The dynamics of physiological properties of ebony (Diospyros celebica Bakh) based on crown position and altitude

S A Paembonan¹,³, S H Lareken², S Millang¹ and Meinardus¹

¹Silviculture Laboratory, Faculty of Forestry, Hasanuddin University, Makassar
²Forestry Biotechnology Laboratory, Faculty of Forestry, Hasanuddin University, Makassar

E-mail: paembonansa@gmail.com

Abstract. Knowledge of the physiological properties of tree species is one of the basic requirements in determining the prescription silvicultural treatment of stands in nature. This study aims to analyze the physiological characteristics of the species of ebony (Diospyros celebica Bakh), as one of the potential endemic species of Sulawesi, based on leaf position in the crown and differences in altitude. Leaf sampling locations were in the Hasanuddin University Educational Forest (507 m asl) and Makassar city (16 m asl). The research variables consist of number of stomata, size of stomata, leaf stomata index, and leaf chlorophyll content. The results show that the physiological characteristics of the leaves were influenced by their position in the tree crown and differences in altitude. The highest number of stomata was found in the leaves exposed to the sunlights with 52.900 stomata / mm² compared to 29.800 stomata / mm² in shaded leaves. Likewise, the highest chlorophyll content was found in leaves exposed to the sunlights of 0.043 mg/L and shaded leaves of 0.034 mg/L. The difference occurs based on differences in altitude as well. The number of stomata in the lower area was 35.733 stomata/mm², while in the high area was 42.667 stomata / mm². The chlorophyll content in the low area was 0.038 mg/L and in the high area it was 0.037 mg/L. The higher a place, the more the number of stomata, this is inversely proportional to the size of the stomata and the stomata index, while the number of chlorophylls is not significantly different.

1. Introduction

Ebony (Diospyros celebica Bakh) is a potential endemic tree species in Sulawesi island that produces beautiful black with brown striated wood. These species require certain environmental requirements to grow and develop properly, including: altitude, light intensity and humidity [1, 2].

Seedlings develop well under a canopy, but ebony saplings prefer small gaps. Ebony regeneration also appears unable to grow solitary. When grown alone, the shoots withers or the stems die, then side shoots grow [2].

The adaptation of each tree species to shaded or sun-exposed conditions based on the growth phase rate plays an important role in determining the rate capacity of photosynthesis. Good photosynthetic abilities play an important role in plant growth and production [3]. Previous studies found that significant
changes in the canopy structure of larger size trees can significantly change the level of light intensity under the canopy [4].

Each species of tree has a different tolerance for sunlight intensity. There are species that do well in the open, on the other hand there are some plants that do well in shade. Likewise, plants require different light intensities for each stage of their growth phase. When the trees are still young they need light with a relatively low intensity and towards maturity they begin to need light with a higher intensity [5].

Physiologically, stomata play a very important role in the processes of photosynthesis, transpiration and respiration. Stomata are places of exchange of CO$_2$ and oxygen (O$_2$) gases from outside and from within the leaf tissue to the atmosphere [6]. In addition, the chlorophyll content in leaves also determines the photosynthetic capacity of the leaves. The ability of plants to absorb CO$_2$ is also influenced by the shape of the canopy, leaf morphology and physiological properties of the plant. Most of the research that has been done so far is only at the tree phase level [7].

The altitude of the place greatly affects plant growth. The higher the place, the lower the air temperature, and vice versa for the lower area. Therefore, the height of a place affects the air temperature and in turn affects physiological processes in plants.

All physiological processes will be affected by temperature and some processes will depend on light. The optimum temperature is needed by plants so that they can be used properly by plants. Temperature that is too high will inhibit plant growth and can even cause death for plants, and vice versa if the temperature is too low. Meanwhile, light is a source of energy for plants. Therefore, this study reveals the differences in the physiological characteristics of trees based on differences in leaf position on the crown and differences in the height of the growing area.

2. Research methods

2.1. Location and time of research
This research was conducted from June to August 2020. Selection of the sample trees were determined in the Hasanuddin University Educational Forest with an altitude of 507 m above sea level (asl) and in the city of Makassar with an altitude of 20 m asl. Research samples were identified and analyzed at the Silviculture Laboratory of the Faculty of Forestry, Hasanuddin University and the Microbiology Laboratory of the Makassar Environmental and Forestry Research and Development Center.

2.2. Research variables and sample measurements methods
The research variables analyzed consisted of: 1) The number of epidermal cells, the number of stomata, the size of the stomata, and the leaf stomata index of the Ebony tree species; 2) Leaf chlorophyll content.

