

Foliar stomata characteristics of tree species in a university green open space

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Manuscript received: 29 November 2021. Revision accepted: 24 February 2022.

Abstract. Susilowati A, Novriyanti E, Rachmat HH, Rangkuti AB, Harahap MM, Ginting IM, Kaban NS, Iswanto AH. 2022. Foliar stomata characteristics of tree species in a university green open space. *Biodiversitas* 23: 1482-1489. Stomata, a gas regulatory system of leaves, provide a great chance to investigate the interaction between plants and their environment. Stomata consist of surrounded by two guard cells. Stomata are found in all parts of the plant that are exposed to the air, especially the leaves. In identifying a plant species, it is necessary to have epidermal characteristics such as stomata to complete the taxonomic data. Several studies have been conducted on the type of stomata on the leaves of some dicotyledonous and monocot plants, but not many have reported similar studies on green space. Universitas Sumatera Utara (USU) campus also plays an important function as green space (GS) in Medan City due to its richness in tree collection number and species. In line with the effort in maximizing the role of trees as the core element of green space, exploring the characteristics of stomata is important to conduct. Therefore, this study aimed to analyze the leaf stomata characteristics of several tree species in the green open space of the USU campus. A total of 83 tree species were taken for their leaves to investigate the stomata characters. Three healthy mature leaves on the lower part of newly grown branches were collected from each plant. The replica and the nail polish method were employed for stomata slice making. The stomata type, length, wide, density and distribution were observed. The result showed that 83 tree species in the USU campus have varied stomata types, with the percentage were highest characteristic found in paracytic (91.46%), followed by anomocytic (6.02%), anisocytic (1.20%), and diacytic (1.20%). The longest stomata were observed in *Antidesma bunius* (32.04 μm). The widest stomata were noticed in *Garcinia mangostana* (37.62 μm). Meanwhile, the shortest and narrowest stomata were found in *Shorea laevis*, which were 5.43 μm and 3.72 μm , respectively. The species with the highest stomatal density was *Schleichera oleosa* (4294 mm^{-2}). According to the study, the tree species at USU generally have high stomata density, length, and width, making them more suitable for green space. Species with a high number and density of stomata and a large size are much more likely to adsorb pollutants such as carbon monoxide.

Keywords: Green open space, stomata characteristic, Universitas Sumatera Utara

INTRODUCTION

The campus of Universitas Sumatera Utara (USU) plays its main role as an academic institution and provides a beneficial function as green open space (GOS) for Medan City. There have been declining in the quality of settlements marked by worsening traffic jams, increased city slums that are vulnerable to flooding, and the loss of GOS, which is essential for human health. Susilowati et al. (2021) stated that the existence of GOS is compulsory to keep a healthy city and environment. A tree is an important element in a city GOS and plays an ecological function to improve environmental quality. GOS plays a role in balancing the ecosystem i.e., microclimate, hydrology function, and clean air availability, as well as an absorbent of carbon dioxide pollutants (Han et al. 2019; Nowak et al. 2014). Aside from those, GOS is also important to reduce noise (Tudorie et al. 2020) and the impacts of increased air temperature (Nowak et al. 2018), to ensure the energy flow (Weber et al. 2013), to absorb heat, as habitat for animals,

and aesthetical aspect (Gulwadi et al. 2013).

The functionality of GOS is determined by the trees growing in its landscape. A tree is composed of several inseparable parts i.e. root, trunk, and leaves, each with respective functions to sustain the tree's life. Of all the tree organs, leaves are deemed as the main organ (Doudou et al. 2021). Stomata are the main channels throughout which plants exchange water and gas with their surroundings, and they are directly linked to plant physiological processes such as photosynthesis, respiration, and transpiration (Torngrern and Leksungnoen 2020). Stomata in leaves are important for gas exchange (Gilner et al. 2015), water transpiration, nutrient resorption and assimilation (Martinez et al. 2016), and respiration (Boer et al. 2018). Although stomata can be found on every part of the plant that is in contact with air such as leaves, stems, and rhizomes (Hubbard and Webb 2016); however, stomata are most abundantly found in leaves (Inoue and Kinoshita 2017). The type of stomata affects the process of leaf transpiration (Khoiroh et al. 2014). Leaf stomata in different plants

differ significantly in density, size, and shape (Khan et al. 2014), while does stomata in plants from the same genus or from different germplasm resources of the same species (Chen et al. 2018). The characteristics of plant epidermal stomata are an important area of research inside the study of plant germplasm resource diversity. Stomata are important organs in plant phylogenetic studies, and their characteristics are important aspects in plant origin, evolution, and classification studies (Brogan et al. 2020).

