Eksakta : Berkala Ilmiah Bidang MIPA
VOLUME 23 NO 02 2022, pp 78-87
ISSN : Print 1411-3724 — Online 2549-7464
DOI : https://doi.org/10.24036/eksakta/vol23-iss02/317

Eksakta
Berkala Ilmiah Bidang MIPA
http://www.eksakta.ppj.unp.ac.id/index.php/eksakta

Article

Leaf Epidermal Comparison of Phasey Bean (*Macroptilium lathyroides*) and Siratro (*Macroptilium atropurpureum*)

I Made Saka Wijaya[1*], Eniek Kriswiyanti[1]

[1] Biology Program, Faculty of Mathematics and Natural Sciences, Universitas Udayana, Bali, Indonesia

Article Info

Article history :

Received April 19, 2022
Revised May 06, 2022
Accepted May 20, 2022
Published June 30, 2022

Keywords :

anatomical comparison, anomocytic, paracytic, stomata, trichomes

Abstract. Phasey bean (*Macroptilium lathyroides*) and siratro (*Macroptilium atropurpureum*) are introduced legumes that become a common species in pastureland. The nutritional content of these legumes has been explored, but on the contrary for the anatomical study. The anatomical traits, especially leaf epidermal, have been used to increase the understanding of taxonomical study. This study aimed to investigate the leaf epidermal variability among phasey bean and siratro. The method used longitudinal section for upper and lower epidermal, then stained in safranin 0,1%. The results show that the upper epidermal in phasey bean has a polygonal epidermal cell, while the lower part and both parts of siratro have irregular-shaped. The type of stomata in the upper epidermal of phasey bean is paracytic and the lower epidermal is paracytic and anomocytic. Both epidermal sides in siratro have paracytic and anomocytic stomata. The index of stomata in phasey bean is higher than siratro, but the index of trichomes in phasey bean is lower than siratro. The trichomes were only absent in the upper epidermal of the phasey bean. Both plants have similar types of trichomes: capitate glandular trichomes and linear non-glandular trichomes.

Corresponding Author :
I Made Saka Wijaya
Biology Program, Faculty of Mathematics and Natural Sciences, Universitas Udayana, Badung, Bali – Indonesia 80361
Email : sakawijaya@unud.ac.id

1. Introduction

Phasey bean (*Macroptilium lathyroides* (L.) Urb.) and siratro (*Macroptilium atropurpureum* (DC.) Urb.) were the two common legumes that were used as a pasture plant. In Indonesia, these two species were introduced from Central and South America [1], and over a long period have been naturalized in Indonesia. These species have high adaptabilities in the tropical region, such as habitat variability and altitudinal distribution from the sea level up to 2,000 m a.s.l. [1, 2].

Phasey bean is an erect legume with a stem up to 1.5 m. Phasey bean is an annual plant (rarely biennial); the stem is woody at the base; leaf pinnately trifoliated, leaflet oval – rhomboid, 3-8 x 1-3.5 cm, margin entire, acute at the base, acute-obtuse at the apex; inflorescence in a raceme, length 15-25 cm (rarely reach 50 cm); flower typical papilionoid, colored in red to red-purple, 1-1.5 cm in diameter; pods in a linear shape, 5.5-12 cm in length, contains 20-30 seeds [1].

Siratro is a climbing legume, rarely forming erect stem; the stem is woody at the base, hairy, height up to 5 m; leaf pinnately trifoliated, middle leaflet ovate – rhomboid, 6-9 x 4-6 cm, lateral leaflet rhomboid, 6-8 x 5-7 cm; inflorescence in a raceme, bearing 6-12 flower; flower typical papilionoid, colored in purple to deep purple; pods in a linear shape, 5-10 cm in length, contains 5-10 seeds [2].

In the botanical study, anatomical observation was widely used to increase the understanding of physiological processes and also in the taxonomical study. The anatomical approach is more convenient to distinguish species with similar morphological traits [3] or to complement the macromorphological traits that might be plesiomorphic or by-products of parallelism [4]. Some of the anatomical traits also show an evolutionary geometric relationship among characters [5], so it can be used to produce a more robust phylogenetic tree.

Leaf was one of the organs that have many variations in anatomical structures that have been considered as important taxonomical data [3, 4, 6], especially the epidermal tissue [7]. The epidermal tissue in the plant is the outermost tissue that plays a major role as a protective barrier to biotic and abiotic threats, while also maintaining the gas and water exchanges [8]. These multiple roles of epidermal tissue are supported by epidermal cells and some epidermal cells that developed certain structures, known as derivates of epidermal.

