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Micromorphometric analysis of five *Begonia* spp. leaves (*Begoniaceae*)

W Suffan¹, D Metusala² and Nisyawati¹

¹Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Indonesia, Depok 16424, Indonesia

²Purwodadi Botanical Garden, Indonesian Institute of Science (LIPI), Pasuruan, East Java 67163, Indonesia

Corresponding author's email: destario.metusala@lipi.go.id

Abstract. *Begonia* is one of the largest plant genera that widely distributed in the tropics and subtropics. This genus has important value and has been cultivated intensively as ornamental plants. Hence, this purpose of this research is to investigate the leaf anatomical characteristic of five *Begonia* species; *B. maculata*, *B. cucullata*, *B. masoniana*, *B. acetosa* and *B. listada*; which are important in horticulture. The mini-microtome with a liquid preservation method has been used to obtain the transverse section. The result showed that the epidermis, hypodermis, and trichome characters are varied, and each species could have a different character's combination. The qualitative and quantitative parameters of five *Begonia* were also described and compared in this study.

Keywords: *Begonia* spp., characters combination, description

1. Introduction

Begonia is one of the biggest plant genera that widely distributed in the tropics and subtropics [1]. This genus is famous as an ornamental plant due to its leaves and inflorescences various color and morphology [2]. Besides that, *Begonia* was also reported could be used as food and medicinal plants [3]. Anatomical studies on leaves in *Begonia* are relatively scarce. Previous studies in *Begonia* mostly focused on morpho-taxonomy, field inventory and exploration, [4]. The leaves of *Begonia* species show morphological diversity, especially in shape and ornamentation.

One of the less popular research in *Begonia* is the anatomical study. Whereas, the anatomical study could give some important clues for the anatomical study further research, such as finding drought-tolerant species to make the conservation rank-scale determination, finding potential ornamental plants for cross breeding purpose and for the completion of the taxonomic description of *Begonia*. The aims of this study are to describe and compare the leaf's micromorphometric characters of five *Begonia* species.

2. Materials and method

Five species of *Begonia* were used as research objects, they are *B. maculata*, *B. cucullata*, *B. masoniana*, *B. acetosa*, and *B. listada*. *Begonia masoniana*, *B. acetosa*, and *B. listada* collected from nurseries in



Bogor, Indonesia with an altitude of 532 m asl. *Begonia maculata* and *B. cucullata* were collected from nurseries in Depok, Indonesia with an altitude of 50–100 m asl. Both of the nurseries have planted the *Begonia* in some plots that shaded by a shade net of 50%. Leaf sections were obtained by scrapping (paradermal-section) and using hand-sliding microtome (cross-section). Cross-section was obtained by cutting the leaf in a 1x1 cm area including its costae. Each leaf piece was placed between a narrow slit of cassava pith, then was cut transversely using a hand-sliding microtome. Furthermore, the obtained sections were preserved in the preservation liquid. All of those steps were explained in detail in Metusala [5]. This study used eight sample units, each sample unit has been observed with three repetitions. The parameters are mentioned in the table 1.

3. Results

3.1. *Begonia maculata*

Leaf is 272.530–348.684 μm thick. Epidermis *adaxial* is slightly radial elongated cells, the entire surface is slightly concave, 1 layer, 98.747–170.952 μm thick; *abaxial* slightly radial elongated cells, the entire surface is slightly concave, 1 layer, 108.591–145.643 μm thick. Hypodermis is absent. Mesophyll palisade cells are conical short shaped; sponge cells are spherical; 38.556–86.519 μm thick in total. Cuticle *adaxial* is 0.964–1.871 μm thick; *abaxial* 0.666–1.331 μm thick. Trichome *glandular* spreads infrequently in adaxial and abaxial; *non-glandular* is absent. Stomata is only in abaxial, single (non-cluster), anisocytic that surrounded by dicyclic subsidiaries; 880.832–1,264.668 μm^2 area per stomata; 12–25 stomata per mm^2 ; 13,288.542–30,385.809 μm^2 area per mm^2 . Vascular bundles are 2 in each costae; 28,426.067–57,235.606 μm^2 area in total. Xylem 14,298.213–38,464.889 μm^2 ; 42.025–67.204 % of total vascular bundles. Phloem is 10,815.749–23,275.453 μm^2 ; 32.796–57.975 % of total vascular bundles. Collenchyma presents below the epidermis of costae, both of lower and upper. Calcium oxalate crystals present in mesophyll cells, druse (figure 1).

