

Morphophysiological Study of Kecapi (*Sandoricum koetjape* Merr.) Seedlings Against Different Artificial Light Spectra and Intensities

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Abstract

Kecapi (*Sandoricum koetjape* Merr.) is a tropical fruit species that belongs to the Meliaceae family. Kecapi trees require 5-7 years to produce fruit when grown from seeds, necessitating efforts to accelerate their growth, including through the modification of light. This research aimed to study kecapi's morphological, physiological, and anatomical responses to light spectra and intensities from light-emitting diodes. The experiment was designed using a two-factor, completely randomized design (CRD), namely LED light spectrum (white and purple) and light intensity (2 and 4 lights). The result showed that morphological and vegetative growth were significantly affected under the high-intensity purple LED treatment. The low-intensity purple LED treatment increased stomatal conductance and chlorophyll-b synthesis. The transpiration rate was highest under the high-intensity white LED treatment. Low-intensity white LED treatment only increased the abaxial stomatal aperture. As the intensity increased, it also increased N-Total content but decreased micronutrient levels in the leaves.

Keywords: chlorophyll, fructose, glucose, photosynthesis, stomata

Introduction

Indonesia, as a tropical country, has an abundant diversity of fruits. This diversity is reflected in variations in taste, shape, color, and aroma, including in the kecapi fruits (*Sandorium koetjape* Merr.). Kecapi is a local fruit with a sweet or slightly sour taste and belongs to the Meliaceae family (Tamin and Anggraini, 2017). Kecapi originated in Brunei,

Cambodia, India, Laos, Malaysia, Myanmar, the Philippines, Thailand and Vietnam. Kecapi was later introduced to Australia and Sri Lanka (Orwa et al., 2009). The nutritional content in 100 g of kecapi fruit includes energy of 100 kcal, 0.6 g protein, 0.1 g fat, 21.1 g carbohydrate, 1.6 g fiber, 7 mg calcium, 7 mg phosphorus, 0.8 mg iron, 340 mg sodium, 150 mg potassium, 5 µm carotene, 0.01 mg vitamin B1, 0.04 mg vitamin B2, 0.5 mg niacin, and 9.3 mg vitamin C (Lim, 2012). In the pharmaceutical field, kecapi fruits are used for reducing low-density lipoprotein levels, preventing diabetes, maintaining dental health, and preventing anemia and Alzheimer's (Taclawan and Pilarta, 2022). However, kecapi trees take 5-7 years to bear fruit if they come from seeds (Orwa et al., 2009), so efforts are needed to accelerate their growth, one of which is through light factors.

Light is an important environmental signal because it affects plant morphology, physiology and anatomy. Light intensity is affected by differences in time, latitude, and season (Folta and Carvalho, 2015). Extreme light differences can cause plant stress and affect photosynthesis (Antal et al., 2013). It is important to understand how the plant responds to artificially regulated light by increasing or decreasing light intensity and certain types of spectrums (Yao et al., 2021), one of which is LED (light emitting diode) lights. The radiation from LED lights is lower, and power consumption is more efficient (Cavallaro and Muleo, 2022). The spectrum and energy of LEDs are similar to sunlight. Commercially, LEDs are widely applied in cultivating green vegetables, herbal plants, and potted flowers (Darko et al., 2014). LEDs can reduce energy use by 70%-80% and increase fruit crop production by 10%-20% (Bang and Kim, 2012).

Light produced by LEDs has a significant impact on changes in plant morphology and physiology. For example, blue LEDs can enhance the growth of stem morphology and leaf area index in lettuce (Snowden et al., 2016), and in perennial plants, they can increase morphological growth (Ma et al., 2021). On the physiological side, blue LED showed the best results in terms of stomatal conductance in tomato (Bian et al., 2018). Meanwhile, the effect of the red LED is to increase chlorophyll content by 36% and increase stomatal conductance by 62% in perennial plants (Ma et al., 2021). Then, white LEDs can promote wider and broader leaf growth in *Eustoma* (Roni et al. 2017), and increase the photosynthesis rate in tomatoes (Ma et al., 2021). The use of white LEDs can also trigger the formation of new shoots in plants and increase the rates of photosynthesis, stomatal conductance, transpiration, intracellular CO₂ concentration, and light received and absorbed in lyre seedlings (Defitrianida et al., 2023). In addition, lighting with a combination of red and blue LEDs on rubber seedlings can increase height, stem diameter, and chlorophyll content (Yao et al., 2021). Stomatal density was higher when leaves were exposed to mixed LED light than high-pressure sodium (HPS) (Palmitessa et al., 2021). Thus, using LEDs with various colors can provide different benefits for plant growth and physiology. This research aims to study kecapi's morphological, physiological, and anatomical responses to different light-emitting diode light spectra and intensities.