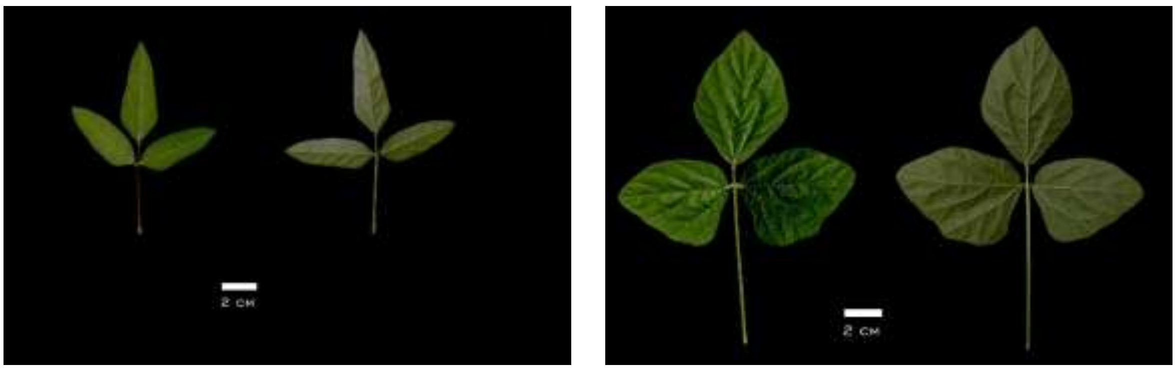
The derivate of epidermal can be observed in form of stomata and trichomes. These two derivates are frequently used for biosystematics studies because of their structure was influenced by the environment [3, 9, 10]. The prior research, trichomes can be used as a diagnostic character in Lamiaceae [11] and tribe Trifolieae of Leguminosae [12], and to provide the taxonomical feature in Caryophyllaceae [7], *Piper* [13], and *Polygonatum* [10]. Trichomes also can be combined with stomata, crystal, and oil cell distribution as complementary data in the systematic study of the genus *Zingiber* [3]. In further research, those epidermal structures, particularly stomata, potentially can be used as a major tool to construct paleoclimatic because of their correlation to functional ecology [14].

Not only the derivates, the epidermal cell also has been studied in many approaches. The pavement of the epidermal cell was correlated with genetic traits that later affect the mechanical process in the formation of the epidermal cell wall [15]. The shape and pavement of epidermal cells can be used to connect the plant evolutionary from fern to eudicots [16]. The epidermal cell also has clade-specific traits such as in Asian *Cinnamomum* [4]. Despite many benefits from the anatomical trait, the anatomical data of the phasey bean and siratro is still insufficient. Then, this leaf epidermal study in phasey bean and siratro aimed to investigate the anatomical characteristic among those plants. In the long term, it could provide the anatomical spectrum of epidermal in the genus *Macroptilium*.

2. Materials and Methods

2.1. Sample collections

Sample of phasey bean and siratro obtained from Tropical Forage Research and Development Center, Universitas Udayana. The samples were mature flowering plants, to assume the consistency of the leaf tissue structure. Three leaves were used for each species, and each leaf used five repetitions.



(a) (b)

Figure 1. Leaf morphological of the samples showing adaxial (left) and abaxial (right) surface (a) phasey bean (*Macroptilium lathyroides*) and (b) siratro (*Macroptilium atropurpureum*)

2.2. Leaf epidermal observation

The leaf epidermal observation used a longitudinal section for upper and lower epidermal tissue. Preparat were made by using hand-section from the prepared sample in alcohol 70%, then stained in 0,1% safranin for 10 minutes and soaked in the aquades for 2 minutes. Preparate observed in a microscope equipped with Optilab (with Optilab viewer 2.2) to capture the images. The cell measurement using Image Raster 3. For a more representative epidermal structure, the observation was added with the illustration. The data collected in the observation was the structure of the epidermal cell, subsidiary cell, type of stomata, and type and structure of trichomes.

2.3. Data analysis

The obtained data were analyzed by descriptive and by index. The descriptive analysis by describing the anatomical structure of the epidermal tissue. The index analysis uses the index of stomata and the index of trichomes with the formula below.

$$Stomatal\ Index\ (SI) = \frac{S}{S+E} \times 100\%$$

S = the number of stomata
E = the number of epidermal cells

$$Trichomes\ Index\ (TI) = \frac{T}{T+E} \times 100\%$$

T = the number of trichomes
E = the number of epidermal cells

3. Results and Discussion

The epidermal component that can be observed in phasey bean and siratro consists of epidermal cells, stomata, and trichomes. The summary of the epidermal variation is shown in Table 1, while the epidermal illustration for the phasey bean is shown in Figure 2 and for the siratro in Figure 3.

