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Physiological and phytochemical characters of *Eleutherine palmifolia* affected by treatment of variation in light intensity and water capacity

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Abstract. *Firdaus NM, Mudyantini W, Sugiarto. 2020. Physiological and phytochemical characters of* Eleutherine palmifolia *affected by treatment of variation in light intensity and water capacity. Cell Biol Dev 4: 26-39.* Bawang dayak (*Eleutherine palmifolia* (L.) Merr.) is one of the tubers widely cultivated in Kalimantan with phytopharmacological potential, but its usage in traditional medicine is still limited. This study aims to ascertain the physiological and phytochemical characteristics of *E. palmifolia* following treatment with varying amounts of water and light intensity. The study used a completely randomized design (CRD) with two components: the treatment of light intensity 50% and 100% and water availability at 50%, 75%, and 100% concentrations, to create six treatment combinations. Flowering pace, leaf number, breadth, length, and blossom number were measured once every three days for one month. Wet weight, dry weight, stomata index, chlorophyll, carotene, vitamin C, flavonoids, and shoot ratio were assessed at harvest. The data were analyzed using ANOVA (Analysis of Variant). If there was a significant difference, a further test was carried out with the Duncan Multiple Range Test with a test level of 5%. The treatment effect on water availability of 75% and light intensity of 50% (K75I50) was significantly different and gave the highest value on leaf width of 0.28 cm, stomata index of 36.84 cm, carotenoid content of 39.70 g/ mL in *E. palmifolia*. The given treatments were not significantly different in the number of leaves, leaf length, the number of flowers, wet weight, dry weight, chlorophyll, vitamin C, flavonoids, and shoot ratio of Dayak roots. Water availability and 100% light intensity (K100I100) are the optimal treatments to accelerate the flowering of Dayak onion plants with a yield of 26.00 days.

Keywords: *Eleutherine palmifolia*, light intensity, physiological characters, phytochemicals, water availability

INTRODUCTION

Eleutherine palmifolia (L.) Merr., or *bawang dayak*, is one type of plant beneficial to health but is used infrequently in the community for treatment. The *E. palmifolia* is a native plant to Indonesia, more precisely to Kalimantan, and may be used as a phytopharmaceutical. The community makes extensive use of this plant on tubers. The tubers of *E. palmifolia* contain a variety of phytochemicals, including alkaloids, glycosides, flavonoids, phenolics, steroids, and tannins (Firdaus et al., 2013). The *E. palmifolia* has been used empirically by indigenous people to treat various diseases, including breast cancer (Sudarmawan et al. 2010), hypertension, diabetes mellitus, cholesterol, ulcers, and colon cancer, as well as to prevent stroke and alleviate abdominal pain following childbirth. Additionally, the leaves of this plant have been shown to increase breast milk production (Galingging 2009).

Water availability is one issue that arises during the long dry season. Groundwater levels will continue to decline in this condition due to high evaporation. Apart from wreaking havoc on the soil, a lack of water has a detrimental effect on plants, as water does not dissolve the nutrients required by plants. As a result, nutrient availability to plants is reduced, resulting in decreased productivity or even wilting. The requirement for water is quite different in plants whose life is sustained by tubers than in plants whose life is supported directly by roots. Tubers and dry matter rely highly on an adequate water supply (Kadayifci et al. 2005).

Light intensity is a critical factor that affects plant growth and development. Too much light has a detrimental effect on plants, specifically the occurrence of chlorosis, chlorophyll damage, and rapid transpiration. On the other hand, when light levels are low, plants consume existing food reserves rather than storing them (Forniawan et al., 2017). The *E. palmifolia*, on the other hand, prefer full sunlight to shaded conditions for growth (Yusuf 2009). Full light intensity results in an increase in photosynthate accumulation in *E. palmifolia* tubers. Some photosynthetic content stored in *E. palmifolia* is in the form of essential compounds such as flavonoids, primarily used by tubers due to their higher biomass production than other organs. Based on these issues, it is necessary to research the physiological and phytochemical characteristics of *E. palmifolia*, as well as the effect of light intensity and water availability variations on its growth. The *E. palmifolia* will be stressed by the combination of the two treatments. Therefore, it is necessary to investigate the most effective methods for increasing the cultivation and the content of beneficial phytochemicals, one of which is flavonoids. Unfortunately, there is no information on the combination of the two treatments. What has been done is to treat each separately without combining them.

The aims of this study were (i) to examine the effect of light intensity and water availability on the physiological and phytochemical characteristics of *E. palmifolia*, and (ii)

to determine the best combination of light intensity and water availability treatment for *E. palmifolia*

MATERIALS AND METHODS

The research was carried out from December 2019 to October 2020. The research was carried out at the Central Laboratory Greenhouse, the Integrated Mathematics and Natural Sciences Laboratory and Biology, Universitas Sebelas Maret, Surakarta, Indonesia.

Research design

This study used a completely randomized design (CRD) with two factors: variations in light intensity at two levels and variations in water administration concentration at three levels, to obtain six treatment combinations. Each treatment was replicated five times in this study. The following treatment parameters were determined: leaf number, leaf width, leaf length, midrib height, flower number, flowering speed, stomata index, crown to root ratio, chlorophyll content, carotenoid content, vitamin C content, and flavonoid content. Rolling once a week for one month was used to administer the combination of treatments. In this study, the following treatments were used in combination:

Water availability factor (A) with three levels, namely:
K100 = control (100% field capacity)
K75 = 75% field capacity
K50 = 50% field capacity
Light intensity factor with two levels, namely:
I100 = control (100%)
I50 = 50% field capacity

The six treatment combinations are shown in Table 1.

Procedure

Selection of tubers samples and preparation of e. palmifolia seeding

The material for this study was freshly harvested *E. palmifolia* tubers of uniform size from Surakarta, Indonesia. First, 60 tubers of *E. palmifolia* were selected in their native state. The tubers are picked based on their size. The tubers of *E. palmifolia* were then weighed using an analytical balance set to a weight range of 5-7 grams. Next, uniformly sized *E. palmifolia* tubers were planted in the prepared media. Tubers of *E. palmifolia* were planted by burying half of the tubers into a media mixture in 11x20 polybags consisting of compost, manure, soil, and husks in a ratio of 1:1:1:1. Seed preparation was carried out for 15 days.

Determination of field capacity

Before use as a planting medium, the gravimetric method is used to determine the soil's field capacity (weighing). The field capacity was determined by mixing soil, husks, manure, and compost in a 1:1:1:1 ratio until the mixture reached a dry weight of 1 kg. After confirming that the media mixture weighs 1 kg, it is placed in a polybag measuring 15 x 20 cm with a capacity of 1 kg and a perforated bottom, then saturated with water until no water drips. The following formula calculates field capacity (FC):

Field Capacity = (Weight of soil + Polybag + Water) – (Weight of soil + Polybag) (Patoni 2000).

