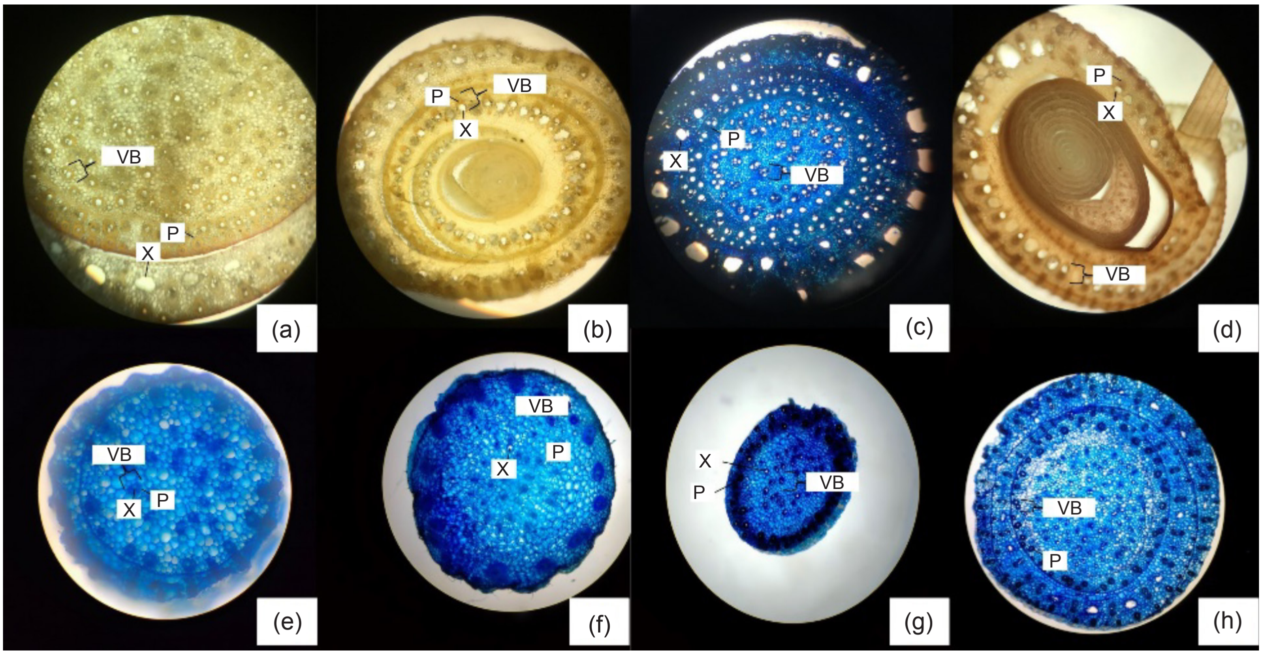

Figure 4 Transverse section on pseudostem of studied species (a) non-limestone *Alpinia javanica*, (b) limestone *A. javanica*, (c) non-limestone *Etlingera punicea*, (d) limestone *E. punicea*, (e) non-limestone *Globba patens*, (f) limestone *G. patens*, (g) non-limestone *Globba leucantha*, and (h) limestone *G. leucantha* Magnification = 4 ×100, microscope type = light microscope, VB = vascular bundle, P = phloem, X = xylem