Examining the air pollution using plants is reliable (Noori et al. 2018). Stomata, as regulating systems for gases entering or exiting from leaves (Antunes et al. 2012), offer a unique opportunity to investigate the interaction between plants and their environment, i.e. the atmosphere and its associated air pollution (Gupta 2016). Plants could change their characteristic stomatal features in short to medium term by affecting stomatal opening and closing, primarily to optimize CO₂ and water vapor exchange, and in the long term, for example, when new leaves are formed (Rai et al. 2011). Because air pollution is assumed to affect stomatal characteristics, changes in the number, distribution, and shape of stomata on a leaf surface may be considered essential plant characteristics (Antunes et al. 2012). Individual atmospheric gases have been shown to influence stomatal characteristics (Gago et al. 2014; Wang et al. 2014). However, the overall impact of gas mixtures is not completely understood, and different gases may have additive, antagonistic and synergetic effects. In order for the GOS to effectively absorb pollutants or reduce pollution, filter air, and detoxification (Nowak et al. 2014), species of the GOS could be selected with regard to stomata characteristics. Thus far, there is no information yet on stomata characteristics of the tree species grown in the GOS of USU. Therefore, this current study observed and examined stomata characteristics of several tree species in the GOS of USU.

MATERIALS AND METHODS

Research location

This research was carried out on 120 hectares at the Universitas Sumatera Utara campus (3.330 N and 98.390 E) in Padang Bulan, Medan, Indonesia. The Padang Bulan campus is located in Medan City at an elevation of 2.5-37.5 meters above sea level. The minimum temperature is 23.2° C/24.3° C, while the maximum temperature is 30.8° C/33.2° C. CO₂ level at the research location ranged from 345-398 ppm. The average air humidity in Medan City is between 84-85%. The average wind speed is 0.48 m/sec, the total evaporation rate is 104.3 mm/ month, and the soil type is inceptisol. The area on the USU campus was rice fields and swamps at the time of its establishment in 1952.

Procedure material collection

Leaf samples of 83 tree species belonging to 30 families were collected from USU campus. The selected trees were collected from a single location on the USU campus in Padang Bulan, with age differences amongst species due to a lack of information data on tree species and planting

years. Three normally growing sample plants from each individual were selected and three pieces of healthy mature leaves on the lower part of newly grown branches were collected from each plant. Five replicates were taken for each sample with similar size and maturity. Replica and nail polish methods (Milstead et al. 2020) were assigned to suit the different characteristics of the stomata. Slides were observed using a stereomicroscope (Nikon SMZ18) and the stomatal characteristics were analyzed and pictured using NIS Element 7.0. For each individual, ten slides were selected and three random pictures were obtained from each slide. The number of stomata in each picture was also counted and the area of each picture was measured using Image-Pro Plus to calculate the stomatal density.

Data analysis

Data were processed using visual and statistical software (SPSS version 25) for getting the stomata type, stomata size, stomata abundance and density. The observation results were visualized as a picture by using the Images Raster Optilab application. Based on preliminary observation on 121 tree species at USU campus, 83 species have abaxial stomata, whereas 38 species have adaxial and both adaxial and abaxial stomata. Eighty three species with abaxian stomata type were observed in this study. The types of stomata were analyzed by observing the arrangement of epidermis cells adjacent to neighboring cells. The stomata type was categorized into 4 (four) different types of stomata, namely anomocytic, anisocytic, paracytic, and diacytic (Da Silva et al. 2014). To determine the density of stomata, the number of stomata was divided by the area of the observed cross-section (Aono et al. 2021). The length and width of stomata were analyzed by using the same application based on the length. The measurement results were grouped into 3 (three) categories, i.e., short (<20 µm), long (20-25 µm), and very long (>25 µm). Based on the width, the stomata were categorized as narrow (<19.42 µm), wide (19.42 µm-38.84 µm), and very wide (>38.84 µm). The density of stomata was measured based on Juairiyah (2014) and classified into three categories, which are low (<300/mm²), medium (300-500/mm²), and high (>500/mm²).