3.2. *Begonia cucullata*

Leaf is 389.345–504.779 μm thick. Epidermis *adaxial* is radial elongated cells, the entire surface is slightly concave, 1 layer, 188.386–260.530 μm thick; *abaxial* is slightly spherical cells, the entire surface is slightly concave, 1 layer, 96.797–167.461 μm thick. Hypodermis is absent.

Table 1. Qualitative and quantitative parameters of leaf anatomy

Characters	Parameter	
	Qualitative	Quantitative
Epidermis	Shape	Number of layer Thickness
Hypoderm	Shape	Number of layer Thickness
Mesophyll	Shape	Number of layer Thickness
Cuticle	-	Thickness
Trichome	Type	Density
Stomata	Type	Area Density
Vascular bundle	-	Area
Collenchyma	Occurrence	-
Calcium oxalate crystal	Type	-

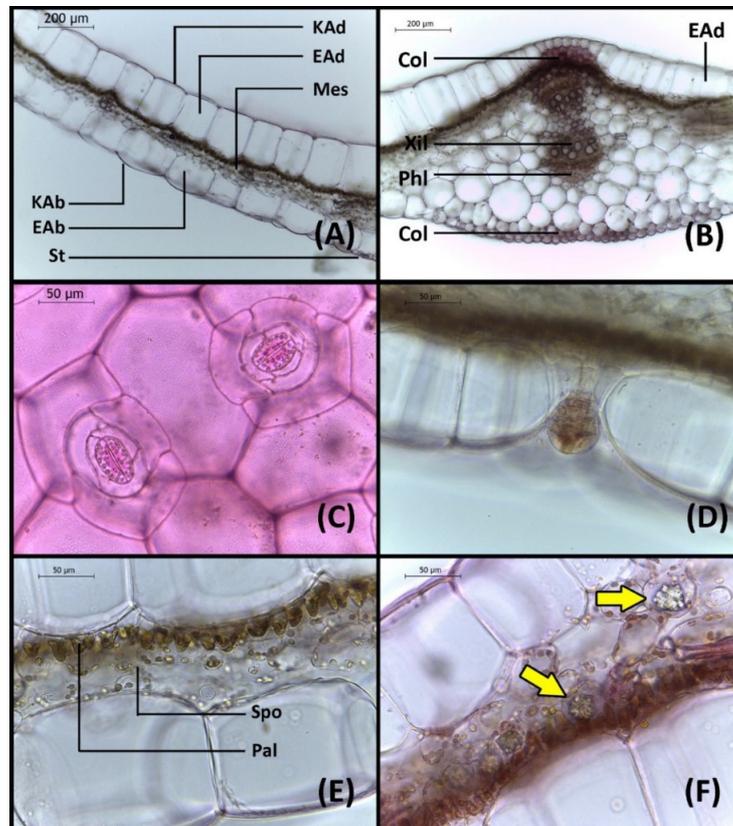


Figure 1. *Begonia maculata* (A) Lamina-CS, (B) vascular bundle-CS, (C) stomata-PS, (D) glandular trichome-CS, (E) mesophyll-CS and (F) calcium oxalate crystals (druse)-CS. CS: cross section, PS: paraderal section, KAd: adaxial cuticle, KAb: abaxial cuticle, EAd: adaxial epidermis, EAb: abaxial epidermis, Mes: mesophyll, St: stomata, Xil: xylem, Phl: phloem, Col: collenchyma, Pal: palisade, Spo: spon.