For epidermal cells, phasey bean have different shapes of epidermal cells between the upper and lower epidermis. The upper epidermis was polygonal-shaped, the cell size is 137.96 ± 27.53 × 59.66 ± 10.79 µm, while the lower epidermis was irregular-shaped with size is 192.92 ± 34.88 µm × 85.84 ± 17.04 µm.

Table 1. Anatomical comparison of leaf epidermal in phasey bean (*Macroptilium lathyroides*) and siratro (*Macroptilium atropurpureum*)

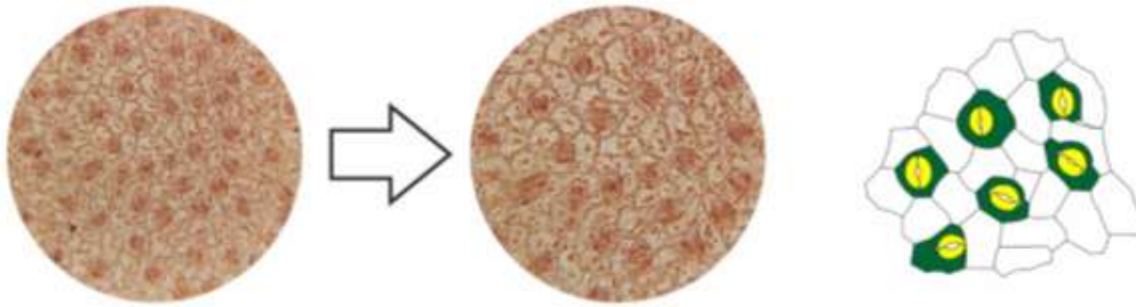
Epidermal component	Characteristic	Phasey bean (*Macroptilium lathyroides*)	Siratro (*Macroptilium atropurpureum*)
Epidermal cell	Shape	U: polygonal-shaped L: irregular-shaped	U: irregular-shaped L: irregular-shaped
	Size (µm)	U: 137.96 ± 27.53 × 59.66 ± 10.79 L: 192.92 ± 34.88 × 85.84 ± 17.04	U: 195.63 ± 36.82 × 88.09 ± 16.83 L: 169.96 ± 36.36 × 70.05 ± 16.37
Stomata	Type	U: paracytic L: paracytic, anomositic	U: paracytic, anomositic L: paracytic, anomositic
	SI (%)	U: 17.55 ± 3.50 L: 20.15 ± 3.29	U: 11.08 ± 3.15 L: 17.73 ± 0.59
Trichomes	Glandular	U: absent L: present. Capitate.	U: present. Capitate. L: present. Capitate.
	Size (µm)	U: - L: 154.27 ± 21.44 × 77.41 ± 12.00	U: 140.06 ± 18.83 × 73.38 ± 12.62 L: 139.39 ± 17.09 × 74.13 ± 11.47
	Non-glandular	U: absent L: present. Linear.	U: present. Linear. L: present. Linear.
	Size (µm)	U: - L: 1124.65 ± 338.88	U: 1195.78 ± 163.85 L: 2002.32 ± 268.48
	TI (%)	U: - L: 1.98 ± 0.36	U: 3.05 ± 0.96 L: 2.90 ± 0.85

Annotations: U=upper epidermis; L=lower epidermis; SI=stomatal index; TI=trichomes index

