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The Effect of Melatonin Application and Watering Frequency on the Growth of Cocoa Seedlings (*Theobroma cacao* L.)

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Abstract

Watering is one of the keys to cocoa plant growth, where proper watering enhances plant development, while infrequent watering can lead to drought stress. Melatonin can help mitigate the negative impacts caused by drought stress. Melatonin is a hormone that regulates various physiological processes and is believed to enhance plant tolerance to stress while stimulating growth. The purpose of this study is to investigate the effect of melatonin application on the growth of cocoa seedlings under different watering frequency. The study was arranged by randomized complete block design with a split-plot arrangement, consisting of two factors with three replications. The main plot represents watering frequency of 3 days, 6 days, and 9 days. The subplot represented melatonin concentrations of 0 μM, 50 μM, 100 μM, and 150 μM. Observational parameters included plant growth based on plant height, stem diameter, leaf number, and leaf area, as well as plant dry weight based on the dry weight of the shoot, roots, and total plant. The results showed that at the 3-day irrigation frequency, melatonin concentrations of 50-100 μM enhanced plant growth parameters including plant height, stem diameter, leaf area, as well as shoot dry weight and total plant dry weight. At the 6-day frequency, melatonin application up to 150 μM reduced growth and had no effect on total plant dry weight, but increased root dry weight. At the 9-day irrigation frequency with application of 150 μM melatonin improved all growth parameters and total plant dry weight. Therefore, melatonin can enhance drought tolerance and promote plant growth under limited irrigation conditions.

Keywords: Melatonin, watering frequency, growth, cocoa

INTRODUCTION

Cocoa (*Theobroma caca*o L.) is one of the key export commodities from the plantation sector, playing a significant role in Indonesia's economy as a source of foreign exchange revenue alongside oil. The development of Indonesia's cocoa production saw a decline during the period from 2021 to 2022. In 2021, cocoa production decreased to 706.50 thousand tons, followed by another drop in 2022, reaching 667.30 thousand tons (BPS, 2021).

The decline in cocoa production can be attributed to global climate change. Climate change affects weather patterns in Indonesia, causing drought in certain regions. This phenomenon is a result of the El Niño event. Cocoa is a crop highly sensitive to climate changes, particularly drought. Originating from the tropical rainforests of the Americas, cocoa cultivation is influenced by critical climatic and soil factors, such as rainfall, temperature, and sunlight. Cocoa plants rely heavily on moderate but evenly distributed rainfall throughout the year, which is crucial for the flowering process (Boer *et al.,* 2009). Based on these considerations, it is essential to adapt cocoa cultivation to changes in weather patterns, particularly through irrigation techniques.

Watering management is a critical aspect of plant maintenance, especially during the early growth stages when cocoa seedlings require adequate water to support root development and overall plant growth. Both water deficiency and excess during the initial growth phase can induce stress in cocoa plants. Therefore, proper management of watering frequency is essential in nursery management, as it can affect soil moisture stability, water absorption efficiency, and seedling resistance to environmental stress (Zakariyya & Indradewa, 2018). Determining the optimal watering frequency aims to maintain a balance of water in the soil, prevent damage caused by over-irrigation or under-irrigation, and ensure that seedlings receive sufficient water for their growth. A watering interval of every 3 days is considered the most optimal, as it maintains a balance between water availability and soil aeration. Daily or every-2-day watering, while capable of keeping the soil moisture high, poses a risk of water saturation in the growing media, which can inhibit root respiration and increase the likelihood of root diseases. In contrast, watering every 3 days provides sufficient water for plant growth without causing excessive soil moisture, while also allowing time for partial drying of the media, thereby promoting healthy root development. Sulastri *et al.* (2022) reported that a 3-day watering interval resulted in greater plant height and dry weight of cocoa seedlings compared to 1-day and 2-day intervals. Meanwhile, a 6-day watering interval begins to show signs of water stress, especially if not supported by external interventions such as the application of bioactive compounds that enhance drought tolerance. Under such conditions, plants generally experience a reduction in leaf number and slower growth. Watering intervals longer than 7 days can cause significant water deficits, inhibit photosynthesis, and drastically reduce both dry weight and plant height. This low-frequency watering is not recommended, as it may lead to increased seedling mortality in cocoa nurseries (Djoah *et al.,* 2022). In cocoa nurseries, the application of appropriate watering strategies that align with local climatic conditions can enhance nursery success and influence the future productivity of cocoa plants. One approach to mitigating drought-induced stress involves the application of hormones, compounds, substances, or specific proteins to help meet the plant's nutritional needs and enhance its resilience.

