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Stomata Density Analysis of Red Chili (*Capsicum annuum* L.) at Different Location

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Abstract

Red chili (*Capsicum annuum* L.) is a commodity of vegetable that has high economic value. Stomata are an important part of plant organs, it a gap that role in the process of photosynthesis and plant transpiration. The purpose of this study to determine differences in the stomata density of red chili (*Capsicum annuum* L.) leaves at different locations, namely locations exposed and shaded from sunlight. This study used a quantitative approach with the type of research used is experiment as by one factor is that sunlight intensity, used two paired treatments and respective of three repetition. The data collection method in this study used direct observation and used analytic method to analyze the stomata density of chili *(Capsicum annuum L)* leaves. The result of his study showed that the stomata density of red chili (*Capsicum annuum* L.) leaves differed according to the planting location in the form of locations exposed to sunlight and locations that were shaded. The stomata density planted in a location exposed to full sun had a higher stomata density at 409.9 cells/mm2, while the stomata density of red chili (*Capsicum annuum* L.) leaves planted in a shaded location had a lower stomata density at 96.9 cells/mm^2

Keywords: light intensity, red chili, stomata density

A. Introduction

Red chili (*Capsicum annuum* L.) is an annual plant belonging to the Solanaceae family, such as cayenne pepper, paprika, and so on. This plant is widely grown in Pakistan, America, Portugal, India, and Indonesia (Shaheen, N., Shahlla, I., Safia, A., Rafi, A. S., Iqbal, A., Zafar, A. M., 2018). Red chili (*Capsicum annuum* L.) is a vegetable commodity that has high economic value. Generally, its used for household consumption needs as a spice and used for herbs or traditional medicines. The chili is consumed both in fresh and as well as processed form (Fitriani, L., Toekidjo, & Setyastuti, P., 2013).

Flowering of chili is strongly influenced by light intensity for a long time (Wati, 2018). This vegetative growth requires proper nutrition, in the form of water, which is a needed component for beginning of growth to the flowers and fruits formation of red chili (*Capsicum annuum* L.) (Tonny and Laksminiwati, 2011). According to Sumarni and Agus (2005), the adaptability of red chili (*Capsicum annuum* L.) is quite extensive, both in the lowlands and highlands, up to 1.400 meters above sea level.

Vegetative growth of plants can accelerate the occurrence of better metabolic processes, especially in the process of photosynthesis (Suharso, 2017). The decrease in leaf water potential is influenced by turgor pressure changes and elasticity of leaf cell walls. This depends on external factors or environmental conditions, one of which is sunlight through the process of photosynthesis to obtain energy (Xiang, L., Wang, R.G., Mao, G., Koczan, J.M., 2006). One part of the plant whose mechanism action is strongly influenced by sunlight is the stomata (Campbell, N.A., Reece, & Mitchell, L.G., 2003). As according to Adisyahputra, Sudarsono, Kukuh, S. (2011) water loss in leaves can occur with decreasing leaf surface area and stomatal conductance. Stomata structure also affects the exchange of gases and water vapor from the leaf to the environment or vice versa. So the regulation of stomata is an important role in controlling water loss in plants.

Stomata are an important part of plant organs, in the form of gaps between the combination of two special epidermal cells called guard cells. These guard cells work to open and close according of plant transpiration (Setiawati and Inneke, 2019). Stomata are found in all plant parts that are exposed to the air, but are more commonly found in leaf organs (Kamaluddin, Gede, A. W., Muhammad, R., 2020). The mechanism of opening and closing stomata in drought-tolerant plants is to avoid water loss through evaporation.

Anatomical approach is important because environmental conditions are increasingly dynamic and each plant has a different response to sunlight stress, such as the ability to open and close stomata (Haryanti and Meirina, 2009). The research results of Haryanti (2010) show that the speed of stomata closing as a stomatal response to changes in the vapor pressure deficit is largely determined by the sensitivity of the stomata. The process of opening and closing stomata is strongly influenced by the sunlight conditions.

Sunlight greatly affects the work of stomata, especially in the opening and closing process of stomata. With the light intensity factor, plants make adaptations to support the continuity of the physiological functions of the plant. One adaptation of plant is the response of stomata formation in shaded and unshaded areas (Campbell, et al., 2003).

Based on the description above, the authors conducted a study to analyze the stomata density on red chili (*Capsicum annuum* L.) leaves at different locations, namely exposed locations and shaded locations.

B. Methology

This study uses a quantitative approach, in the data is analysis of stomatal density. The type of research used is an experiment consisting of a single factor, namely the sunlight intensity, using two treatments and three repetitions each.

