

## Foliar application of melatonin alleviates the effects of drought stress in rice (*Oryza sativa* L.) seedlings

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### Abstract

Melatonin (*N*-acetyl-5-methoxytryptamine) plays an essential role in abiotic stress in plants, but its mechanism in drought tolerance is unclear. To better understand the protective roles of melatonin against drought stress, we investigated the effect of foliar application with exogenous melatonin on plant growth, physiological responses, and antioxidant enzyme activities in rice seedlings under drought stress. Rice seedlings were grown for 21 days, and foliar sprayed with 0, 50, 100, 200, and 300  $\mu$ M melatonin. The control plant was watered daily, while the others were subjected to drought stress by withholding water for seven days. The results showed that drought stress significantly reduced plant growth, relative water content, and chlorophyll content. Electrolyte leakage, malondialdehyde (MDA) content and hydrogen peroxide ( $H_2O_2$ ) were also negatively affected by drought stress. Application of melatonin alleviated the effects of drought stress by increasing plant growth, improving relative water content and chlorophyll content, and decreasing electrolyte leakage, MDA, and  $H_2O_2$ . Foliar application with melatonin also increased antioxidant enzyme activities, including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (GPX). In addition, melatonin also enhanced proline and total soluble sugar accumulation during drought stress. It is, therefore, suggested that foliar application with 100  $\mu$ M melatonin was the most effective for reducing the adverse effects of drought stress in rice plants.

**Keywords:** antioxidant enzymes; drought stress; foliar application; melatonin

### Introduction

Rice (*Oryza sativa* L.) is a staple crop widely cultivated in Thailand and exported worldwide (Siriphollakul *et al.*, 2017). One of the most famous rice in Thailand is Thai jasmine rice or 'Khao Dawk Mali 105' (KDML 105) rice because of its aroma and tender cooking properties. Unfortunately, drought stress is still a significant limitation to rice production in Thailand, especially in the Northeastern region of Thailand, where this cultivar is popularly cultivated (Polthanee *et al.*, 2014). Drought stress is one of the major abiotic stresses restricting crop growth and yield because it adversely affects many biochemical and physiological processes in plants. Drought stress induces stomatal closure, resulting in a lower transpiration rate and reduced photosynthesis (Farooq *et al.*, 2009). Also, drought stress induces the generation of reactive oxygen species (ROS), leading to lipid peroxidation and ultimately causes membrane dysfunction (Li *et al.*, 2011; Liang *et al.*, 2019). In order to maintain the ROS balance and alleviate cellular oxidative injuries, plants have evolved

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antioxidant defence mechanisms, either non-enzymatic antioxidant such as glutathione, ascorbic acid carotenoids or enzymatic antioxidants, including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (GPX) (Apel and Hirt, 2004; Mittler *et al.*, 2004). These antioxidant mechanisms are essential in plants, as they help maintaining the stability and integrity of the cell membrane. Moreover, the accumulation of compatible solutes, such as soluble sugar, glycine betaine and proline also play a vital role in drought tolerant mechanisms. Proline accumulation is believed to play multiple roles beyond osmotic adjustment in the adaptation to drought stress by acting as an antioxidative defence molecule and a signalling molecule (Verbruggen and Hermans, 2008; Hayat *et al.*, 2012).

Recently, plant growth regulators have been widely used to regulate plant growth and improve plant stress tolerance. Melatonin (*N*-acetyl-5-methoxytryptamine) is an indole hormone widely present in plants and animals. It was identified and quantified for the first time in plants in 1995 (Dubbels *et al.*, 1995). It performs various functions in plant growth, development, and stress responses (Shi *et al.*, 2016). Melatonin plays multiple roles in plants, including increasing seed germination, promoting lateral root development, delaying leaf senescence, protecting plants from stress, and increasing crop yield (Zhang *et al.*, 2013; Ahmad *et al.*, 2020). Moreover, melatonin has been demonstrated as an effective plant biostimulant against biotic and abiotic stresses (Arnao and Hernández-Ruiz, 2021). Melatonin triggers the accumulation of compatible solutes such as total soluble sugars and proline content (Sharma *et al.*, 2020). Many studies reported that exogenous melatonin could enhance plant tolerance to drought stress (Wei *et al.*, 2015; Zhang *et al.*, 2015). For example, melatonin alleviates oxidative damage during drought stress by directly scavenging ROS resulted in decreases in electrolyte leakage and MDA content and by enhancing antioxidant enzyme activities (Ye *et al.*, 2016; Cheroni *et al.*, 2021). Melatonin also has crosstalk with other plant growth regulators such as gibberellin, jasmonic acid, and abscisic acid to regulate various physiological processes in plants under drought stress. In addition, melatonin regulates the transcription of various essential genes involved in antioxidative defense mechanisms (Sharma *et al.*, 2020).