Materials and Methods

Planting, maintenance, and morphological observations and measurements were carried out in the screen house of Leuwikopo experimental station 6°33'45.15" N and 106°43'11.91" E at an altitude of 178.6 m above sea level, IPB Campus, Dramaga District, Bogor Regency, West Java, Indonesia. In contrast, physiological and anatomical analyses were

carried out at the Integrated Laboratory of the Seed Center, Department of Agronomy and Horticulture.

The average temperature and humidity were 27.9°C and 86.6% respectively. The minimum temperature in the screen house was 19°C, and the maximum was 39.9°C. Meanwhile, the minimum humidity was 41.6%, and the maximum was 99.9%. LED light was quantified with a Light Sensor Logger LI-1500 from before sunrise to sunset to measure the intensity, Irradiation flux, and PPFD value.

The study utilized a purple and white LED full-spectrum light source, a LI-1500 Light Sensor Logger, a LI-6800 Portable Photosynthesis System, a Multiskan Sky Microplate Spectrophotometer (Thermo Fisher), a K-FRGLQR D-Fructose/D-Glucose Assay Kit (Megazyme), and ethanol, methanol, and chloroform. Elitech GSP-6 Temperature & Humidity Data Logger, GLOTECH Centrifuge, CX-23 Olympus binocular microscope. The plant material used was 4-month-old kecapi seedlings, planted in November 2023, from seeds extracted from fruits collected in Curug District, Serang (6°11'45.9" S, 106°11'03.9" E). Seeds were collected from the yellow, over-ripe fruits (Figure 1).

Light Treatment

The experiment was designed using a two-factor, completely randomized design; the first factor is the LED spectrum (white and purple), and the second is light intensity (2 and 4 lights). This study used a control to compare the effects of using sunlight under a 60% screen house. LED irradiation was carried out every day for 21 hours, starting from 04.00 to 01.00 in the morning, based on a previous study on strawberries (Adrian, 2021). A black cloth was placed between the treatments. Observations made were morphological, physiological, and anatomical responses.

Maintenance included watering, fertilizing, and pest and disease control. Environmental conditions are

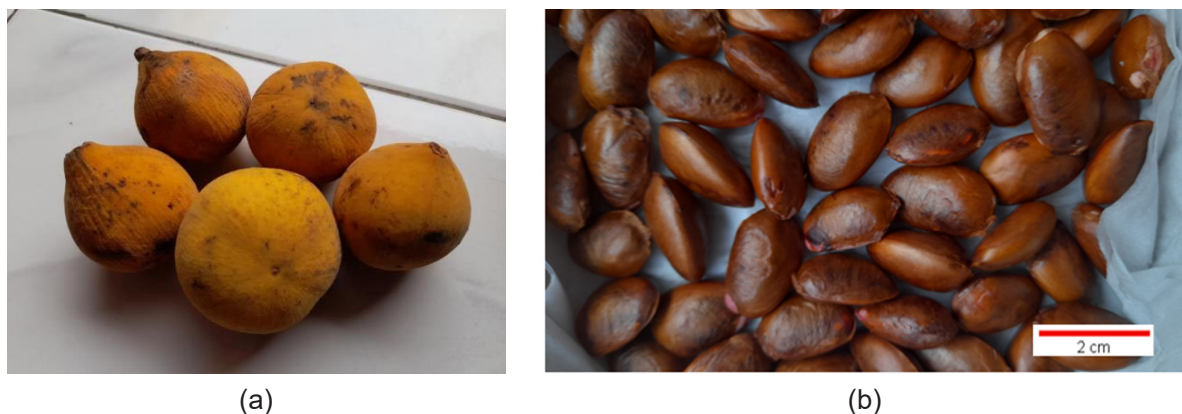


Figure 1. Kecapi overripe fruits (a) and seeds (b)

measured by temperature and humidity using an Elitech GSP-6 Temperature & Humidity Data Logger digital thermometer. Meanwhile, LED light conditions are quantified with the LI-1500 Light Sensor Logger every two hours before sunrise until sunset (05.00 - 19.00 WIB).