The application of exogenous growth regulators, such as melatonin, has been proven to enhance drought tolerance in plants (Arnao & Hernandez-Ruiz, 2019). Melatonin itself is a natural pleiotropic biomolecule widely found in both plants and animals, and it has been shown to improve productivity (Ren *et al.,* 2020). Melatonin belongs to the class of tryptophan derivatives and functions as a potent antioxidant. As an alternative to mitigating stress in cocoa plants, the use of melatonin can be considered. The purpose of this study is to examine the effects of exogenous melatonin application on growth and stress tolerance under different irrigation frequencies.

MATERIALS AND METHODS

The research was conducted from April to July 2024, a period that includes the transition from the rainy season (April), with maxium temperatures 26.5 °C and minimum temperatures 25.2 °C, to the early dry season (May–July), during which maximum temperatures 30.8 °C and minimum temperatures 27.2 °C. The study took place in the greenhouse of the Indonesian Coffee and Cocoa Research Institute, Kaliwining, Jember, East Java. The cocoa seedlings used in this study were ICCRI 06H variety, 6 months old after planting. The planting medium was prepared using 25 x 25 cm polybags, with a mixture of soil, manure, and sand in a 2:1:1 ratio (Sugianto & Jayanti, 2021), with each polybag weighing 2.5 kg.

The experimental design used was Completely Randomized Design (CRD) with split plot design consisting of two factors: watering frequency and melatonin concentration. The combination of treatments was repeated three times, resulting in 36 experimental units. The main plot was watering frequency, which included three treatments: watering every 3 days, 6 days, and 9 days, with 450 mL of water applied every polybag (Indri, 2020) each morning. The sub plot was melatonin concentration, with four treatments: 0 µM (control), 50 µM, 100 µM, and 150 µM.

The melatonin solution was prepared by first creating a stock solution with DMSO as the solvent and stirring until fully dissolved. Then, a liter of distilled water (aquadest) was added, and the solution was diluted to the desired concentrations. Melatonin was applied foliar spraying the solution onto the cocoa leaves, with a volume of 10 mL per plant every two weeks in the morning.

The seedling growth includes plant height, stem diameter, and leaf number were observed every 9 days over the 3-month experimental period. Other measurements, such as leaf area and the drymass of the plant (including shoot, roots, and total dry weight), were taken at the end of the study. Stress tolerance index was calculated by using formula ($\frac{Ypi \times Ysi}{Yp^2}$), where Y_{pi} represents the seedling dry weight of 3-days watering frequency. Y_{si} represents the seedling dry weight of 6- and 9-days watering frequencies, and Y_p represents the average cocoa dry weight of 3-days watering conditions (Bhandari *et al.*, 2024). Soil moisture was measured using a moisture meter both before and after watering to determine changes in soil water content as a result of the watering treatments. Data obtained from the research then was analyzed using analysis of covariance (ANCOVA), and if significant differences were found between treatments, a Duncan's Multiple Range Test will be performed at a 5% significance level.

RESULTS AND DISCUSSION

Plant growth variables, including plant height, stem diameter, and leaf number, were observed 10 weeks after treatments (WAT). Increasing the watering frequency from every 3 days to every 6 days and 9 days suppressed plant growth in terms of height, stem diameter, and the number of leaves at the end of the observation period. The increase in watering frequency from every 3 days to every 6 days and 9 days reduced plant height by 19.9% and 28.9%, respectively. ANCOVA results showed an interaction between water frequency with melatonin application in the plant height, stem diameter, number of leaves, and leaf area. All of growth variables includes plant height, stem diameter, number of leaves, and leaf area showed polynomial pattern. At the 3-day watering frequency, the application of 100 µM melatonin enhanced plant height and stem diameter. Meanwhile, at the 9-day watering frequency, the application of 150 µM melatonin significantly improved all growth variables of the plants.