The role of melatonin under drought stress has been reported in many plant species. Its mechanism and effective concentration in each species, however, remain unclear. Therefore, it is intriguing to investigate the effects of melatonin on 'Khao Dawk Mali 105' rice which is the major economic crop of Thailand. This study aimed to investigate the efficacy and appropriate concentration of exogenous melatonin in alleviating the negative effects of drought in rice. Physiological responses and antioxidant enzyme activities were also determined to better understand the mechanism of melatonin in drought tolerant mechanism.

## Materials and Methods

### *Plant material and growth conditions*

Rice seeds (*Oryza sativa* L. cv. 'Khao Dawk Mali 105') were surface sterilized in 3% sodium hypochlorite for 10 min and rinsed with distilled water. The seeds were then soaked in distilled water for 48 h and germinated on moistened filter paper for three days in the dark. After that, the germinated seedlings were transferred into black plastic pots (diameter: 20 cm, height: 15 cm) filled with 4 kg of soil (loamy sand; pH 6.04; electrical conductivity 0.04 dS m<sup>-1</sup>; organic matter 0.24%; total nitrogen 0.02%; total phosphorus 36.65 mg kg<sup>-1</sup>, and total potassium 234.50 mg kg<sup>-1</sup>), with ten seedlings per pot. The seedlings were watered daily with distilled water (500 ml/pot/day) and allowed to grow for 21 days in the greenhouse under natural light with an average temperature of 28.90 °C and average humidity of 68.36% at Khon Kaen University, Khon Kaen, Thailand. The seedlings were then divided into six groups, including the control group (well-watered), MT0 (foliar spray with distilled water), foliar spray with 50 (MT50), 100 (MT100), 200 (MT200), and 300 (MT300) μM melatonin. The plants were sprayed to a runoff in the late morning (10.00 a.m.) and repeated for three days. The seedlings were then subjected to drought stress by withholding water for seven days or until rice leaves roll in O-shaped or scale 7 according to 0-9 leaf rolling scale of standard evaluation system for rice

(International Rice Research Institute, 2002) except the control group which was watered daily with distilled water.

#### *Measurement of plant growth*

After withholding water for seven days, the seedlings were uprooted, washed with tap water, and lightly wiped with a paper towel to remove excess water. The plants were separated into the shoot and root parts. Shoot length, root length, shoot fresh weight, and root fresh weight was recorded. For dry weight analysis, the samples were dried in a hot air oven at 80 °C for three days.

#### *Chlorophyll content*

Chlorophyll content was determined following the method described by Arnon (1949). About 20 mg of leaf tissues were extracted with 5 ml of 80% acetone. Chlorophyll extract was measured spectrophotometrically at 645 and 663 nm.

#### *Relative water content*

Fresh leaf was cut into the size of 1.5×1.5 cm<sup>2</sup> and immediately weighted to obtain fresh weight (FW). The leaf piece was subsequently floated on deionized water for 4 h and weighed as turgid weight (TW). After that, the leaf piece was dried at 70 °C for 48 h and weighed as dry weight (DW). Relative water content (RWC) was calculated according to the formula (Turner, 1981):  $RWC = [(FW-DW) / (TW-DW)] \times 100$ .

#### *Electrolyte leakage*

Approximately 0.1 g of leaf tissue was immersed in 10 ml of distilled water at room temperature for 24 h. The electrical conductivity of the samples was measured with a conductivity meter (model FE30 FiveEasy™, Mettler Toledo, USA) and recorded as EC<sub>1</sub>. The sample was then boiled at 100 °C for 30 min. After cooling down, the electrical conductivity of the samples was measured and recorded as EC<sub>2</sub>. Electrolyte leakage (EL) was calculated using the following formula (Dionisio-Sese and Tobita, 1998):  $EL = (EC_1 / EC_2) \times 100$ .

#### *Hydrogen peroxide content*

Approximately 0.1 g of leaf sample was extracted with 5 ml of 0.1% trichloroacetic acid (TCA) and then centrifuged at 12,000 rpm for 15 min. Then, 0.5 ml of supernatant was combined with 0.5 ml of 10 mM phosphate buffer (pH 7.0), and 1 ml of 1 M potassium iodide (KI). The absorbance was determined at 390 nm, and H<sub>2</sub>O<sub>2</sub> content was calculated using a standard curve plotted with the known concentration of H<sub>2</sub>O<sub>2</sub> (Sergiev *et al.*, 1997).

#### *Malondialdehyde content*

Approximately 0.1 g of leaf tissue was extracted with 5 ml of 0.1% trichloroacetic acid (TCA) and centrifuged at 10,000 rpm for 10 min. Then 0.5 ml of supernatant was combined with 1.5 ml of 0.5% thiobarbituric acid (TBA) in 20% TCA. The mixture was incubated in a water bath at 95 °C for 30 min. The reaction was terminated by rapid cooling on ice for 10 min, and the samples were then centrifuged at 10,000 rpm for 5 min. The absorbance was measured at 532 and 600 nm using a spectrophotometer. The concentration of TBA reacting substance (TBARS) was calculated using a molar extinction coefficient of 155 mM<sup>-1</sup> cm<sup>-1</sup> (Heath and Packer, 1968).