Planting and sampling of this research were located in the Biology Garden and Green House of Biology Education, and stomata observations at the Laboratory of Biology Education, Muhammadiyah University of Parepare. It was held from July 17th to August 31st 2021. This study used red chili (*Capsicum annuum* L.) as the research subject. The research sample used each one of third leaf, from three red chili (*Capsicum annuum* L.) at the age 28 days after planting.

The data collection method in this study was direct observation to determine environmental conditions and morphology of red chili (*Capsicum annuum* L.) leaves based on different locations, namely those exposed and shaded from sunlight, and using analytical methods to calculate stomata density of red chili (*Capsicum annuum* L.). This study used one red chili variety with two different treatments and made three observations on each treatmen.

1. Research Preparation

a. Planting preparation

Preparation for planting begins with preparing the selected seeds in advance by soaking for 3 hours, then sowing. Seeds that grow 3-4 leaves are then transferred to an exposed location and a shaded location, until the age of 28 days after planting.

b. Observation Preparation

This research prepares tools and materials in the form of a microscope, camera, lux meter (application), thermohygrometer, cover slip, object glass, transparent tape, scissors, label paper, transparent nail polish, and leaf samples from red chili plants.

2. Reserch Step

The research steps were carried out by observing the stomata density of red chili leaf (*Capsicum annuum* L.) with the replica or mold methods, that using transparent tape and transparent nail polish. After that, it is placed on a slide and then observed under a microscope.

Preparations for observation of stomata density was carried out using the mold or replica method. Where is the manufacture of stomata replicas on red chili leaves as:

a. Prepare tools and materials needed to conduct research.
b. Take a sample of leaf blades and clean it from dirt.
c. Apply transparent nail polish on the surface of the center of the leaf and allow it to dry.
d. Paste transparent tape on the surface of the leaf that has been smeared with transparent nail polish, by gently massaging the surface of the leaf using your fingers, so that the nail polish sticks well.
e. Peel off the tape slowly and stick it on the slide.
f. Give label paper to the corner of the slide and provide information.
g. Observing the density of stomata until the object can be observed clearly.
h. Make similar observations for each sample from two different locations at 11.00 AM on three repetitions, for more accurate of observation results. (Source: Humami, D. W., Puput, A. W. S., Iska, D., 2020).

3. Final Stage

The final stage in this research is to analyze data based on the stomata observations results. Stomata in the field of view of the microscope will be counted for each cell. Then it is entered into the formula for calculating stomatal density. The data obtained from observations of stomatal density were made in the graphs and explain the description. The results of this study will be drawn a conclusion and published.

4. Data Analysis Technique

Research the density of stomata on leaf blades of red chili (*Capsicum annuum* L.), then the data analysis technique used is descriptive data analysis and quantitative description, namely: presenting the results of analysis based on facts displayed with pictures (photos) and explain the description, so the results of the study can be returned directly to the data obtained.

After getting the results of descriptive data from images, then analyzing the percentage of stomata density using the formula. The formula for measuring stomatal density:

$$\text{Stomata density} = \frac{\text{number of stomata}}{\text{wide field of view}}$$

Measurement of stomatal density using 400x magnification with a broad field of view from a microscope measured by the formula:

$$\begin{aligned}\text{Area of view} &= \tfrac{1}{4}\,\pi\, d^2 \\ &= \text{x } 3.14 \text{ x } (0.5 \text{ mm})^2 \\ &= 0.19625 \text{ mm}^2\end{aligned}$$

C. Result and Discussion

Environmental conditions based on different planting locations of red chili, show that conditions not have high significance. Sampling was carried out at 11.00 AM, with the location for planting shading in the Biology Education Green House which only gets little sunlight intensity, while the location is exposed to sunlight which is around the Green House of Biology Education.

Observations showed that environmental conditions exposed to sunlight had an average temperature is 30^0C with 67% humidity. The results of the observation of light intensity as measured by Lux Meter, which is 65.536 Lux. In the shaded environment, the average temperature is $28,4^0$C with 69% humidity. The light intensity obtained from the measurement results is 32.834 Lux. These results indicate that the higher the light intensity received by chili plants, has the higher temperature and lower the humidity.

The results from observations of red chili plants in shaded locations and exposed to sunlight, have clear morphological differences. The results of observations on red chili leaf blades in shaded and exposed locations also have differences in leaf size and leaf texture. The size of the shaded red chili leaves has an average leaf width is 1.5 cm and average leaf length 2.8 cm, while the size of the red chili leaves at the exposed location has an average leaf width is 1.7 cm and a length 2.2 cm.

