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PHYSIOLOGICAL CHARACTERS OF VANILLA PLANT (*Vanilla planifolia* Andrew) IN VARIOUS TYPES OF SHADE PLANTS

Sri Suryanti [1*], Hangger Gahara Mawandha [1], Herawati Oktavianty [2]

[1] *Agrotechnology Study Program, Faculty of Agriculture, INSTIPER Yogyakarta, Jl. Nangka II, Maguwoharjo, Depok Sleman, Yogyakarta.*
[2] *Agricultural Product Technology Study Program, Faculty of Agricultural Technology, INSTIPER Yogyakarta, Jl. Nangka II, Maguwoharjo, Depok Sleman, Yogyakarta.*
[*] *Correspondence email : ntie@instiperjogja.ac.id*

ABSTRACT

Vanilla plants are CAM plants and are very sensitive to direct sunlight, so vanilla plants need shade trees as shade plants to reduce the intensity of sunlight. Research on the physiological characteristics of vanilla plants on various types of shade plants was carried out to finding out the influence of types of shade plants on the physiological characteristics of vanilla plants. The research was conducted using a randomized completely block design with four groups. Planting was carried out using four types of shade plants, namely *Gliricidia sepium, Syzygium aromaticums, Erythrina variegata* and *Leucaena leucocephala* plants. To determine microclimate conditions, temperature, air humidity and sunlight intensity were observed three times a day (morning, afternoon and evening). Physiological observation parameters include content of chlorophyll A, chlorophyll B levels, total chlorophyll, proline, relative water content. The results of the research showed that temperature, air humidity and the intensity of sunlight received are different for each type of shade plant. Types of shade plants significantly influence content of chlorophyll A, chlorophyll B, total chlorophyll, proline and relative water content. The conclusion was the type of shade plant affects the microclimatic conditions of the vanilla plant, thereby influencing the physiological properties of the vanilla plant.

Keywords : Physiological; Shade Plant; Vanilla

INTRODUCTION

Vanilla plant (*Vanilla planifolia* Andrews) is a tropical plant and widely cultivated in Madagascar, Indonesia, China and Mexico (Rahman et al., 2019). Vanilla plants can grow optimally at temperatures between 20 and 30 ° C (Parada-Molina et al., 2022), minimum rainfall 2.000 mm per year (Fouché & Jouve, 1999), air humidity 65 – 75 % (Tombe et al., 2001), sunlight intensity 30 – 50 %, air humidity 60 – 75 %, dry months 2 – 3 months and wet months 7 – 9 months (Rosman, 2015).

Vanilla plants are very sensitive to direct sunlight, so vanilla plants need a shade plant to reduce the intensity of sunlight. Excessive shading on vanilla plants can be destructive, vine stems become small, susceptible to disease (mildew and root rot) and yields drop (Fouché and Jouve, 1999). Land and climate suitability, especially light intensity, is very important so it needs adjustment of land, climate, and cultivation technology measures (Rosman, 2020).

Vanilla plants can perform phytosynthesis effectively and grow at a sunlight intensity of 300-800 $\mu Em^{-2}s^{-1}$ whereas only plants that receive a light intensity of 600-800 $\mu E\ m^{-2}s^{-1}$ are able to effectively transport accumulated carbon into the fruit structure. Therefore the productivity of vanilla plants is greatest at light intensity 600-800 $\mu E\ m^{-2}s^{-1}$, while at sunlight intensity 300-600 $\mu E\ m^{-2}\ s^{-1}$ is found to support vegetative growth. Sunlight above 800 $\mu E\ m^{-2}s^{-1}$ decreases plant productivity. The increased intensity of sunlight causes the accumulation of proline and carotenoids in vanilla plants to increase. The mechanism of using protective plants to reduce the intensity of sunlight is not enough to protect vanilla plants from photoinhibitor damage. This is indicated by high levels of lipid peroxidation and malondialdehyde (MDA) levels, low chlorophyll content and low plant productivity when exposed to sunlight above 800 $\mu E\ m^{-2}s^{-1}$. The use of protective trees as shade plants with good development will be able to reduce the side effects of photoinhibition (Puthur, 2005). The quantum photochemical yield of PSI and PSII of vanilla plants was mostly reduced in the afternoon compared to the morning. This suggests that CO_2 assimilation is very low in the afternoon. Meanwhile, non-photochemical quantum in PSII and PSI increased markedly to protect PSI and PSII (Wang et al., 2022). The high relative due to excessive shading decreased yield of Vanilla planifolia (Andrade et al., 2023).