#### *Proline content*

The proline content was assayed as described by Bates *et al.* (1973) with minor modifications. Approximately 100 mg of leaf tissue was homogenized in 5 ml of 3% sulfosalicylic acid. The extract was filtered through Whatman filter paper No.1, and 2 ml of supernatant was mixed with 2 ml of glacial acetic acid and 2 ml of ninhydrin reagent. The reaction mixture was boiled at 100 °C for 1 h. After cooling by immersion in an

ice bath to stop the reaction, 4 ml of toluene was added, and the test tube was well vortexed. Then, the absorbance was measured at 520 nm, and the proline content was calculated from a standard curve plotted with the known concentration of proline.

#### *Total soluble sugar content*

Total soluble sugar content was assayed by phenol-sulfuric method described by Dubois *et al.* (1956). Approximately 50 mg of leaf tissue was extracted with 10 ml of 80% ethanol. The reaction mixture consisted of 100  $\mu$ l of extracted sugar solution, 500  $\mu$ l of 5% (W/V) phenol and 1 ml of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) was incubated at room temperature for 10 min and homogeneously mixed. The reaction was continuously incubated at room temperature for 30 min. Finally, the absorbance was measured at 490 nm, and total soluble sugar content was calculated using the standard curve plotted with known concentration of glucose.

#### *Antioxidant enzyme activities*

About 0.2 g of fresh leaf sample was ground with 4 ml of grinding buffer containing 50 mM phosphate buffer (pH 7.8), 0.4 mM EDTA, and 1 mM ascorbic acid. The extracts were centrifuged at 12,000 rpm at 4 °C for 30 min, and the crude extract was used for enzymatic activity assays. Superoxide dismutase (SOD) activity was assayed by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium chloride (Gong *et al.*, 2005). The reaction mixture contained 50 mM phosphate buffer (pH 7.8), 0.4 M methionine, 2 mM NBT, 16 mM EDTA, 1 mM riboflavin, and 50  $\mu$ l of enzyme extract in a final volume of 2 ml. The test tubes were exposed to fluorescence light for 30 min, and the absorbance was measured at 560 nm. Catalase (CAT) activity was assayed by measuring the rate of disappearance of H<sub>2</sub>O<sub>2</sub> using the method described by Hamurcu *et al.* (2013). The reaction mixture contained 50 mM phosphate buffer (pH 7.0), 0.5 M H<sub>2</sub>O<sub>2</sub>, and 100  $\mu$ l of enzyme extract in a final volume of 2 ml. The decrease in H<sub>2</sub>O<sub>2</sub> was followed by a decline in absorbance at 240 nm. Ascorbate peroxidase (APX) activity was determined according to Sunohara and Matsumoto (2004). The reaction mixture contained 50 mM phosphate buffer (pH 7.0), 16 mM EDTA, 20 mM ascorbic acid, 0.5 M H<sub>2</sub>O<sub>2</sub>, and 100  $\mu$ l of enzyme extract. H<sub>2</sub>O<sub>2</sub> dependent oxidation of ascorbate was followed by a decrease in the absorbance at 290 nm. Guaiacol peroxidase (GPX) activity was determined by the method described by Zhang *et al.* (2005). The reaction mixture consisted of 50 mM phosphate buffer (pH 7.0), 0.5 M H<sub>2</sub>O<sub>2</sub>, and 3% guaiacol. The reaction was started by adding 100  $\mu$ l of enzyme extract to the reaction mixture at 25 °C. The increase in absorbance at 470 nm due to guaiacol oxidation was recorded for 2 min.