Morphological and Physiological Responses

Measurements were conducted on canopy diameter, chlorophyll, glucose, and fructose levels. Canopy diameter was measured horizontally and vertically on all kecap seedlings. Leaf chlorophyll a and b were measured monthly after LED application using a spectrophotometer (Warren, 2008). Absorbance values were read using a Multiskan Sky Microplate Spectrophotometer with 652 nm and 665 nm wavelengths. Leaf glucose and fructose was measured monthly after LED application, referring to Lanoue et al. (2019), using Megazyme's KFRGLQR (D-Fructose/D-Glucose Assay Kit) procedure via a Multiskan Sky Microplate Spectrophotometer at 340 nm wavelength.

Photosynthetic parameters, which include transpiration and stomatal conductance, were measured once a month after LED light treatments using the LI-6800 Portable Photosynthesis System.

Leaf mineral nutrient content, including macro and micronutrient content, was measured at the end of the study, 4 months after treatment. The samples used weighed ± 50 g.

Leaf Anatomy Measurement of leaf thickness and stomata was done once a month. Measurement of leaf thickness was performed using a microscope (Olympus Binocular Microscope Cx23) with a 100x objective lens magnification. Measurement of stomata in the form of stomatal density and a stomatal aperture on the leaf's abaxial and adaxial parts using a microscope with an objective lens magnification of 400x.

Data Analysis

Morphological, physiological, and anatomical data were analyzed with the F test using Statistical Analysis System (SAS) software and Microsoft Excel. If the interaction analysis was significantly different, further tests were carried out with the Duncan Multiple Range Test (DMRT) at $\alpha < 0.05$.

Results

The highest level of light intensity in the high-intensity purple LED treatment was 592.22 lux, followed by the high-intensity white LED treatment (584.63 lux), low-intensity purple LED (394.47 lux), low-intensity white LED (373.87 lux), and control (249.15 lux). This shows that the more lights are used, the brighter the light becomes, resulting in the same amount of radiation emitted. The highest Irradiation flux was found in the high-intensity purple LED treatment (26.43 W.m^{-2}), followed by the high-intensity white LED treatment (24.25 W.m^{-2}), the low-intensity purple LED (19.08 W.m^{-2}), the low-intensity white LED (18.18 W.m^{-2}), and the control (20.27 W.m^{-2}). PPFD (photosynthetic photon flux density) is the number of photons emitted per unit area and unit time ($\mu\text{mol.m}^{-2}.\text{s}^{-1}$) (Sato and Tsukada, 2017). The mean PPFD values of the low-intensity purple LED, high-intensity purple LED, low-intensity white LED, high-intensity white LED, and control treatments were $77 \mu\text{mol.m}^{-2}.\text{s}^{-1}$, $106 \mu\text{mol.m}^{-2}.\text{s}^{-1}$, $129 \mu\text{mol.m}^{-2}.\text{s}^{-1}$, $202 \mu\text{mol.m}^{-2}.\text{s}^{-1}$, and $76 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ (Figure 2), respectively. White LEDs are full-spectrum (Figure 3a) with higher blue and green spectral characteristics than violet LEDs. Blue light functions for cell elongation by increasing vertical growth, thus accelerating growth (Huché-Thélier et al., 2016). Meanwhile, purple LEDs (Figure 3b) are superior in the red spectrum. Red light functions for plant physiological improvements, such as increasing the rate of photosynthesis (Tsaballa et al., 2023).

Morphological Responses

Horizontal canopy expansion of the kecap treated with the high-intensity purple LED was 10.14% higher at 14 WAT and vertically was 8.41% higher than the control at 18 WAT (Figure 4).

Physiological Responses

Transpiration rate decreased at 3 months after treatment, but high-intensity white LED treatment increased from 3 to 4 months after treatment (Figure 5a). At the same time, stomatal conductance at 4 months after treatment increased significantly with the low-intensity purple LED treatment (Figure 5b). Figure 6 shows that the high intensity white LED treatment resulted in the highest chlorophyll a accumulation at 1 month after treatment by 15.30% compared to the control. However, in terms of chlorophyll b content, the control was not significantly different. At the end of observation (4 months after treatment), although not significantly different between treatments, the low-intensity purple LED treatment had the best accumulation compared to the control.