Treatment determination

Variations in light intensity and water availability were considered in this study. The water availability treatment was applied when the *E. palmifolia* plants had 2-4 leaves. Water was sprayed at 100%, 75%, and 50% of the field capacity, respectively (Nasir et al. 1996). Watering is performed every four days (to ensure that treatment conditions remain consistent with the level of water availability being tested) (Haryati et al., 2010). In the Green House, polybags are placed at two different light intensities, 50%, and 100%. Wickramasinghe et al. (2015). Weeding is accomplished by removing weeds from the plant's immediate vicinity. Each treatment was repeated five times, focusing on field capacity. After exactly one month, the same water and light intensity volume was applied to *E. palmifolia* plants to be analyzed.

Daily observation

Daily observations were made using thread and a ruler to determine the height of the midrib, the number of leaves, the width of the leaves, the length of the leaves, the speed of flowering, and the number of flowers. In addition, all treatment polybags were observed every three days for one month. After exactly one month, *E. palmifolia* plants were treated with the same volume of water and light intensity to be analyzed.

Relative growth calculation

The Relative Growth Rate (RGR) is the essential crop strategy indicator in crop productivity under environmental stress. Since the beginning of growth at a specific time interval, the relative growth rate increases the relative size of the existing plant. The calculation of the relative growth rate is as follows.

$$RGR = \frac{Wfinal - Winitial}{Winitial}$$

Where:
RGR : Relative Growth Rate
Wfinal : final day growth parameters
Winitial : initial day growth parameters

Table 1. Combination of Treatment of water availability and light intensity on *E. Palmifolia*

	I100	**I50**
K100	K100I100	K100I50
K75	K75I100	K75I50
K50	K50I100	K50I50

Stomata index measurement

The stomata index was determined by incision of the epidermal leaf of the *E. palmifolia* plant. The lower epidermal leaves were sampled. The imprinting method is used to determine the stomatal index. From a total of 30 pots, one leaf each was selected and smeared on the underside of the leaf using clear nail polish. After applying, the polished area is left for 1 hour until it dries completely. When the nail polish dries, clear tape is applied to the area where the polish has been applied and then slowly removed until all the polish is removed and sticks to the clear tape. The clear tape containing the print is then placed on a glass object and observed under a digital microscope to observe the index. The formula for calculating the stomata index is as follows.

$$\text{stomata index} = \frac{\text{number of stomata}}{\text{number of epidermal cells - number of stomata}} x100$$

Wet and dry biomass measurement

Plant biomass measurements were carried out for wet and dry biomass. The crown and roots were measured separately, whereas the total biomass was determined by adding the shoot and root biomass, both dry and wet. After two months of maintenance, wet biomass was weighed. Dry biomass was prepared by drying it in an oven at 60°C until it reached a constant weight and weighed.

Measurement of the root-to-top ratio

The ratio of roots and shoots was obtained by comparing the biomass of roots and shoots when wet and dry.

Testing for photosynthetic pigment levels

Leaves weighing 0.5 grams were macerated using a mortar and pestle and dissolved in 5 mL of 80% PA (Pro Analysis) acetone intermittently while slowly mashed. Then the smooth leaves were filtered using a glass funnel in a test tube using Whatman 42 filter paper. The filtered filtrate was chlorophyll extract. All processes are carried out in conditions protected from sunlight (Prasetyo and Laili, 2015). First, the obtained filtrate is poured into the cuvette until 2/3 of the cuvette is filled. Next, the 80% PA (Pro analysis) acetone solution was poured into the cuvette until 2/3 of the cuvette was filled for the blank solution on the UV-Vis spectrophotometer. Furthermore, the content of chlorophyll and carotenoids was measured using a UV-Vis spectrophotometer at wavelengths of 480 nm, 645 nm, and 663 nm. After obtaining the absorbance value, the chlorophyll content can be calculated by the following formula:

Chlorophyll a mg/g leaf weight = (12.7 x A663) – (2.69 x A645 x 10^{-1})
Chlorophyll b mg/g leaf weight = (22.9 x A645) – (4.68 x A663 x 10^{-1})
Total chlorophyll mg/g leaf weight = Klorofil a + Klorofil b

$$\text{Carotenoids } \mu\text{mol/g leaf weight} = \frac{(A480 + 0.114 \text{ x } A663 - 0.638 \text{ x } A645) \text{ x } V \text{ x } 103}{112.5 \text{ x } 0.1 \text{ x } 10}$$

Photosynthetic pigment data processing was carried out in the last week of observation.

Measurement of vitamin C levels

Determination of pure vitamin c calibration curve

Pure ascorbic acid was weighed as much as 0.02 mg, 0.04 mg, 0.06 mg, 0.08 mg, 0.012 mg, and 0.016 mg and dissolved in 10 mL of aquabides to the mark so that the solution concentration was 2 ppm, 4 ppm, 6 ppm, 8 ppm, 12 ppm, and 16 ppm. Then the maximum absorption was measured at a wavelength of 265 nm with a UV-Vis spectrophotometer using an aquabides blank (Karinda and Citraningtyas 2013). After analyzing using a UV-Vis spectrophotometer, a calibration curve was made using excel.

Determination of vitamin C levels in samples

Tubers of *E. palmifolia* were weighed 5 g and crushed and smoothed; 10 mL of aquabides were added and homogenized. Next, the solution is filtered. The filtrate was put into a 10 mL measuring cup, then aquabides were added to the mark and homogenized. Finally, the absorption was measured at a wavelength of 265 nm (Karinda and Citraningtyas, 2013).

Measurement of flavonoid level

A total of 2 g samples of dried *E. palmifolia* tubers were put into a maceration container. Then added with ethanol PA (Pro Analysis) 96% 10 mL until the entire sample was submerged, closed, and left for 24 hours. Next, the macerate was filtered using Whatman 42 filter paper. The filtrate was obtained through filtering with a funnel, and then the pulp was macerated again with 96% PA (Pro Analysis) 10 mL ethanol so that the filtrate was almost colorless. Finally, all filtrates were combined and evaporated using a fan until there was no more liquid PA 96% ethanol to obtain an ethanolic extract of *E. palmifolia* tubers. The thick extract of *E. palmifolia* tuber obtained was used for further analysis by UV-Vis spectrophotometer.

Quercetin standard curve creation

In the making of quercetin solution concentration, weighed as much as 0.06 mg, 0.08 mg, 0.010 mg, 0.012 mg, and 0.014 mg of quercetin standard and dissolved in 10 mL of aquabides, so the quercetin solution concentration was 6 ppm, 8 ppm, 10 ppm, 12 ppm, 14 ppm. Then 1 mL of 2% AlCl3 and 1 mL of 120 mM potassium acetate were added. Samples were incubated for one hour at room temperature. Finally, the absorbance was determined using the UV-Vis spectrophotometric method at a maximum wavelength of 435 nm (Stankovic 2011).

Determination of total flavonoid content of Eleutherine palmifolia *tubers*

The extract *Eleutherine palmifolia* tubers were dissolved with 1 mL of 96% PA (Pro Analysis) ethanol to dissolve the remaining extract still attached to the porcelain cup. Then, according to the treatment, the solution was put into a test tube, and 1 mL of 2% $AlCl_3$ solution and 1 mL of 120 mM potassium acetate were added. Samples were

incubated for one hour at room temperature. Finally, the absorbance was determined using the UV-Vis spectrophotometric method at a maximum wavelength of 435 nm (Stankovic 2011).