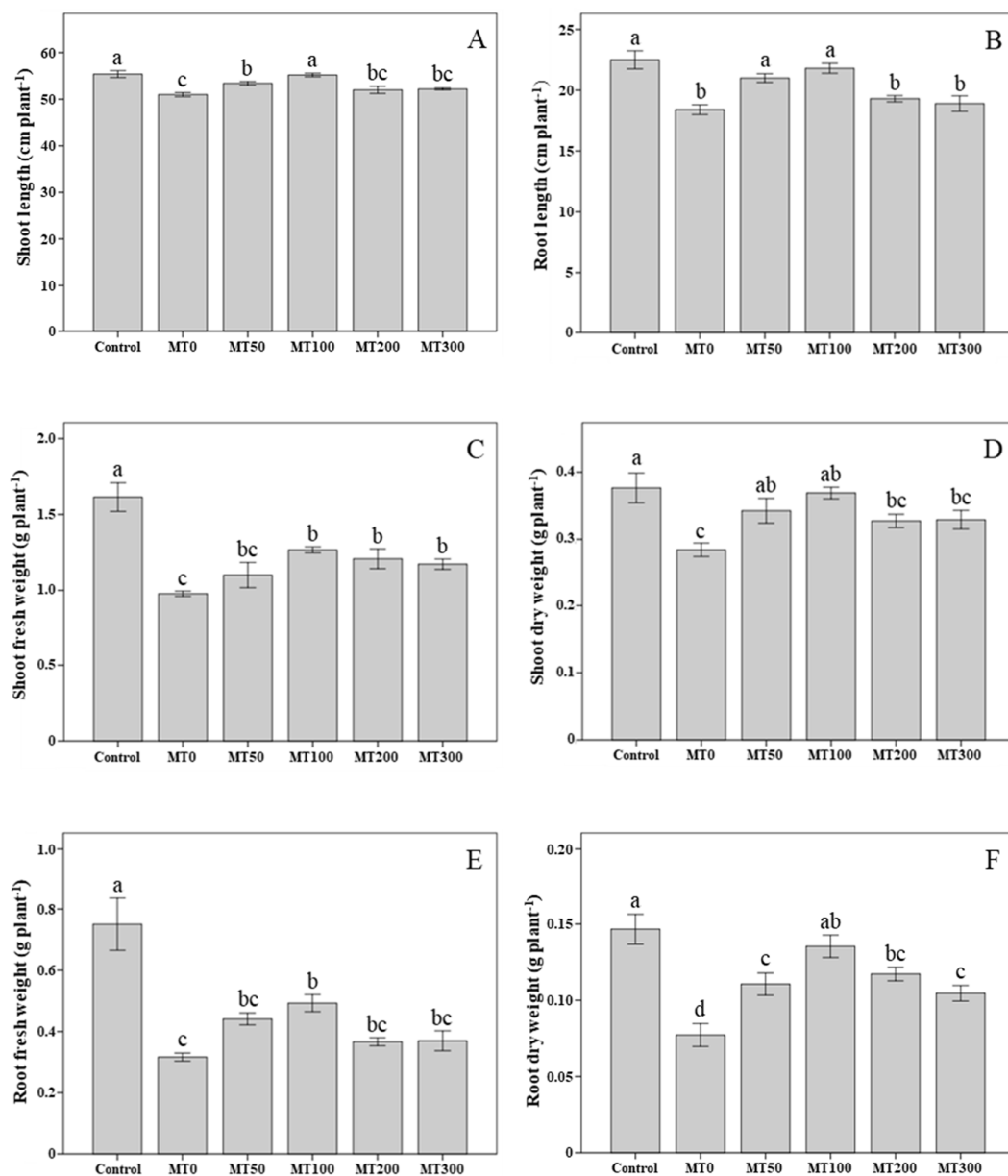
#### *Statistical analysis*

The experiment was designed as a completely randomized design with five replicates. All data were analysed via IBM SPSS statistics 24, and values were presented as the mean  $\pm$  standard error. The data were evaluated by one-way ANOVA using Duncan's multiple range tests ( $P < 0.05$ ).

## **Results**

#### *Effects of exogenous melatonin on rice seedling growths under drought stress*

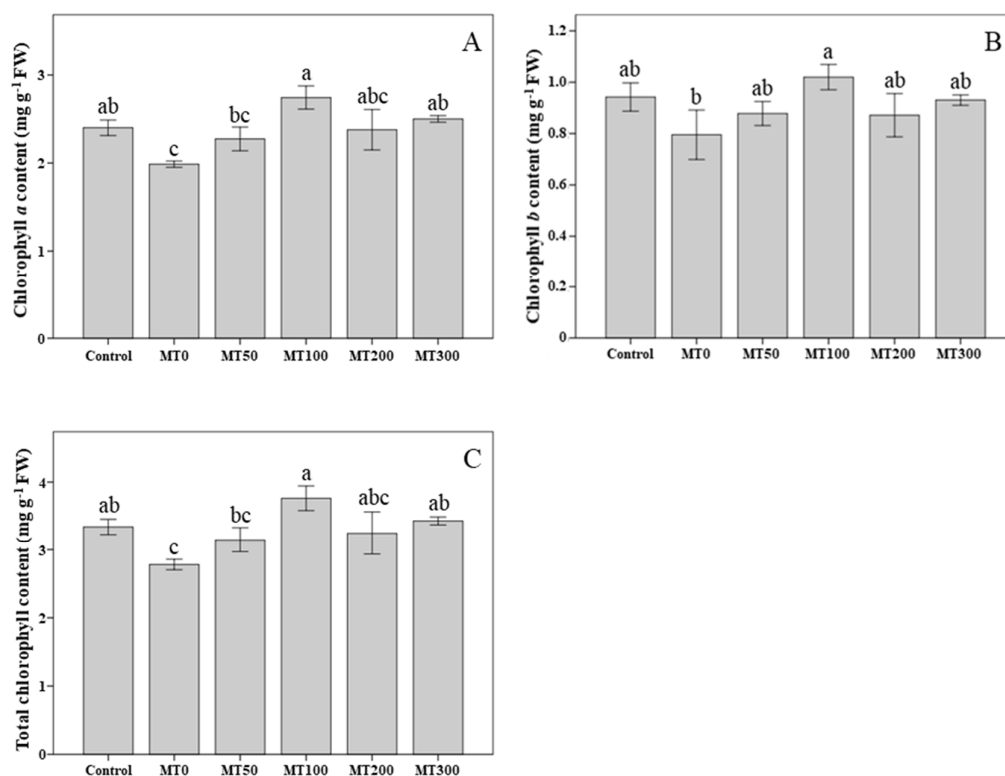
Drought stress reduced plant growth significantly, as evidenced by decreases in shoot and root length, shoot and root fresh weight, and shoot and root dry weight. Foliar application with 50 and 100  $\mu$ M melatonin showed significantly higher shoot and root length compared to the untreated group (Figure 1A, 1B). Similarly, treating the plant with 50 and 100  $\mu$ M melatonin also resulted in higher shoot fresh and dry weight, as well as higher root fresh and dry weight compared to the untreated group (Figure 1C, 1D, 1E, 1F). High concentrations of melatonin, i.e., 200 and 300  $\mu$ M, had no beneficial effects, and plant growth was nearly similar to the untreated plant.