The results of stomata observations of red chili (*Capsicum annuum* L.) leaves also attention to the shape and parts of the stomata. Based on the shape of the stomata found in the observations, the stomata in red chili (*Capsicum annuum* L.) belong to the Anisocytic type.

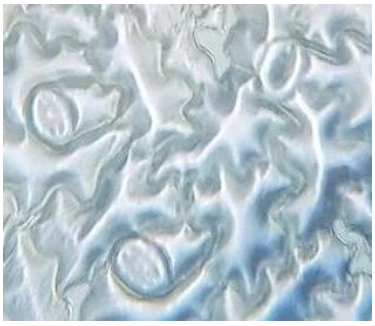

Figure 1. Stomata Observations of Red Chili (*Capsicum annuum* L.)

The observations results of stomatal density obtained from the calculation of stomata and the surface area of the microscope's field of view, were then analyzed for each sample. The results of observations of stomatal density can be seen in Table 1.

Table 1. Stomata Density Observation Results

Treatment	Stomata Density Observation Result		
	Sample	Stomata Result	Stomata Density (cells/mm2)
Shaded	A1	19	96.9
	A2	14	71.4
	A3	24	122.4
Exposed	B1	88	448.9
	B2	82	418.3
	B3	71	362.5

The results showed that the stomata were in two different locations, namely shaded and exposed to sunlight. In samples A1, A2, and A3 are the shaded leaves conditions. Sample A1 had 19 stomata with a stomata density of 96.9 cells/mm2, sample A2 had 14 stomata with a stomata density of 71.4 cells/mm2, sample A3 had 24 stomata with a density of 122.4 cells/mm2. In samples B1, B2, and B3 are the exposed leaves to sunlight. Sample B1 had 88 stomata with a

density of 448.9 cells/mm2, sample B2 had 82 stomata with a density of 418.3 cells/mm2, and sample B3 had 71 stomata with a density of 362.5 cells/mm2.

The average density of stomata in shaded locations is 96.9 cells/mm2, while the average density of stomata in exposed to sunlight area is 409.9 cells/mm2. The results of the analysis of stomatal density at the shaded location included the category of low stomatal density. Meanwhile, at the exposed location has moderate of stomatal density analysis.

Based on the paired sample test (Paired sample test) with SPSS as shown in Table 4.3. obtained the value of Sig ≠ 0 (0.013), it can be concluded that H0 is rejected and H1 is accepted. Therefore, it was concluded that light intensity had an effect on stomatal density.

1. Stomata Condition of Red Chili Leaves (Capsicum annuum L.)

Stomata sampling on red chili (*Capsicum annuum* L.) leaves was carried out at 11.00 AM, and stomata observations were immediately carried out using the replica method. So that it is possible that there is no stomata damage that occurs in the leaf samples to be observed. Stomata observations of red chili (*Capsicum annuum* L.) leaves were carried out to determine the parts of the stomata and the characteristics of the stomata types (Figure 4.3.). The difference in the light intensity of received also affects the temperature and humidity. The sunlight intensity of received in the shaded area is lower, so it has a lower temperature and high humidity. Meanwhile, in areas exposed to sunlight, the temperature is high and the humidity is low.

Based on the shape of the stomata found in observations, the stomata in red chili (*Capsicum annuum* L.) belong to the *Anisocytic* type. As found that has neighboring cells that are not the same size. *Anisocytic* or *Cruciferous* type, each guard cell is surrounded by 3 neighboring cells of unequal size, found in *Crucifera* and *Solanaceae* family (Kamaluddin, et al., 2020).

Structure and distribution of stomata in each plant is different. Likewise, the distribution of stomata on red chili (*Capsicum annuum* L.) leaves in shaded locations and exposed to sunlight, has the same distribution pattern, which adjusts to the morphology of the pinnate leaves, so that the distribution pattern is also spread out.

2. Stomata Density in Red Chili (Capsicum annuum L.) Leaves

The level of stomatal density is influenced by environmental factors such as the sunlight intensity, water availability, temperature and humidity. The increase in light intensity is proportional to the increase in environmental temperature, namely the red chili (*Capsicum annuum* L.) planting location exposed to full sun has high temperature with low humidity. Otherwise, in shaded planting location, it will receive a little light intensity, so that it has a low temperature with a high level of humidity (Meriko and Abizar, 2017).

3. Shaded Location

Observations of the environmental conditions that are shaded location still receive exposure to the morning sun, namely at 07.00 until 09.00 AM. Sampling was carried out at 11.00 AM, with received sunlight intensity of 32.834 Lux. The location of planting red chili (*Capsicum annuum* L.) in the shade showed the number of stomata in the microscope field of view, namely 19.14 and a maximum of stomata cells is 24. This is a stomata response to the environment, because of the shade which results in the low intensity of sunlight obtained, so the number of stomata is small.