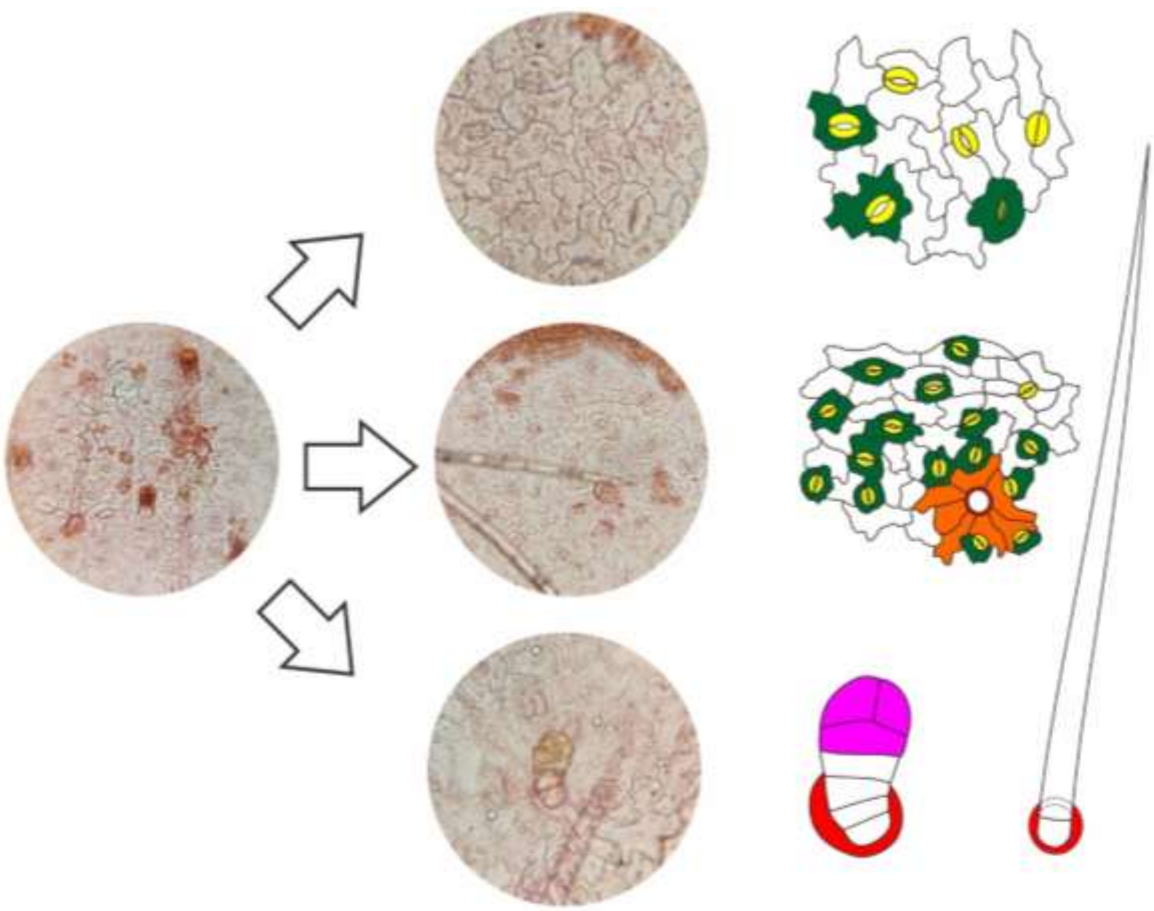
In siratro, both upper and lower epidermal cells are irregular-shaped with more grooves than lower epidermal cells of phasey bean. The irregular-shaped epidermal cell showed an identical shape to the typical epidermal cell in *Solanum* [17]. The average size of upper epidermal cells is wider than the lower epidermal cells, about 195.63 ± 36.82 × 88.09 ± 16.83 µm for upper epidermal and 169.96 ± 36.36 × 70.05 ± 16.37 µm for lower epidermal.

The shape and size of the epidermal cell can be affected by environmental conditions, such as temperature, soil, nutrient, and humidity [8]. The shape of epidermal cells in the upper and lower epidermal can have a similar shape or might be different. In phasey bean, the lower epidermal have an irregular-shaped epidermal cell, while the upper epidermal have polygonal-shaped epidermal cell. In the different shapes of the epidermal cell, the irregular-shaped (or also known as undulated-shaped) epidermal cell was more often found in the lower part of the leaf [16].

The undulations have increased the research curiosity because of the pattern in higher taxa. The epidermal cell in the fern has a strongly undulating margin, monocots have weakly undulating margins, and eudicots have no particular degree of undulation [16]. The high undulation epidermal cell is commonly found in drought-tolerant species [11]. There is some hypothesis about the undulation structure, which might be to increase the cells' contact to improve the efficiency of chemical signaling, to increase the epidermal integrity, or it might be caused by their growth dynamics [18].



(a)



(b)

Figure 2. The epidermal characteristics of phasey bean (*Macroptilium lathyroides*) (a) the upper epidermal that only has stomata as its epidermal derivates; and (b) the lower epidermal that has more varied epidermal derivates, such as two types of stomata, glandular trichome, and non-glandular trichomes.
The coloration in illustration indicates particular cells: epidermal cell = without shade; guard cells = yellow; subsidiary cells = green; base of trichomes = red; cells

around trichomes = orange; apical cell in glandular trichome = purple.