**Figure 1.** Effects of exogenous melatonin on shoot length (A), root length (B), shoot fresh weight (C), shoot dry weight (D), root fresh weight (E), and root dry weight (F) of rice seedling under drought stress. Data represent mean  $\pm$  SE of 5 replicate samples. Different letters indicate significant differences according to Duncan's multiple range tests ( $P < 0.05$ ).

*Effects of exogenous melatonin on chlorophyll content in rice seedling leaves under drought stress*

Drought stress had a detrimental effect on the chlorophyll *a*, chlorophyll *b*, and total chlorophyll levels in rice seedling leaves. Foliar application of melatonin at a concentration of 100  $\mu$ M resulted in a significant increase in chlorophyll content relative to untreated plants, whereas other concentrations including 50, 200, and 300  $\mu$ M resulted in slight increases in chlorophyll content. (Figure 2A, 2B, 2C).

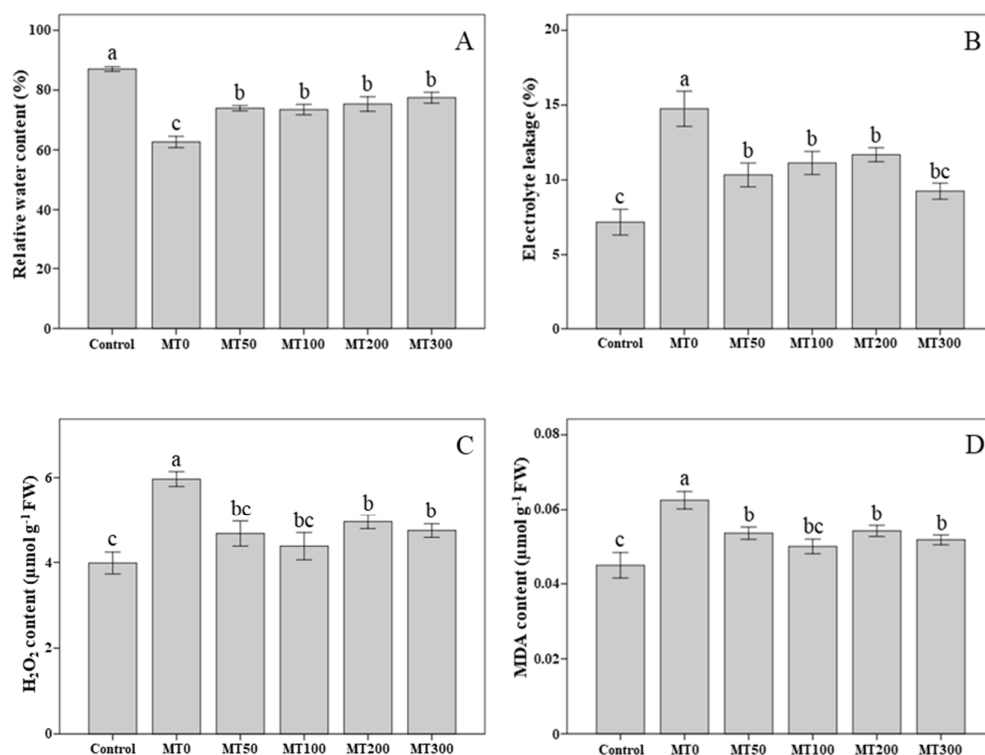


**Figure 2.** Effects of exogenous melatonin on chlorophyll *a* content (A), chlorophyll *b* content (B), and total chlorophyll content (C) in rice seedling leaves under drought stress

Data represent mean  $\pm$  SE of 5 replicate samples. Different letters indicate significant differences according to Duncan's multiple range tests ( $P < 0.05$ )