RESULTS AND DISCUSSION

On the leaf surface, stomata may occur on both sides (amphistomatous leaves) or on either surface alone, usually the lower surface (hypostomatous leaves). Amphistomatous leaves are most commonly found in arid environments, whereas leaves with stomata only on the underside seem to be more common in plants of mesophytic habitats (Parkhurst 1978). On the other hand, although less common in nature, leaves with stomata only on the adaxial (upper) surface (epistomatous or hyperstomatous leaves) can be found in some floating plants, such as water lilies (Lawson 2009). On forest trees species, stomata were located only on the abaxial surface (hypostomatous leaves) with different observed parameter values. Generally, forest tree species have hypostomatous leaves, with abaxial stomata

(Lüttge and Buckeridge 2020)

Types of stomata

Foliar stomata of the plant have great variety (Shahzad et al. 2015). In determining the type of stomata, the presence of the epidermis cell and the neighboring cell is an important observation. The position, number, and shape of both cells also determine the type of leaf stomata (Ullah et al. 2019). Stomata are classified into several types based on the relationship between the position of the porous and guard cells and the position of neighboring cells. The classification of stomata shape differs from the classification of stomata shape based on its development. The position structure and layout of stomata cells can be used for taxonomy studies, though it does not rule out the possibility of different types of stomata in families, or even within the same plant species. Stomata patterning is classified based on shapes and arrangement of subsidiary cells and distribution patterns in dicotyledonous arboreal and tropical species. Based on the guard epidermal cell arrangement neighboring the guard cell, more than 25 main types of stomata in dicot have been recognized (Metcalfe 1972). The observation on the 83 leaves samples in USU GOS, 4 (four) types of stomata were found, namely paracytic, anomocytic, anisocytic, and diacytic with varies in the number of an individual tree (Figure 1). The most abundant type of stomata found in the USU campus was paracytic (91.46%) followed by anomocytic (6.02%), anisocytic (1.20%), and diacytic (1.20%).

The paracytic stomata are characterized by joining the guard cell with one or more of the adjacent neighboring cells with the longitudinal axis parallel with the axis of the neighbor cell and the aperture (Aono et al. 2021). This type of stomata was found in 76 species, including *Hura crepitans*, *Acacia mangium*, *Persea americana*, *Elaeocarpus grandiflorus*, and *Garcinia atroviridis*. The anomocytic was typified by guard cells that are surrounded with cells with similar shape, size, and arrangement as the rest of epidermis cells. In USU GOS this type found in bungur (*Lagerstromia speciosa*), gaharu (*Aquilaria malaccensis*), kapuk (*Ceiba pentandra*), kelor (*Moringa oleifera*), and sukun (*Artocarpus altilis*). The anisocytic an diacytic were the lowest. The anisocytic type is marked by the guard cells surrounded by different sizes of three neighbor cells and found in kesambi (*Scheichera oleosa*). The diacytic stomata are recognized by the guard cells that are surrounded by two neighbor cells with the cell wall at the right angle to the longitudinal axis of the stomata (Shahreen et al. 2010) and found in *Gnetum gnemon*. Those four types of stomata have particular features and appearance in the position, number, and presence of epidermis and neighbor cells (Figure 2).