Data analysis

Observational data of *Eleutherine palmifolia* tubers were analyzed by statistical analysis. In addition, quantitative data on daily observations (number of flowers, flowering speed, number of leaves, leaf width, stem height, and leaf length) and observations at harvest (dry and fresh biomass, flavonoid test, vitamin C test, photosynthetic pigment test, root shoot ratio, and index stomata) were analyzed by One Way ANOVA (Analysis of Varian). If there was a significant difference between treatments, proceed with DMRT at the 5% test level.

RESULTS AND DISCUSSION

Physiological character is closely related to the growth and productivity of its environment. The relationship shows that the physiological character formed can support plant growth needs with a larger sink so that the productivity of a plant can be adequately achieved. The ability of the source and sink will determine the potential yield of plants. The source is estimated as the total available energy and carbohydrates derived from the photosynthesis process after flowering and accumulation before flowering (Nurchayati et al., 2019). The sink results from photosynthesis stored as food reserves (Mastur 2015). Environmental factors also have a core role in influencing the formation of physiological characters. The environment plays a role in influencing plant behavior to regulate physiological processes to achieve comparable conditions between the environment and plant internals (Soverda 2012).

The phytochemical content in *E. palmifolia* plants has a vital role in plants defending themselves from stress, both drought and light stress. In addition, one of the secondary metabolites produced by plants is to defend themselves from unfavorable environmental conditions such as temperature, climate, pests, and plant diseases (Dwidjoseputro 1992).

Number of leaves

The number of leaves of *E. palmifolia* increased in the overall treatment for 30 days. The given interaction triggers the *E. palmifolia* plant to maintain the turgidity of plant cells, which impacts cell enlargement, stomata opening, and protoplasm formation. According to Felania (2017), water contributes to the turgidity of plant cells by acting as a constituent of protoplasmic cells and regulating plant temperature. Therefore, the increase in the relative growth of the number of leaves on *E. palmifolia* plants was triggered by the fact that the given water aided in the formation of protoplasmic cells, which could result in an increase in the formation of leaf organs on *E. palmifolia* plants subjected to all treatments. Additionally, the treatment interactions carry out photosynthesis, which results in the formation of tuber weight, which is useful for providing nutrients for growth (Figure 1).

The results of variance (Table 2) on the relative growth of the number of leaves of *E. palmifolia* showed that the overall effect of the treatments given was not significantly different. The relative growth of the number of effective leaves on 50% water availability and 50% light intensity treatment was 31.20 strands. This treatment resulted in fewer leaves because the chlorophyll in the leaves of *E. palmifolia* could absorb excess light, causing damage to the center of the chloroplast and resulting in decreased photosynthesis. The decrease in photosynthate production, especially the formation of vegetative organs, will also be hampered. According to Shao et al. (2014), plants exposed to excessive light damage photosynthetic equipment, particularly chloroplasts, resulting in decreased photosynthetic activity.

The combination given affects the response of *E. palmifolia* plants to drought stress which closes stomata to slow down water loss through the transpiration process. The slowing of water loss causes a tendency to produce materials (polysaccharides, lignins, proteins, lipids, amino acids, and other elements) widely used for canopy production. According to Mebrahtu et al. (2018), in the presence of water stress, the process of stomata closure to promote slow transpiration may occur in shallot plants. Plants directly suited to reward will respond by allocating dry matter for crown growth (Grime 1979).

Table 2. Relative growth of the number of the leaf of *E. palmifolia* after treatment with water availability and light intensity for 1 month (blades)

Treatment		Result
Water availability (%)	**Light intensity (%)**	**(blade)**
50	100	28.40
75	100	25.40
100	100	28.60
50	50	31.20
75	50	29.20
100	50	30.60

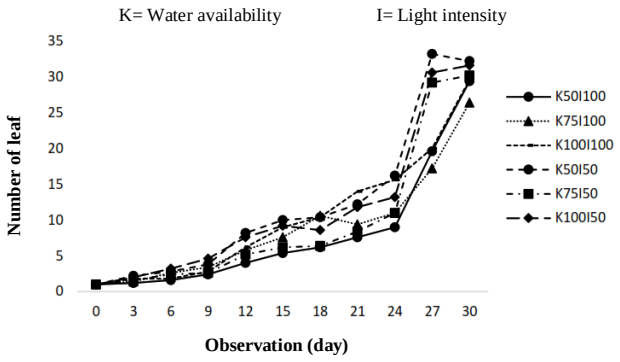

Figure 1. Daily leaf number growth of *E. palmifolia* after treatment with various light intensity and water availability combinations

The number of leaves in the formation correlates with the value of the root crown ratio. In addition, 50% water availability helps roots maximize nutrient absorption into the media to absorb the nutrients needed by *E. palmifolia* plants to make the necessary dry matter, and carotenoids become part of *E. palmifolia* plants to protect PSII from ROS during the process.

Leaf width

On *E. palmifolia* plants, the relative growth of leaf width increased from day 0 to day 18 and lowered from day 18 to day 30. When the water conditions in the planting media and the roots of *E. palmifolia* were reduced, photosynthate synthesis decreased, resulting in a gradual increase in leaf width. According to Bizuneh (2019), eliminating water leads the onion to grow until the root system's available water is fully depleted. All available nutrients generate photosynthate during the early phase of plant growth, which is later consumed and utilized by *E. palmifolia* plants to form new vegetative organs (Figure 2).

The variance analysis (Table 3) reveals a significant difference between water and light availability provision and the relative growth of leaves on *E. palmifolia* plants. The combination of 75% water and 50% light intensity resulted in the highest average value of 0.28 cm for *E. palmifolia* leaf width. The range of used treatments increased leaf surface area. The cells on the surface of the leaves grow more rapidly and expand more significantly as a result of maximizing light capture in shaded conditions for photosynthesis. According to Semida et al. (2017), its leaf surface increases when a plant is given shade. Due to photosynthesis, shade enables cells to grow in size and quantity. The best mixture facilitates carbon uptake by Dayak onion plants in *E. palmifolia* plants. As a result, *E. palmifolia* plants are capable of efficient photosynthesis, which enables the creation of vegetative organs to occur optimally. Shi et al. (2018) report that dryness can impede plant growth by lowering water loss and carbon assimilation. The interaction of the two (light intensity and water availability) leads to a photosynthetic cycle that generates, assimilates, and consumes plants to increase the size of *E. palmifolia* plant cells (cells that play a role in forming leaf width). Shade improves the chloroplasts' ability to capture light and assimilate it, which is advantageous for developing *E. palmifolia* plant cells (cells that support the formation of leaf width). Carotenoids have a critical function in the development of leaf width. Carotenoids absorb light that chlorophyll cannot absorb in darkened situations, speeding up the photosynthesis process. The condition of 75% water availability contributes to providing nutrients and light to *E. palmifolia* plants.

Leaf length

The graph depicts the relative increase in leaf length (strands) following the comprehensive treatment. The rise shows that *E. palmifolia* plants are becoming more tolerant of the overall treatment. When sufficient nutrients are available, photosynthesis occurs efficiently, and the produced photosynthate is also sufficient for leaf development. According to Manan and Machfudz (2015), optimal water availability is intimately tied to how plants absorb nutrients during their metabolic processes. Therefore, plants increase their leaf area in response to increased water availability. Furthermore, the combination of treatments resulted in an additional tolerance in increased chlorophyll content, indicating that this *E. palmifolia* plant got additional light energy that was subsequently employed optimally to generate photosynthate, specifically leaf length. According to Niinemets (2010), the low-light tolerant plant species in shadowed situations increase their chlorophyll content more than they do in full-light conditions (without shade) (Figure 3).