Vanilla plants do not require full sun (*shade-loving plants*) (Supriadi et al., 2014). Protective plants on vanilla plantings are absolutely necessary. The types of protective trees used on vanilla plants are so diverse that they will affect the intensity of sunlight the plant receives. Protective plants that meet the requirements are *Gliricidia sepium*, *Erythrina variegata*, *Leucaena leucocephala* (Henuhili, 2004). The vanilla plant is a CAM plant. High solar radiation can inhibit the process of photosynthesis and the growth of vanilla plants. However, in the long term vanilla plants show higher photosynthesis and growth at intermediate radiation levels (Moreno & Gantiva, 2017). In CAM plants, stomata open at night when conditions are relatively cold and moist. PEP carboxylase is react at night, binding inorganic carbon to C_4 acid which is saved in big vacuoles. At noon, the stomata close to save water, and the C_4 acid is decarboxylated, emit CO_2 which after that fixed by Rubisco in C_3 photosynthesis phatway.

Light quality has an main role in photosynthesis and plant morphogenesis. The physiological response of plants to light quality varies depending on growing conditions (Kwak et al., 2011). At an altitude of 825 m asl, vanilla plants produce better generative growth and vanilla yield components, but vegetative growth decreased. Climatic conditions around the plant, especially sunlight intensity, air temperature, and the cause may be due to different nutrients soil nutrients (Supriadi et al., 2014). The flowering *of Vanilla planivolia* required strong sunlight (Kitai & Lahjie, 2016). The research results showed that blue color light with a wavelength of 460 nm and red color light with a wavelength of 660 nm markedly improved stem elongation and chlorophyll synthesis of vanilla plants. Blue light markedly improves root elongation, number of roots and number of leaves of vanilla plant (Ramírez-Mosqueda et al., 2017).

Vanilla plant can adapt to their light environments through morphological and physiological characteristic (Zhang et al., 2018). Changes in the absorption of sunlight by plants result in morphological and physiological changes. Adjustment to the leaves is carried out through filtration effect, light distribution and adaptation to environmental conditions. The difference in the content of chlorophyll in shaded and unshaded plants makes the change photosynthesis photon flow density. The transmission of light is carried out through vacuoles to shorten the distance that electrons must travel in the electron transfer chain. Physiological acclimatization is done by changing the chlorophyll arrangement and PSII/PSI ratio. Sheltered plants have a high PS II/PSI ratio and a/b chlorophyll ratio to increase the light-capture complex for more efficient photosynthesis (Yustiningsih, 2019). Shade conditions can improve gas exchange, reduce leaf temperature and promote chlorophyll synthesis (Gómez-Bellot et al., 2023).

MATERIALS AND METHODS

a. Time and Place of Research :

The research was carried out on vanilla farmer land in Sinogo, Pagerharjo, Samigaluh, Kulonprogo from May to November 2023.

b. Research Materials and Tools:

The ingredients used in this experiment were vanilla plants, toluent, 80% alcohol 3% sulfosalicylic acid solution, ninhydrin acid, glacial acetic acid, phosphoric acid, aquades, NaOH, 70% alcohol. The tools used in this experiment are scales, lux meters, thermometers, hygrometers and spectrometers.

c. Experiment design

The study was carried out with a randomized completely block design with 4 blocks. Treatment is a type of shading plant which includes 4 types of shade trees namely *Gliricidia sepium, Syzygium aromaticum, Erythrina variegata* and *Leucaena leucocephala.* The number of plant samples was 5 plants on each different type of shade tree. The physiological parameters observed include content of chlorophyll A, chlorophyll B, total chlorophyll, proline and relative water content. Data analysis was carried out using *analysis of variance* (ANOVA) at the level of 5% and next with *Duncan Multiple Range Test* (DMRT) and correlation regression analysis. Measurements of sunlight intensity, temperature and air humidity under the canopy of shade trees were carried out daily for each plant sample. The time of measurement of light intensity, temperature and air humidity is 6.00, 12.00 and 18.00.