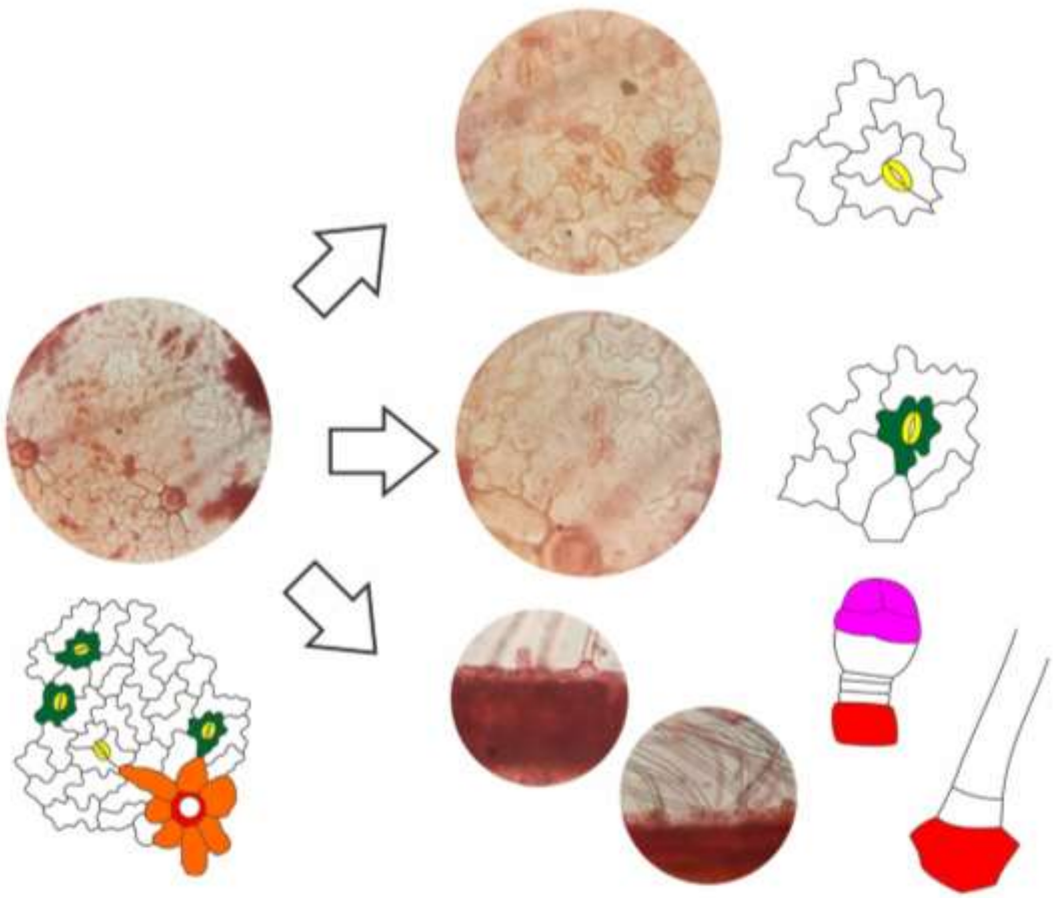


Figure 3. The epidermal characteristics of siratro (*Macroptilium atropurpureum*) have the same structure for upper and lower epidermal, such as two types of stomata (anomocytic and parasitic), glandular trichome, and non-glandular trichomes.
The coloration in illustration indicates particular cells: epidermal cell = without shade; guard cells = yellow; subsidiary cells = green; base of trichomes = red; cells around trichomes = orange; apical cell in glandular trichome = purple.

The most interesting part of the phasey bean and siratro is the stomata. Stomata is a derivate of epidermis that plays a major role in water and gas exchange between the plant and the atmosphere [14, 19]. The functionality of stomata is related to the two guard cells that are responsible for pore formation. In the majority of the plant, there is also an integral part of stomata called subsidiary cells that flanked those guard cells. The subsidiary cells are defined as cells that are adjacent to guard cells and are distinct (have a unique morphology or molecular signature) from other epidermal cells [20]. In some plants, such as grasses (Poaceae), the stomata consist of two dumbbell-shaped guard cells with parallel subsidiary cells associated with faster movement to increase the efficiency of water and gas exchange [21]. That typical characteristic of stomata in grasses is known as the Halter type.

