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Enhancing Drought Stress Tolerance in Prilep Tobacco Plants with Melatonin Treatment

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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Original Research Article

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ABSTRACT

In this work, we studied the effect of treatment with melatonin at concentrations (50, 100 and 150 micromolar) on some biochemical and production characteristics of Prilep tobacco plants under conditions of applied drought stress (15%, 30% and 45%).

Chlorophyll content in leaves decreased under conditions of drought stress, and H_2O_2, proline, MDA and Total Soluble Sugars increased steadily with increasing applied stress, while Chlorophyll, total soluble sugars content in leaves increased when sprayed with melatonin, especially at low concentrations (50 micromolar).

The treatment with the reduced concentration of melatonin and the applied stress outperformed all treatments and the control for all indicators studied. Therefore, it is recommended to use melatonin, especially at a concentration of 50 micromolar, on tobacco plants because of its role in improving chemical traits under conditions of drought stress.

Keywords: Tobacco seeds; Prilep; melatonin; drought stress.

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1. INTRODUCTION

"Abiotic stress greatly limits the growth and yield of plants. With the increase in global temperatures, the effect of drought stress is particularly prominent, and has become the main environmental factor limiting the growth and development of plants worldwide" [1].

"Drought stress increases the production of reactive oxygen species (ROS) such as H_2O_2. Membrane lipid peroxidation is triggered when the balance between ROS accumulation and the free radical scavenging systems is disrupted, causing damage to the membrane system and leading to an imbalance in plant metabolism" [2].

Drought stress has been shown to have a harmful effect on plant performance [3] including plant growth [4], leaf water content [5] and redox characteristics [6].

"Drought is among the most critical environmental concerns in agriculture today, especially in dry and semi-dry climates including the Mediterranean area" [7]. "Plant biologists have focused their efforts on studying the deleterious effects of drought on plants as well as techniques for mitigating those effects" [8].

"It has been reported that under water deficit stress, compared with no treatment, the application of exogenous hormones can significantly alleviate growth and developmental impairments and improve the activity of antioxidant enzymes" [9].

Melatonin (N-acetyl-5 methoxytryptamine), also known as the pineal hormone [10], was first detected in plants by Hattori [11].

"One important function of melatonin is as an antioxidant that can alleviate plant damage under adverse conditions. Such antioxidant effects have been reported in several plants (e.g., maize, wheat" [12,13].

"Research has shown that under water stress, melatonin can directly scavenge ROS and also improve the activity of antioxidant enzymes to alleviate the oxidative damage caused by water stress in cells" [14].

Therefore, this research aims to improve the biochemical properties of tobacco plants (Prilep) by foliar spraying with melatonin when exposed to drought stress conditions.

2. MATERIALS AND METHODS

The experiment was carried out during the 2024 season. The field experiment was conducted in the village of Jinghil- Lattakia- Syria.

Tobacco seeds were grown on an agricultural medium containing compost. The seedlings were transferred to plastic bags (60 × 40) cm forty days after germination.

Melatonin treatments: Melatonin solution was prepared by dissolving melatonin in ethanol (50 mg of melatonin in 1 ml of ethanol), after preparing the solution, it was diluted with deionized water to obtain concentrations (0, 50, 100 and 150) micromolar for use in foliar spraying for four days in the dark [15].

Drought stress treatments: To induce drought stress, polyethylene glycol (PEG-6000) was used at concentrations (15, 30 and 45 %) equivalent to an osmotic pressure (-0.7, -1.4 and -2.1) MPa [16]. PEG 6000 causes obvious water stress to plants without any toxic effects [17]. Through irrigation at a rate of two irrigations of 200 ml per plant at each treatment, Irrigation was done to the bottom of the plant stem, with an interval of two weeks between one irrigation and the second, during the critical growth period of the plant, which corresponds to the stage of active vegetative growth, about a month after transplanting.

- D_0: Plants were irrigated with water only.
- D_1: The plants were irrigated with a solution at a concentration of 15%, equivalent to an osmotic pressure of -0.7 MPa
- D_2: The plants were irrigated with a 30% solution equivalent to an osmotic pressure of -1.4 MPa.
- D_3: The plants were irrigated with a solution at a concentration of 45%, equivalent to an osmotic pressure of -2.1 MPa.

