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THE EFFECT OF AIR POLLUTION ON THE STOMATA CHARACTERISTICS PIGEON ORCHID (*Dendrobium crumenatum*) LEAVES, IN THE TASIKMALAYA

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ABSTRACT

This study aims to determine the effect of air pollution on the stomata characteristics in Pigeon Orchid (Dendrobium crumenatum) leaves in Tasikmalaya. This research is quantitative. The Samples were taken through survey method with purposive sampling technique in the Singaparna Bus Station as an exposed area with the highest air pollution, Sukaraja-Mangunreja street as an exposed area with the moderate air pollution, and Mount Galunggung as an exposed area with the the lowest air pollution. The characteristics of the stomata observed using an Optilab camera type Professional Model Series MTN001 which already has the Image Raster application and previously calibrated with an object glass micrometer at 400 x magnifications. The Data analysed quantitatively and descriptively using one way ANOVA test. The results showed that different levels of air pollution could affect the characteristics of the stomata. Stomata in areas with high air pollution have the highest stomata index and stomata density, namely 8.2% and 326.11/mm^2, with the category of medium density and oval stomata shape. Stomata in areas with moderate air pollution have the lower stomata index and stomata density, namely 7.3% and 256.47/mm^2, with the low-density category and round stomata shape. Meanwhile, stomata in areas with low air pollution have the lowest stomata index and stomata density, namely 5.7% and 256.47/mm^2, which indicate the category of low density and the shape of the stomata is round.

INTRODUCTION

The environment is interpreted as a combination of physical conditions that include natural resources such as air, soil, solar energy, minerals, flora and fauna. As the human population increases, so do human needs, primary, secondary and tertiary needs, which are obtained from the environment. The continuous degradation of natural resources effects in a large number of problems that occur in the environment and cause environmental pollution.

Environmental pollution which is currently in becomes the public spotlight and affecting the world's climate is air pollution. Wardhana (in Hikmiyah, 2018) states that air pollution is the presence of foreign materials or substances in the air which can cause changes in the composition of air from its normal condition. The presence of foreign materials or substances in a certain amount and in quite a long time will disrupt the life of humans, animals and plants.

One of the most common air pollution factors in daily life is transportation. Tasikmalaya is one of the regions where the uses of transportation keep increasing; consequently, air pollution affect air quality. On August 20, 2020, the Air Quality Index for Tasikmalaya was recorded at 93 US AQI by means of moderate levels of air pollution (IQAir, 2020). If it keeps increasing, it will have a negative impact to the environment and disrupt the survival of humans, animals and plants in it.

One of the plants that is often found on the side of main road is Pigeon Orchid (*Dendrobium crumenatum*) which is a group of epiphytic plants that attaches to trunks or trees, branches, or tree branches, both of which are still alive but do not damage or harm the host, or have died (Purwanto, 2016). Orchid plants are attractive ornamental plants and can be enjoyed visually because of their beautiful flowers and distinctive smell. According to (Widyastuti, 2018) one of the functions of ornamental plants is to cool the soul and preserve the environment, they produce O_2 which is needed by living beings to breathe and absorb CO_2 which is not needed by humans and as the lungs of the environment. (Palupi, 2016) stated that orchid plants act as neutralizers to reduce air pollution. According to (Purwanto, 2016) in their natural habitat, orchids can thrive under tall trees in dense forests, but orchids can also survive in dry conditions.

Based on the research conducted by (Juma'ani & Munawwaroh, 2017) concluded that the characteristics of stomata of Bamboo Rejeki plant (*Dracaena reflexa*) in air

polluted location have an oval shape with parallel neighboring cells and are have diasitic type and the number of stomata is less than that of the location which is not polluted. However, some researchers have evidence that the number of stomata changes in face of changes in the atmosphere in different locations, one of which is the results of research from (Mutaqin et al., 2016) which states that stomata of mango leaves on the side of the road have a higher density of stomata. It is arger than the stomata of Mango leaves in nature reserves. Thus, an anatomical approach is important to support morphological and physiological approaches in determining plant genotypes that are sensitive and able to adapt to environmental conditions with different levels of pollution.