The role of melatonin, as shown in Figures 1a, 1b, 1c, and 1d, at a 9-day watering frequency with the addition of 150 µM melatonin, indicated that melatonin can enhance the growth of plants under drought stress. This finding was in line with research by Arnao & Hernández-Ruiz (2017), who reported that melatonin application at concentrations ranging from

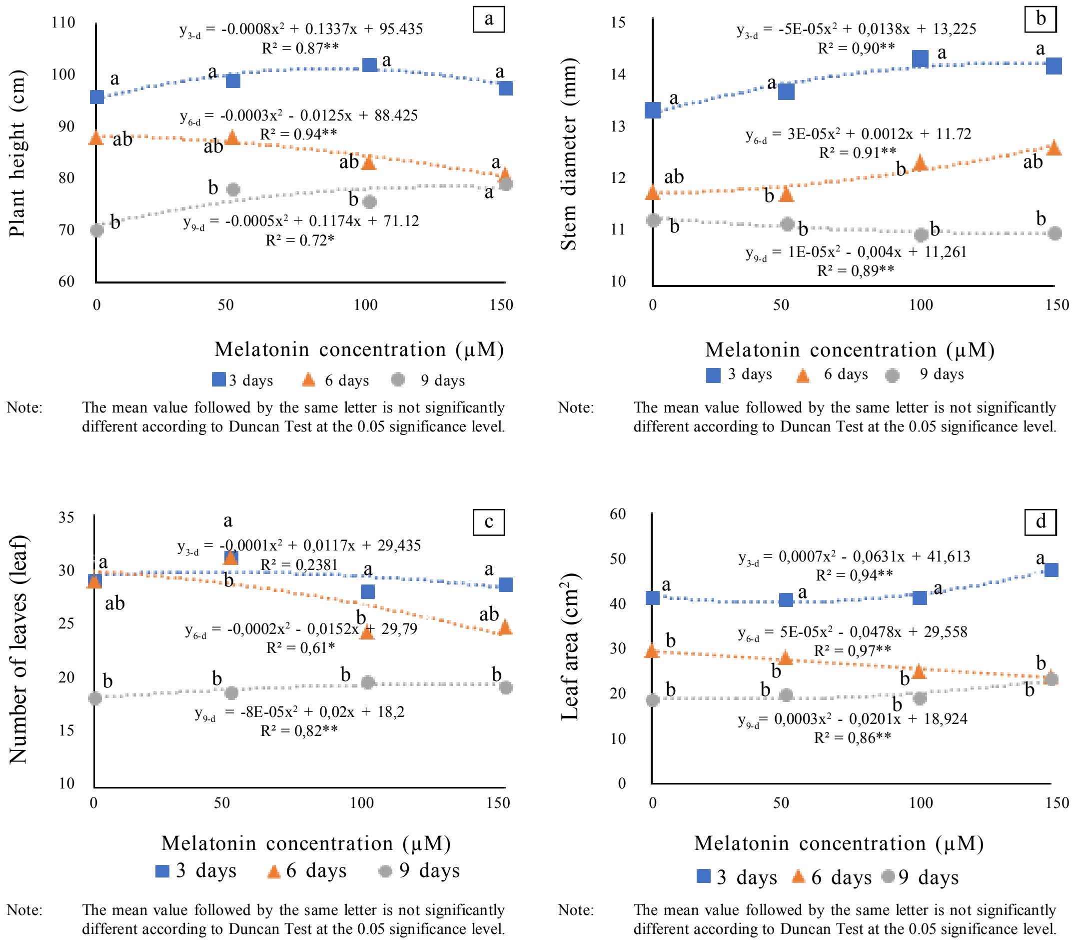
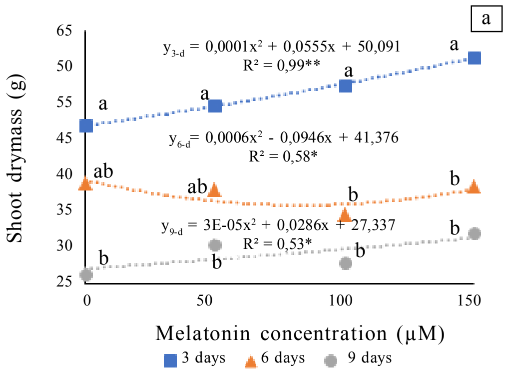


Figure 1. Analysis of covariance (ANCOVA) to the growth variables: plant height (a), stem diameter (b), number of leaves (c), and leaf area (d)

