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Stomata Diversity Profile of Several Coffee Species at the Indonesian Coffee and Cocoa Research Center

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Abstract

Stomata are a special part of plants, namely a number of leaf epidermal cells, both the upper and lower surface epidermis of the leaves. Stomata function as a place for CO2 to enter from the air in the process of photosynthesis, where respiration and transpiration take place so that stomata are an important tissue in plant metabolic processes. Observing stomata using a microscope is an effective and easy way to determine the type of stomata in angiosperm plants. This research was conducted on November 19 2022 with the aim of determining the type and diversity of stomata on several types of coffee plants at the Indonesian Coffee and Cocoa Research Center. The method used in this research is an exploratory survey method, while the replica method is used to look at leaf stomata, namely applying nail polish to the leaf surface and identifying the type of stomata using a qualitative descriptive method. The survey found that there were 9 types of coffee plants at the Indonesian Coffee and Cocoa Research Center. The results of the research show that the type of stomata on coffee plants based on the number and arrangement of neighboring cells is classified as a parasitic type. This can be seen from the presence of two neighboring cells surrounding the guard cell and the location of these cells parallel to the guard cell.

Keywords: stomata types, coffee plants, indonesian coffee and cocoa research center

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INTRODUCTION

Stomata are small pores in the epidermal cells of leaves or stems which function to regulate the release of water in the transpiration process and the absorption of CO_2 in photosynthesis. The role of stomata is to connect the external and internal environments of the plant. Therefore, in plants, stomata function as a place for gas exchange, respiration and transpiration. Stomata can be found in all plants (except the roots), but the majority are found in the leaves. Stomata are gaps in the epidermis surrounded by guard cells. These guard cells can have a similar or different shape

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compared to other epidermal cells. Based on the shape of the epidermal cells, stomata consist of several types, namely anomocytic, anisocytic, parasitic, diacytic, actinocytic and cyclocytic. Knowledge of stomata types can be used to understand relationships between species and is useful in the field of taxonomy [1].

Stomata are specialized epidermal cells commonly found on leaves but occasionally present on stems. They consist of two guard cells containing chloroplasts. Each guard cell is fortified with suberin and wax substances, imparting water resistance and enhancing the closure of the stoma. Stomata play a crucial role in regulating gas exchange, both in photosynthesis and water absorption. Carbon dioxide diffuses through stomata into chloroplasts for use in the dark reactions of photosynthesis, while oxygen, a byproduct of photosynthesis, exits through stomata. Stomata also allow water vapor to escape from the leaves. In most plants, stomata open during the day when photosynthesis occurs, leading to a significant loss of water through stomatal openings due to the heat from the sun [2].

The epidermis is a layer of outer cells that continuously covers every organ of a plant (leaves, stems, and roots). These cells may form a single layer or more. The epidermis serves as a protective barrier for the underlying tissues from the external environment, regulating the amount of water exiting the cells due to the presence of cuticles, wax, and hairs on the surface of the epidermal cells [3]. The epidermal tissue develops and undergoes modifications based on its function and structure. One form of modification in the epidermal tissue is the presence of stomata (pores/hole), which function as regulators of water content in leaves. Stomata are known as the epidermal openings, each delimited by two guard cells. In Greek, "stoma" means "mouth," and this term is often used specifically for stomatal pores. Stomata are most commonly found on the leaves of plants and can also be present on stems, fruits, and flowers, albeit in fewer numbers. Stomata provide a direct pathway between the leaf and the atmosphere, facilitating gas exchange (CO_2 and O_2) as well as water (H_2O). Stomatal density is influenced by the water pressure on the leaves and is affected by light intensity. Water pressure affects the size of the epidermis, while light intensity influences stomatal density and the stomatal index [4].