Seeing the ability of the *Dendrobium crumenatum* to neutralize and reduce air pollution and able to survive in extreme locatios means it can live in a wet environment even in a dry environment. Therefore, a study was conducted to see the effect of air pollution on the characteristics of stomata in the leaves of the *Dendrobium crumenatum*. Leaf samples were obtained from several areas in Tasikmalaya, which is: Singaparna Bus station, Mangunreja-Sukaraja main road and Mount Galunggung. These three places were taken based on initial observations made in the morning by calculating motorized vehicles and environmental assumptions. The calculation of traffic density at the Singaparna bus station which was carried out with a duration of 10 minutes consisted of mostly small vehicles such as motorcycles, and large vehicles such as public transport cars, family cars, trucks, buses and containers, as many as 370 vehicles were recorded as passing. The density of traffic in this bus station is the largest number compared to the Sukaraja-Mangunreja main road and Mount Gunung Galunggung and the condition of the Singaparna bus station is a lot of pollution so that it can be interpreted as a high polluted area. Meanwhile, on Sukaraja-Mangunreja main road, within 10 minutes, 167 vehicles were recorded, consisting of mostly small vehicles, namely motorcycles and large vehicles, namely public transportation and family cars. The area of Mount Galunggung is an area that is still leafy. There is extensive forest and a large enough oxygen producer, with vehicle calculations carried out in the morning within 10 minutes. It was recorded that not a single vehicle passed, so the Mount Galunggung area is considered as low polluted area . Therefore, the purpose of this study was to determine the effect of air pollution on the characteristics of stomata on the leaves of the *Dendrobium crumenatum* in Tasikmalaya.

MATERIALS AND METHODS

Research Subject

The subject of this research was *Dendrobium crumenatum* which is found in several areas in Tasikmalaya. Preliminary observations show that the species of *Dendrobium crumenatum* is the most dominant plant species in Tasikmalaya, especially on the roadsides where many motorized vehicles pass. Thus, the samples in this study were the leaves of *Dendrobium crumenatum* taken directly from three areas in Tasikmalaya, namely Singaparna Bus station, Mangunreja-Sukaraja main road and Mount Galunggung. Three leaf samples were taken from each area point of a *Dendrobium crumenatum* plant, each sample consisting of the abaxial surface of the leaf.

The samples used were determined based on the presence of plants, the level of air pollution or areas frequented by motorized vehicles, the characteristics of the plants taken were having 10-20 leaves and the leaf sample size was relatively the same. The plants used are assumed to be of the same age. The leaves are taken from the center of the plant or selected with a leaf position among 3 to 5 from the top of each plant facing the road. The sampling method for each leaf refers to Taulu (in Palit, 2008) which was modified by the researchers, namely taking samples from the middle of the leaf with a length x width of 1.0 x 0.5 cm.

Tools and Materials Used

The tools needed are a Multifunction Air Detector, Altimeter, Lux Meter, Olympus Binocular Microscope CX-23 type, Optilab Camera type Professional Series Model MTN001 which has the Image Raster application and has previously been calibrated with the help of Object Glass Micrometer, Image-J application, Scissors, Object Glasses, Rulers, Transparent Tape, Transparent Nail Polish, and Labels. While the materials used in this study were the leaves of *Dendrobium crumenatum* taken from three areas with different levels of air pollution in Tasikmalaya.