Phasey bean and siratro have stomata in the upper and lower epidermal, also known as amphistomatic. The amphistomatic leaves are associated with the fast-growing species that have high adaptability in the dry environment [22]. The main concern of those stomata is the variation in subsidiary cells that led to different types of stomata. In phasey bean, the upper epidermal has paracytic-type stomata that are indicated by two subsidiary cells that paralleled with the guard cells. The size of subsidiary cells was smaller than the epidermal cells. The subsidiary cells also have different sizes among them, the smaller ones and the larger ones.

The lower epidermal in phasey bean has two types of stomata, the paracytic and the anomocytic. The anomocytic-type of stomata is characterized by the undifferentiated shape of the subsidiary cells, so it looks like did not have subsidiary cells. In Fig. 2b, the top sequence showed the anomocytic-type of stomata. The cells that flanked around guard cells vary in number, ranging from 2 to 4 cells. In the middle sequence of Fig. 2b, the parasitic-type stomata also have different shapes of subsidiary cells, the near leaf nerve has polygonal-shaped subsidiary cells, while the leaf blade has irregular-shaped subsidiary cells.

In siratro, the upper and lower epidermal show the same structure of stomata. Both sides have parasitic and anomocytic stomata and irregular-shaped subsidiary cells. The epidermal that has more than one type of stomata was rarely reported. Some species of wild tomato (*Solanum* spp.) have two types of stomata, anisocytic and anomocytic [17]. In bananas (*Musa acuminata*), the type of stomata can be classified as paracytic and hexacytic [20].

The type of stomata is defined by the shape and formation of subsidiary cells, so it can be used as a unique morphological trait in the taxonomical study. In Pteridaceae, the shape of the subsidiary cell has the highest value to distinguish among clades [6]. As the morphological structure reflect the functional system, the different type of subsidiary cells might be correlated with the environment. Subsidiary cells support the function of the guard cell in water and ions regulation or enhance the particular morphology that affects the gas exchange [20]. Although the specific water and gas regulation in every type of stomata is unclear, the occurrence of two types of stomata in phasey bean and siratro can be observed in the elaborated study.

The stomata that occurred in the upper and lower epidermis have a different index of stomata (SI). In the upper epidermis, the SI value for phasey bean is 17.55 ± 3.50 %, while the lower epidermis has a higher SI value, 20.15 ± 3.29 %. In siratro, the SI value is lower than phasey bean, but has the same trend, which is the upper epidermal tends to have lower SI than the lower epidermal. In siratro, the SI value for the upper epidermal is 11.08 ± 3.15 %, while for the lower epidermal is 17.73 ± 0.59 %. The SI value correlates with the plant adaptation to drought. The drought-tolerant have more SI values, such as phasey bean and siratro. For a plant that needs high humidity, such as *Zingiber*, the SI value of the upper epidermal was less than 3 %, while for the lower epidermal was less than 10 % [3].

The trichomes in phasey bean are only found in the lower epidermal. The glandular trichomes are shaped in capitate, while the non-glandular trichomes have a linear shape. The length of non-glandular trichomes is 1124.65 ± 338.88 μm, while the size of non-glandular trichomes is 154.27 ± 21.44 × 77.41 ± 12.00 μm. Siratro has glandular and non-glandular trichomes in both upper and lower epidermal. The glandular trichomes are shaped in capitate, the same shape as the phasey bean. The size of glandular trichomes on both sides is quite the same, 140.06 ± 18.83 × 73.38 ± 12.62 μm in upper epidermal and 139.39 ± 17.09 × 74.13 ± 11.47 μm in lower epidermal. The non-glandular trichomes also have a linear shape as same as phasey bean. The size of non-glandular trichomes in siratro was longer than phasey bean and easily recognized morphologically. The upper epidermal has shorter non-glandular trichomes (1195.78 ± 163.85 μm) than the lower epidermal trichomes (2002.32 ± 268.48 μm).

Despite the SI value being higher in phasey bean than siratro, the index of trichomes (TI) is showing the contrary. The trichomes only occur in lower epidermal with a TI value is 1.98 ± 0.36 %. In opposition, the trichomes in siratro occur on both sides. The upper side of the epidermal has a TI value of 3.05 ± 0.96 %, while in lower epidermal has a little lower at 2.90 ± 0.85 %.

Trichomes might be the derivate of epidermal that have specialization against biotic and abiotic threats [23]. The glandular and non-glandular trichomes have different roles in plant protection. The glandular trichomes secret exudates as protection for herbivory or pathogen [24], while the non-glandular trichomes tend to deal with abiotic stress [10, 23]. The high amount of non-glandular trichomes indicated adaptability in the xeromorphic environment [7, 11]. In the early stage of development, the non-glandular trichomes have produced phenolic compounds that are similar to glandular trichomes, but then diffused to the cell wall at the maturation [23]. So, the non-glandular trichomes also have a role in UV protection, especially against UV-B radiation damage [23].