1. Chlorophyll

   Chlorophyll content were observed by taking samples of leaves that had opened completely. Chlorophyll was extracted from fresh leaves using 80% acetone and filtered, then read with Spectronic 21 at wavelengths of 645 and 663 nm according to Lichtenthaler (1987).

2. Proline

   Proline content were measured using a modified method (Bates, et al. 1973). The plant material to be measured is leaves that have opened completedly. The leaves are mashed with a grinder, taken as much as 0.5 g then finely ground with mortar in a 3% sulfosalicylic acid solution as much as 10 ml. The impact results are filtered with Whatman 2 filter paper. A solution of ninhydrin acid is prepared by heating 0.50 g of ninhydrin in 30 l of glacial acetic acid and 29 ml of 6 M phosphoric acid until the solution is mixed. As much as 0.5 ml a filtrate was reacted with 2 ml of ninhydrin acid in a test tube, then shaken and heated at 100 ^{0}C for one hour. The mixture was extracted with 5 ml of toluene then cornered with a stinger for 15 seconds. After about 24 hours, the absorbent separates at the top and is then aspirated with a pipette. The absorbent of the solution is read spectronic 21 D at a wavelength of 520 nm.

3. Relative Water Content

The relative water content of the leaves is observed by measure the fresh weight of the sample leaves, then the leaves are saturated in water for 24 hours and weighed to get the turgid weight. After soaking, the samples were dried quickly with filter/tissue paper and immediately weighed to get fully turgid weight (TW). Samples are then oven dried at 80^{0}C for 24h and weighed to obtain dry weight (DW).

The relative moisture content (RWC) is calculated by the formula:

$$RWC\ (\%) = \frac{\text{fresh weight} - \text{dry weight}}{\text{turgid weight} - \text{dry weight}} \qquad 1)$$

RESULTS AND DISCUSSION

a. Microclimate of vanilla plants

Vanilla plants should use shade to reduce the intensity of sunlight. The influence of shade plants has different influences on the parameters of temperature, air humidity and sunlight intensity (Table 1).

Table 1. Microclimate Data on Various Shade Plants

Types of Shade	Temperature (^{0}C)	Air Humidity (%)	Intensity of sunlight (%)
Gliricidia sepium	29	75	62
Syzygium aromaticum	25	67	59
Erythrina variegata	25	65	31
Leucaena leucocephala	25	67	49

Vanilla plants require a sunlight intensity of 30-50% (Fouché and Jouve, 1999), temperature 20-30 ^{0}C and humidity 65 – 75 % (Tombe et al., 2001). The results of measurements of microclimate conditions show differences in temperature, air humidity and sunlight intensity. The use of shading plants already the requirements for growing temperatures because temperatures range from 25 – 29 ^{0}C. The temperature is highest in the shade of *Gliricidia sepium*, while the use of *Syzygium aromaticum, Erythrina variegata* and *Leucaena leucocephala* shade plants has the same temperature. Air humidity between 65 – 75% so that the use of four kinds of shade also meets the requirements for growing vanilla plants. The intensity of sunlight in the shade of *Erythrina variegata* plants 31%, *Leucaena leucocephala* 49%, *Gliricidia sepium* 59% and shade of *Syzygium aromaticum* plants 62%. This shows that *Erythrina variegata* and *Leucaena leucocephala* plants are suitable types of plants for vanilla plant shade, because vanilla plants require 30-50% sunlight intensity. This result in line (Rosman, 2020), that *Erythrina variegata* and *Leucaena leucocephala* trees qualify as shading plant of vanilla.

Sunlight intensity is a factor that influences the growth and production of vanilla plants. In the vegetative phase, lower sunlight intensity is needed than during the generative phase. The intensity of ray influences the temperature and humidity of the air. There is a close relationship between the intensity of sunlight received by vanilla plants with air humidity and temperature. This is shown by the results of correlation analysis, where there is a positive correlation between the sunlight intensity and air humidity ($r = 0.712$) and between the intensity of rays and temperature ($r = 0.56$). The results of the correlation analysis between temperature factors and air humidity factors also showed a positive correlation ($r = 0.977$). This shows a close relationship between temperature and air humidity.

b. Chlorophyll

Chlorophyll levels are one of the main factors affecting the photosynthetic ability of plants. The use of shade in the form of *Erythrina variegata* plants can markedly increase levels of chlorophyll A, chlorophyll B and total chlorophyll. There was a marked difference between chlorophyll A, chlorophyll B and total chlorophyll levels when shading *Erythrina variegata* plants with *Leucaena leucocephala, Syzygium aromaticums* and *Gliricidia sepium* (Table 2).