The paracytic type was found to dominate the leaf stomata of the tree species in USU campus. A similar finding was also found by Tripathi and Mondal (2012), where 64.1% of the 45 observed species had paracytic stomata type. The species found with the same type of stomata were mostly from the same family. The paracytic type was found in 23 plant families in the GOS (Figure 3). Stomatal type has long been regarded as one of the

persistent and diagnostic features for different taxa and has well been utilized by different workers to establish the groups or to eliminate the controversy about the taxonomic group (Mitra et al. 2015). However, the occurrence of different types of stomata in the same species is rare. The finding of the same stomata types in several species from the same genus indicated the close relationship of those species. Those species were also appeared to have similar physiological characteristics.

Stomata dimension

Stomatal number, size and density show a variation that depends on the plant's species, cultivar, clones and cultivation conditions (Ahmet et al. 2014). The dimension of stomata was determined by measuring the length and width of the observed stomata. Based on the length, the observed stomata were grouped into three categories, short, long, and very long (Figure 4.A). Based on the width, the observed species in the GOS were only fell into two categories, narrow and wide (Figure 4.B).

The longest stomata were observed in species *Antidesma bunius* (32.04 μm), while the shortest is *Shorea laevis* (5.43 μm). The widest stomata found in *Garcinia mangostana* (37.62 μm) and the narrowest is *Shorea laevis*, measuring 3.72 μm . *Antidesma bunius* is a common plant in the GOS region and can be found in several GOS in Indonesia. Zandalinas et al. (2018) and Khoiroh et al. (2014) noted that the dimension of leaf stomata tends to vary, and is influenced by internal and external factors, i.e., genetic and environmental. Several species with larger stomata are considered suitable to assign as GOS plants. Large stomata would accommodate a higher rate of plant transpiration which is in line with the rate of water and nutrient uptake, aside from the avoidance of excess turgor (Salisbury and Ross 1995). Water in photosynthesis also plays a role in the leaf CO_2 fixation; therefore, increasing water uptake would increase CO_2 uptake by the leaves. The CO_2 gas could result from various anthropogenic activities such as transportation, household electricity consumption, and waste (Baraldi et al. 2019). The dense urban population contributes to the high production of pollutant gases in city areas. Thus, plant species with large stomata in the GOS could fixate the pollutant gases and reduce CO_2 emissions (Alushi and Veizi 2020).