Observations were recorded *viz,* Chlorophyll and carotenoid, H_2O_2, proline, MDA and sugar content in leaves.

- **Determination of Chlorophyll content in leaves**

Chlorophyll content determination Pigments were extracted from the leaves. Th e extraction of leaf pigments was performed with 80% acetone, and

the absorbance at 663 and 645 nm was measured with an Amersham spectrophotometer (Amersham Biosciences, Piscataway, NJ, USA). The total chlorophyll quantities were calculated according to the method of Arnon [18]. Total carotenoid content was measured at 470 nm. The pigment concentrations were expressed as μg g^{-1} fresh weight (FW).

- **Determination of H_2O_2 content in leaves**

The H_2O_2 level was measured colorimetrically as described by Jana and Choudhuri [19]. H_2O_2 was extracted by homogenizing 50 mg leaf tissue with 3 mL of phosphate buffer (50 mM, pH 6.5).

- **Determination of proline content in leaves:**

The proline content was determined according to Bates *et al*. [20]. "Frozen leaf tissue (0.5 g) was homogenized with 10 mL of 3% sulfosalicylic acid at 4 °C. Th e extract was filtered with Whatman No. 2 filter paper. In a test tube, 2 mL of fi ltrate, 2 mL of acid-ninhydrin, and 2 mL of glacial acetic acid were mixed and incubated at 100 °C for 1 h. Th e reaction was terminated on ice, and the reaction mixture was then extracted with 4 mL of toluene. The chromophore-containing toluene was separated from the hydrated phase. The absorbance at 520 nm was spectrophotometrically determined with toluene as the blank. The proline concentration was calculated based on a standard curve and was expressed as μmol proline g^{-1} FW". Bates et al. [20].

- **Determination of malondialdehyde content in leaves:**

"Extract the MDA from 50 mg plant material using 1 ml 0.25% thiobarbituric acid (TBA) dissolved in 10% trichloroacetic acid (TCA). 2. Collect the extract in a 1.5-ml eppendorf tube, heat the mixture at 85 C for 30 min, and then quickly chill it on ice. Proceed by the same way for the blank sample but without plant material. 3. Centrifuge the mixture at maximum speed for 10 min to pellet the particles. 4. Transfer the supernatant in a 1-ml plastic cuvette for spectrophotometric measurement. 5. Read the absorbance first at 532 nm (the peak of MDA–TBA complex) and second time at 600 nm (nonspecific absorption). As blank, use 1 ml 0.25% TBA in 10% TCA. Calculate A (532–600)" (Heath and Packer, 1968). 6. Estimate the MDA concentration using the Beer–Lambert–Bouguer law, MDA extinction coefficient e532 155 mM1 cm1, calculate the amount of MDA, and normalize the values to the fresh weight of each sample.

- **Determination of total soluble sugars in leaves (%):**

Quantification of Sugars Samples for sugar determinations were harvested in the morning, between 3 and 4 h into the photoperiod. Sugars were extracted in 80% ethanol and determined enzymatically as described by Scholes *et al*. [21].

Statistical Analysis: Statistical analyses were performed by the analysis of variance (ANOVA) with Tukey. All data are presented as means ± standard deviation (SD) of three replicates. Differences at $P < 0.05$ were considered to be significant.

3. RESULTS AND DISCUSSION

Effect of melatonin and drought stress on chlorophyll content in leaves: Data in Fig. 1 indicate that there are significant differences ($P<0.05$) between the studied treatments in terms of the chlorophyll content in leaves.

Drought stress led to a decrease in chlorophyll content in leaves, While treatment with melatonin increased chlorophyll content in leaves compared to the control.

Treatment with melatonin at a concentration of 50 micromolar under drought conditions also outperformed all other parameters and the control.

"In general, drought stress causes a series of physiological and biochemical reactions such as stomatal closure, decreased chlorophyll content, decreased transpiration, and reduced antioxidant capacity" [22].