*Effects of exogenous melatonin on relative water content, electrolyte leakage, H<sub>2</sub>O<sub>2</sub> content, and MDA content in rice seedling leaves under drought stress*

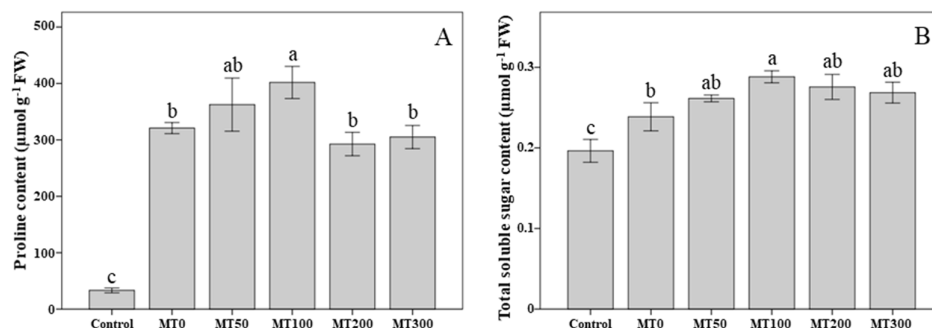
Drought stress resulted in a significant decrease in the leaf relative water content. Relative water content was reduced from 87.05% in the control plants to 62.64% in the untreated plants. Foliar applications with melatonin significantly increased relative water content and showed higher relative water content than the untreated plants. However, the values are not significantly different across melatonin concentrations (Figure 3A). On the other hand, electrolyte leakage increased approximately two-fold when the plant was subjected to drought stress. Exogenous application of melatonin resulted in a significant reduction in electrolyte leakage compared to the untreated plant (Figure 3B). The values, however, were not significantly different between each concentration of melatonin treatment. Under drought stress, H<sub>2</sub>O<sub>2</sub> content in the leaf increased significantly when compared to the control plant. The seedling treated with exogenous melatonin showed significantly lower H<sub>2</sub>O<sub>2</sub> than the untreated plant (Figure 3C). However, H<sub>2</sub>O<sub>2</sub> content was not significantly different across the concentrations of melatonin. Similarly, MDA, a product of membrane lipid peroxidation, showed a significant increase when the plant was subjected to drought stress. Treating the plant with melatonin also alleviates membrane damage, as evidenced by the reduction in MDA content (Figure 3D).



**Figure 3.** Effects of exogenous melatonin on relative water content (A), electrolyte leakage (B), H<sub>2</sub>O<sub>2</sub> content (C), and MDA content (D) in rice seedling leaves under drought stress  
Data represent mean ± SE of 5 replicate samples. Different letters indicate significant differences according to Duncan's multiple range tests ( $P < 0.05$ )

*Effects of exogenous melatonin on proline content and total soluble sugar content in rice seedling leaves under drought stress*

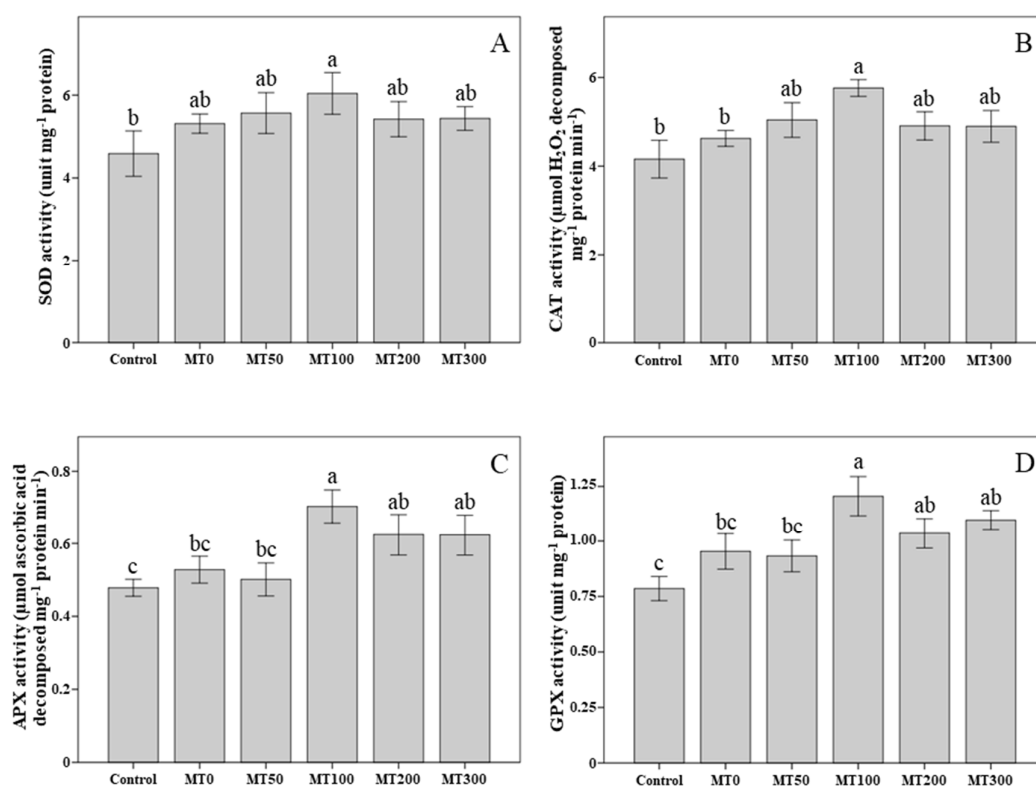
Under drought stress, proline content in the leaf increased approximately 10-fold compared to the control plant. Treating the plant 100 μM melatonin resulted in a significantly higher proline accumulation, while the other concentrations showed no difference in proline content compared to the untreated plant (Figure 4A). In addition, total soluble sugar was also significantly increased by drought stress. Exogenous application of melatonin increased sugar accumulation at all concentrations, with 100 μM melatonin resulting in the highest total soluble sugar content (Figure 4B).