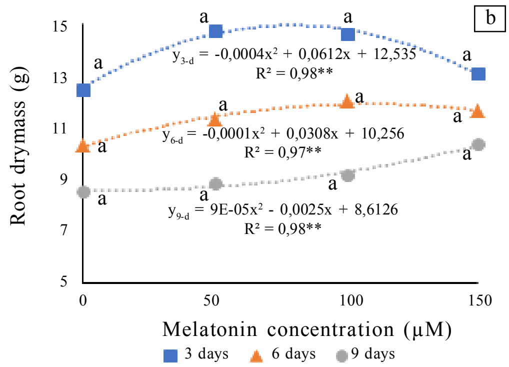
100 µM to 200 µM increased plant drought resistance, including in *Arabidopsis thaliana* and *Oryza sativa*. Another study by Zhang *et al.* (2019) also demonstrated that melatonin at concentrations of 100 µM to 150 µM improved growth in *Oryza sativa* under drought stress by increasing chlorophyll content and improving cell turgor. Additionally, Tan *et al.* (2016) reported that melatonin application could enhance the drought tolerance of *Arabidopsis thaliana* by increasing antioxidant capacity and improving hormone regulation, supporting the finding that higher melatonin doses are more effective under severe drought conditions.

The application of various concentrations of melatonin and watering frequencys showed different effects on seedling dry weight variables, depending on the applied drought conditions. At a 3-day watering frequency, the addition of melatonin at 50 µM to 100 µM increased the dry weight of the shoots and total plant dry weight but reduced root dry weight. This suggested that melatonin stimulates the growth of the shoot parts (leaves and stems), which is associated with enhanced photosynthesis and other metabolic activities. However, the decrease in root dry weight may be attributed to an imbalance between shoot growth and the root system's ability to expand, as more frequent watering (every 3 days) supports faster above-ground growth than root development. At a 6-day irrigation, the addition of melatonin up to 150 µM had no effect on the total drymass of the plants and actually reduced growth (plant height, number of leaves, and leaf area) as shown in Figure 1. However, concentrations ranging from 100 µM to 150 µM resulted in an increase in root dry weight (Figures 2b). This indicates that melatonin can stimulate root development in response to moderate drought. The increase in root dry weight reflects the role of melatonin in modulating various plant hormones involved in stress responses, particularly by enhancing the activity of growth hormones like auxins, which support root cell division and elongation (Yang *et al.,* 2020).

At a 9-day watering frequency, the application of melatonin at concentrations ranging from 50 µM to 150 µM increased the total plant drymass, shoot drymass, and root drymass, indicating that melatonin is effective in stimulating overall plant growth under more severe drought stress conditions. Although, the soil moisture at a 9-day watering interval (101,6 %) was slightly higher than that of the 3-day (100,4%) and 6-day (96,1%) treatments, the prolonged interval between water applications likely imposed cumulative water stress over time. This indicates that the observed increase in root drymass was not solely attributable to soil moisture levels at a single time point, but rather to physiological adjustments triggered by the extended intervals between watering. These conditions also suggest that



Note: The mean value followed by the same letter is not significantly different according to Duncan Test at the 0.05 significance level.



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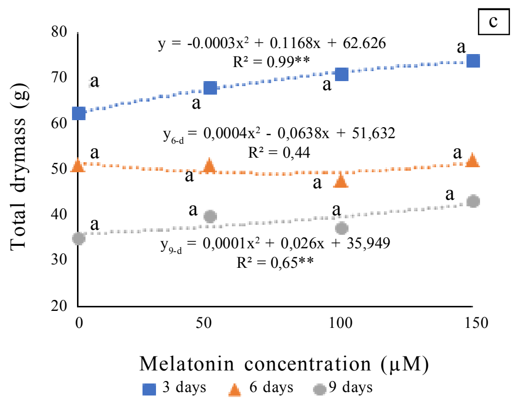

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Figure 2. Analysis of covariance (ANCOVA) to the biomass: shoot drymass (a), root drymass (b), and total drymass (c)