The planting location has an influence on stomata conditions, namely the average stomata density obtained is 96.9 (cells/mm2). Red chili (*Capsicum annuum* L.) plants planted in shaded locations will get less light intensity, so the stomatal density found is in the low category according to (Table 1) indicating the stomatal density category. This is due to the physiological adaptation of plants under shade, causing the distribution of stomata on the leaf surface to scatter so that the received sunlight can be distributed efficiently for their survival. As explained in research of Martins, S.C.V., Jeroni, G., Paulo, C.C., Lucas, F.P., Marilia, C.V., Fabio, M.D. (2014), that plants grow in shaded areas will make adaptations by making chlorophyll enveloped, thus providing a wide distance between stomata to optimize their metabolic processes.

At least the intensity of sunlight received by red chili (*Capsicum annuum* L.), will affect performance and processes that occur such as photosynthesis and transpiration (Haryanti, 2010). The results of observations on the morphology of red chilies showed that the average leaf condition was 1.5 cm wider and leaf length 2.8 cm compared to chili leaves at the exposed location. This is due to the adaptation of red chili leaves to the received light intensity by increasing the leaf surface area in order to avoid light deficits, so that can receive light optimally for the photosynthesis process.

Leaves of red chili (*Capsicum annuum* L.) planted in shaded locations were thinner and greener (Table 1) than those in exposed locations. This is because the light intensity received is lower, resulting in reduced mesophyll tissue in the leaves in order to optimize light transmission in the leaves. That conditions of low light intensity make plants adapt by reducing the formation of mesophyll cells which results in a decrease of leaf thickness. While the color of the leaves of red chili (*Capsicum annuum* L.) in the shaded location is greener, due to the low intensity of sunlight affecting the increase in chlorophyll in the leaves. As according to Yustiningsih (2019), a high chlorophyll will also increase the optimization of the photosynthetic process if plants are able to adapt of shaded conditions.

4. Exposed Location

Locations exposed to sunlight have environmental conditions that receive full sunlight from 08.00 to 17.00 AM without any barrier or shade. At the time of sampling, which was conducted at 11.00 AM, the light intensity was 65.536 Lux. The planting location has an influence on the condition of the stomata, which has a fairly high number of stomata in the microscope field of view, namely 71, 82, and a maximum of 88 stomata cells. This is because the red chili leaf surface exposed to more intense sunlight will significantly increase the number of stomata, due to an increase rate of CO_2 diffusion.

The results of the analysis of the total of stomata in field of view on a microscope found that red chili planted in exposed locations had an average stomata density of 409.9 (cells/mm2). The high level of stomata density found in red chili (*Capsicum annuum* L.) leaves grown in exposed locations was included in the medium density category according to (Table 1) the category of stomata density level. These results indicate that the high intensity of sunlight received makes the leaves adapt by increasing the amount of stomata which is in line with the increase the level of stomatal density. As the research results of Yustiningsih (2019), plants that are able to adapt to the environment will produce structures, morphology, and physiology that are suitable for the environment in which they grow. This allows plants to makes specification to adjust the environmental differences, including the intensity of sunlight received.

Red chili leaves (*Capsicum annuum* L.) planted in sunlight exposed locations have thicker leaf morphology, small surface area, and yellowish color. The high intensity of light received makes the leaves thicken because it increases the number of mesophyll cells, and the small leaf surface area because the leaves have received the maximum light intensity make the leaves no longer cover the surface to reception streamline of sunlight. Plants that receive high of light intensity produce leaves that are smaller, thicker, more compact with a cuticle layer and thicker cell walls, smaller intercellular spaces, and hard leaf texture.

Yellowish color occurs on the leaves of red chili (*Capsicum annuum* L.) due the possible influence of the presence of weeds around the planting site exposed to sunlight. Explained by Zulkarnain (2013) that the growth of red chili (*Capsicum annuum* L.) can be disrupted due to lack of hydronation and aeration during its early growth period, as well as disturbances from pests and weeds. The optimal soil conditions for planting red chili are as long as the soil has good drainage and aeration, as well as available water and sufficient amounts during the plant growth period (Zulkarnain, 2013).

D. Conclusion

Based on the results of the analysis and discussion, it was found that the planting location of red chili (*Capsicum annuum* L.) in a place that was shaded and exposed to sunlight had a significant effect on differences in stomatal density. Differences in stomata density of red chili (*Capsicum annuum* L.) leaves grown in exposed full sun locations had higher stomata density. Meanwhile, the stomata density of red chili (*Capsicum annuum* L.) leaves planted in shaded locations had a lower stomata density

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