**Figure 4.** Effects of exogenous melatonin on proline content (A) and total soluble sugar content (B) in rice seedling leaves under drought stress  
Data represent mean ± SE of 5 replicate samples. Different letters indicate significant differences according to Duncan's multiple range tests ( $P < 0.05$ )

*Effects of exogenous melatonin on antioxidant enzyme activities in rice seedling leaves under drought stress*

Antioxidant enzyme activities, including SOD, CAT, APX, and GPX, were determined to understand the effects of drought and exogenous melatonin on the enzymatic antioxidant system. SOD activity increased slightly after the plant was subjected to drought stress. Treating with exogenous melatonin showed no significant difference in SOD activity compared to the untreated plant, except in the 100  $\mu\text{M}$  treatment which resulted in a slight increase in SOD activity (Figure 5A). CAT activity in the droughted plant was not significantly different compared to the control plant. Treating with exogenous melatonin showed increases in CAT activity, with 100  $\mu\text{M}$  resulting in the highest CAT activity (Figure 5B). Drought stress had no effect on APX activity, which remained comparable to that of the control plant. Treating with 100, 200, and 300  $\mu\text{M}$  melatonin showed higher APX activity than the untreated plant, while 100  $\mu\text{M}$  yield the most increased APX activity (Figure 5C). Similarly, foliar application with 100, 200, and 300  $\mu\text{M}$  melatonin also increased GPX activity compared to the untreated plant, with 100  $\mu\text{M}$  being the most effective concentration to increase GPX activity (Figure 5D).



**Figure 5.** Effects of exogenous melatonin on antioxidant enzyme activities in rice seedling leaves under drought stress represented by the activities of antioxidant enzymes namely superoxide dismutase (SOD) (A), catalase (CAT) (B), ascorbate peroxidase (APX) (C), and guaiacol peroxidase (GPX) (D) Data represent mean  $\pm$  SE of 5 replicate samples. Different letters indicate significant differences according to Duncan's multiple range tests ( $P < 0.05$ )



## Discussion

Drought stress inhibits rice seedling growth due to lower turgor pressure, reduction in photosynthesis, and cellular damage caused by ROS. However, our findings indicate that exogenous melatonin can alleviate the inhibition of plant growth caused by drought stress. Foliar application with 100  $\mu\text{M}$  melatonin presents higher shoot and root growth in rice seedlings, whereas 200 and 300  $\mu\text{M}$  melatonin results in decreased root growth. The effect of melatonin on the improvement of plant growth was also reported in maize (Ahmad *et al.*, 2019) and kiwifruit seedlings (Liang *et al.*, 2019). Melatonin is a plant growth regulator and plays a vital role in regulating plant growth and development (Arnao and Hernández-Ruiz, 2015). One of the most intriguing aspects of research on melatonin in plants is the postulated similarity between its function and that of IAA (indole 3-acetic acid). IAA is an auxin involved in various physiological processes, the most notable of which is its role as a growth promoter (Arnao and Hernández-Ruiz, 2006). It is believed that melatonin and IAA can have a co-regulatory impact on plant growth by working in a combined or similar fashion to promote root morphogenesis (Murch *et al.*, 2001). Depends on the concentration of melatonin, it can either promote or inhibit root growth which affects the water uptake process. Melatonin-induced root growth leads to a more efficient acquisition of soil water and nutrients, therefore enhancing the growth in the aerial part of the plant (Arnao and Hernández-Ruiz, 2017).