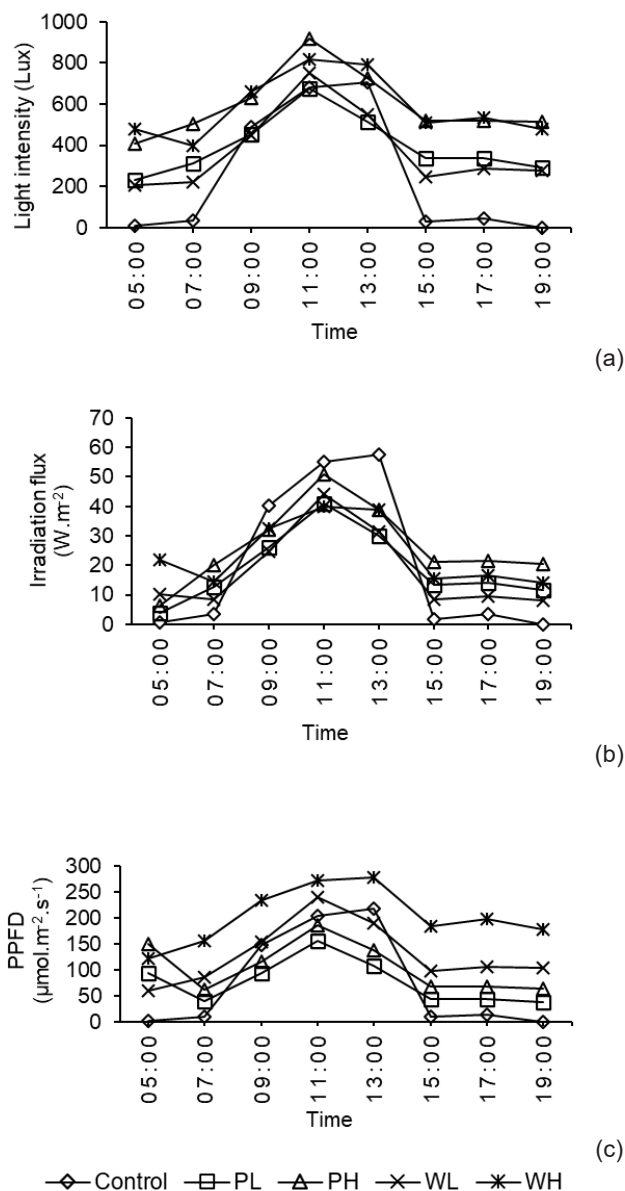


Figure 2. LED light intensity (a), irradiation flux (W.m^{-2}) (b), and PPFD ($\mu\text{mol.m}^{-2}.\text{s}^{-1}$) of each LED light spectrum (c). PH = high-intensity purple LED, WL = low-intensity white LED, PL = low-intensity purple LED, WH = high-intensity white LED.



Figure 3. Wavelength measurement of the light spectrum of (a) white LED, (b) purple LED using a light meter

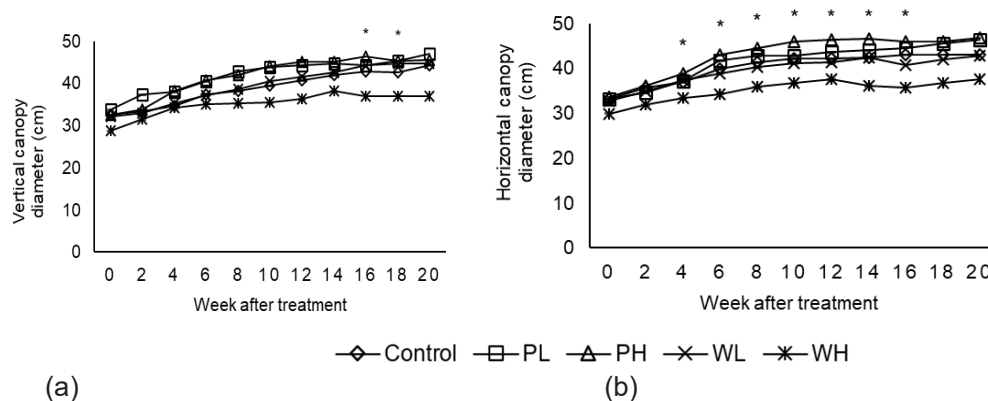


Figure 4. Growth of canopy diameter (a) horizontal, and (b) vertical on different LED light spectra and intensities. PH = high-intensity purple LED, WL = low-intensity white LED, PL = low-intensity purple LED, WH = high-intensity white LED. *= significant at $\alpha=0.05$ according to DMRT.