Mesophyll palisade cells are conical short shaped; sponge cells are spherical; 61.212–171.944 μm thick in total. Cuticle *adaxial* is 0.653–1.485 μm thick; *abaxial* 0.549–1.198 μm thick. Trichome *glandular* spreads infrequently in adaxial and abaxial; *non-glandular* is absent. Stomata is only in abaxial, non-contagious cluster (2–5 stomata per cluster), anisocytic that surrounded by dicyclic subsidiaries; 930.744–1,731.268 μm^2 area per stomata; 31.235–84.334 stomata per mm^2 ; 31,811.760–117,294.284 μm^2 area per mm^2 . Vascular bundles is 1–2 in each costae; 9,765.794–21,281.858 μm^2 area in total. Xylem is 5,905.361–13,741.587 μm^2 ; 57.589–64.569 % of total vascular bundles. Phloem is 3,860.433–7,559.216 μm^2 ; 35.431–42.411 % of total vascular bundles. Collenchyma presents below the lower epidermis of costae. Calcium oxalate crystals present in parenchyma cells of costae, druse (figure 2).

3.3. *Begonia masoniana*

Leaf is 213.335 – 268.042 μm thick. Epidermis *adaxial* is slightly radial elongated cells, the entire surface is slightly concave, 1 layer, 58.687–97.190 μm thick; *abaxial* is slightly spherical cells, the entire surface is slightly concave, 1 layer, 66.590–108.101 μm thick. Hypodermis is absent. Mesophyll palisade cells are conical short shaped; sponge cells are spherical; 73.260–132.484 μm thick in total.

Cuticle *adaxial* is 0.472–1.024 μm thick; *abaxial* 0.506–1.079 μm thick. Trichome glandular spreads infrequently in adaxial and abaxial. Non-glandular trichome, *adaxial* is tapering multiseriate, 8–20 trichome per cm^2 ; *abaxial* is tapering multiseriate that spread along the veins. Stomata is only in abaxial, single (non-cluster), anisocytic that surrounded by dicyclic subsidiaries; 543.238–829.835 μm^2 area per stomata; 22–47 stomata per mm^2 ; 14,908.347–29,699.256 μm^2 area per mm^2 . Vascular bundles is 4–5 in each costae; 83,611.780–169,168.948 μm^2 total area. Xylem is 50,735.078–117,732.834 μm^2 ; 47.179–69.595 % of total vascular bundles. Phloem is 31,373.176–65,732.701 μm^2 ; 30.405–52.821 % of total vascular bundles. Collenchyma presents below the epidermis of costae, both of lower and upper (figure 3).

3.4. *Begonia acetosa*

Leaf is 610.498–710.364 μm thick. Epidermis adaxial, lens-shaped cells, 1 layer, 43.271–68.939 μm thick; abaxial, tangent elongated cells, 1 layer, 20.531–50.693 μm thick. Hypodermis adaxial, slightly spherical cells up to radial elongated cells, 2–3 layers, 247.966–375.814 μm thick in total; abaxial, radial elongated cells, 2 layers, 139.121–198.655 μm thick in total. Mesophyll palisade cells are conical short shaped; sponge cells are spherical; 65.250–153.863 μm total thick.