Table 2. The Effect of Shade on the Physiological Properties of Vanilla Plants

Note : Numbers followed by the same letters in the column show a significance difference in the

Types of Shade	Chlorophyll A (unit)	Chlorophyll B (unit)	Total Chlorophyll (unit)	Proline content (μmol/g)	Relative water content (%)
Gliricidia sepium	14,087 b	3670,191 c	26,203 c	0,066 c	20,899 b
Syzygium aromaticum	13,568 d	1885,965 d	22,254 d	0,157 a	13,797 d
Erythrina variegata	14,607 a	7484,309 a	31.910 a	0,059 d	29,163 a
Leucaena leucocephala	14,401 c	4999,367 b	28.542 b	0,073 b	20,825 c

confidence level of 5%.

The levels of chlorophyll a, chlorophyll b and total chlorophyll levels are opposite to the intensity of sunlight the vanilla plant receives. This is shown by the results of correlation analysis which shows a negative correlation between the intensity of sunlight with chlorophyll A levels (r = - 0.787), chlorophyll B (r = - 0.917) and total chlorophyll levels (r = - 0.865). The intensity of sunlight in the shade of *Erythrina variegata* is 31%, *Leucaena leucocephala* 49%, *Syzygium aromaticums* 59% and *Gliricidia sepium* 62%. This result is in line with the results of previous studies on pineapple plants. Pineapple plants are CAM plants like vanilla, and high light intensity in pineapple plants also reduces chlorophyll levels (Rodríguez-Escriba et al., 2015). Different research results are found in plants *Aralia errata* which is a C_3 plant, which is manifestly picmen photosynthesis of chlorophyll A, chlorophyll B and total chlorophyll decreases when the degree of shade increases or light intensity decreases (reduction in chlorophyll content is directly follow to reduce light intensity) (Gao et al., 2019). The environmental factors such as light intensity have different effects on the arrangement and role of the photosynthetic organs from C3 and C4 species (Hu et al., 2023). The results of this study are the same with research conducted by (Juhaeti et al., 2020) that chlorophyll levels increase to 50% in the presence of 50% shade and decrease when shade is 75%.

Chlorophyll B occurs due to the adaptation of chlorophyll A to shaded plant conditions. Chlorophyll B occurs from chlorophyll A which undergoes oxidation so that the CH_3 group in ring II in chlorophyll A turns into an aldehyde group on chlorophyll molecule B. The important differences between chlorophyll A and B is its usefull in photosynthesis, namely chlorophyll A is the main pigment that plays a role in photosynthesis while chlorophyll B is an supporting pigment to collect energy and be converted into chlorophyll A. Chlorophyll B is needed to stabilize the main light-chatching chlorophyll binding protein. Chlorophyll B is synthesized from chlorophyll A and catabolized after being converted back into chlorophyll A (Tanaka & Tanaka, 2011). The results showed that in vanilla plants chlorophyll A levels and chlorophyll B levels markedly increased at low light intensity (*Erythrina variegata* tree shade). The intensity of sunlight received by plants becomes lower if using shade trees with increasingly dense leaves or large leaves. *Erythrina variegata* plants have wider leaves so that the intensity of sunlight received by vanilla plants is lower. Low light intensity causes air temperature and humidity to decrease (Table 1). Environmental conditions such as air temperature and humidity greatly affect plant chlorophyll levels. Under low light intensity conditions, chloroplasts of CAM plants contain more photosynthetic picmen per unit volume. According to (Sopandie, 2013) In an effort to increase the efficiency of light capture, namely at low light intensity, plants will reduce the light transmitted, namely by increasing the number of chloroplasts and increasing the pigment content per chloroplast. According to (Ko et al., 2020) *Vanilla planifolia* is very susceptible to high radiation. Vanilla chloroplasts started to decreased when blue light was higher than 20 μmol $m^{-2}s^{-1}$.

The high intensity of sunlight increases the temperature. Increased temperature also decreases chlorophyll A levels, chlorophyll B and total chlorophyll levels.