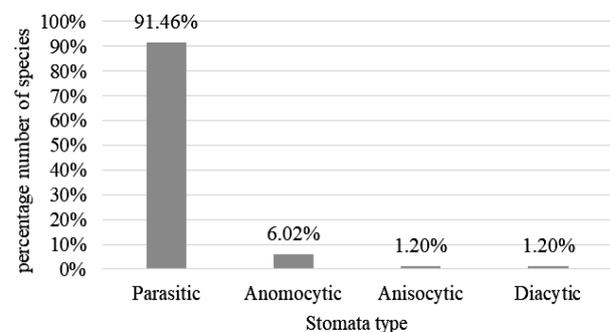


Figure 1. Stomata type of 83 tree species in USU green space

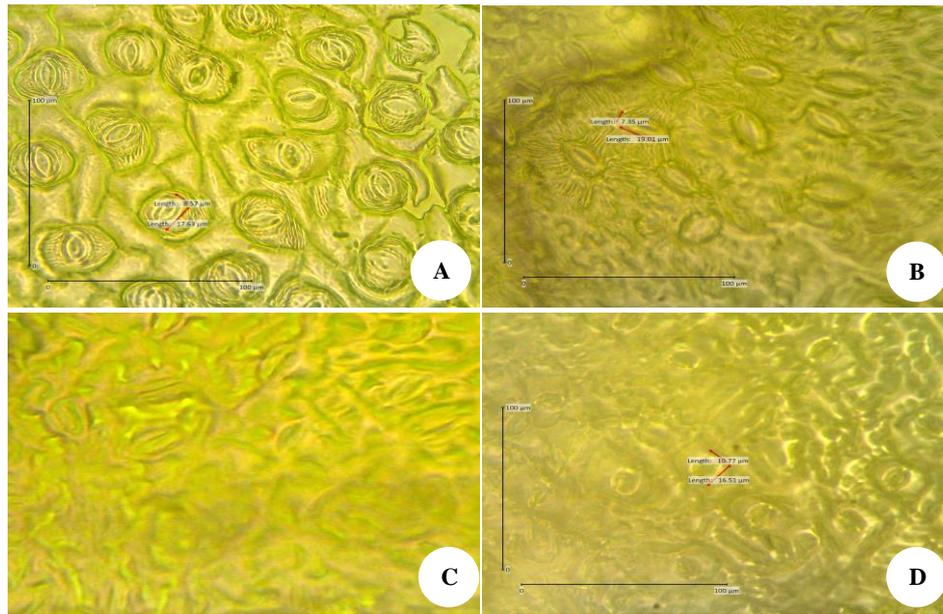


Figure 2. The type of stomata found in 83 observed leaves species in the GOS of USU green space, Indonesia: A. *Hura crepitans* with paracytic type, B. *Artocarpus altilis* with anomocytic type, C. *Schleicheria oleosa* with anisocytic type, and D. *Gnetum gnemon* with diacytic type

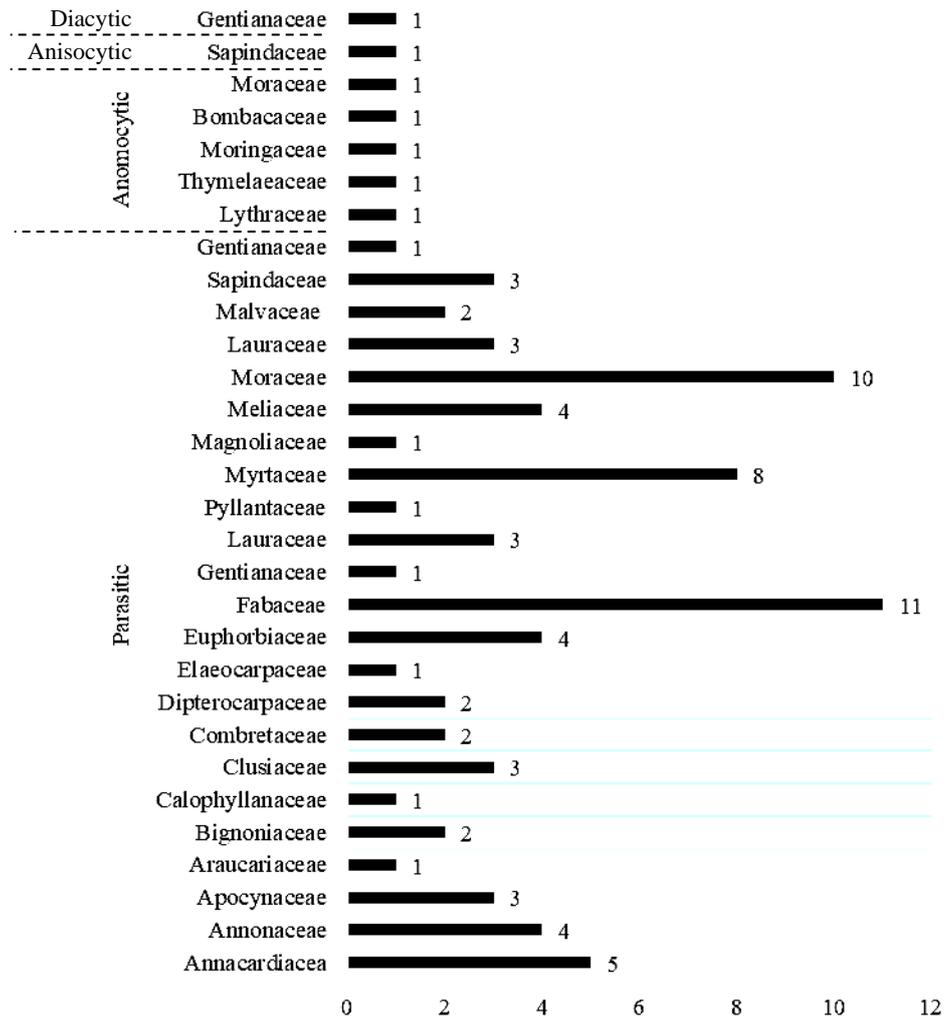


Figure 3. Stomata type based on the family distribution of 83 tree species in USU green space, Indonesia