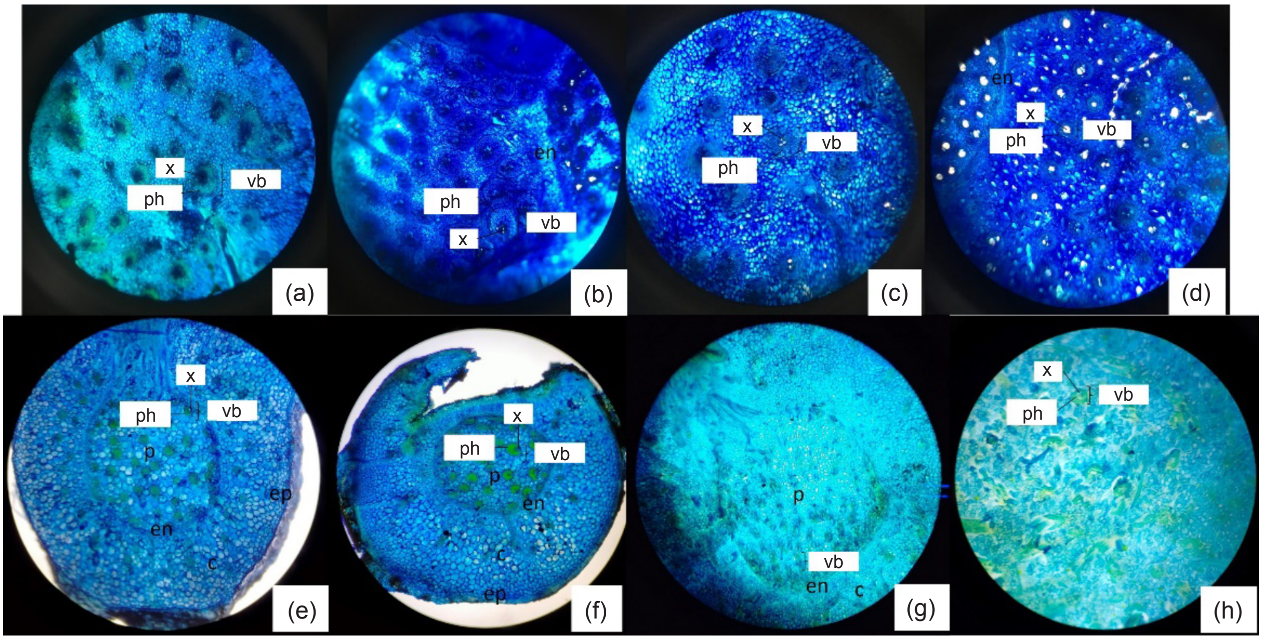

Figure 5 Transverse section on rhizome of studied species (a) non-limestone *Alpinia javanica*, (b) limestone *A. javanica*, (c) non-limestone *Etlingera punicea*, (d) limestone *E. punicea*, (e) non-limestone *Globba patens*, (f) limestone *G. patens*, (g) non-limestone *Globba leucantha* and (h) limestone *G. leucantha* Magnification = 4 × 100, microscope type = light microscope, ph = phloem, x = xylem, vb = vascular bundle, p = pith, c = cortex, en = endodermis, ep = epidermis

the base and later changed into tuberous roots which might become fibrous roots (Khuankaew et al. 2009) whereby adventitious roots could be differentiated from rhizome in the tribe Alpineae (Uma & Muthukumar 2014). Moreover, species belongs to tribe Globbae exhibit small roots that pulled the rhizome deeper into the ground for protection purposes (Barclay 2002).

Based on visual observation, xylem in non-limestone origin gingers was denser (Figure 6) and more concentrated compared to limestone origin gingers (Figure 5). The small hole in specimen shown was xylem which functioned to transport water for plants. However, concentration of phloem was higher in limestone origin gingers might be due to presence of calcium carbonate in limestone soil. Future work could also focus on the comparison of the macro and micronutrients soil content of these two habitats.

Factors influencing the anatomical structure between limestone and non-limestone gingers

The changes of anatomical structures in plant might be due to the microclimate. Microclimate is an important for plant adaptation because phenotypic and genotypic changes are influenced by the organisms' actual environmental factors. Microclimate refers to any optimal growth in a relatively small area involving only a few meters or less either above or below the Earth's surface and within soil layers. Microclimate influences the plant physiology such as photosynthesis, respiration, transpiration, and translocation ultimately determine plant productivity and growth (Nassar et al. 2018). The present study which compared selected species of wild gingers collected from limestone and non-limestone habitat in Kelantan showed several significant differences through their anatomy which indicated microclimate influence and accumulation of plant adaptation effect. Gobilik et al. (2003) has mentioned that limestone areas lack dispersion, water and insufficient light source. Thus, due to that reason the anatomical features in limestone and non-limestone gingers showed clear differences because of the hilly limestone areas in Kelantan. The microclimate condition especially water amount and irrigation frequency in highland and lowland is different. Therefore, the limestone gingers mostly showed water deficiency. However, the microclimate in