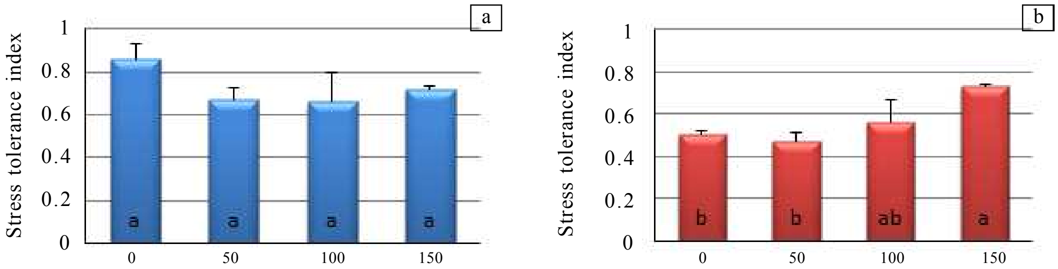

Figure 3. Stress Tolerance Index: a) 6 days watering frequency and b) 9 days watering frequency

melatonin may enhance the plant's water uptake efficiency by promoting root development, which constitutes a critical adaptation for survival under long-term drought stress. This study was consistent with the research by Arnao & Hernández-Ruiz (2017), who found that melatonin application could improve drought tolerance by enhancing root function and increasing overall plant dry weight. Similarly, a study by Zhang *et al.* (2019) showed that melatonin concentrations of 100 µM to 150 µM increased the dry weight of *Oryza sativa* plants subjected to drought stress.

The stress tolerance index was calculated based on the dry weight under three watering conditions: every 3 days, every 6 days, and every 9 days, as shown in Figures 3a and 3b. Soil moisture levels prior to irrigation showed a decreasing trend with increasing irrigation intervals, measured at 46.8% for the 3-day, 42.8% for the 6-day, and 33.8% for the 9-day. Following irrigation, soil moisture increased significantly to 100.4%, 96.1%, and 101.6% for the 3-day, 6-day, and 9-day watering intervals, respectively. Under the 6-day watering frequency, the application of melatonin did not significantly affect the stress tolerance index. However, the effect of melatonin became evident under the 9-day watering frequency. The application of 150 µM melatonin signi-ficantly increased the stress tolerance index from 0.51 to 0.73. This demonstrated that melatonin can help plants better withstand more extreme drought conditions, where plants must cope with longer periods of stress due to less frequent watering. This result aligned with the research by Arnao & Hernández-Ruiz (2017) on *Arabidopsis thaliana*, which showed that melatonin plays a role in enhancing plant resistance to abiotic stress, including drought, by regulating hormone balance and increasing antioxidant activity in plants. Another study by Zhang *et al.* (2019) also reported that melatonin application in drought-stressed plants could improve their physiological condition and enhance their adaptive capacity to water stress.

Melatonin functions as a protective molecule that helps plants survive drought conditions through various mechanisms, including reducing oxidative stress, regulating hormones involved in stomatal opening, enhancing photosynthetic efficiency, increasing osmolyte content, and improving root structure (Arnao & Hernández-Ruiz, 2020). Drought stress induces oxidative stress, which damages plant cells, including membranes, proteins, and nucleic acids, due to the presence of free radicals. Melatonin acts as an antioxidant by enhancing the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (Sharma *et al.*, 2020), as well as increasing ascorbic acid and glutamine content in plants (Huang *et al.,* 2019). In this way, melatonin helps protect plant cells from oxidative damage during drought stress.

Additionally, melatonin works by modulating various plant hormones involved in stress responses, such as auxins to promote root growth, cytokinins to maintain cellular metabolic balance, and gibberellins. Moreover, melatonin increases the production of abscisic acid (ABA), a key hormone in drought response, which regulates stomatal closure and reduces water evaporation, thus helping the plant conserve water during drought (Sharma *et al.*, 2020).

CONCLUSIONS

Based on the results of this study, the application of melatonin can significantly enhance plant growth and dry weight across different watering frequencys. At a 3-day watering frequency, melatonin concentrations ranging from 50 to 100 µM positively affected growth variables, including plant height, stem diameter, leaf area, as well as shoot dry weight and total plant dry weight. Meanwhile, at the 6-day irrigation, although it reduced growth and had no effect on the total plant dry weight, concentrations of 100-150 µM were found to enhance root dry weight. Furthermore, the application of 150 µM melatonin under the 9-day watering frequency led to significant improvements in all growth variables and total plant dry weight. Additionally, melatonin at concentrations of 100-150 µM was shown to significantly increase the stress tolerance index, highlighting its potential in enhancing plant resistance to drought stress associated with less frequent watering. These findings suggest that melatonin can effectively improve drought tolerance and promote plant growth under water-limited conditions.

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