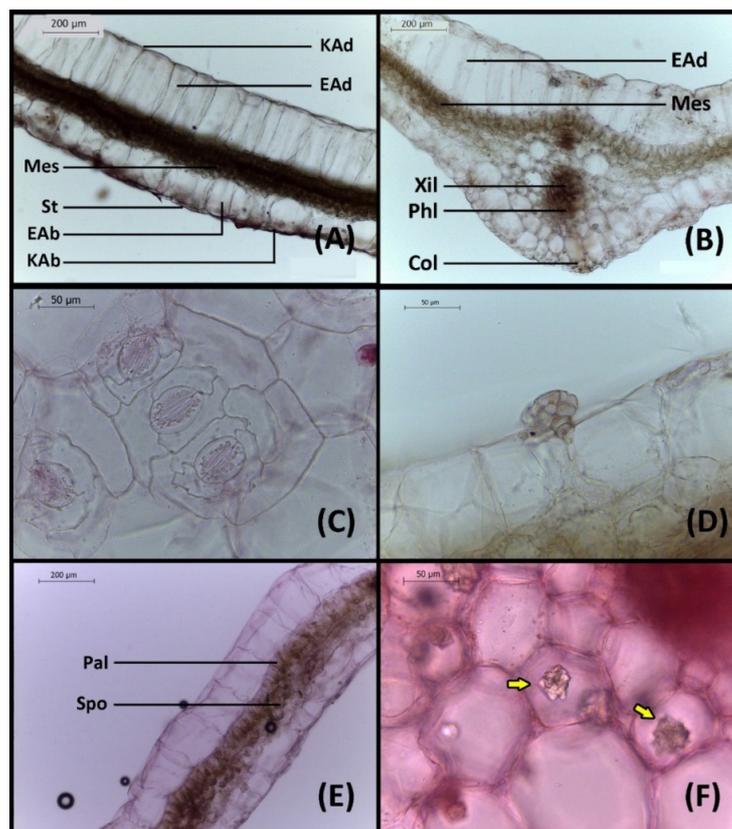


Figure 2. *Begonia cucullata* (A) Lamina-CS, (B) vascular bundle-CS, (C) stomata-PS, (D) glandular trichome-CS, (E) mesophyll-CS and (F) calcium oxalate crystals (druse)-CS. CS: cross section, PS: paraderal section, KAd: adaxial cuticle, KAb: abaxial cuticle, EAd: adaxial epidermis, EAb: abaxial epidermis, Mes: mesophyll, St: stomata, Xil: xylem, Phl: phloem, Col: collenchyma, Pal: palisade, Spo: spongs.

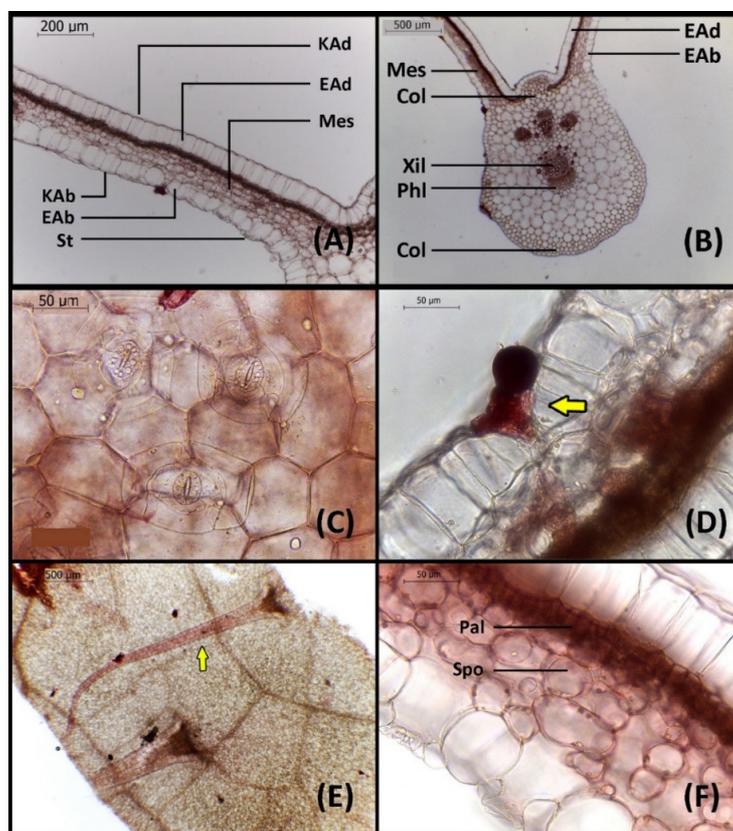


Figure 3. *Begonia masoniana* (A) Lamina-CS, (B) vascular bundle-CS, (C) stomata-PS, (D) glandular trichome-CS, (E) non-glandular trichome in the adaxial side-PS and (F) mesophyll-CS. CS: cross section, PS: paraderal section, KAd: adaxial cuticle, KAb: abaxial cuticle, EAd: adaxial epidermis, EAb: abaxial epidermis, Mes : mesophyll, St: stomata, Xil: xylem, Phl: phloem, Col: collenchyma, Pal: palisade, Spo: spones.