Research procedure

To obtain the data in this study, physical data was collected, namely measuring the average concentration levels of the chemical pollutant HCHO (Formaldehyde) and TVOC

(Total Volatile Organic Compounds), temperature, humidity, sunlight intensity, and calculating the total number of motorized vehicles crossing the point. sampling area (measurements were carried out 3 times at each study location, namely in the morning at 06.00 - 08.00 am, afternoon at 12.00 -14.00 p.m and in the afternoon at 16.00-18.00 p.m) and took samples for study using the replica method. The steps for making fresh preparations using the replica method are as follows (Haryanti, 2010):

a. Leaves are taken from plants which already present at the sampling point, grow naturally and without any control.
b. The leaves have been taken are cleaned on the lower surface using a tissue to remove any dust or dirt.
c. The lower surface of the leaves is smeared with transparent nail polish and left for 10 minutes so that the nail polish dries.
d. After the nail polish has dried, a transparent tape is attached and leveled.
e. The tape is slowly peeled off and then affixed to the object glass.
f. Leveled and then labeled with a description of the sampling location.
g. Calibrate the microscope camera and Image Raster with the help of a glass micrometer object.
h. At the end, take measurements on the observed objects and observe the characteristics of the stomata for each location per field of view using a microscope with the same magnification (10 x 40) and record some of the parameters needed in the study.

Data Analysis and Interpretation

The Observational data were analyzed using numbers and descriptively by showing pictures and tables in order to provide information about the characteristics of the stomata on the leaves of *Dendrobium crumenatum*. Stomatal density data obtained were grouped into categories: [low density <300/mm^2], [medium density 300-500/mm^2], and [high density >500/mm^2]. The Calculation of the stomata index was using the formula for the total number of stomata divided by the total number of epidermal cells plus the total number of stomata multiplied by 100% (Widianti et al., 2017).

$$Stomata\ index = \frac{Total\ number\ of\ stomata}{Total\ number\ of\ epidermal\ cells\ +\ Total\ number\ of\ stomata} x\ 100\%$$

Stomata density is calculated by adopting (Anisa, 2017) formula:

$$Stomatal\ density = \frac{Total\ number\ of\ stomata}{Field\ of\ view\ area\ (mm^2)} x\ 100\%$$

The calculation results from the formula were averaged. After the results were known, they were analyzed using the analysis of variance test (ANOVA) using the SPSS program. The data analysis used is one way analysis of variance test (one way ANOVA), which is to see the average comparison of several groups, usually more than two groups with the assumptions that must be fulfilled, namely the data must be normally distributed and have the same variance (homogeneous), independent or each group is not related to each other, and additive, which means that the data being analyzed is interval/ratio data. If one does not meet these assumptions, the Kruskall-Wallis nonparametric statistical analysis is used (Tyastirin & Hidayati, 2017).

RESULTS AND DISCUSSION

The results of measurements of average physical data or environmental factors and observations of stomatal characteristics including stomatal index, stomatal density, stomatal size and stomatal shape on the leaves of *Dendrobium crumenatum* in the Tasikmalaya region (**Table 1, Figure 1**).

Table 1. Results of Physical Data Measurement of Environmental Conditions and Observation of Stomata Characteristics of Pigeon Orchids during the Research

No	Aspects that are measured	Singaparna Bus Station	Sukaraja-Mangunreja Main Road	Mount Galunggung
1	Vehicles (/hour)	1408 ± 563	606 ± 248	-
2	HCHO (mg/m^3)	0,106 ± 0,01	0,066 ± 0,02	0,017 ± 0,01
3	TVOC (mg/m^3)	0,646 ± 0,02	0,457 ± 0,16	0,115 ± 0,04
4	Temperature (^{0}C)	27,7 ± 2,86	25,5 1,36	29,5 ± 3,90
5	Humidity (%)	77,6 ± 10,2	83 ± 2,00	71,6 ± 17,2
6	Sunlight Intensity (Lux)	870 ± 201,11	728,6 ± 290,85	1327 ± 460,98
7	Stomatal Index (%)	8,18 ± 0,19	7,26 ± 0,38	5,12 ± 0,54
8	Stomatal Density (/mm^2)	326,11 ±13,49	256,47 ± 19,29	256,47 ±51,03
9	Stomata Length (µm)	21,85 ± 4,19	18,98 ± 2,12	17,53 ± 3,37
10	Stomata Width (µm)	18,93 ± 1,58	14,72 ± 0,30	16,68 ± 1,89