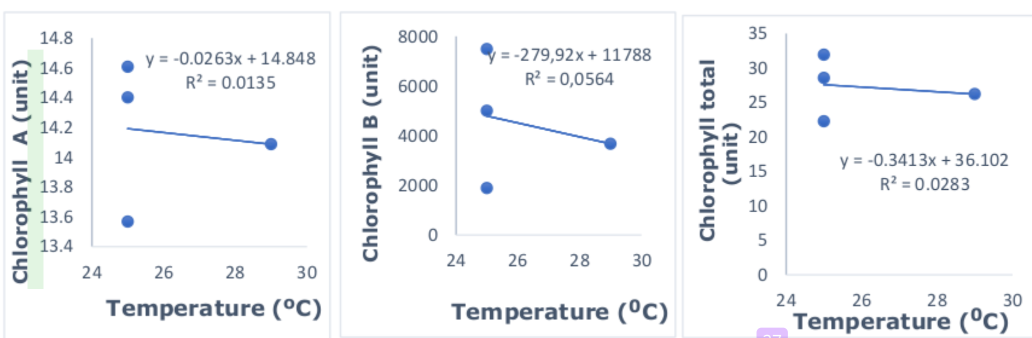

Figure 1. Graph of the Relationship between Temperature and Content of Chlorophyll A, Chlorophyll B and Total Chlorophyll.

This is shown by the results of regression analysis between temperature and chlorophyll A levels (y = - 0.0263 x + 18.848), chlorophyll B levels (y = -

279.92 x + 11788) and total chlorophyll levels (y = - 0.3413 x + 36.102) (Figure 1). The results of this study are different form the results of research on orchid plants which are CAM plants. Most orchids, like vanilla plant can adapt to their light environments through morphological and physiological characteristic (Zhang et al., 2018). Blue red photoselective shade netting increased chlorophyll content and maximum quantum yield of photosystem II of vanilla plant In orchid plants, low temperatures further reduce chlorophyll A, chlorophyll B levels and the higher the air temperature chlorophyll levels higher (Daems et al., 2022).

c. Proline

Proline is an osmolytic compound so that plants become resistant to drought stress (*drought tolerance*) through osmotic adjustment mechanism. Light is a factor that greatly influences the growth, development and physiological processes of plants. Plants have the ability to adjust metabolism to environmental changes such as light by accumulating proline. When the light intensity is very high, the accumulation of proline is three times greater than when the light intensity is normal (Kovács et al., 2019). The results showed that the use of *Syzygium aromaticum* shade increased proline levels and was significantly different from *Erythrina variegata*, *Gliricidia sepium*, and Leucaena leucocephala shade. The intensity of sunlight received by vanilla plants was lower when given *Erythrina variegata* shade (31%) so that proline levels were lower. Conversely, the shade of *Syzygium aromaticum* plants causes the intensity of sunlight received by vanilla plants to be high so that proline levels are higher. There is a close relationship between sunlight intensity and proline levels. The results of the correlation analysis showed a positive correlation between the intensity of sunlight and proline levels (r = 0.484). These results are not in line with the results of research conducted by (Kwak et al., 2011) that oak plant proline accumulates more at very low sunlight intensities, as well as in plants *Aralia errata* indicates that the maximum proline content at 80% shade (Gao et al., 2019). Proline content in leaves increased in the CAM orchids after 7 weeks of drought under moderate light (Tay et al., 2019).

The results of regression analysis showed a positive linear regression between light intensity and proline levels (y = 0.0016x + 0.0087). This suggests that an increase in sunlight intensity is followed by an increase in proline levels, whereas a decrease in sunlight intensity will be followed by a decrease in proline levels (Figure 2).

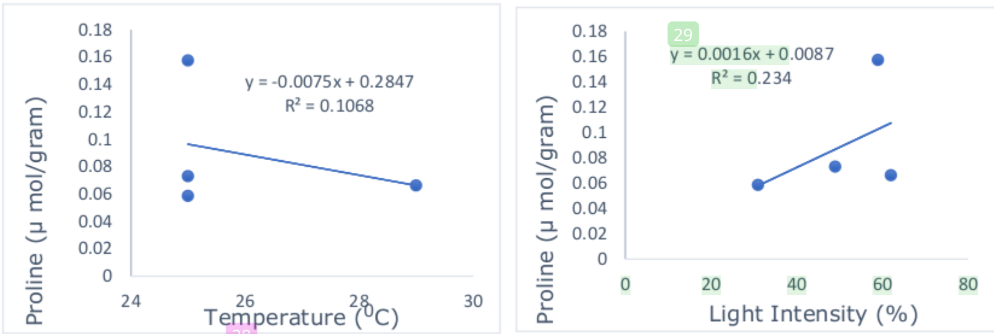

Figure 2. Graph of the Relationship between Temperature and Light Intensity with Proline Levels