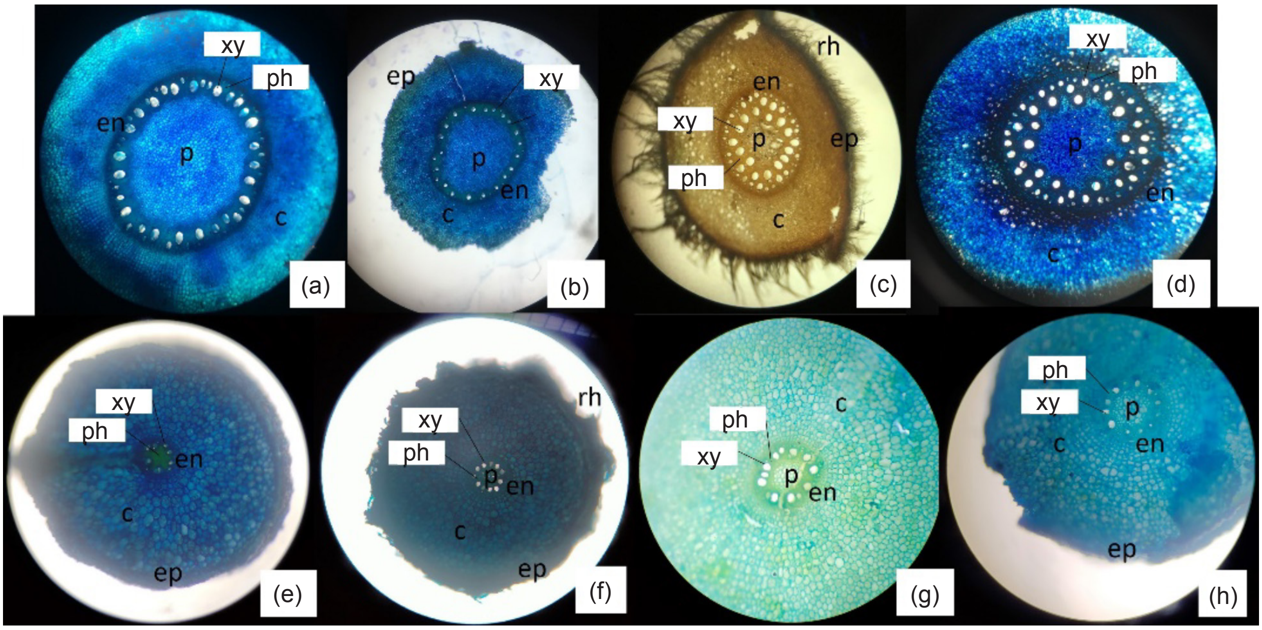

Figure 6 Transverse section on roots of studied species (a) non-limestone *Alpinia javanica*, (b) limestone *A. javanica*, (c) non-limestone *Etlingera punicea*, (d) limestone *E. punicea*, (e) non-limestone *Globba patens*, (f) limestone *G. patens*, (g) non-limestone *Globba leucantha* and (h) limestone *G. leucantha* Magnification = 4 × 100, microscope type = light microscope, ph = phloem, xy = xylem, p = pith, c = cortex, en = endodermis, ep = epidermis, rh = root hairs

natural environments is beyond human control and therefore, the wild ginger needs adaptive anatomical features in order to survive in those areas.

From the study, the ginger species collected from limestone and non-limestone areas clearly showed differences in their anatomical features even though the limestone and non-limestone species look morphologically similar. Limestone soil contains sedimentary rocks consist of minerals such as calcite (calcium carbonate, $CaCO_3$) and dolomite [$CaMg(CO_3)_2$] (Tan 2002) and the water resources in the limestone area are scarce. The weakening of soil drainage makes the soil in limestone becoming clay colloids and high pH level more than 8.5. Plants at that area are stressed and disrupted growth also due to the presence of pedogenic carbonate in calcium carbonate ($CaCO_3$) in limestone soil (Pal 2016). Therefore, wild ginger plants need to make adjustment to ensure they can adapt to the conditions of limestone habitat. The study proved that the thickness of these ginger cells and tissues were different because they were influenced by these factors. Limestone also could affect the pH level which gave plant the opportunity to enhance the nutritional level (Zellweger et al. 2020) and may lead to thickness of phloem in limestone dwelling gingers.

Based on visual observation, the xylem in limestone gingers were smaller compared to the non-limestone species due to the limited water resources. Jaafar et al. (2012) mentioned that ginger grew best in steep and well-drained environments. In addition, natural disturbance, substrate, dispersion, canopy gaps and pollination are the primary elements that could change the composition of gingers on the limestone ridges. Herbivory and pathogens were also suggested to be major influences and very severe in gingers, even though their effects in the limestone area had never been quantified (Gobilik et al. 2003). According to Zellweger et al. (2020), climate conditions near to the ground (2 m) or coupled with vertical forest profiles at relatively fine spatiotemporal resolutions, such as over spatial dimensions of centimeters to meters and temporal dimensions of minutes or less, are referred to as microclimate. The microclimate variables include temperature, precipitation, humidity, wind and radiation regimes (Zellweger et al. 2020). Therefore, the microclimate in their habitat play important role in the differences between the limestone and non-limestone species.

CONCLUSION

The study listed four fertile ginger species from different localities of limestone and non-limestone areas in Kelantan. All the gingers species had the capability to grow in both limestone and non-limestone area thus a general comparison was made between limestone and non-limestone gingers. The comparison of the anatomical features achieved the objective of comparing limestone and non-limestone species, as the result showed the differences in anatomical features of the same species in different habitat areas. The study showed that the morphological features of the species were almost the same but there some differences in the anatomical features especially a slight difference in terms of the thickness of the cell, the arrangement of the cell and ability to survive and adapt with the surroundings. However, some cells still demonstrated the same criteria and size even they were of different areas of origin. The utilization of microscope scale in microscopic images assisted in differentiating and measuring the cell thickness between limestone and non-limestone ginger species. However, there were few limitations that could be improved in future study such as the determination of the age of collected plants and distribution of rainfall in the area as well as the calculation of the stomatal density on the epidermal cells.

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