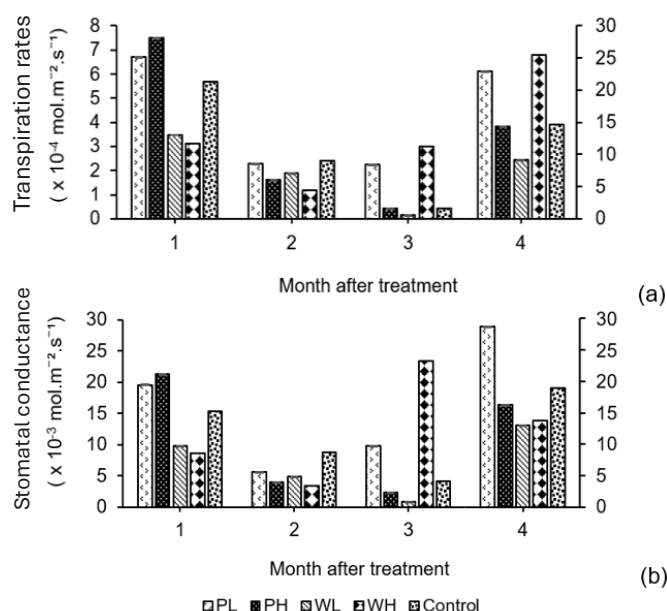


Figure 5. Transpiration rates (a) and stomatal conductance (b) at different LED light spectra and intensities. The right axis shows values 1, 2, and 3 months after treatment; the left axis shows values 4 months after treatment. PH = high-intensity purple LED, WL = low-intensity white LED, PL = low-intensity purple LED, WH = high-intensity white LED.

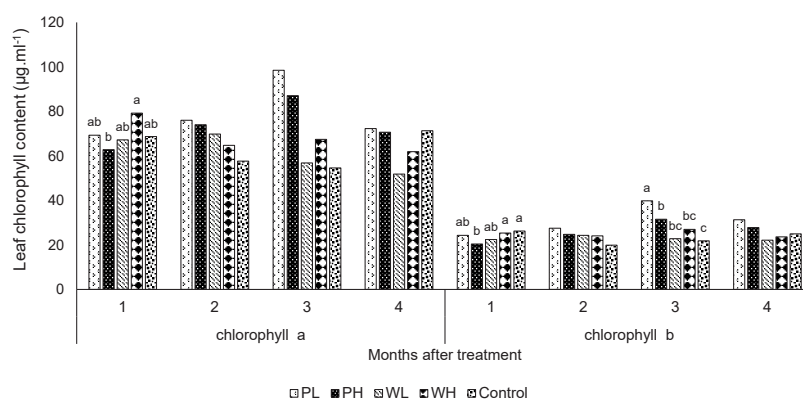


Figure 6. Leaf chlorophyll a and b content at different LED light spectra and intensities. PH = high-intensity purple LED, WL = low-intensity white LED, PL = low-intensity purple LED, WH = high-intensity white LED.

Figure 7 shows that the high-intensity white LED treatment accumulated the highest amount of glucose (78.41%) and fructose (16.59%) compared to the control at 3 months after treatment. However, these amounts then dropped the following month (4 months after treatment). Figure 8 shows that Ca, Mg, S, Fe, Zn, and B nutrients accumulated less (low-intensity purple LED, high-intensity purple LED, low-intensity white LED, and high-intensity white LED) compared to the control. However, N-total levels were higher as light intensity increased (high-intensity purple LED and high-intensity white LED). While in white LED (high-intensity white LED), P nutrient levels also increased when intensity was high, but at low intensity. In contrast, the low-intensity purple LED treatment produced the highest K nutrient levels.

Leaf Anatomy

The effect of different LED spectra and intensities on leaf thickness was not significantly different, although the high-intensity purple LED treatment had thicker leaves until the end of observation (Figure 9). The interaction of spectrum type and LED intensity had no significant effect on stomatal density. However, high-intensity purple LED and high-intensity white LED yielded superior results compared to other treatments, primarily due to the higher PPFD value of the treatment.