In general, the types of stomata can vary between plant species, even within the same family. This variation is evident in a study conducted by Sarjani et al. [5], which observed stomatal types in the Piperaceae family by comparing five different species within the same family. Despite belonging to the same family, each species exhibited distinct stomatal types. However, within a single family, some plants may share the same stomatal type, as seen in the Nymphaeaceae family, specifically in the genus Nymphaea. Research on various species within the Nymphaeaceae family, particularly the Nymphaea genus, revealed that all observed plant types had the same anomocytic stomatal type.

On the other hand, the Rubiaceae family is known to possess stomata. This family comprises about 450 genera with approximately 6,500 species distributed in tropical and subtropical regions. Common characteristics of this family include leaves with flat margins, paired leaves with supporting leaves located between leaf stalks, and sometimes divided into threes. Eight well-known genera within this family include Dentella, Geocardia, Mussaenda, Gardenia, Morinda, Guettarda, Ixora, Pavetta, and Coffea.

In stomatal identification, several efficient methods are required, and one of them is stomatal printing. Stomatal printing, or replication, is a technique for creating stomatal preparations using transparent nail polish as a printing agent on the epidermal part of leaves. Applying transparent nail polish to the underside of the leaf (abaxial surface) can produce imprints of the leaf's epidermal surface through clear plaster castings, which are

then observed under a microscope [6]. Based on this method, the identification of stomatal types in various leaves was conducted using stomatal printing. With this method, it is expected to obtain clear stomatal preparations efficiently, facilitating the identification of stomatal types. This research aims to determine stomatal types based on the number and arrangement of neighboring cells surrounding the guard cells and the diversity of stomata in the sampled area taken from 9 types of coffee plants collected by the Indonesian Coffee and Cocoa Research Center.

METHODS

The research aims to identify the types and diversity of stomata in nine coffee plant species, namely Arabica coffee, Robusta coffee, Liberica coffee, Kawisari B coffee, Kawisari D coffee, Black coffee, Excelsa coffee, Kalimas coffee, and Sumbertelogo coffee, which are part of the collection at the Indonesian Coffee and Cocoa Research Center. The method employed in this study is an explorative survey. To observe leaf stomata, the printing or replica method is utilized, involving the application of clear nail polish on the leaf surface.

The preparation of stomatal specimens follows the stomatal printing or replica method. This involves thinly applying clear nail polish containing acetone to the abaxial (lower surface) part of the leaf, allowing it to dry, and subsequently covering it with clear tape. The tape is then carefully peeled off, ensuring that the abaxial epidermis adheres to the tape. The tape containing the abaxial leaf section is then affixed to a microscope slide. The stomatal preparations on the slide are observed under a microscope until clear stomatal images are obtained for easy analysis. Once clear and high-quality stomatal images are acquired, documentation is performed using a camera, followed by the identification of stomatal types using descriptive qualitative methods. The research results indicate the stomatal types and the diversity of their numbers in various coffee plant species present at the Indonesian Coffee and Cocoa Research Center.

The research data involves the identification of stomatal types in Robusta, Arabica, Liberica coffee species, and several collection plants owned by the Indonesian Coffee and Cocoa Research Center, suspected to be the result of crosses between them, along with 9 other coffee species, including collection plants at the Indonesian Coffee and Cocoa Research Center. Each of the nine coffee species is represented by one type of coffee (with 2 repetitions). The observed parameters are measured using Optilab as an application that connects the microscope lens with the monitor, and raster image as an application for measuring stomatal sample research. The data sources in this study include primary and secondary data. Primary data is obtained directly from the research location, namely the Indonesian Coffee and Cocoa Research Center, through observations for direct observation and documentation. On the other hand, secondary data is obtained from various literature sources that are relevant to the research, serving as supporting information to enrich the dataset.