Schleichera oleosa has the most stomata; those were 2229, while *Baccaurea macrocarpa* has the fewest, six stomata. A large number of stomata affected transpiration rate, but it has beneficial to the urban space area. In plants, transpiration is basically fresh evaporation of water that transports mineral salts from the soil. In combination with the utilization of sunshine, transpiration is also beneficial in some tree species. A harmful rise in temperature can be avoided since part of the radiated sunlight is utilized for water evaporation (Raharjo et al. 2015; Samiyarsih et al. 2020).

Stomata density and distribution

Stomata is more abundant in leaves than in the other plant organs (Camargo and Morenco 2013). They are present both on the abaxial and adaxial surface, although sometimes could only be found on the abaxial side (Lüttge and Buckeridge 2020). Therefore, in this current study, the stomata were solely observed on the abaxial side. The observation revealed that all samples have widely distributed stomata with various densities that are

categorized into high, medium, and low densities (Table 1). The highest stomata density was found in *Schleichera oleosa* (4294 mm⁻²), followed by *Psidium guajava* Red Malaysia (1880 mm⁻²) and *Syzygium malaccense* (1514 mm⁻²) (Table 1). Meanwhile, *Baccaurea macrocarpa* showed the lowest density of stomata (11 mm⁻²).

Variation in size and density of stomata may arise due to genetic factors and/or growth under different environmental conditions. A negative correlation has frequently been suggested between these two stomatal traits (Varshney et al. 2018). The density of stomata is affected by the number of stomata on the leaf surface (Bertolino et al. 2019). The distance between stomata is necessary because it would facilitate the maximum function of gas exchange so as to avoid an overlap in the gas exchange (Rudall et al. 2017). The dense stomata could increase the cooling evaporation in the leaves, and accelerate CO₂ assimilation during the period of stomata opening in the morning and evening. However, the water availability during daylight could limit the increase in the daily C assimilation (Wang et al. 2018).