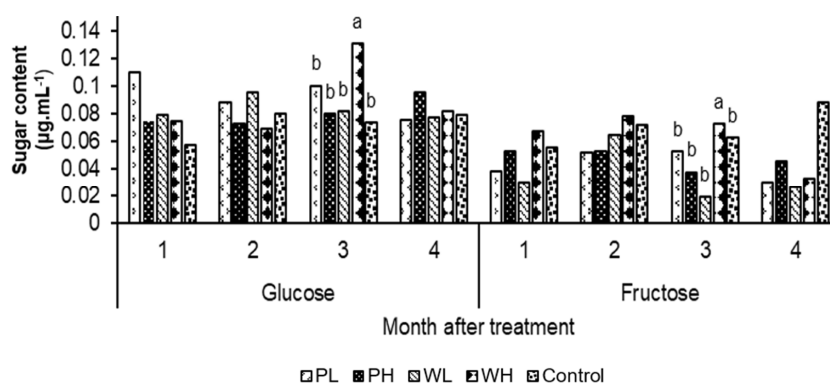


Figure 7. Leaf glucose and fructose content at different LED light spectra and intensities. PH = high-intensity purple LED, WL = low-intensity white LED, PL = low-intensity purple LED, WH = high-intensity white LED.

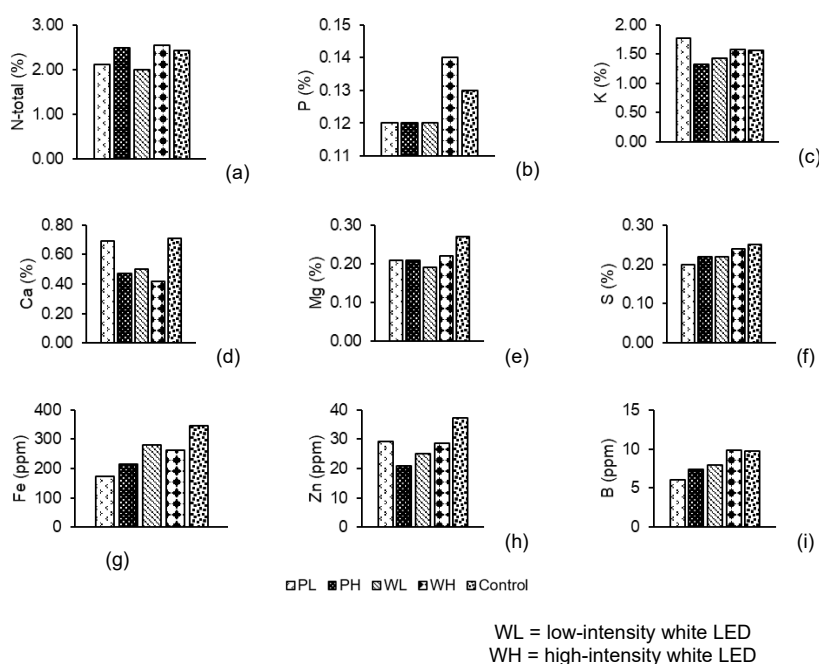


Figure 8. Leaf nutrient content (a) N, (b) P, (c) K, (d) Ca, (e) Mg, (f) S, (g) Cu, (h) Zn, (i) B against different LED light spectra and intensities. PH = high-intensity purple LED, WL = low-intensity white LED, PL = low-intensity purple LED, WH = high-intensity white LED.