The area of this research involves identifying the diversity of stomata in various coffee plant species, where the coffee samples used in this study were obtained from the Indonesian Coffee and Cocoa Research Center. The materials utilized in this study include coffee leaf samples, articles, books, catalogs, and magazines that have been published. The research materials encompass the process of collecting stomata and measuring stomata based on the parameters to be investigated, following the procedures outlined by the Indonesian Coffee and Cocoa Research Center. The tools employed in this research include a Smartphone (Hp), scissors/cutter, glass slides, cover slips, compound microscope, laptop, clear nail polish or nail varnish,

transparent tape, and writing tools. This study adopts a descriptive method to illustrate and interpret the epidermal cell structure and leaf stomata of various plants from the Rubiaceae family. Stomata sampling is conducted in the morning, where samples from 9 coffee plant species are taken from the Indonesian Coffee and Cocoa Research Center plantation. The replica/printing method is employed on leaves with a pristine surface, meaning leaves not affected by leaf characteristic viruses or similar issues. After obtaining stomata samples, further research is conducted in the breeding laboratory to examine the collected stomata using a microscope connected to OptiLab and a monitor, enabling a visual representation of stomata magnified 40x for human observation. The parameters observed include stomatal types in the 9 coffee plant species and the diversity of stomatal numbers within the sampled area.

RESULTS AND DISCUSSION

The observation results of stomata types based on the number and arrangement of neighboring cells surrounding the guard cells can be seen in the following figures:

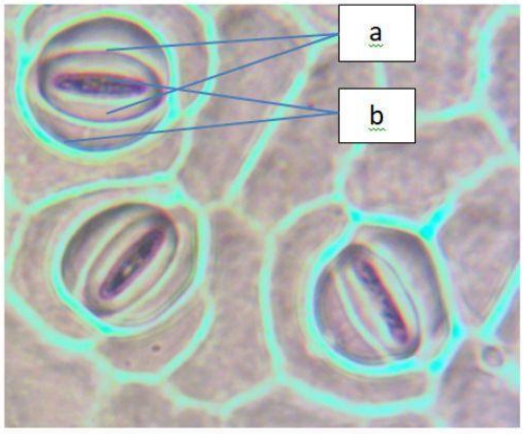

Figure 1. Stomata type (a) guard cells, (b) neighboring cells

From the above picture, the stomata type of Arabica coffee (*Coffea arabica*), Robusta coffee (*Coffea canephora* or *Coffea robusta*), Liberica coffee (*Coffea liberica* varietas Liberica), Kawisari B coffee, Kawisari D coffee, Black coffee, Excelsa coffee, Kalimas coffee, and Sumber telogo coffee, when viewed based on the number and arrangement of neighboring cells surrounding the guard cells, is parasitic. This type is evident from the presence of two neighboring cells surrounding the guard cell, and the position of these cells is parallel to the guard cell. This aligns with the statement that the parasitic stomatal type involves the guard cell accompanied by one or more neighboring cells located parallel to the axis of the guard cell and the stoma. These guard cells, in each stomata, are kidney-shaped and irregularly distributed (Figure 2).

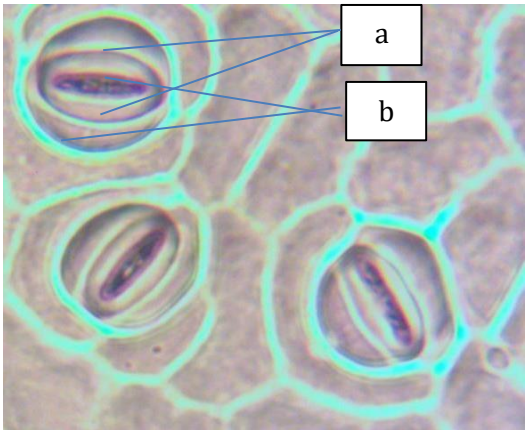

Figure 2. Parasitic cells type (a) Guard cells, (b) Neighboring cells

The observation results obtained the diversity of stomata numbers in 9 types of coffee at the Indonesian Coffee and Cocoa Research Center, using a microscope with a 40x magnification and a leaf area of 0.5cm x 1.5cm for each sample, with each leaf sampled twice. Repetition 1 can be seen in Figure 3, Table 1.