The variance analysis revealed that the effect of the combined treatment of water availability and light intensity on the relative leaf length growth of *E. palmifolia* plants was not substantially different (Table 4). Bozkurt and Keskin (2018) also found that the amount of water applied to cucumber plants had no significant effect on leaf length increase. Treatment with 75% water availability and 50% light intensity yielded a yield of 1.80 cm. The combination of these treatments was sufficient for *E. palmifolia* plants to avoid over-saturation due to the enormous volume of stagnant water in the root zone, allowing the *E. palmifolia* root system to function normally and grow rapidly. According to Boskurt and Keskin (2018), plants grown under stagnant water conditions could not perform regular respiration, consequently impeding plant growth.

Table 3. Relative growth of leaf width of *E. palmifolia* after treatment with water availability and light intensity for one month (cm)

Treatment		Result (cm)
Water availability (%)	Light intensity (%)	
50	100	0.05^{ab}
75	100	- 0.25^{a}
100	100	0.08^{ab}
50	50	0.03^{ab}
75	50	0.28^{b}
100	50	0.14^{b}

Note: the numbers followed by the same letter are not significantly different at the 5% DMRT test level

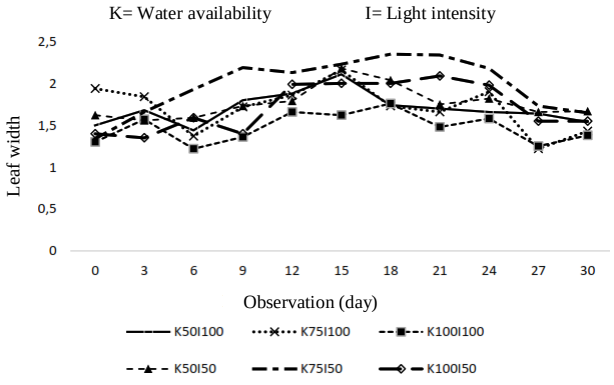

Figure 2. The relative growth of daily leaf width of *E. palmifolia* after the treatment of several combinations of light intensity and water availability

Optimal treatment can help reduce sun radiation exposure. Decreased exposure to solar radiation causes total chlorophyll (a and b) to be protected from excess damage (chlorophyll table) so that the formation of leaf organs can be optimal. Semida et al. (2017) stated that the shading effect could reduce radiation exposure and temperature around *E. palmifolia* plants. Lettuce that grew in the shade had a higher content of chlorophyll a and b than lettuce that was not exposed to shade. The shading effect also allows carotenoids to maximize light capture, which chlorophyll does not.

Midrib height

All treatments increased the relative growth of midrib. Even when subjected to various treatments, *E. palmifolia* plants exhibit great production and resistance to the creation of vegetative organs used for life support. The availability of water and the amount of light could signal that there are controls in place that promote the activity of photosynthesizing, which is required for plants to survive (Figure 4).

The variance analysis results (Table 5) show that when water availability and light intensity were combined, there was no significant effect on the relative development of midrib height in *E. palmifolia* plants. Moreover, 75% water availability and 100% light intensity were the ideal treatment for *E. palmifolia* plants to retain water potential and increase physiological and metabolic activities while maintaining a 7.50 cm yield. The interplay of the treatments aids in providing oxygen and carbon dioxide required for proper respiratory activity, ensuring that *E. palmifolia* plants continue to perform physiological and metabolic functions. According to Rachmawati and Retnaningrum (2013), when plants are submerged in water, their oxygen and carbon dioxide supply is lowered, interfering with photosynthesis and respiration.

The physiological process accelerated the growth of the midrib of *E. palmifolia* under conditions of 75% water availability and 100% light intensity. According to Sopandie (2014), light is involved in various physiological activities in plants, including photosynthesis, respiration, nutrition and assimilation transfer, growth and development, leaf opening and closure, and plant movement. When exposed to full sunshine intensity, the height of the midrib increased but was not accompanied by an increase in leaf number or width. Furthermore, it was determined that plants that received complete light treatment were more effective at stimulating midrib development acceleration than plants that received shade treatment. However, according to Hamdani et al. (2018), providing excessive shade to potato plants decreases growth rates. It is due to the low efficiency of photosynthesis caused by reduced CO_2, inhibited by a decrease in light intensity.

Table 4. Relative growth of *E. palmifolia* leaf length after treatment with water availability and light intensity for one month (cm)

Treatment		Result (cm)
Water availability (%)	Light intensity (%)	
50	100	1.24
75	100	1.04
100	100	1.48
50	50	1.07
75	50	1.80
100	50	1.56

Table 5. Relative growth of midrib height of *E. palmifolia* after treatment with water availability and light intensity for one month

Treatment		Result (cm)
Water availability (%)	Light intensity (%)	
50	100	5.51
75	100	7.50
100	100	2.88
50	50	3.87
75	50	3.94
100	50	4.83

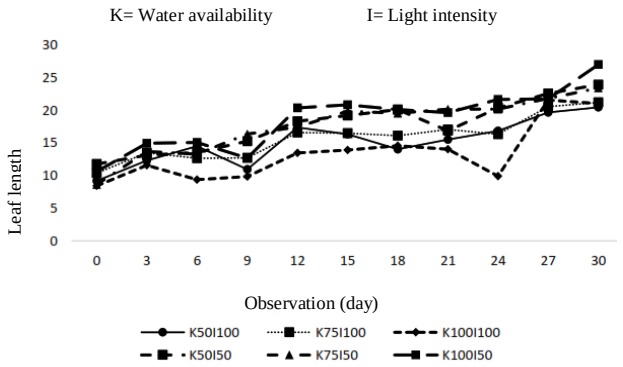

Figure 3. After treatment, the daily leaf length of *E. palmifolia* with several combinations of light intensity and water availability

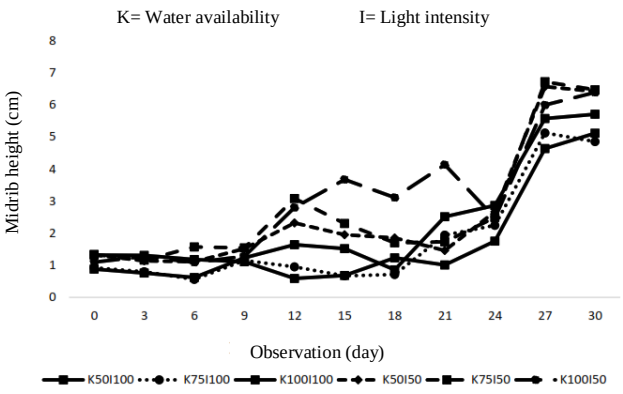

Figure 4. Daily midrib height of *E. palmifolia* after treatment with several combinations of light intensity and water availability

The wet weight of 22.50 grams (wet weight table) supported optimal growth of the midrib. The correlation between midrib height and wet weight indicates that potassium (a compound stored in *E. palmifolia* tubers and one of which affects the wet weight of tubers) is one of the nutrients absorbed by plants with tubers (Woldetsadik 2003). Potassium regulates the translocation of newly formed carbohydrates (Akhtar et al. 2002) and accelerates plant tissue growth (Hakim et al. 1986) so that the height of the midrib can be formed optimally.