"Drought stress inhibits plant growth and yield by reducing its photosynthetic capacity and results in an imbalance in the cellular redox modules, with antioxidant defences failing to counterbalance the increased creation of reactive oxygen species (ROS)" [23]. "This leads a cascade of oxidative damage, impairing development and growth" [24] melatonin inhibit chlorophyll degradation, and increase leaf photosynthetic rates and the accumulation of dry matter [25].

Effect of melatonin and drought stress on H2O2 content in leaves: Data in Fig. 2 indicate that there are significant differences (P<0.05) between the studied treatments in terms of the H_2O_2 content in leaves.

Drought stress led to an increase in H_2O_2 content in leaves, While treatment with melatonin decreased H_2O_2 content in leaves compared to the control.

Treatment with melatonin under drought conditions at low concentration (50 micromolar) also led to an decrease in the H_2O_2 compared to the remaining treatments and the control.

"Stress increases the active oxygen yield and causes damage to cells. The H_2O_2 is an important signaling molecule for the plant stress response, and is widely involved in plant physiological and cross-resistance processes" [26].

Therefore, melatonin can directly scavenge excess ROS [27], and it can also promote the accumulation of other antioxidants and antioxidant enzymes to indirectly remove ROS [28].

Effect of melatonin and drought stress on proline content in leaves: Data in Fig. 3 indicate that there are significant differences (P<0.05) between the studied treatments in terms of the proline content in leaves.

drought stress led to an increase in proline content in leaves, While treatment with melatonin decreased proline content in leaves compared to the control.

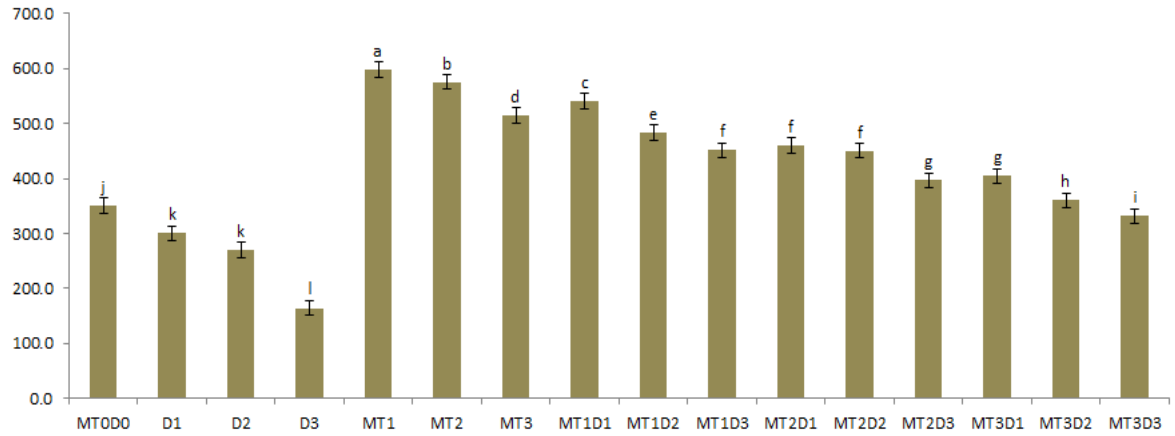

Fig. 1. Effect of melatonin on the chlorophyll content in tobacco leaves under drought stress