In Table 1 it can be seen that each region generates variety calculation numbers for the intensity of motorized vehicles, high vehicle intensity will result in high exhaust emissions of motorized vehicles into the air, the entry of contaminants in the form of gas or small particles in a long period of time can change the quality the air becomes abnormal

and disrupts life and the lives of the creatures in it. The process of incomplete combustion of fuel oil in motorized vehicles produces chemical elements that pollute the air. These chemical elements include: Carbon Monoxide (CO), Sulfur Oxides (SO_x), Nitrogen Oxides (NO_x) , Hydrocarbons (HC), Particulates and Lead (PB) (Wakhid, 2018). The process of incomplete oxidation of hydrocarbon content can produce Formaldehyde (HCHO) and Volatile Organic Compounds (VOC) as the main emission products from combustion, so the higher the intensity of vehicles in an area, the higher the levels of HCHO and TVOC pollutants will also be (Ekananda et al., 2016).

The stomatal index calculation results in Table 1 show that the average percentage number of stomata index for *Dendrobium crumenatum* leaves in the Singaparna bus station area is 8.2%, which is the largest value compared to the other two regions. Same with the concentrations of HCHO and TVOC which are also the highest numbers among all sampling areas, this proves that the higher the average number of air pollutants HCHO and TVOC, the higher the Stomata Index on the leaves of *Dendrobium crumenatum* at Singaparna bus station.

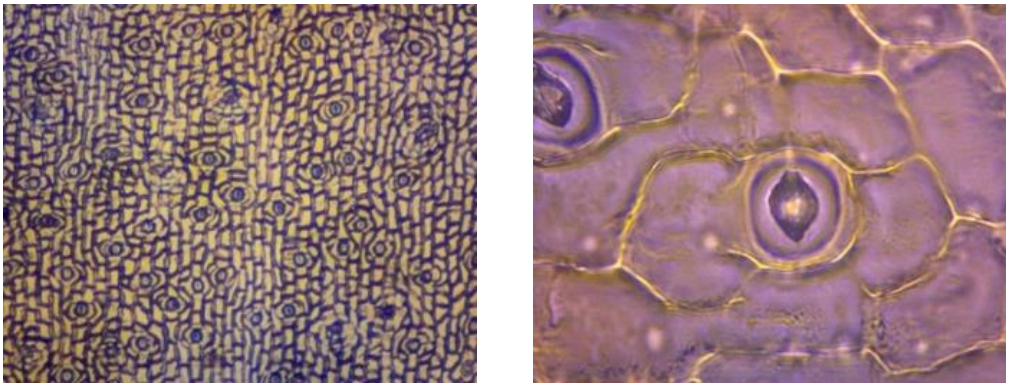
In the Sukaraja-Mangunreja road area it shows an average percentage number of 7.3%, one level lower than the Singaparna bus station area, the same as the average concentration of HCHO and TVOC in the Sukaraja-Mangunreja road area which is also in the second highest position, this proves that the concentration levels of HCHO and TVOC on Sukaraja-Mangunreja road affect the high and low Stomata Index of *Dendrobium crumenatum* leaves on Sukaraja-Mangunreja road. Meanwhile, in the Galunggung Mountain region, the average percentage of stomata index is 5.7%, which is the smallest number among the other two regions. The presence of HCHO and TVOC air pollutants in the Mount Galunggung area is also very low and this proves that the lower level of air pollution, the lower the Stomata Index of *Dendrobium crumenatum* leaves in that area. Based on the results stated above, it can be concluded that there is an effect of air pollution on increasing the stomata index of *Dendrobium crumenatum* leaves in the Tasikmalaya.