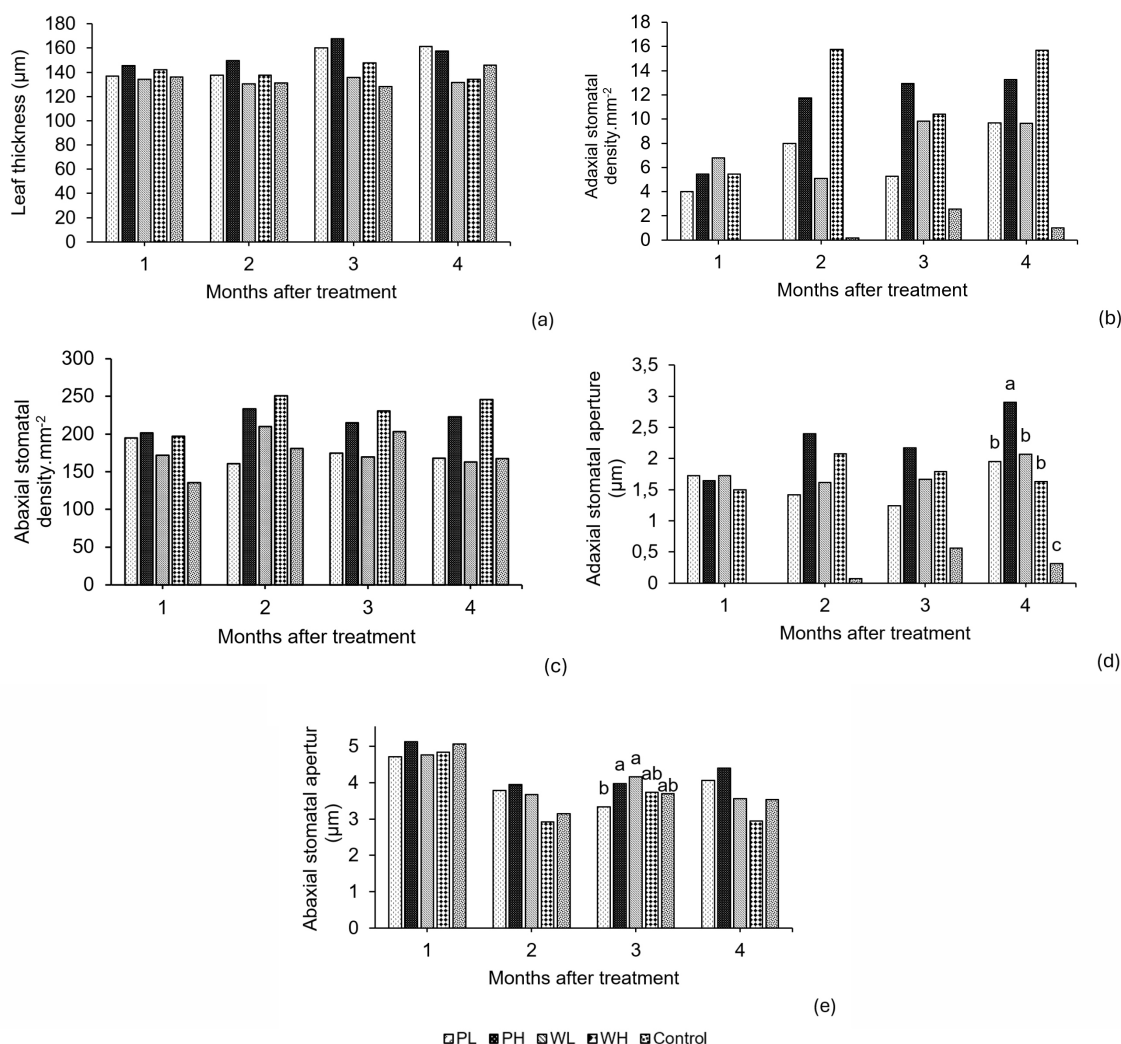


Figure 9. Kecapi leaf anatomy: leaf thickness (a), adaxial stomatal density (b), abaxial stomatal density (c), adaxial stomatal aperture (d), abaxial stomatal aperture (e). PH = high-intensity purple LED, WL = low-intensity white LED, PL = low-intensity purple LED, WH = high-intensity white LED.

Discussion

Morphological Responses

The addition of artificial light significantly increased vegetative growth, especially canopy diameter horizontally and vertically (Figure 2). The high-intensity purple LED treatment has a higher ratio of the red spectrum, causing an increase in leaf length (Dong et al., 2023). In addition, the red spectrum can absorb light optimally for chlorophyll and phytochrome synthesis (Jung et al., 2021). As time goes by, there is a decrease in leaf area, presumably the leaves have entered a senescent phase. The red spectrum can delay leaf senescence (Sakuraba, 2021). In apple, low light intensity delays growth and development (Choi et al., 2022).

Physiological Responses

Over time, the transpiration rate decreased, but the high-intensity white LED treatment increased from 3 months after treatment to 4 months after treatment (Figure 3a). Increasing intensity causes an increase in stomatal aperture (Feng et al., 2019), thus increasing the photosynthetic and transpiration rates. Meanwhile, stomatal conductance at 4 months after treatment increased dramatically in the low-intensity purple LED treatment (Figure 3b) likely due to differences in leaf structure and chloroplast accumulation in the mesophyll, as reported in Izzo et al. (2021).