Flowering speed

The study discovered that the interaction effect of water availability and light intensity on the rate of emergence of *E. palmifolia* flowers was not substantially different (Table 6). On the 26th day, the combination of water availability treatment and 100% light intensity produced optimal results for the flowering speed of *E. palmifolia* plants. The E. palmifolia plant completed its life cycle on that day by transitioning from the vegetative to the generative phase. This change happened due to the *E. palmifolia* plant utilizing certain nutrients (carbohydrates) to hasten flowering before waterlogging stress and extreme light intensity. In species that are not resistant to flooding, the interplay of treatment with water availability and 100% light intensity results in inundation and excess radiation, which can hinder the commencement of flower buds, flower blooms, fruit set, and fruit enlargement (Ezin et al. 2010). On the flooding stress, the carbohydrate content produced by photosynthesis drops. According to Ye et al. (2018), high waterlogging decreases carbohydrate content in *Arundinella anomala* plants. In contrast, it is well established that carbohydrates function in the flowering process (Fauzi et al., 2017).

The treatment interaction (K100I100) affected the plant's ability to survive in the presence of drought. Drought stress induces resistance in *E. palmifolia* plants. Therefore, *E. palmifolia* plants are categorized as escape plants, which means they finish their life cycle before being subjected to severe drought stress. Typically, this is accomplished by accelerating flowering and fruiting (Lestari 2006).

The considerable chlorophyll content combined with 100% light intensity optimally created carbohydrates, a necessary component for flower formation in *E. palmifolia* plants (chlorophyll table). As a significant element produced, Carbohydrates are kept as food reserves, and some are used to expedite the flowering process by *E. palmifolia* plants.

Amount of flower

The graph of the relative growth of flower rates increased from the 24th day to the 30th day. The *E. palmifolia* plants completed their vegetative phase on the 24th day and entered the generative phase on the same day. Flowering on the 24th day is influenced by the availability of water, light conditions, or the interaction of the two. The treatment given to *E. palmifolia* plants indicated they completed their reproductive cycle quickly on the 24th day by efficiently using and storing food reserves before the more severe drought and light stress. Barnabas et al. (2008) stated that this type of escape strategy is a strategy for plants to overcome drought by completing their reproductive cycle before severe drought stress occurs. Through a short life cycle, higher growth rate, efficient storage, and use of food reserves.

Research on the number of flowers showed that the effect of treatment on water availability and light intensity was not significantly different on the number of flowers. Treatment of 75% water availability and 50% light intensity showed the optimal interaction value in influencing the number of flowers compared to treatments with as many as 6.20 strands (Table 7). The interactions that occur produce products in the form of high carbohydrates to support flower initiation considering that flowering requires abundant energy. Increased carbohydrate status at the time of bud will stimulate the flowering process. These results follow the research of Pingping et al. (2017) on *A. carambola* plants, who reported that plants subjected to drought stress gave faster flowering results. Drought stress stimulated carbohydrate accumulation to initiate flower buds of *A. carambola*. Fauzi et al. (2017) also confirmed that in *Mangifera indica*, the canopy conditions with high carbohydrate accumulation supported the initiation of flowering, of course, in conditions that supported flowering. A supportive environment, such as sufficient water and light, stimulates carbohydrate accumulation in the shoots during the late vegetative phase to increase flower bud formation.

Table 6. The *E. palmifolia* flower emergence speed after treatment with variations in light intensity and water availability (days)

Treatment		Result (day)
Water availability (%)	Light intensity (%)	
50	100	26.67
75	100	27.00
100	100	26.00
50	50	27.00
75	50	27.00
100	50	27.00

Table 7. The number of *E. palmifolia* flowers after treatment with water availability and light intensity for one month (strands)

Treatment		Result (blade)
Water availability (%)	Light intensity (%)	
50	100	5.40
75	100	1.40
100	100	2.80
50	50	3.60
75	50	6.20
100	50	3.40

The optimal treatment altered the red light (Pr) received by *E. palmifolia* plants and converted it to long red light (Pfr) (660 nm) to accelerate generative development in *E. palmifolia* plants. According to Utami (2016), the generative phase in short-day plants begins with phytochromes receiving red light (Pr) and converting it to long red light (Pfr). Phytochromes are homodimers and polypeptide groups in *E. palmifolia*, each containing a prosthetic group called a chromophore. The phytochrome is physiologically affected by the light-absorbing chromophore. Phytochrome 2 is more active when plants are exposed to strong light. Pf and Pfr are formed from Phytochrome 2. Pfr is not generated in the absence of light. If Pfr exceeds Pr, apical dominance is lost, and the plant is induced into the generative phase. Phytochromes are found in the nucleus and throughout the cytoplasm of the cell. Shade circumstances maintain a healthy equilibrium between pf and pfr, producing good and optimal flower production.

Additionally, the quantity of blooms is proportional to the amount of carotenoids present. Carotenoids contribute to the absorption of light that is not absorbed by chlorophyll. Thus, the photosynthetic process can optimize the production of carbohydrates for future usage as flowering substrates.

Stomata index

The treatment of water availability and light intensity on the stomata index of *E. palmifolia* plants gave significantly different effects (Table 8). 100% water availability and 50% light intensity resulted in the best stomata index compared to other treatments, 36.89 cm. *E. palmifolia* plants responded by increasing the number of stomata on the epidermal surface. An increase in the stomata index indicates that the *E. palmifolia* plant is optimal in carrying out photosynthesis or metabolism and indicates that the plant is still surviving, which is indicated by the normal process of water loss and increased net CO_2 uptake in the leaves so that the photosynthesis process can take place optimally and increase productivity. According to Subantoro (2014), through his research, plants with drought stress environmental growth conditions will reduce the number of stomata, thereby reducing the rate of water loss followed by stomata closure and decreased net CO_2 uptake in leaves. MAPKs play an important role for *E. palmifolia* plants in maintaining drought stress conditions ranging from moderate to extreme levels. Plant resistance to drought stress due to MAPKs signaling activity. MAPKs consist of several sub-enzymes, namely MKK4/MKK5-MPK3/MPK6, which play an important role in controlling stomata development according to environmental conditions. The control carried out by several sub-enzymes of MAPKs is optimizing the ratio of differentiation between stomata and epidermal cells on the lower surface of the leaf according to environmental conditions. The lower the level of drought stress in an environment, the performance of MAPKs decreases. Research conducted using the plant Arabidopsis thaliana showed that MKK4/MKK5-MPK3/MPK6 had dual functions for stomata development and environmental stress response pathways. In stomata development, the function of this module is the transduction of endogenous and exogenous signals to target cells. Specifically, it optimizes plants' differentiation ratio of stomata and epidermal cells (Wang et al. 1998).