The increase in air temperature that occurs when the intensity of the rays is higher causes the proline levels of vanilla plants to be lower even though the decrease is not too large. This is shown by the results of regression analysis between air temperature and proline levels (y = - 0.0075 x + 0.2847 (Figure 2). The results of the study are not in line with the results of research on tomato plants which are C3 plants, where in tomato plants proline levels are higher when the temperature is higher (Suminar et al., 2021).

d. Relative Water Content

Relative water content is related to cell volume and can indicate a balance between the amount of water absorbed and the amount of water transpired. Plants that have a high relative moisture content are more resistant to drought stress. The results showed that the type of shade significantly affects the relative moisture content. The results of the correlation analysis show a negative correlation between the intensity of light and the relative moisture content (r = - 0.839), an increase in sunlight intensity will reduce the relative moisture content of leaves. This result is in line with previous research on moderate to high light intensity and low to moderate relative moisture content can increase plant growth and yield *Dendrobium nobile* Lindl (Li et al., 2017).

The results of regression analysis showed a negative linear regression between light intensity and relative water content (y = - 376.88 x + 40110).

This shows that an increase in light intensity is followed by a decrease in relative moisture content, and conversely a decrease in light intensity is followed by an increase in relative water content (Figure 3).

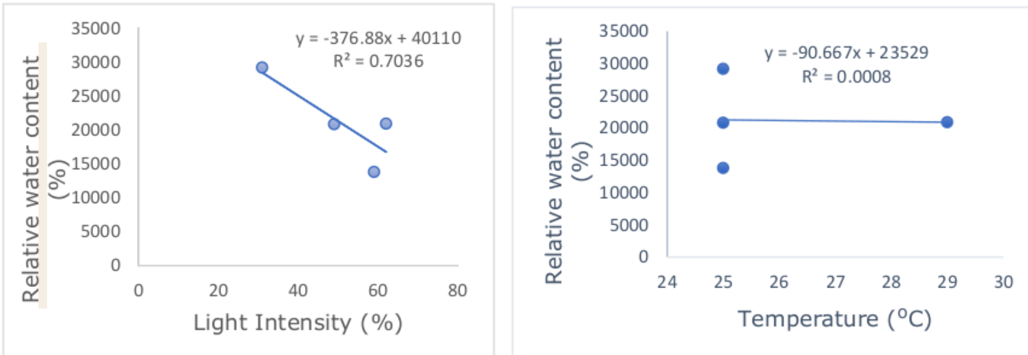

Figure 3. Graph of the Relationship between Light Intensity and Relative Water Content

In CAM plants with succulent leaves such as vanilla plants, inside the leaves there are large vacuoles so that they can store more water (Hu et al., 2023). It is a strategy of plants to store water in unfavorable conditions such as lack of water. The relative water content indicates the water status inside the plant. The higher the relative water content, the more water stored in the network. In conditions of lack of water, the relative water content is getting lower (Arena, 2020). The intensity of sunlight received by vanilla plants with high shade of *Syzygium aromaticum* plants (59%) can increase air temperature so that evaporation is higher and can reduce the relative water content in the leaves. Conversely, in the shade of *Erythrina variegata* plants, the intensity of sunlight received by vanilla plants is low (31%) so that the air temperature is lower, evaporation is smaller and the moisture content is large leaf ratio. This is shown by the results of regression analysis between temperature factors and relative water content (y = - 90.667 x + 23539). The results of regression analysis, it shown that the increase in air temperature causes the relative water content to be lower (Figure 3).

CONCLUSION

Erythrina variegata and *Leucaena leucocephala* are plants that qualify as shading plants for *Vanilla planifolia*. The use of shading plants increased chlorophyll A content, chlorophyll B, total chlorophyll levels and relative water content but decreased vanilla plant proline levels. Higher sunlight intensity decreases chlorophyll A, chlorophyll B, total chlorophyll and relative water content but high sunlight intensity increases vanilla plant proline levels.

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