The resulting stomatal index number will determine the stomatal density number on the leaves. The difference in the average stomatal index percentage number in each region determines stomatal density differently. In the Singaparna bus station, the average stomata density of three leaf samples was 326.11/mm^2, which indicates stomata density

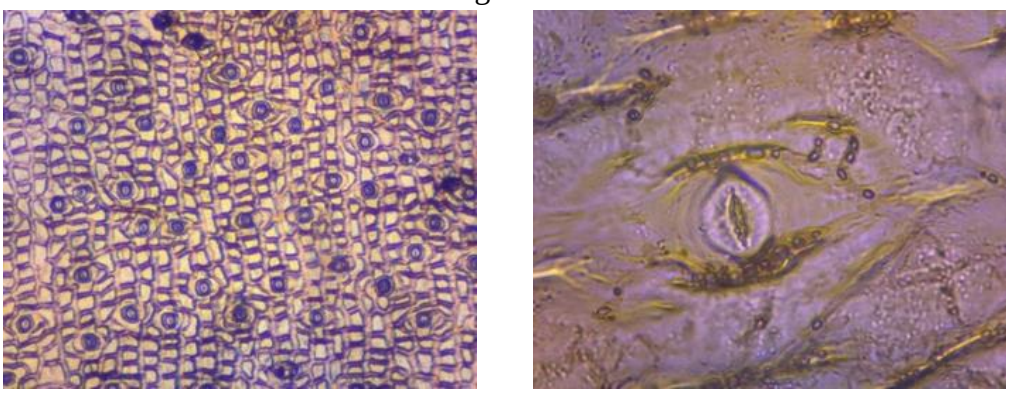
is in the medium density category. In the area of Sukaraja-Mangunreja road the average number of stomata density of three leaf samples is 256.47/mm^2 which indicates that stomata density is included in the low-density category. Meanwhile, the average stomata density of three samples of pigeon orchid leaves in the Galunggung Mountain area was also at 256.47/mm^2 which indicates a low-density category.

Plants adapt by increasing the number of stomata so they can survive environmental stress due to increased pollutant levels. By decreasing the number of epidermis then increasing the index and density of stomata can increase CO2 absorption and reduce the diffusion of pollutants into plant tissues, resulting in increasing photosynthetic efficiency and plants can survive even though they are in environmental stress.

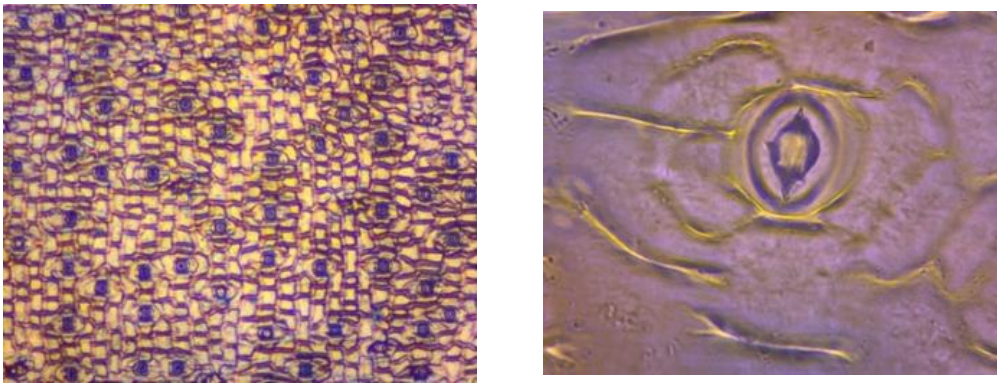
The results of this study are in line with those stated by (Munir et al., 2019) that there are differences in stomata density of plant leaves that live in different places, where leaves are exposed to air pollution have a greater density of stomata compared to the not exposed one to pollution.



Gunung Mountain



Sukaraja-Mangunreja



Singaparna Bus Station

Figure 1. Observation results of stomatal anatomy observed through a microscope magnification 40x (left) and 400x (right)

Based on the observations in **Figure 1** regarding the characteristics of the stomata of *Dendrobium crumenatum* located in several areas in Tasikmalaya including pollutant concentrations and their effect on stomata type, stomata shape and stomata size. Samples of leaf stomata in each region were taken in the morning at 10.00 a.m, it can be seen clearly in the picture that leaves of *Dendrobium crumenatum* have the same type of stomata in three sample test areas, namely stomata of Anomocytic where the stomata do not have neighboring cells or the neighboring cells have the same shape as other epidermal cells (Salira & Moralita, 2021). The guard cell shape in the observed stomata has the form of a guard cell in the form of a kidney, and the porous state of the stomata observed in the three regions is in the open position. Stomata have an adjustment mechanism to changes in soil water content, if the osmosis value in the cover cells increases it will cause water to enter from neighboring cells into the guard cells, this effect in turgor on the walls of cover cells which are thin (porous) and stomata will open (Taluta et al., 2017).