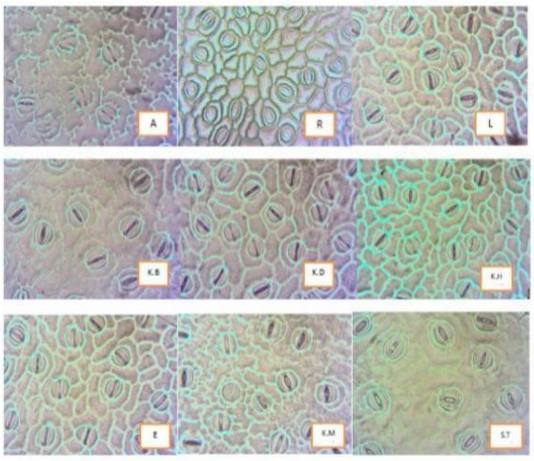

Figure 3. Replication 1. (A) Arabica, (R) Robusta, (L) Liberica, (K.B) Kawisari B, (K.D) Kawisari D, (K.H) Black Coffee, (E) Excelsa, (K.M) Kali Mas, (S.T) Sumber telogo).

Table 1. Differences in the diversity of the number and distance between stomata, Repetition 1.

No.	Coffe type	Mean distance between stomata (µm)	Total
1	Arabika	21,88	6
2	Robusta	28,52	17
3	Liberika	15,66	14
4	Kawisari B	27,80	8
5	Kawisari D	37,97	17
6	Kopi Hitam	21,92	13
7	Excelsa	25,83	13
8	Kali Mas	40,15	13
9	Sumber telogo	39,56	10

Based on the observation and comparisons among several types of coffee with the same area in the first repetition of the experiment: 1) Arabica coffee has a total of 6 stomata and an average distance between stomata of 21.88 µm. 2) Robusta coffee has a total of 17 stomata and an average distance between stomata of 28.52 µm. 3) Liberica coffee has a total of 14 stomata and an average distance between stomata of 15.66 µm. 4) Kawisari B coffee has a total of 8 stomata and an average distance between stomata of 27.80 µm. 5) Kawisari D coffee has a total of 17 stomata and an average distance between stomata of 37.97 µm. 6) Black coffee has a total of 13 stomata and an average distance between stomata of 21.92 µm. 7) Excelsa coffee has a total of 13 stomata and an average distance between stomata of 25.83 µm. 8) Kalimas coffee has a total of 13 stomata and an average distance between stomata of 40.15 µm. 9) Sumber telogo coffee has a total of 10 stomata and an average distance between stomata of 39.56 µm.

The observation reveal the diversity of stomata number in 9 types of coffee at Indonesia Coffee and Cocoa Research Center, using a microscope with a 40x magnification and a leaf area of 0.5 cm x 1.5 cm for each sample, with each leaf sampled twice. Repetition 2 shown in Figure 4, and Table 2.

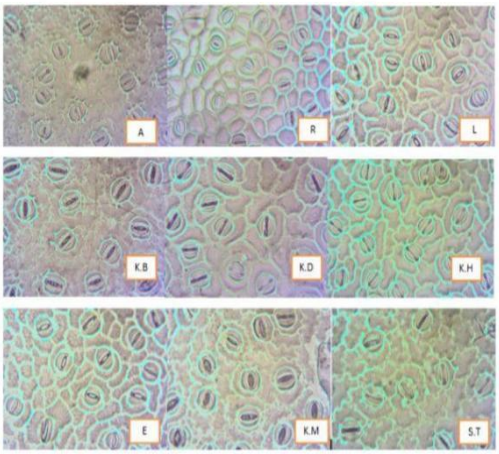

Figure 4. Replication 2. (A) Arabica, (R) Robusta, (L) Liberica, (K.B) Kawisari B, (K.D) Kawisari D, (K.H) Black Coffee, (E) Excelsa, (K.M) Kali Mas, (S.T) Sumber telogo).

Table 2. Differences in the diversity of the number and distance between stomata in repetition 2.