The interaction of the two treatments affects the genetic activity of SDD1 (Stomatal Density and Distribution). SDD1 is required for the beginning of stomata, the number of stomata, and the level of stomatal density per leaf area. Shade activates the SDD1 gene, resulting in the abaxial leaf's active production of stomata and epidermal cells. In addition, the SDD1 gene plays a role in developing stomata, producing protodermal cells that originate from stomata and epidermal cells (Berger and Thomas 2000).

Furthermore, the presence of a high stomata index in the presence of 100% water availability and 50% light intensity affects the gas exchange activity of *E. palmifolia* plants. As a result, *E. palmifolia* plants use the gas exchange to increase photosynthetic activity, which results in the assimilate formation process occurring properly. The assimilate is channeled to the phloem, one of which goes to the tubers to form the optimal wet weight under 100% water availability and 50% light intensity (wet weight table).

Wet and dry weight

The interaction impact of water availability and light intensity on the wet weight of *E. palmifolia* plants was not significantly different. The treatment with 75% water availability and 100% light intensity decreased the wet weight value for *E. palmifolia* plants. In contrast, the treatment with 100% water availability and 50% light intensity increased 29.77 grams in wet weight for *E. palmifolia* plants (Table 9). Ratri et al. (2015) showed that when turmeric plants were subjected to water stress, the intensity of the light had no discernible influence on fresh weight. It is believed that the plant has a tolerance for shade and water stress to maintain proper metabolic function. It does not affect the turgor pressure in plant cells, allowing plants to survive and avoid severe withering when light intensity is high and water availability in the soil drops. The absence of wilting shows that the *E. palmifolia* plant's primary productivity is in good health. A high wet weight indicates the presence of nutrients in tubers, roots, and other plant parts that *E. palmifolia* plants utilize to generate vegetative organs.

Table 8. Abaxial stomata index (lower leaf surface) of *E. palmifolia* after treatment with variations in water availability and light intensity for one month (cm)

Treatment		Result (cm)
Water availability (%)	Light intensity (%)	
50	100	32.52[a]
75	100	36.19[b]
100	100	36.30[b]
50	50	34.93[ab]
75	50	36.84[b]
100	50	36.89[b]

Note: The numbers followed by the same letter are not significantly different at the 5% DMRT test level

According to Table 10, the effect of the combination of water availability and light intensity on the dry weight of *E. palmifolia* plants was not significantly different. The combination of 100% water availability and 50% light intensity led to the dry weight of *E. palmifolia* plants aggregating to 7.56 grams. According to Ratri et al. (2015), the dry weight of turmeric plants had no discernible effect on shadow and drought stress. Therefore, it is believed that shading and water stress did not affect the photosynthetic activity or photosynthate translocation. Plants can also maintain a balance between water loss and absorption, thereby reducing the amount of water in plant cells.

Root canopy ratio

The interaction effect of water availability and light intensity on the root crown ratio of *E. palmifolia* was not significantly different (Table 9). Anggraini et al. (2015), in the study of black locust plants, stated that the root crown ratio increase occurred because the biomass allocation to the roots of *E. palmifolia* plants decreased and was shifted to crown growth. It is reinforced by Nejad et al. (2010), who noted that the water deficit decreased the root crown ratio in maize. In drought conditions, the allocation of biomass to roots is usually increased to be used as an effort to access water sources (Zlatev and Fernando 2012).

The optimal treatment interaction obtained played a role in increasing the root temperature (Table 11). An increase in root temperature causes an increase in root activity to absorb nutrients in the growing media. The application of treatment caused a decrease in temperature, which resulted in the allocation of biomass being directed towards the goal of crown growth. Increased root activity in absorbing nutrients would greatly impact photosynthate formation activity so that the canopy increase can be optimally carried out. Wilson (1988) stated that increasing root temperature increased root activity in absorbing nutrients so that it could accelerate root formation. Akmalia and Suharyanto (2017) said that the lowest light intensity would allocate the biomass towards the canopy so that the value of the root-crown ratio is smaller.

Chlorophyll content

The effect of light intensity and water availability on chlorophyll a, b, and total chlorophyll was not significantly different (Table 12). Chlorophyll a, b, and total were formed in *E. palmifolia* at a light intensity of 8.79 g/mL, 13.06 g/ml, and 21.85 g/mL, respectively, with 100% water availability. This condition implies that the *E. palmifolia* plant is still tolerant, allowing for good metabolism and photosynthesis. The treatment of water availability and 100% light intensity resulted in a blue color spectrum that accelerated the *E. palmifolia* plant's reaction to chlorophyll creation, resulting in increased chlorophyll production. The reaction results in chlorophyll synthesis from the glutamate molecule, which is deaminated to create α-ketoglutarate. α-ketoglutarate is transformed into the amino acid levulinate in the presence of sunlight via transaminases with the assistance of ATP and NADPH. The release of levulinate amino acids occurs sequentially into the water, NH_3, and CO_2, resulting in the formation of protoporphyrinogen. The chlorophyll synthesis process is continued with the production of Mg^+ to Mg^-protoporphyrin monomethylester. The presence of magnesium in protoporphyrin monomethylester affects chlorophyll's ability to absorb light. Chlorophyll a is formed when Mg-protoporphyrin monomethylester reacts with H+ ions. The creation of chlorophyll b begins with the synthesis of methyl oxidation in chlorophyll a. The enzyme CAO aids in the formation of chlorophyll b. (Chlorophyll and Oxygenase). This enzyme is responsible for transferring electrons from the methyl group in chlorophyll a to chlorophyll b (Tanaka et al. 2005) (Figure 5).

Table 9. Total wet weight of *E. palmifolia* after treatment with variations in water availability and light intensity for one month (grams)

Treatment		Result (gram)
Water availability (%)	Light intensity (%)	
50	100	26.44
75	100	22.50
100	100	26.17
50	50	26.36
75	50	25.42
100	50	29.77

Table 10. The total dry weight of *E. palmifolia* after treatment with variations in water availability and light intensity for one month (days)

Treatment		Result (gram)
Water availability (%)	Light intensity (%)	
50	100	6.52
75	100	6.12
100	100	6.68
50	50	6.64
75	50	6.63
100	50	7.56

Table 11. Root crown ratio of *E. palmifolia* after treatment with variations in water availability and light intensity for 1 month (cm)

Treatment		Result (cm)
Water availability (%)	Light intensity (%)	
50	100	1.65
75	100	0.96
100	100	1.84
50	50	1.23
75	50	1.33
100	50	1.18

Table 12. The content of chlorophyll a, b, and total of *E. palmifolia* after treatment with variations in water availability and light intensity for one month (g/mL)

Treatment		Chlorophyll a	Chlorophyll b	Total chlorophyll
Water availability (%)	Light intensity (%)			
50	100	6.14	9.84	15.98
75	100	5.05	9.07	14.12
100	100	8.79	13.06	21.85
50	50	7.26	8.78	16.04
75	50	8.29	9.68	17.97
100	50	6.92	9.85	16.80

The ideal treatment combination (100% water availability and 100% light intensity) (Table 12) enhanced the chloroplast's ability to avoid gene expression reprogramming, resulting in chlorosis or programmed cell death. Reduced H_2O_2 levels protect *E. palmifolia* plants from undergoing programmed cell death. According to Wei et al. (2015), the decrease in chlorophyll content under drought stress could result from ROS activity damaging the chloroplasts. ROS generation in chloroplasts in response to abiotic stress (such as drought) can also result in gene expression reprogramming, causing cells to enter chlorosis or programmed cell death (Lee et al. 2007).