Apart from the increasing index and stomatal density, the size of stomata also increased (**Table 1**), the increased length of stomata is also an indication of plant adaptation to air pollution. The longer size of stomata will help plants absorb CO2 to photosynthesize and maintain themselves. In a study conducted by (Anisa, 2017) stated that stomata size can be used as a bioindicator and air biomonitoring, the larger the size of the stomata, the better the absorption of air pollution.

The overall shape of stomata in the Galunggung Mountain as the area with the lowest pollutant is round with an average stomata length of 17.52 µm and an average

stomata width of 16.68 µm. The mean number of stomata size is the smallest number among all sample test areas. Stomata of *Dendrobium crumenatum* leaves are surrounded by 4 guard cells located on the right and left sides of the stomata and two other cells located above and below the stomata or the end of each porus. In the Galunggung Mountain, stomata look very good and clear with white spots which it is possible that the nuclei of cells in plants are located on the edge of the epidermis. The epidermal cells are elongated, rectangular, pentagonal and also irregular. Overall, epidermal cells of *Dendrobium crumenatum* leaves in the Galunggung Mountain are seen in an elongated arrangement from top to bottom with no space between cells between one epidermis and another. This is in accordance with (Anu et al., 2017) statement that in leaves with parallel bones, stomata are arranged into elongated rows and the formation of stomata starts from the tip to the base of leaf or basipetals.

The results of observing the characteristics of *Dendrobium crumenatum* stomata leaves located on Sukaraja-Mangunreja road as an area with moderate air pollution show the characteristic shape of the stomata which are also round with an average stomata length of 18.98 µm and an average stomata width of 14.72 µm. The average number of stomata size is at the middle or second lowest. Stomata have 4 neighboring cells with size and shape not much different from other epidermal cells. The shape of epidermal cells in the samples in this area is not many rectangular epidermal cells as is the case in Galunggung Mountain, most of the epidermis is rectangular, pentagonal and irregular in shape, but the location of the epidermis is still in an orderly row. There is no visible cell nucleus in the image and there is no space among cells between one epidermal cell and another epidermal cell.

The results of observing the characteristics of *Dendrobium crumenatum* stomata leaves located on Singaparna bus station have a different shape compared to samples in the other two areas. The shape of stomata in the Singaparna bus station area is oval with an average stomata length of 21.85 µm, an average stomata width of 18.93 µm, the average stomata size number is one of the highest numbers of three regions. Epidermal cells in samples from this area show only a few cells with elongated shapes, most of which are rectangular, pentagonal and irregular. It can also be observed in the figure that in the Singaparna bus station area there are several damaged stomata and not completely formed. This could be caused by stomatal responses that are not resistant to environmental

stress, especially air pollution conditions in the region. The differences in the size and shape of stomata in the research that has been carried out is in line with research conducted by (Munir et al., 2019) which concluded that the size of stomata in environments exposed to pollution is smaller than in environments not exposed to air pollution.

The characteristics of stomata described above are not only influenced by air pollution levels, this is also caused by differences in environmental conditions where plants grow and genetic factors which greatly affect plant morphogenesis. Adequate water availability, sunlight intensity, temperature and humidity are also factors that strongly support plant growth so that they can affect stomata density.

In **Table 1** it can be seen that the data on temperature, humidity and sunlight intensity have varying average numbers. The average nmber is obtained from measuring the intensity of sunlight in the morning, afternoon and evening. In the Singaparna bus station the average number of sunlight intensity is 870 Lux by means the lowest number compared to the Sukaraja-Mangunreja road which has an average number of 729 Lux and the highest average number is in the Mount Galunggung which is 1327 Lux.