Cuticle adaxial is 0.577–0.997 μm thick; abaxial 0.726–1.072 μm thick. Trichome glandular spreads adjacent to non-glandular trichome in adaxial and abaxial. Non-glandular trichome, *adaxial* is tapering multiseriate, 49–200 trichome per cm^2 ; *abaxial* is tapering multiseriate, 99–500 trichome per cm^2 . Stomata is only in abaxial, non-contagious cluster (2–5 stomata each cluster), anisocytic that surrounded by dicyclic subsidiaries; 570.404–874.911 μm^2 area per stomata; 53–116 stomata per mm^2 ; 46,461.320–85,934.566 μm^2 area per mm^2 . Vascular bundles are 6 in each costae; 93,233.114–179,833.827 μm^2 area in total. Xylem is 51,066.057–117,780.085 μm^2 ; 54.772–67.572 % of total vascular bundles. Phloem is 35,291.044–62,053.742 μm^2 ; 32.428–45.228 % of total vascular bundles. Collenchyma presents below the epidermis of costae, both of lower and upper. Calcium oxalate crystals present in parenchyma cells of costae, druse and prismatic (figure 4).

3.5. *Begonia listada*

Leaf is 414.366–498.913 μm thick. Epidermis adaxial, lens-shaped cells, 1 layer, 31.545–70.775 μm total thick; abaxial, tangent elongated cells, 1 layer, 15.771–32.703 μm thick. Hypodermis adaxial, radial elongated cells, 1 layer, 209.785–266.799 μm thick; abaxial, slightly spherical cells, 1 layers, 73.799–142.906 μm thick. Mesophyll palisade cells are conical short shaped; sponge cells are spherical;

48.275–81.547 μm thick in total. Cuticle adaxial is 0.436–0.796 μm thick; abaxial 0.611–1.031 μm thick. Trichome glandular spreads infrequently in adaxial and abaxial. Non-glandular trichome, *adaxial* is tapering multiseriate, 49.976–249.879 trichome per cm^2 ; *abaxial* is Y-shaped, 199–350 trichome per cm^2 . Stomata is only in abaxial, single to non-contagious cluster (2 stomata each cluster), anisocytic that surrounded by dicyclic subsidiaries; 524.240–792.615 μm^2 area per stomata; 21–53 stomata per mm^2 ; 13,958.961–34,824.278 μm^2 area per mm^2 . Vascular bundles are 2 in each costae; 14,820.619–26,498.745 μm^2 area in total. Xylem is 9,488.839–16,957.926 μm^2 ; 58.397–65.449 % of total vascular bundles. Phloem is 5,120.709–9,816.612 μm^2 ; 34.551–41.603 % of total vascular bundles. Collenchyma presents below the lower epidermis of costae (figure 5).

4. Discussion

Begonia in the present study showed diverse anatomical leaf characteristics. In the epidermis, for example, there were three different epidermis shapes observed from five species: lens-shaped, sub-spherical, and radial elongated. Lens-shaped cells in the epidermis of *B. acetosa* and *B. listada* are probably used to increase the energy capturing efficiency [6].