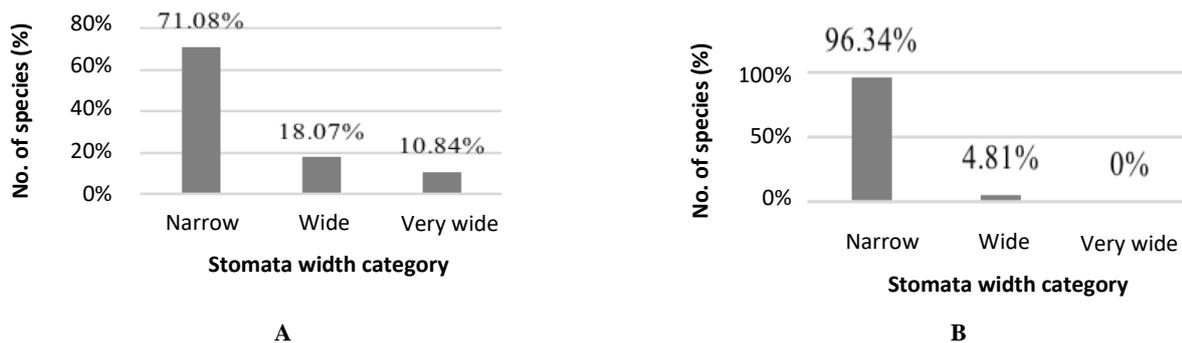


Figure 4. A. Stomata length, B. Stomata width

Table 1. Stomatal density category of tree species in USU green space, Indonesia

Stomata density category	Species
High	<i>Schleichera oleosa</i> , <i>Psidium guajava</i> "Red-Malaysia", <i>Syzygium malaccense</i> , <i>Swietenia microphylla</i> , <i>Psidium guajava</i> , <i>Swietenia macrophylla</i> , <i>Syzygium paniculatum</i> , <i>Theobroma cacao</i> , <i>Syzygium polyanthum</i> , <i>Mangifera odorata</i> , <i>Cinnamomum verum</i> , <i>Mangifera foetida</i> , <i>Shorea macrophylla</i> , <i>Filicium decipiens</i> , <i>Shorea laevis</i> , <i>Ficus ampelas</i> , <i>Delonix regia</i> , <i>Manilkara zapota</i> , <i>Anacardium occidentale</i> , <i>Syzygium cumini</i> , <i>Polyalthia longifolia</i> , <i>Ficus racemose</i> , <i>Ficus lyrata</i> , <i>Flacourtia rukam</i> , <i>Bouea macrophylla</i> , <i>Spathodea campanulate</i> , <i>Dialium indum</i> , <i>Morinda citrifolia</i> , <i>Premna corymbosa</i> , <i>Cinnamomum burmannii</i> , <i>Syzygium aqueum</i> , <i>Mangifera indica</i> , <i>Eucalyptus globulus</i>
Medium	<i>Acacia mangium</i> , <i>Tamarindus indica</i> , <i>Magnolia alba</i> , <i>Hevea brasiliensis</i> , <i>Persea americana</i> , <i>Hura crepitans</i> , <i>Artocarpus heterophyllus</i> , <i>Plumeria alba</i> , <i>Ficus variegata</i> , <i>Artocarpus integer</i> , <i>Cerbera manghas</i> , <i>Instia bijuga</i> , <i>Lagerstroemia speciosa</i> , <i>Annona montana</i> , <i>Cynometra cauliflora</i> , <i>Artocarpus altilis</i> , <i>Garcinia dulcis</i> , <i>Aquilaria malaccensis</i> , <i>Elaeocarpus grandiflorus</i> , <i>Ficus hispida</i>
Low	<i>Delonix regia</i> , <i>Ficus benjamina</i> , <i>Ceiba petandra</i> , <i>Gnetum gnemon</i> , <i>Alocasia macrorrhiza</i> , <i>Moringa oleifera</i> , <i>Stelechocarpus burahol</i> , <i>Aleurites moluccana</i> , <i>Terminalia cattapa</i> , <i>Adenanthera pavonina</i> , <i>Salix babylonica</i> , <i>Ficus elastic</i> , <i>Ficus microcarpa</i> , <i>Sterculia foetida</i> , <i>Annona muricata</i> , <i>Handroanthus chrysotrichus</i> , <i>Pterocarpus indicus</i> , <i>Erythrina cristagalli</i> , <i>Lansium domesticum</i> , <i>Melia azedarach</i> , <i>Mimusops elengi</i> , <i>Garcinia atroviridis</i> , <i>Plumeria alba</i> , <i>Terminalia mantaly</i> , <i>Calophyllum inophyllum</i> , <i>Garcinia mangostana</i> , <i>Agathis dammara</i> , <i>Leucaena leucocephala</i> , <i>Antidesma bunius</i> , <i>Baccaurea macrocarpa</i>