Measurement of chlorophyll content and stomata analysis based on leaf position in the crown and differences in altitude. At each crown position, 10 leaves exposed to sunlight each, for the east and the west position, were taken and 10 leaf samples from the shaded crown. Sampling was done purposively by paying attention to the position of the outer leaves and shaded leaves in the crown. Leaf samples were taken at 09.00-10.00 am when the stomata were fully open.

2.3. Leaves sampling for stomata measurements
The lower leaf surface was smeared with nail polish, as soon as the leaves were plucked [7]. The dried stomata mold was then covered with clear insulation then pulled and attached to the object glass. These stomatal preparations were observed under an electron microscope with a 200x magnification. Stomata characteristics observed were: number of stomata, size of stomata and index of stomata. While the 400x magnification is for observing the length, width, opening of the stomata, and stomata type. The sample that has been observed is then photographed under the electron microscope.

The stomata index (IS) is calculated based on the formula of [7] and the stomata size was calculated according to the following equation:

$$Stomata \ size = stomata \ length \times stomata \ width$$  (1)
2.4. Chlorophyll content measurements
The value of leaf chlorophyll content was calculated directly in the field after being measured by averaging the chlorophyll content at the base, middle and tip of the leaves (mg / L).

Light intensity was measured using a light meter (lux meter). Measurements of light intensity were carried out inside and outside the stands. Measurement of light intensity aims to determine how much light enters each tree canopy. Air temperature and humidity were measured using a Digital Thermometer Max-Min model No: AZ-HT-02. [7]. Temperature measurement is carried out to determine the maximum and minimum temperature under the wood stands. Meanwhile, air humidity measurements were carried out outside and inside of the stands.

2.5. Data analysis
Data analysis used variance (ANOVA) at the 5% level to determine the differences between treatments. Tukey’s further test at the 5% level is carried out if there is a significant effect on the observed variables. The data were processed using the R Statistics program.

3. Results and discussion

3.1. Number of stomata
The results show that there was a difference in physiological characteristics of the leaves based the different in altitudes and canopy directions.

![Figure 1](image)

**Figure 1.** Number of leaf stomata based on differences in height and position of leaves in the crown. Different alphabet of a, b, and c means significantly different based on Tukey’s Test at the 5% levels.

The results showed that the physiological characteristics of the leaves based on the altitude showed a higher number of stomata in the education forest location, which an average of 42.667 stomata/mm² compared to the location of Makassar which is only 35.733 stomata/mm². Meanwhile, based on the position of the canopy, it shows the highest number of stomata is in the direction of the eastern canopy, namely 52.900 stomata/mm² and the lowest is in the shade canopy of 29.800/mm². This is in accordance with [8] that there is an increase in the number of plant stomata based on altitude, especially in mountainous areas in humid environmental conditions, so that plants adapt to the environment in terms of transpiration by increasing the number of stomata [9]. In addition, in the abaxial (lower) layer of the cuticle (protective layer) that covers the epidermis is thinner or there is only a slight barrier to transpiration through the stomata [10].
3.2. Stomata size
The results showed that the size of the stomata on the leaves with the east side of the canopy was greater than that of the west and shaded leaves.

![Figure 2. Size of leaf stomata based on differences in altitude and leaf position in the crown. Different alphabet of a, b, and c means significantly different based on Tukey’s Test at the 5% levels.](image)

Meanwhile, based on the altitude, it shows that the size of the stomata is greater at a lower location than at a higher location. This is reversed by the number of stomata. Stomata size affects the efficiency associated with solar energy, because leaf stomata play a role in the absorption of CO₂ which is used in the photosynthesis process. According to [7], the size of the stomata is less if: size <20 μm, long if: size 20-25 μm and very long if the size is> 25 μm. The length and width of the stomata are closely related to the size of the stomata porus, the bigger the size of the stomata, the bigger the stomata porus. This results in a high rate of transpiration, thereby increasing the uptake of nutrients and water from the soil. The absorbed water is used for the photosynthesis process which is then used for plant growth and development [11].

3.3. Stomata index
The results of analysis of variance for the stomata index of wood leaves at two different locations and the direction of the crown had a significant effect (p> 0.05).