The non-glandular trichomes in phasey bean were only found in the lower epidermal, while in siratro were found on both sides of the leaf. These differences might be implied that siratro has more adaptability in drought environments, or it might be a form of compensation for the broader leaf. In drought stress or low water potential, plants tend to produce reduced-size of leaf to maintain the stomatal conductance [19], or increase the density of non-glandular trichomes [10]. Siratro has a broader leaf than phasey bean which caused the higher rate of evapotranspiration, but it is also in accordance with the higher IT value.

The leaf anatomical traits were widely used as supporting data in plant taxonomy. In this study, the leaf epidermal traits in phasey bean and siratro provided nine characters, and some of them (such as the type of stomata, shape of the epidermal cell, and trichomes distribution) were the most varied characters. Based on the epidermal characteristics, both phasey bean and siratro show the drought adaptation, especially in form of amphistomatic leaf irregular-shaped epidermal cells with high curved, and the presence of non-glandular trichomes. Trichomes and stomata have been known as valuable taxonomical traits [10], but in this study, the shape of the upper epidermal cell was more informative to distinguish the leaf between phasey bean and siratro. This study only used epidermal traits, so for further research, the use of anatomical traits in stem and root nodules can be considered as the main variables.

4. Conclusion

In conclusion, the lower epidermal of phasey bean and siratro have relatively similar traits, such as the shape of the epidermal cell, type of stomata, and type of trichomes. The quantitative measurement of size and index (stomata and trichomes) was varied. The upper epidermal provides more distinct characters. The upper epidermal in phasey bean only have anomocytic stomata, polygonal-shaped epidermal cells, and without any trichomes, while siratro have paracytic and anomocytic stomata, irregular-shaped epidermal cells, and have glandular and non-glandular trichomes.

5. Acknowledgement

Thanks to The Institution of Research and Community Services Udayana University for the research grant in Penelitian Unggulan Program Studi scheme with contract number 023.17.2.677526/2021. We also thank the Faculty of Mathematics and Natural Sciences Udayana University, Adi Ariyanto Wibisono, Komang Kartika Indi Swari for their support and assistance.

References

[1] Cook, B. G., Pengelly B. C., Brown, S. D., Donnelly, J. L., Eagles, D. A., Franco, M. A., Hanson, J., Mullen, B. F., Partridge I. J., Peters, M., & Schultze-Kraft, R. (2005). Tropical forages. *CSIRO, DPI&F(Qld), CIAT and ILRI*. Brisbane

[2] Heuzé, V., Tran, G., Hassoun, P., and Lebas, F. (2015). Siratro (*Macroptilium atropurpureum*). *Feedipedia INRAE, CIRAD, AFZ and FAO*.

[3] Zhao, H., Xiao, M., Zhong, Y., and Wang, Y. (2022). Leaf epidermal micromorphology of *Zingiber* (Zingiberaceae) from China and its systematic significance. *PhytoKeys*, *190*, 131–146.

[4] Gang, Z., Liu, B., Rohwer, J. G., Ferguson, D. K., & Yang, Y. (2021). Leaf epidermal micromorphology defining the clades in *Cinnamomum* (Lauraceae). *PhytoKeys*, 182, 125–148.

[5] Zhang, F.-P., Murphy, M. R. C., Cardoso, A. A., Jordan, G. J., & Brodribb, T. J. (2018). Similar geometric rules govern the distribution of veins and stomata in petals , sepals and leaves. *New Phytologist*, 219, 1224–1234.

[6] Shah, S. N., Celik, A., Ahmad, M., Ullah, F., Zaman, W., Zafar, M., Malik, K., Rashid, N., Iqbal, M., Sohail, A., & Bahadur, S. (2018). Leaf epidermal micromorphology and its implications in systematics of certain taxa of the fern family Pteridaceae from Northern Pakistan. *Microscopy Research & Technique*, 1–16.

[7] Ullah, F., Zafar, M., Amhad, M., Sultana, S., Ullah, A., Shah, S. N., Butt, M. A., & Mir, S. (2018). Taxonomic implications of foliar epidermal characteristics in subfamily Alsinoideae (Caryophyllaceae). *Flora*, 242, 31–44.