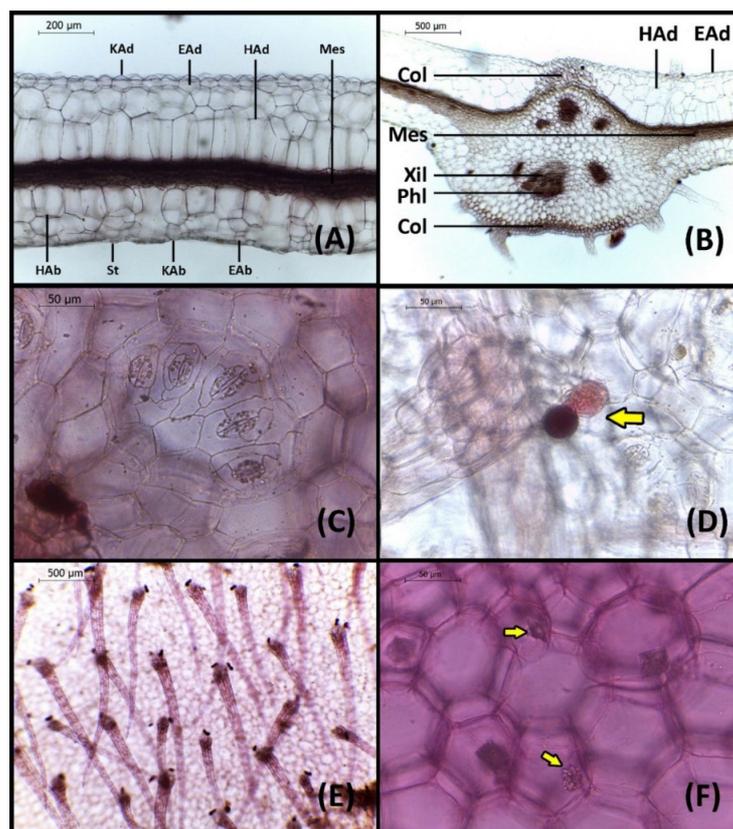


Figure 4. *Begonia acetosa* (A) Lamina-CS, (B) vascular bundle-CS, (C) stomata-PS, (D) glandular trichome-CS, (E) mesophyll-CS and (F) calcium oxalate crystals (druse)-CS. CS: cross section, PS: paraderal section, KAd: adaxial cuticle, KAB: abaxial cuticle, EAd: adaxial epidermis, EAb: abaxial epidermis, Mes : mesophyll, St: stomata, Xil: xylem, Phl: phloem, Col: collenchyma, Pal: palisade, Spo: spon.

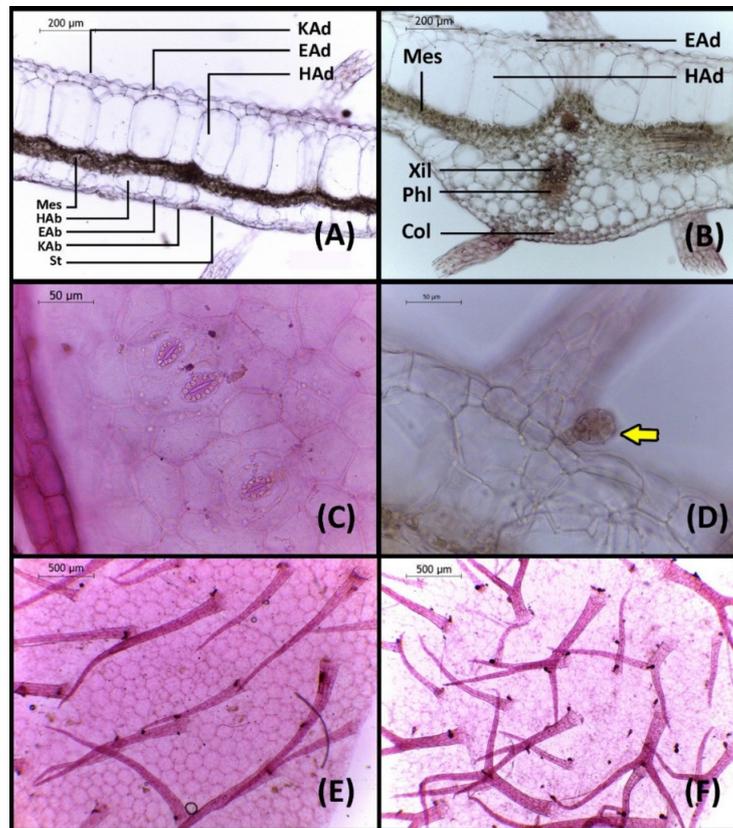


Figure 5. *Begonia listada* (A) Lamina-CS, (B) vascular bundle-CS, (C) stomata-PS, (D) glandular trichome-CS, (E) non-glandular trichome in adaxial side-PS and (F) non-glandular trichome in abaxial side-CS. CS: cross section, PS: paraderal section, KAd: adaxial cuticle, KAb: abaxial cuticle, EAd: adaxial epidermis, EAb: abaxial epidermis, HAd: adaxial hypodermis, HAb: abaxial hypodermis, Mes : mesophyll, St: stomata, Xil: xylem, Phl: phloem, Col: collenchyma.

The species with thickest epidermis is *B. cucullata* (260.530 μm thick in the adaxial side, 167.461 μm thick in the abaxial side) and the thinnest one is *B. listada* (31.545 μm thick in the adaxial side, 15.771 μm thick in the abaxial side).