The chlorophyll a, b, and total content in the treatment of water availability and 100% light intensity resulted in an increase in chlorophyll activity with the help of vitamin C, which was sufficient to reduce ROS levels in *E. palmifolia* plants, allowing photosynthesis to occur quickly and reliably. In addition, since chlorophyll b has a larger molecular weight (907.49 g/mol) than chlorophyll a (839.51 g/mol), it resulted in higher average content.

Carotenoid level

Based on the variance (Table 13), the interaction effect of water availability and light intensity on the carotenoid content of *E. palmifolia* plants was significantly different. The best treatment was 75% water availability and a 50% light intensity of 39.70 g/mL. This treatment was able to increase the value of the best carotenoid levels. The optimal interaction affects the activity of carotenoid formation and the extension of the antenna for light absorption. The function of carotenoids is to give plants the ability to reduce singlet oxygen (oxygen which has high energy and is more reactive to organic compounds) PSII which, if left unchecked, will trigger oxidative reactions that can damage the PSII reaction center complex and the core of PSII in the photosynthesis process. In addition, when the carotenoid content is high, there is a reduction in the content of ROS (Reactive Oxygen Species) which, when high, causes cell and tissue damage. According to Van Gorkom and Schelvis (1993), the carotenoids in the PSII reaction center in photosynthesis reduce singlet oxygen, which protects against oxidative damage. Uarrota's (2018) research also corroborates that carotenoids play an important role in converting singlet oxygen that can damage PSII into less reactive triplet oxygen.

The best treatment produced helps the light-harvesting activity increase due to the antenna activity performance of the carotenoids to increase energy in light absorption. Carotenoids help plants expand the light capture area, which will later function for the smooth process of photosynthesis. It can be seen that the result of photosynthesis involving carotenoids is flower formation which occurs more quickly in *E. palmifolia* when it is given shade treatment (Table 7). Carotenoids accelerate flowering and give color to flowers. Strazlka et al. (2003) suggested that carotenoids have an impact on accelerating the formation and coloring of flowers. Strazlka et al. (2003) stated that carotenoids could act as energetic antennae under low light conditions, harvesting light at wavelengths not absorbed by chlorophyll and transferring excited electrons to the phytochemical reaction center. Under these conditions, carotenoids play a role in expanding the absorption of light in photosynthetic activity.

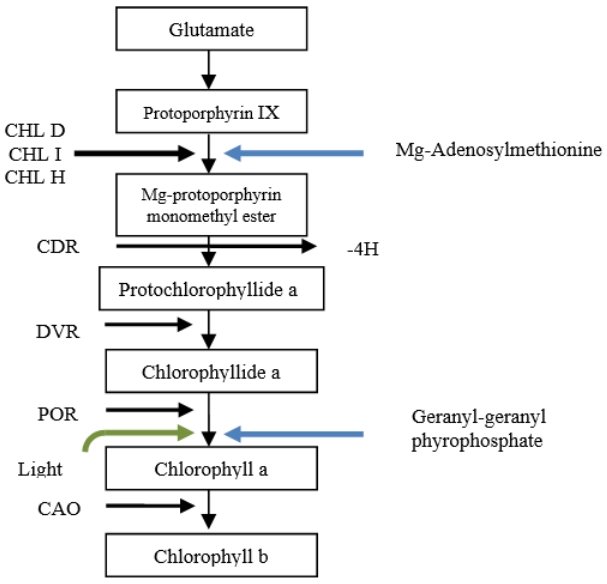

Figure 5. Mechanism of biosynthesis of chlorophyll a and b

Vitamin content

The linear regression line equation for ascorbic acid absorption is Y = 0.0004x + 0.0662 with an R2 value of 0.6773. The range of data obtained from validating the ascorbic acid level test method demonstrates a linear response. The test's regression coefficient was 0.6773 (67.73 %) (Figure 6).

This study (Table 14) demonstrated no significant difference in the effect of light intensity treatment and water availability on the vitamin C content of *E. palmifolia* plants. Vitamin C concentrations ranging from 0.06754 to 0.06760 g/mL in *E. palmifolia* tubers enabled *E. palmifolia* plants to survive under drought and light stress. Ascorbic acid protects *E. palmifolia* plants from various environmental stressors, including dryness and excessive light (Venkatesh and Se 2014).

The interaction between water availability and light intensity resulted in an optimal vitamin C concentration of 0.06760 g/mL in the 100% water availability and light intensity treatment (Table 14). These results are obtained because flooding causes hypoxia, a condition where the oxygen level (O^2) used for metabolic processes is too low. The oxygen content in the growing media is used by microorganisms more quickly than diffusion to the roots. Hypoxia triggers genetic activity in plants to prepare *E. palmifolia* plants to acclimatize to stressful environments. Hypoxia triggers ROS formation (Reactive Oxygen Species) as a signal that receives "information" from the stressed environment. The ROS produced in the *E. palmifolia* plant comes from the RBOH (Respiratory Burst Oxidase Homolog)/NADPH oxidase system as an important protein that produces ROS that functions as a signal for regulating growth, development, and stress response. If excessive, ROS produced by *E. palmifolia* plants will damage cells and tissues in *E. palmifolia* plants.

The coordination between RBOH, ROS, and ascorbic acid produced was able to carry out aerobic respiration without significant damage to plant cells and tissues due to inundation stress. Shasidaran et al. (2018) stated that during floods/excessive puddles, a signaling process occurs in the form of ROS (Reactive Oxygen Species) induction via RBOH (Respiratory Burst Oxygen Homolog). Therefore, to provide stimulation so plants can acclimate to stagnant conditions, antioxidants in ascorbic acid play an important role in controlling ROS so that cells and tissues during the acclimatization process to inundation stress are not damaged (Ullah et al., 2017).

The interactions that occur (Table 14) affect *E. palmifolia* plants' genetic activity. According to Massot et al. (2013), the activity of APX (ascorbate peroxidase) in symplast has about the same ability to regulate ascorbic acid synthesis. Moreover, GME, GPP1, and GLDH can synthesize ascorbic acid, which is greatly influenced by the quantity and quality of light. Full light conditions create an atmosphere with a temperature that is more conducive to enhancing the activity of the three genes. According to Massot et al. (2013), there was no discernible change in the ascorbic acid concentration of shaded and unshaded plants. This situation was created by a little rise in ascorbic acid level in the shade, induced by modestly enhanced expression of the GME, GPP1, and GLDH genes at 120C. The effective vitamin C content in the treatment of water availability and 100% light intensity suggested an interaction that resulted in a vitamin C content sufficient to lower ROS levels in *E. palmifolia* plants.