![Figure 3. Stomata index based on differences in altitudes (left) and canopy direction (right). Different alphabet of a, b, and c means significantly different based on Tukey’s Test at the 5% levels.](image)
From figure 3, it can be seen that the stomata index value is higher in the Makassar location, namely 2.6% compared to the Bengo location with a stomata index of only 1.9%. Likewise, the stomata index based on the direction and position of the crown was significantly different, where the direction of the canopy to the east was greater than that of shaded leaves.

3.4. Upper and lower epidermal cells of leaves
The results of the analysis of variance (ANOVA) of the upper epidermis showed that based on the study location, it was significantly different (p> 0.05), but the direction of the crown showed insignificant differences (p <0.05). Meanwhile, the lower epidermis shows significant differences for the two variables.

![Figure 4. Average number of upper and lower leaf epidermis based on study location (left) and canopy direction (right). Different alphabet of a and b means significantly different based on Tukey’s Test at the 5% levels.](image)

In figure 4 it can be explained that the average number of upper epidermis is greater in the Bengo-bengo area, namely 2957.6 epidermis / mm² compared to the Makassar location which is only 2133.1 epidermis / mm² and is significantly different from one another. Different conditions occur in the canopy direction where the mean of the upper epidermis is not significantly different but the lower epidermis is significantly different.

3.5. Chlorophyll content
The results showed the highest chlorophyll content in leaves with the canopy to the east compared to shaded leaves. Meanwhile, based on altitude, it shows the highest chlorophyll content in the lower area in Makassar compared to higher locations in Educational Forest, although the difference is not significant.
Figure 5. Leaf chlorophyll content. Different alphabet of a and b means significantly different based on Tukey’s Test at the 5% levels.

The effect of different sunlight intensity at each location is one of the great effects of the chlorophyll content in the leaves. Decreasing chlorophyll content is one of the physiological responses of water-deficient plants also reduces the rate of plant photosynthesis [12]. The difference in the greenness of the leaves is due to the fact that the leaves have a greener color which directly provides an indicator of the amount of chlorophyll. [12] stated that chlorophyll synthesis is strongly influenced by heredity, light and certain mineral supplies. [13] stated that plants are influenced by environmental factors such as sunlight, temperature, humidity, competitors, shade, soil nutrients, and growth forms. Furthermore, [13] stated that the intensity of sunlight can affect the condition of the leaves, in which chlorophyll is produced. Thus environmental factors, especially light intensity, greatly affect the chlorophyll content of a plant. As stated by [14] that leaves from open and shaded positions, or from tolerant and intolerant plants, have very varied morphologies. Leaves that are exposed are smaller, thicker and more skin-like than leaflets of the same age and species.

Leaf green color is closely related to chlorophyll content. In general, the older the leaves, the green the leaf color, the higher the chlorophyll content. The difference in leaf color also shows the different types of pigments contained in the leaves. The chlorophyll in young leaves is still a protochlorophyll and the leaves turn green after the protochlorophyll transformation [15]. Furthermore [15] stated that the amount of chlorophyll is not only affected by the amount of pigment, but also by the leaf surface area. Furthermore, the size of the leaf area also has a role in the photosynthesis that occurs in leaves. Photosynthesis yield per plant unit is determined by leaf area. With a larger leaf surface area, it is possible to capture better light so that it has a higher value for photosynthesis. As the leaves age, the chlorophyll content and leaf area also increase. This is consistent with the statement of [16] that chlorophyll content is also influenced by the morphological and anatomical structure of a plant. The larger the leaf size of a plant, the more chlorophyll content and vice versa. However, the older the leaves are, the less their ability to photosynthesize because of the reduced function of chlorophyll. [15] stated that chlorophyll increases in line with the development of leaf area where the amount of chlorophyll per unit leaf area will reach a maximum level before the leaves eventually stop growing.

3.6. Epidermis and stomata form
The shape and arrangement of epidermal cells varies widely. Epidermal cells are pentagon, hexagon, elongated, and some even irregular. The position of the epidermal cells is irregular with one another and there are spaces between cells. Epidermal cells are found in the nucleus which is located in the middle and some are located at the edge of the cell (figure 6).
Figure 6. The upper epidermis (a), and the lower epidermis along with the stomata (b) with 200x magnification.

Figure 7. The shape of stomata in the seedling stage (a) and tree phase (b) magnification of 400x.