[8] Ercan, F. E. Z., Mikola, J., Silfver, T., Myller, K., Vainio, E., Słowinska, S., Slowinski, M., Lamentowicz, M., Blok, D., & Wagner-cremer, F. (2021). Effects of experimental warming on Betula nana epidermal cell growth tested over its maximum climatological growth range. *PLoS ONE*, 16(5), 1–12.

[9] Zagoto, A. D. P., & Violita, V. (2019). Leaf Anatomical Modification in Drought of Rice Varieties (*Oryza sativa* L.). *Eksakta : Berkala Ilmiah Bidang MIPA*, 20(2), 42–52.

[10] Ali, M., Bahadur, S., Hussain, A., Saeed, S., Khuram, I., Ullah, M., Shao, J-W., & Akhtar, N. (2020). Foliar epidermal micromorphology and its taxonomic significance in *Polygonatum* (Asparagaceae) using scanning electron microscopy. *Microscopy Research & Technique*, 3, 1–10.

[11] Gul, S., Ahmad, M., Zafar, M., Bahadur, S., Celep, F., Sultana, S., Begum, N., Zaman, W., Shuaib, M., & Ayaz, A. (2019). Taxonomic significance of foliar epidermal morphology in Lamiaceae from Pakistan. *Microscopy Research & Technique*, 5, 1–22.

[12] Rashid, N., Zafar, M., Ahmad, M., Khan, M. A., Malik, K., Sultana, S., & Shah, S. N. (2018). Taxonomic significance of leaf epidermis in tribe Trifolieae L. (Leguminosae; Papilionoideae) in Pakistan. *Plant Biosystems*, 3504, 1–11.

[13] Nugroho, L. H., Sutikno, Susandarini, R., Yuliati, I. R., Priyono, Y., Munawaroh, E., & Astuti, I. P. (2019). Comparative leaf and stem anatomy of ten Piper species from Indonesia. *Asian Journal of Agriculture and Biology*, 7(3), 434–441.

[14] Liu, C., Li, Y., Xu, L., Li, M., Wang, J., Yan, P., & He, N. (2021). Stomatal Arrangement Pattern: A New Direction to Explore Plant Adaptation and Evolution. *Frontiers in Plant Science*, 12, 1–7.

[15] Sapala, A., Runions, A., & Smith, R. S. (2018). Mechanics, geometry and genetics of epidermal cell shape regulation: different pieces of the same puzzle. *Current Opinion in Plant Biology*, 47, 1–8.

[16] Vofely, R. V, Gallagher, J., Pisano, G. D., Bartlett, M., & Braybrook, S. A. (2019). Of puzzles and pavements: a quantitative exploration of leaf epidermal cell shape. *New Phytologist*, 221, 540–552.

[17] Sampaio, V. S., Araújo, N. D., & Agra, M. F. (2014). Characters of leaf epidermis in Solanum (clade Brevantherum) species from Atlantic Forest of Northeastern Brazil. *South African Journal of Botany*, 94, 108–113.

[18] Sapala, A., Runions, A., Routier-Kierzkowska, A. L., Gupta, M. Das, Hong, L., Hofhuis, H., Verger, S., Mosca, G., Li, C. B., Hay, A., Hamant, O., Roeder, A. H. K., Tsiantis, M.., Prusinkiewicz, P., & Smith, R. S. (2018). Why plants make puzzle cells, and how their shape emerges. *eLife*, 7, 1–32.

[19] Time, A., & Acevedo, E. (2021). Effects of Water Deficits on Prosopis tamarugo Growth , Water Status and Stomata Functioning. *Plants*, 10(53), 1–11.
[20] Gray, A., Liu, L., & Facette, M. (2020). Flanking Support: How Subsidiary Cells Contribute to Stomatal Form and Function. *Frontiers in Plant Science*, 11, 1–12.
[21] Nunes, T. D. G., Zhang, D., & Raissig, M. T. (2020). Form, development and function of grass stomata. *The Plant Journal*, 101(4), 780–799.
[22] Richardson, F., Brodribb, T. J., & Jordan, G. J. (2017). Amphistomatic leaf surfaces independently regulate gas exchange in response to variations in evaporative demand. *Tree Physiology*, 37(7), 869–878.
[23] Karabourniotis, G., Liakopoulos, G., Nikolopoulos, D., & Bresta, P. (2020). Protective and defensive roles of non - glandular trichomes against multiple stresses: structure – function coordination. *Journal of Forestry Research*, 31, 1–12.
[24] Schuurink, R., & Tissier, A. (2020). Glandular trichomes: micro-organs with model status? *New Phytologist*, 225(6), 2251–2266.