The data collection process was only carried out one day in each region. At that point, the average number taken is the value that appears only during the environmental conditions that day. The weather conditions at the time of data collection were erratic because the data collection process was carried out during the transition season or the transition from the rainy season to the dry season, so that the average measurement results varied greatly. Data collection in the Singaparna bus stsation in the afternoon experienced rainy weather so that the average intensity of sunlight was the lowest number among the other two areas, due to the weather experienced there was a decreasing temperature and humidity in this area. Data collection in the Sukaraja-Mangunreja road experienced pretty good weather with no rain at all. Whereas in the area of Mount Galunggung is a mountain which is at an altitude of 2,168 masl with low humidity levels, but high temperatures and high intensity of sunlight.

At the time of data collection, there were no tall trees covering the ground surface of the mount Galunggung crater and the sun was shining brightly so that the sun's rays hit the ground directly in the area. Facts in the field occur that are different from the theory previously carried out by Lakitan (in Prakoso, 2018) stating that variations in air

temperature in the Indonesian archipelago depend on the altitude, the air temperature will be lower at higher altitudes, if the temperature is high, the humidity will be low and vice versa.

Sallisbury and Ross (in Sihotang, 2017) stated that sunlight has a major role in the processes of germination, growth, development, photosynthesis, closing and opening of stomata and metabolism of green plants. One of the factors that affect the intensity of sunlight reaching the leaves is shade, leaf samples taken from the Singaparna bus station live attached to trees with the position of *Dendrobium crumenatum* leaves shaded by the tree which is the host of plant *Dendrobium crumenatum*. Thus, in the Sukaraja-Mangunreja road the leaf samples also live as epiphytes on trees that are large enough so that the leaves of *Dendrobium crumenatum* leaf samples are shaded naturally. While the leaf samples in the Galunggung Mountain also live as epiphytes, but the location of the plants is not too high so they are still exposed to direct sunlight and are located at the top of the mountain. The existence of *Dendrobium crumenatum* that live as epiphytes in each region is presented in **Figure 2**.

pigeon orchids at Singa-parna bus station | pigeon orchids at Sukaraja-Mangunreja road | pigeon orchids at mount Galunggung

Figure 2. The existence of Pigeon Orchids in each sampling area

The existence of shade, both natural and artificial, decreasing intensity of sunlight that will be received by the surface of the leaves of *Dendrobium crumenatum*, Thus, the *Dendrobium crumenatum* plant will adapt to the environment in which the plant grows. As a statement from Gregoriou et.al (in Sihotang, 2017) that plants live with low sunlight intensity will adapt by maximizing sunlight capture both changing the anatomy and morphology of leaves for efficient photosynthetic processes, adaptations can be made

because plants have the ability to detect the presence, direction, intensity and wavelength of light.

In order to survive, every living being must adapt, as well as *Dendrobium crumenatum* plant, which adapts so that it supports the continuity of the physiological functions of the plant body. One form of adaptation is the response to the formation of stomata, this is supported by (Budiono et al., 2016) that stomata density is influenced by environmental factors such as temperature, light intensity and humidity.

CONCLUSION

There is an effect of air pollution on the characteristics of stomata in the leaves of *Dendrobium crumenatum* in the Tasikmalaya. It is evidenced by the results of statistical analysis, the higher the concentration level of HCHO and TVOC, the higher the density and index of stomata in areas with high pollutant levels as a result of the increased intensity of motorized vehicles. Stomata Characteristics of *Dendrobium crumenatum* has an anomocytic type of stomata. Based on the stomata density category, the leaves of *Dendrobium crumenatum* in Singaparna bus station are included in the medium density category, namely 326.11/mm^2 (300-500/mm^2), while in the Sukaraja-Mangunreja Road and Mount Galunggung are included in the low category i.e. 256.47/mm^2 < 300/mm^2. The enhancements occur is indicated as a response or adaptation of stomata to the stress of environmental conditions due to increased pollutant levels.

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