Hypodermis only presents in two of five observed species, *B. acetosa* and *B. listada*. Metcalfe et al. [7], have pointed out that these cells function as water storage. In addition, hypodermis strengthens the leaf [8], stores the starch [9] and is common in xerophytes [10]. According to the information, the species that have this cell, *B. acetosa* and *B. listada*, are likely potential as drought tolerant parent plants. This study also observed that *B. acetosa* has thicker hypodermis than *B. listada*, both of adaxial and abaxial sides. The thickness of adaxial and abaxial hypodermis are 209.785 μm up to 375.814 μm and 73.799 μm up to 198.655 μm , respectively.

Mesophyll in *Begonia* is rather less developed than other leaf's tissues. The thickness of *Begonia*'s mesophyll that we observed are 38.556 μm up to 171.944 μm . The mesophyll is relatively thinner compared to the leaf thickness. The mesophyll thickness is probably linked to the photosynthesis efficiency or their habitat pressure that favours evolution toward this character. Li et al. [11] mentioned that the *Begonia* species which are living in shady and humid habitat usually showed thinner mesophyll

than the *Begonia* from full sun or xerophyte habitat. *Begonia cucullata* has the thickest mesophyll (171.944 μm thick) from the rest of the studied species. And the thinnest one is *B. maculata* (38.556 μm thick).

Cuticle in five *Begonia* has been observed. One of the cuticle functions is to reduce the transpiration rate [12, 13]. The *Begonia* that has the thickest cuticle is *B. maculata*, both of adaxial and abaxial sides. *B. listada* and *B. masoniana* have the thinnest cuticle, in the adaxial and abaxial parts, respectively. It is probably showed that *B. maculata* relatively have a better capability to reduce the transpiration.

This study showed that three out of five *Begonia* have trichome. The presence of trichome of these *Begonia* can be found on the surface of lamina or only on the vein of their leaves. The types of the trichome in this study can be classified into glandular trichome and non-glandular trichome. Based on their morphology, there are two types of trichome, tapering multiseriate and “Y”-shaped trichome. The highest non-glandular trichome density on the adaxial side was found in *Begonia listada* compared to *B. acetosa* on the abaxial side. Werker (2010) said that the function of many trichomes are either presumed or entirely unidentified. Some of the suggested function of trichomes are protection (herbivore, extensive light, excessive water loss, etc.), water absorption, salt secretion or allure [14].

Stomata in *Begonia* reported by some researches were found as cluster [7, 15]. The fact was also confirmed in this study. Clustered stomata were found in *B. cucullata*, *B. acetosa*, and *B. listada*. The clustered stomata found are classified as non-contagious cluster. The previous study showed that the clustered stomata are significantly increased along the drought/salt treatment [16]. The further research is needed to understand why some of the humid-shady living species of *Begonia* developed the clustered stomata.

The quantitative parameters of stomata are also described in this study, such as the number of stomata in each cluster, the density and the area of stoma. Each non-contagious cluster contains two up to five stomata. The highest number of stomata density is 116 stomata per mm^2 (*B. acetosa*) and the lowest number of stomata density is 12 stomata per mm^2 (*B. maculata*). The species with largest and smallest area of stoma are *B. cucullata* and *B. listada*, respectively. These parameters probably can predict the leaf capability to regulate its transpiration. Lower stomatal area is more capable to regulate the excessive water loss through stomatal aperture [17].

Vascular bundles in *Begonia* are differentiated into xylem and phloem. The xylem is always in the inner side of the bundle. In each costae, the number of vascular bundles are various, one up to six vascular bundles. In this observation, *Begonia* that has the largest total area of vascular bundles is *B. acetosa* (179,833.827 μm^2 area) and the smallest one is *B. cucullata* (9,765.794 μm^2 area). The percentage ratio of xylem and total vascular bundles are 42.025–69.594 % and the percentage ratio of phloem and total vascular bundles are 30.405–57.974 %. *Begonia* that has the highest percentage ratio of xylem and phloem are *B. masoniana* and *B. maculata* respectively. Some of species have collenchyma tissue in their costae, either in the adaxial or abaxial side. Bercu (2015) stated that the collenchyma tissue, placed between the group of vascular bundle and epidermis in costae, strengthens the lamina [18].

5. Conclusion

Five *Begonia* observed showed that the characters of epiderm, hypoderm, stomata, and trichome are varied, and each species could have different character's combination.

Acknowledgments

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