Glucose has a critical role as an energy source, a carbon supply, and a signaling molecule that regulates gene expression during the formation of secondary metabolites. Secondary metabolites can be formed in response to carbon sources, and secondary metabolites can be maintained at high carbohydrate concentrations. Carbohydrates influence the synthesis of secondary metabolites in plant cells via glycolysis and the Krebs cycle. The produced glucose will subsequently enter the vitamin C manufacturing process via the D-glucuronic acid and L-gulonic acid pathways, which will convert it to ascorbic acid. However, the phases of vitamin C production via the D-glucuronic and L-gulonic acid routes begin with the conversion of glucose-6-phosphate to glucose-1-phosphate and end with the formation of ascorbic acid, do not always proceed in the same direction or the same order. Because of D-glucuronate and L-gulonate, there are additional phases, one of which is an oxidation process, which results in vitamin C degradation. Oxidation is a chemical reaction in which a molecule, an atom, or an ion releases electrons (Syefanis et al., 2019).

Table 13. Carotenoid content of *E. palmifolia* after treatment with variations in water availability and light intensity for one month (g/mL)

Treatment		Result (g/mL)
Water availability (%)	Light intensity (%)	
50	100	19.24[ab]
75	100	7.39[a]
100	100	28.19[ab]
50	50	38.87[b]
75	50	39.70[b]
100	50	21.49[ab]

Note: the numbers followed by the same letter are not significantly different at the 5% DMRT test level

Table 14. After treatment, the vitamin C content of *E. palmifolia* with variations in water availability and light intensity for one month (g/mL)

Treatment		Result (g/mL)
Water availability (%)	Light intensity (%)	
50	100	0.06756
75	100	0.06754
100	100	0.06760
50	50	0.06756
75	50	0.06758
100	50	0.06758

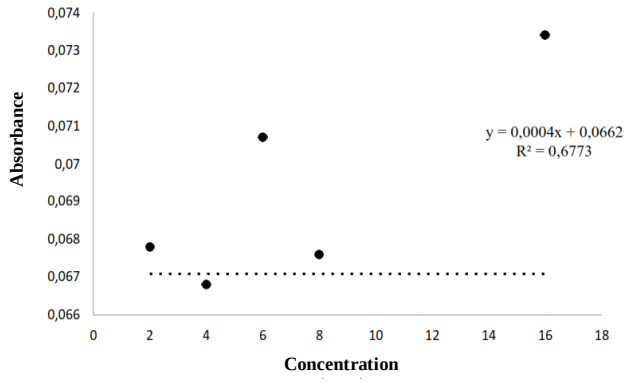

Figure 6. Graph of standard curve of vitamin C solution

Table 15. The flavonoid content of *E. palmifolia* after treatment with variations in water availability and light intensity for one month (g/mL)

Treatment		Result (g/mL)
Water availability (%)	Light intensity (%)	
50	100	2.517×10^{-1}
75	100	2.500×10^{-1}
100	100	2.515×10^{-1}
50	50	2.505×10^{-1}
75	50	2.508×10^{-1}
100	50	2.515×10^{-1}

Flavonoid content

The analysis of variance revealed that the combined effect of water availability and light intensity on the flavonoid content of *E. palmifolia* plants was not substantially different. Compared to other treatments, the combination of 50% water availability and 100% light intensity (Table 15) offered effective treatment with a yield of $2,517 \times 10^{-1}$ g/mL. It demonstrates that the *E. palmifolia* plant has an efficient photosynthetic and metabolic mechanism. Flavonoids produced in adequate amounts during drought and light stress could minimize the quantities of free radicals that can damage cell and tissue organelles, impairing the life of *E. palmifolia* plants. The Sufficient flavonoids enable Arabidopsis thaliana plants to survive by boosting their ability to minimize free radical levels (Shojaie et al. 2016). Drought stress affects increasing the flavonoid content in the tubers and roots of *E. palmifolia*, which plays a critical role in the control of ROS (Reactive Oxygen Species), which, if allowed to accumulate, will damage the cell organelles and plant tissues of *E. palmifolia*, impairing metabolic processes in *E. palmifolia* plants. According to Brown et al. (1998), plants generate flavonoids to combat oxidative damage to cells and tissues during drought, thereby reducing the generation of excess reactive oxygen species (ROS). Therefore, sufficient flavonoid content can aid in the response of plants to drought stress. Under drought stress, *E. palmifolia* plants execute phenolic (flavonoid) production more efficiently than under normal climatic conditions. Drought stress inhibits flavonoid biosynthesis pathways, hence protecting plants from harmful impacts. The function of PAL enzymes (enzymes involved in membrane integrity and canopy formation) and CHS in the phenylpropanoid pathway (the primary pathway for the synthesis of phenolic compounds (flavonoids)) is critical for the continuance of flavonoid formation under stressful circumstances. Under stressful conditions, a rise in the enzymes PAL (Phenylalanine ammonia-lyase) and CHS (Chalcone synthase) encouraged an increase in the synthesis of flavonoids, such as kaempferol and quercetin.

Additionally, an increasing the transcript level of genes encoding essential enzymes in phenolic biosyntheses such as F3H, CHI, FLS (Flavono Synthase), and FGT occurred in parallel with the increase in both enzymes (Flavonol glycosyltransferase). Both substances are categorized as flavonoids, which help prevent the oxidative stress response from increasing (Kurepa et al., 2019). According to Sharma et al. (2019), plants that grow under extreme stress have a greater capacity for flavonoid biosynthesis than plants that grow under normal conditions. The biosynthesis of phenolics, particularly flavonoids, enhanced PAL and CHS enzymes under stress conditions. Increases in these enzymes level were also accompanied by increases in the levels of gene transcripts encoding essential enzymes involved in phenolic biosynthesis, including F3H, CHI, FLS (Flavono Synthase), and FGT (Flavonol glycosyltransferase). The treatment with 50% water availability and 100% light intensity induced the roots of *E. palmifolia* plants to produce more primary photosynthate; this primary photosynthate was then employed as a substrate for *E. palmifolia* to produce flavonoids in the epidermal cells. *E. palmifolia* plants utilize antioxidants in the form of flavonoids to prevent excessive oxidative responses. It is consistent with the research of Warren et al. (2003), who found that increased light intensity increases the creation of primary photosynthate, and primary photosynthate produces phenolics. Antioxidants are believed to be created as a response to protect plants from oxidative stress. Numerous studies have demonstrated increased flavonoid content in various taxa that grow in bright sunshine rather than shade (Karimi et al., 2013).

The production of flavonoids begins with phenylalanine, which is formed when glucose is converted to pyruvic acid and then transferred to the acetoacetyl-CoA pathway via the Krebs cycle. Next, the acetoacetyl-CoA pathway generates phenylalanine, which is converted into flavonoid compounds such as quercetin and kaempferol via the phenylpropanoid biosynthesis pathway (Penuelas and Marc 1998).

This study can draw the following conclusions: (i) The interaction of light intensity treatment and water availability substantially affected leaf width, stomata index, and carotenoids. However, the quickest flowering time was 26 days in the treatment with 100% water availability and light intensity and was not substantially different in the other treatments. In addition, leaf quantity, length, midrib height, flower count, root crown ratio, wet and dry weight, chlorophyll content, vitamin C content, and flavonoids were not substantially different. (ii) The combination treatment of

75% water availability and 50% light intensity (K75I50) increased the leaf width, and carotenoid content of *E. palmifolia* plants the most. In contrast, the 100% water availability treatment and 50% light intensity (K100I50) combination treatment is the best treatment to increase the stomatal index.

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