No.	Coffe type	Mean distance between stomata (µm)	Total
1	Arabica	41,15	14
2	Robusta	34,53	18
3	LiberiCa	40,17	15
4	Kawisari B	36,12	12
5	Kawisari D	37,18	12
6	Black Coffe	23,13	15
7	Excelsa	27,15	12
8	Kalimas	32,12	12
9	Sumber telogo	41,19	8

Based on the observation results and comparisons among several types of coffee with the same area in the second repetition of the experiment: 1) Arabica coffee has a total of 14 stomata and an average distance between stomata of 41.15 µm. 2) Robusta coffee has a total of 18 stomata and an average distance between stomata of 34.53 µm. 3) Liberica coffee has a total of 15 stomata and an average distance between stomata of 40.17 µm. 4) Kawisari B coffee has a total of 12 stomata and an average distance between stomata of 36.12 µm. 5) Kawisari D coffee has a total of 12 stomata and an average distance between stomata of 37.18 µm. 6) Black coffee has a total of 15 stomata and an average distance between stomata of 23.13 µm. 7) Excelsa coffee has a total of 12 stomata and an average distance between stomata of 27.15 µm. 8) Kalimas coffee has a total of 12 stomata and an average distance between stomata of 32.12 µm. 9) Sumber telogo coffee has a total of 8 stomata and an average distance between stomata of 41.19 µm.

Stomata, or leaf pores, are components of the epidermal cells of leaves. The function of stomata is as a passage for the entry and exit of CO2, O2, and H2O during the process of photosynthesis and respiration. Stomata are often used as a genetic characteristic for selection because they are related to a plant's production and resistance to pests and diseases. Besides being influenced genetically, the development and quantity of stomata are affected by the environment. Plants that grow in arid environments with high light intensity tend to have numerous but smaller stomata compared to plants in

wet and sheltered environments. Generally, stomata are elongated in shape, and the number and size per unit area vary among plant species and even among leaves within the same plant. Stomata can be found on either the upper or lower surface of leaves, depending on the plant species [7].

The quality of the visible stomata is also influenced by the success of making preparations using the replica method. Samples with smooth leaf surfaces can result in an imperfect depiction of stomatal anatomy because not all transparent nail polish applied adheres to the isolation. Wilted leaves as samples also affect the level of adhesion to the isolation. The nail polish may not adhere perfectly when applied to a smooth leaf surface, resulting in residual blocks on some parts of the leaf surface. The success of isolating is also influenced by the thickness of the leaf. Pulling isolation from thin leaves often causes all tissues below the epidermis to adhere to the isolation, causing the leaf to tear.

Environmental factors that can affect the size, quantity, and type of stomatal distribution include light intensity, air temperature, and soil pH. The epidermis has different shapes and varying numbers of stomata and subsidiary cells and guard cells. The frequency of stomata in each plant varies, and the type of stomata affects the transpiration process in leaves. The diffusion of CO2 in plants depends on the opening of stomatal pores. The opening and closing of stomata depend on the turgor of the guard cells, and the availability of water in plant cells allows stomata to open optimally [8].

CONCLUSION

Based on the research results obtained, it can be concluded that stomata in various plant species, both in dicotyledonous and monocotyledonous plants, differ. This study identified stomata types in 9 coffee plant species, including arabica, robusta, liberika, kawisari B, kawisari D, black coffee, excelsa, kopi kalimas, and sumber telogo, which belong to the coffee plant family Rubiaceae. Based on the number and arrangement of neighboring cells surrounding the guard cell, the stomata are parasitic. This type is characterized by the presence of two neighboring cells surrounding the guard cell, and their position is parallel to the guard cell. Plants that grow in dry environments with high light intensity tend to have numerous stomata.

The research results show a variety of stomata numbers and density distances. Environmental factors that can affect the size, quantity, and type of stomatal distribution include light intensity, air temperature, and soil pH. In conclusion, this research hopes to pave the way for future studies with more diverse and comprehensive presentations.

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