4. Conclusion

- Physiological characteristics of leaves based on crown position and differences in elevation of Ebony (Diospyros celebica Bakh) differ in the number of stomata, stomata size, stomata index and leaf chlorophyll content.
- The higher the altitudes, the greater the number of stomata but inversely proportional to the size of the stomata and the stomata index. The number of stomata is greater in leaves exposed to sunlight than leaves that are shaded.
- Meanwhile, the chlorophyll content was higher at a low elevation compared to a higher one, although it was not significantly different. The chlorophyll content in leaves exposed to sunlight in the east is higher than in shaded leaves.

Acknowledgment

The highest gratitude is conveyed to the Rector of Hasanuddin University through the Institute for Research and Community Service (LP2M) who has provided research funding assistance to enable this research to be carried out.

References

[1] Kurniawan E 2013 Strategi penyelamatan eboni (Diospyros celebica Bakh) dari ancaman kepunahan Info Teknis Eboni 10 (2) 99-106
[2] Seran D, Lempang M, Allo M K, Sumardjito Z, Paembonan S and Ginoga B 1988 Aspek ekologi eboni (Diospyros celebica Bakh) di cagar alam Kalena, Kabupaten Luwu, Propinsi Sulawesi Selatan Jurnal Penelitian Kehutanan 2 (1) 1-8

[3] Suherman, Millang S and Asrl L 2016 Respon morfofisiologi, fenologi, dan produksi tanaman kopi terhadap berbagai naungan dalam sistem agroforestri di Kabupaten Enrekang J. Sains & Teknologi 16 (2) 197 – 202 ISSN 1411-4674

[4] Campbell L 2012 Biophysical Drivers of Tree Crop Performance in Shade Agroforest Systems: The Case of Coffee in Costa Rica (Toronto: Department of Geography, University of Toronto)

[5] Farida E 1995 Pengaruh intensitas cahaya, mikoriza dan serbuk arang pada pertumbuhan alam Dryobalanops sp. Buletin Penelitian Nomor 29 FakultasKehutanan Universitas Gadjah Mada Yogyakarja

[6] Santrucek J, Vrablova M, Simkova M, Hronkova M, Drtinova M, Kveton J, Vrbl D, Kubasek J, Mackova J, Wiesnerova D, Neuwithova J and Schreiber L 2014 Stomatal and pavement cell density linked to leaf internal CO2 concentration Ann. of Bot. 114 191-202

[7] Tambaru E 2012 Potensi Absorpsi Karbon Dioksida pada Beberapa Jenis Pohon Hutan Kota di Kota Makassar [Theses] (Makassar: Program Studi Ilmu Pertanian Program Pascasarjana Universitas Hasanuddin)

[8] Ura’ R, Paembonan S A and Malamassam D 2017 Analisis vegetasi tanaman bawah berkasiat obat pada sistem agrisilvikultur di Lembang Sereale Toraja Utara Jurnal Ilmu Alam dan Lingkungan 8 (16) 45-5

[9] Elina N, Fitmawati and Iryani D 2012 Karakterisasi Anatomi Stomata Daun Sagu (Metroxylon sagu Rottb.) Pada Tahap Anakan dan Nyorong (Riau: FMIPA Universitas Riau)

[10] Papuangan, Nurhasanah N and Djurumudi M 2014 Jumlah dan distribusi stomata pada tanaman penghijauan kota Ternate Jurnal Bioedukasi 3 (1) 287-292

[11] Putri F M, Suedey S W A and Darmayanti S 2017 Pengaruh pupuk nanosilika terhadap jumlah stomata, kandungan klorofil dan pertumbuhan padi hitam Buletin anatomi dan fisiologi 2 (1) E ISSN 2541-0083

[12] Salisbury F B and Ross C W 1992 Fisiologi Tumbuhan Jilid 3 (Bandung: Penerbit ITB)

[13] Fitter A H and Hay R K M 1994 Fisiologi Lingkungan Tanaman (Yogyakarta: Gadjah Mada University Press)

[14] Daniel T W, Helms J A, Baker F S 1992 Prinsip-prinsip Silvikultur Translated ed Marsono D (Yogyakarta: Gadjah Mada University Press)

[15] Sumenda L, Henny L R and Feky R M 2011 Analisis kandungan klorofil daun mangga (Mangifera indica L.) pada tingkat perkembangan daun yang berbeda Jurnal Bioslogos 1 (1) 20-24

[16] Musyarofah N, Susanto S, Aziz S A and Kartosoewarno S 2006 Respon Tanaman Pegagan (Centella asiatica L. Urban) Terhadap Pemberian Pupuk Alami di Bawah Naungan (Bogor: Sekolah Pasca Sarjana Institut Pertanian Bogor)