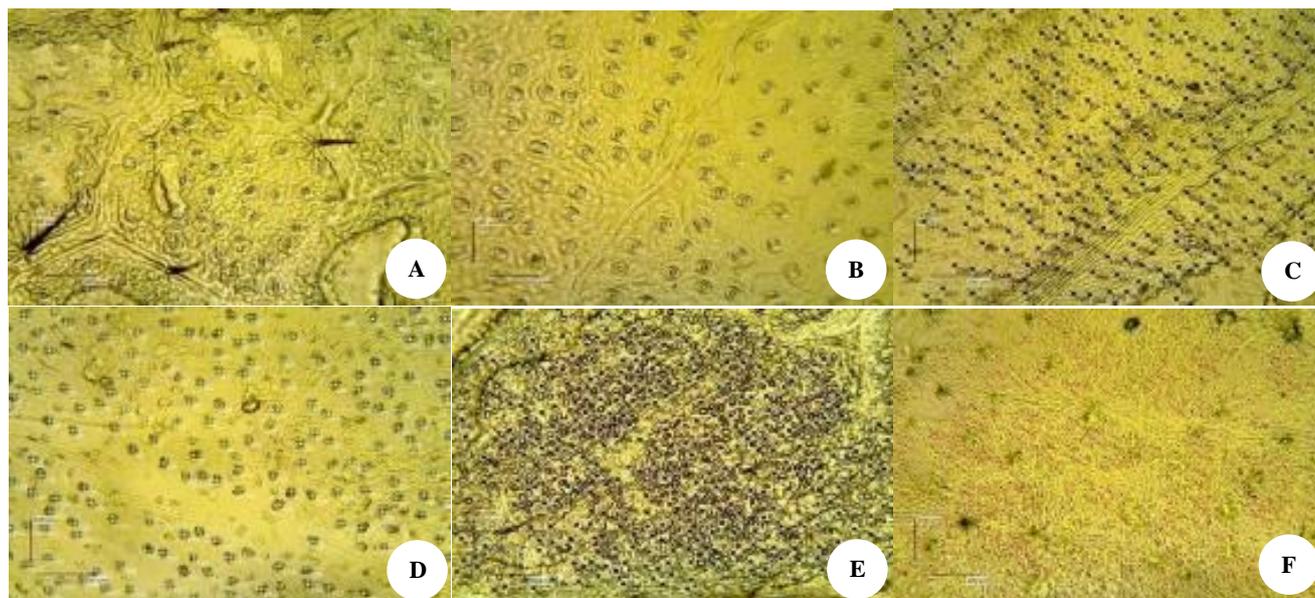


Figure 5. The image overlay of the observation on numbers and stomata density of tree species in the GOS of USU green space, Indonesia; A. *Pterocarpus indicus* and B. *Garcinia atroviridis*, which had low-density stomata, C. *Acacia mangium* and D. *Elaeocarpus grandiflorus*, which had medium density, E. *Dialium indum* and F. *Shorea laevis* that had a high density

The various stomata density found in the plants growing in the GOS was affected by environmental factors e.g., air temperature, light intensity, and CO₂ concentration (Zhao et al. 2017). The stomata are affected by the air quality surrounding the plant. Stomata serve as a conduit for gas and water vapor exchange between plants and their surroundings (Lüttge and Buckeridge 2020). Stomatal density varies substantially in one unit of leaf surface area, according to Lawson et al. (2014). This is due to variations in the environment in which they grow, and genetic factors that have a significant impact on the formation of stomata. Stomatal density is affected by elements such as air availability, light intensity, temperature, and CO₂ content. The tree species in the GOS of the USU were found to have a dispersed and irregular distribution of stomata (Figure 5). The dispersed stomata distribution was assumed due to the genetics of dicotyledonous plants with pinnate leaves. The pinnate leaves, such as in the dicotyledonous plant, have a dispersal stomata distribution. In contrast, the monocotyledonous plants with parallel venation tend to have stomata arranged in a row (Faunirakis et al. 2015).

To conclude, there are 4 (four) types of stomata found in the green open space of the USU campus, which are paracytic, anomocytic, anisocytic, and diacytic, with the highest percentage found in paracytic type. The longest stomata were observed in *Antidesma bunius* with a length of 32.04 μm . The widest stomata were in *Garcinia mangostana* with a width of 37.62 μm . The shortest and narrowest stomata species was *Shorea laevis*, with 5.43 μm and 3.72 μm for each attribute, respectively. The species with the highest stomatal density was *Schleichera oleosa* (4294 mm^{-2}). All species in the GOS showed a random stomata distribution. According to the findings of this study, a large percentage of the tree species at USU have high stomata density, length, and width, making them more

suitable for green space. Species with a high number and density of stomata and a large size are much more likely to adsorb pollutants such as carbon monoxide.

ACKNOWLEDGEMENTS

This research was supported by the Indonesian Directorate of Research and Community Service, Ministry of Research and Technology and National Research and Innovation Agency, Republic of Indonesia (12/E1/KP.PTNBH/2021) through *Penelitian Dasar Unggulan Perguruan Tinggi* (PDUPT) Scheme 2021.

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