Based on Figure 4, the highest accumulation of chlorophyll a in the high-intensity white LED treatment at 1 month after treatment was 15.30% compared to the control. Still, the control was not significantly

different in chlorophyll b content. Following previous research, the increase in light intensity in kiwi plants leads to a continuous increase in chlorophyll content (Xiaoying et al., 2022). At 3 months after treatment, low-intensity purple LED treatment significantly increased chlorophyll b synthesis by 82.46% compared to the control. Similar to the previous study, purple LED can maintain chlorophyll content in broccoli plants (Xie et al., 2022). The increase in chlorophyll a and b indicates a better photosynthetic system. These pigments are responsible for capturing light for photosynthesis by converting light energy into chemical energy, so the quality directly affects photosynthesis (Ye et al., 2017) which determines the formation of soluble sugars such as glucose and fructose (Yindeesuk et al., 2021).

Figure 5 shows that the high-intensity white LED treatment has the highest accumulation ability of glucose (78.41%) and fructose (16.59%) compared to the control at 3 months after treatment, then the following month decreased. White LED has a higher blue spectrum ratio that can encourage the translocation and utilization of soluble sugars in chloroplasts (Li et al., 2017). In addition, white LEDs are full-spectrum light; the combination of red, green, and blue spectra can increase fructose content in wheatgrass plants (Yindeesuk et al., 2021). The high-intensity purple LED treatment at 4 month after treatment showed better fructose accumulation than the previous month, although it did not significantly affect other treatments. This is because increasing light intensity increases the area of light absorption so that it can increase the dissolved sugar content due to increased photosynthetic activity (Feng et al., 2019),

Based on Figure 6, the accumulation of nutrients Ca, Mg, S, Fe, Zn, and B was lower (low-intensity purple LED, high-intensity purple LED, low-intensity white LED, and high-intensity white LED) than in the control. This is due to differences in irradiation duration (Son et al., 2018) and light intensity (Liu et al., 2020) between the LED and the control. However, N-total levels were higher with increasing light intensity (high-intensity purple LED and high-intensity white LED) similar to the previous study with an intensity of $450 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ (Song et al., 2020). P nutrient levels also increased when the high-intensity white LED treatment was applied to lettuce plants (Amoozgar et al., 2017). Meanwhile, the accumulation of K nutrient levels was highest in the low-intensity purple LED treatment compared to other treatments. This is because the red spectrum affects the metabolic pathways of plants, increasing the levels of mineral elements in the leaves through water absorption (Amoozgar et al., 2017).

Leaf Anatomy at Different Light Spectra and Intensities

The increase in leaf thickness is due to an increase in spongy, palisade, and grana tissues that develop better under high-intensity light (Yao et al., 2017). In addition, LED exposure increases mesophyll length in cucumber plants (Kowalczyk et al., 2022). More red light also increased leaf thickness and spongy and palisade tissue in tea plants (Zhang et al., 2023).

Stomatal density changes with longer duration of irradiation at 4 months after treatment (Figure 7). Stomatal density is related to the regulation of water and carbon assimilation (Palmitessa et al., 2021). Previous research reported that increasing intensity to $250 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ resulted in higher stomatal density in petunia (Sakhonwasee et al., 2017). Adaxial stomatal aperture increased significantly under the high-intensity purple LED treatment at 4 months after treatment. Treatments under low-intensity white LED and high-intensity purple LED significantly increased the abaxial stomatal aperture width by 12.64% and 7.63%, respectively, compared to the control at 3 months after treatment. The stomatal aperture, which functions in gas exchanges (Roni et al., 2017) indicates the plant's response to the light period (Sakhonwasee et al., 2017).

Conclusions

The addition of LEDs in the screen house affected the growth and development in morphology, physiology, and anatomy of lyre seedlings. Kecapi under high-intensity purple LED treatment, accumulates more fructose and total N and has an increased leaf thickness and wider stomatal aperture. Low-intensity purple LED treatment increased stomatal conductance, chlorophyll b synthesis, and nutrient K accumulation. Under high-intensity white LED, plants accumulate more glucose, synthesize more chlorophyll, and have more dense stomata and wider apertures. Extending the duration of irradiation and higher light intensity decreased micronutrient levels in the leaves.

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