Drought stress impairs various physiological processes, including chlorophyll degradation, lower relative water content, and cellular membrane damages, as evidenced by increased electrolyte leakage and MDA content. The results in this study indicated that an appropriate concentration of exogenous melatonin could mitigate the detrimental effects of drought stress and improve many physiological parameters. Drought stress stimulates the production of ROS in plant cells, resulting in chlorophyll degradation (Yordanov *et al.*, 2000). Chlorophyll degradation is also accelerated by the chlorophyllase enzyme, and the transcription level of the gene encoding this enzyme is upregulated in response to drought stress (Wang *et al.*, 2013; Ma *et al.*, 2018). Our findings indicate that exogenous melatonin can improve chlorophyll content in the drought-stressed plant. Additionally, previous research has demonstrated that exogenous melatonin can reduce the drought-induced chlorophyll degradation and improve photosynthetic rate in tomato, cucumber, and maize (Liu *et al.*, 2015; Wang *et al.*, 2016; Ahmad *et al.*, 2019). Melatonin treatment may help prevent chlorophyll degradation by down-regulating the gene encoding the chlorophyllase enzyme. (Sharma *et al.*, 2020).

Drought-induced ROS overproduction in plant cells leads to oxidative stress and lipid peroxidation, which causes cellular damage, membrane dysfunction, and death. Our results indicate that drought induces  $\text{H}_2\text{O}_2$  accumulation as well as increases electrolyte leakage and MDA content. Exogenous application of melatonin contributes to the reduction of ROS accumulation and membrane damage, as evidenced by the decrease in  $\text{H}_2\text{O}_2$ , electrolyte leakage, and MDA content. Additionally, melatonin has been shown to reduce electrolyte leakage and MDA content in *Cucumis sativus* L. and Moldavian balm under moderate and severe drought stress (Zhang *et al.*, 2013; Naghizadeh *et al.*, 2019). Melatonin also acts as a powerful scavenger of ROS in plants (Arnao and Hernández-Ruiz, 2015). In the present study, melatonin application reduces  $\text{H}_2\text{O}_2$  content in the leaf, which is consistent with several previous studies (Zhang *et al.*, 2013; Ye *et al.*, 2016; Liang *et al.*, 2018). Moreover, plants have evolved scavenging systems against ROS, including antioxidant enzymes, to maintain a balance between ROS production and removal. In this study, our results indicate that the activities of the antioxidant enzymes, including SOD, CAT, APX, and GPX, were higher when treated with melatonin under drought stress. The role of melatonin in regulating oxidative stress and ROS in plants, through redox enzymes including SOD, CAT, Peroxidase (POX), APX, and glutathione reductase (GR) has been demonstrated (Arnao and Hernández-Ruiz, 2019). Similar results were reported in rapeseed seedlings (Li *et al.*, 2018), naked oat seedlings (Gao *et al.*, 2018), maize seedlings (Su *et al.*, 2019), and Chinese hickory plants (Sharma *et al.*, 2020), which showed the capability of melatonin to enhance antioxidant enzyme under drought stress. The increased antioxidant enzyme activity may help reduce ROS accumulation, lower lipid peroxidation, and cellular damage, resulting in higher relative water content and lower membrane damage.

In addition to the response of the antioxidant system, the ability of plants to withstand drought involves the accumulation of compatible solutes to reduce the osmotic potential and maintain turgor pressure. The over-accumulation of proline is an important indicator of drought stress tolerance in higher plants (Ashraf and Iram, 2005). The results in our study show that proline increased several folds when the plant was under drought stress. Melatonin application, particularly at concentrations of 50 and 100  $\mu\text{M}$ , increases proline accumulation even more when the plant is under drought stress. The effect of melatonin on improving proline accumulation has also been reported in *Zea mays* L. (Ahmad *et al.*, 2019) and *Coffea arabica* L. (Campos *et al.*, 2019). In a previous study, Ding *et al.* (2018) proposed that melatonin increases the expression of *P5CS*, a key gene for proline biosynthesis, and stimulated pyrroline-5-carboxylate synthase (*P5CS*) activity in poplar leaf under methyl viologen stress, indicating that melatonin mediates the biosynthesis of proline during oxidative stress. Our result also shows that melatonin increased sugar accumulation when the plant was under drought stress. Melatonin stimulates the biosynthesis of soluble sugars which is responsible for maintaining the turgor and osmotic pressure of plant cells growing under drought stress (Chen *et al.*, 2020).

### **Conclusions**

To summarize, this study suggests that foliar application of melatonin could alleviate the adverse effects of drought stress in rice seedling. Melatonin increases plant growth, relative water content as well as chlorophyll content. Foliar spray with exogenous melatonin also increases total soluble sugar content and proline content which may function as compatible solutes. Moreover, melatonin also enhances the antioxidant enzyme activities, thus reducing the drought-induced oxidative damage, as indicated by lower electrolyte leakage, MDA, and  $\text{H}_2\text{O}_2$  content. Melatonin at a concentration of 100  $\mu\text{M}$  was found to be the most effective concentration at alleviating the effects of drought stress in rice seedlings.

### **Authors' Contributions**

Both authors read and approved the final manuscript.

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### **Conflict of Interests**

The authors declare that there are no conflicts of interest related to this article.

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