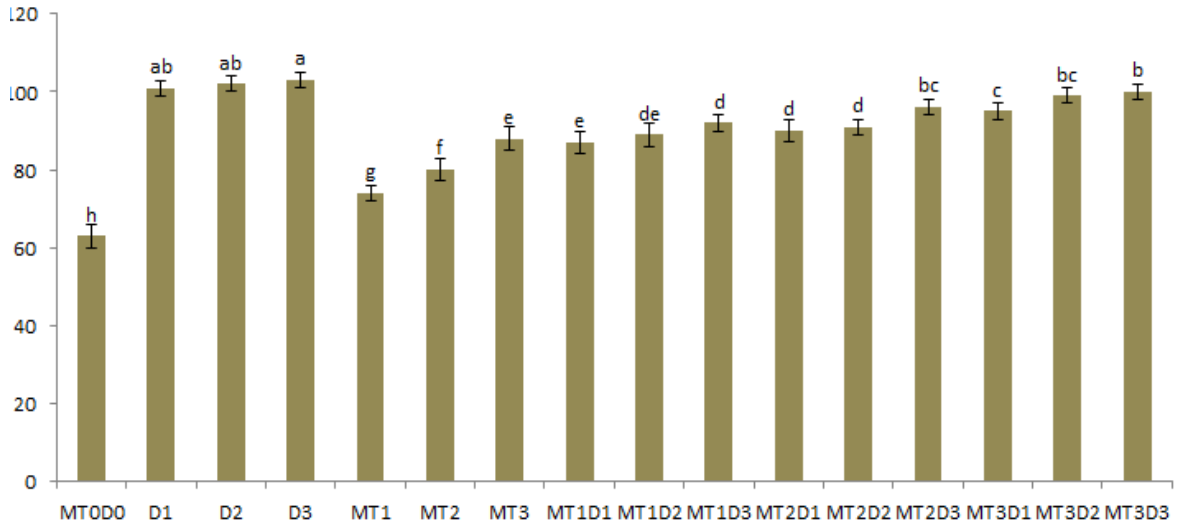

Fig. 2. Effect of melatonin on the H_2O_2 content in tobacco leaves under drought stress

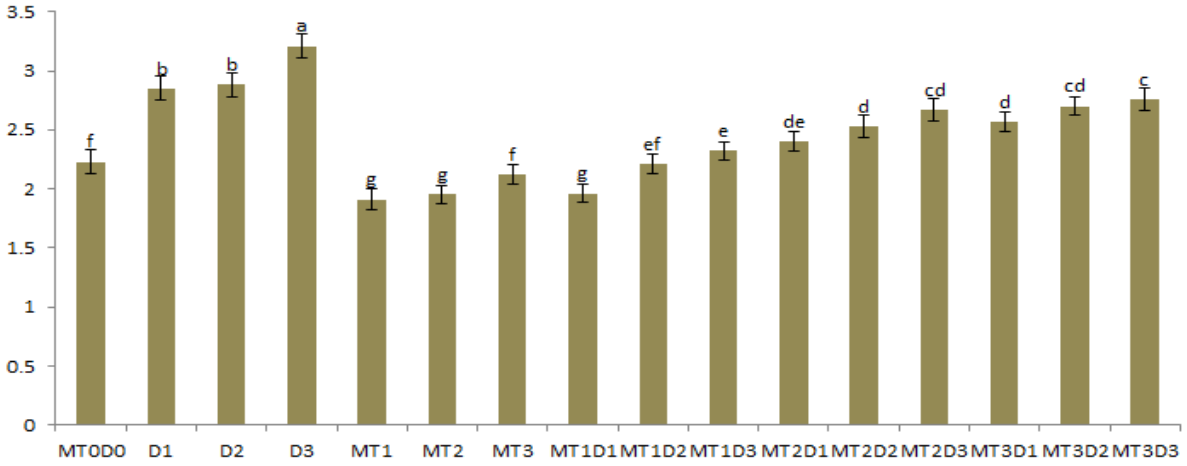

Fig. 3. Effect of melatonin on the proline content in tobacco leaves under drought stress

Treatment with melatonin under drought conditions at low concentration (50 micromolar) also led to an decrease in the proline compared to the remaining treatments and the control.

The results of current study showed that proline concentrations increased in response to drought, which is in agreement with prior research conducted by Cao et al. [29].

“This finding supports the notion that increased accumulation of free amino acids and proline is a common response of plants to water deficit conditions. Proline also protects plant cells from oxidative damage and effectively neutralises ROS in addition to acting as an osmoprotectant” [30].

Effect of melatonin and drought stress on malondialdehyde content in leaves: Data in Fig. 4 indicate that there are significant differences (P<0.05) between the studied treatments in terms of the malondialdehyde content in leaves.

drought stress led to an increase in malondialdehyde content in leaves, While treatment with melatonin decreased malondialdehyde content in leaves compared to the control.

Treatment with melatonin under drought conditions at low concentration also led to an decrease in the malondialdehyde compared to the remaining treatments and the control.

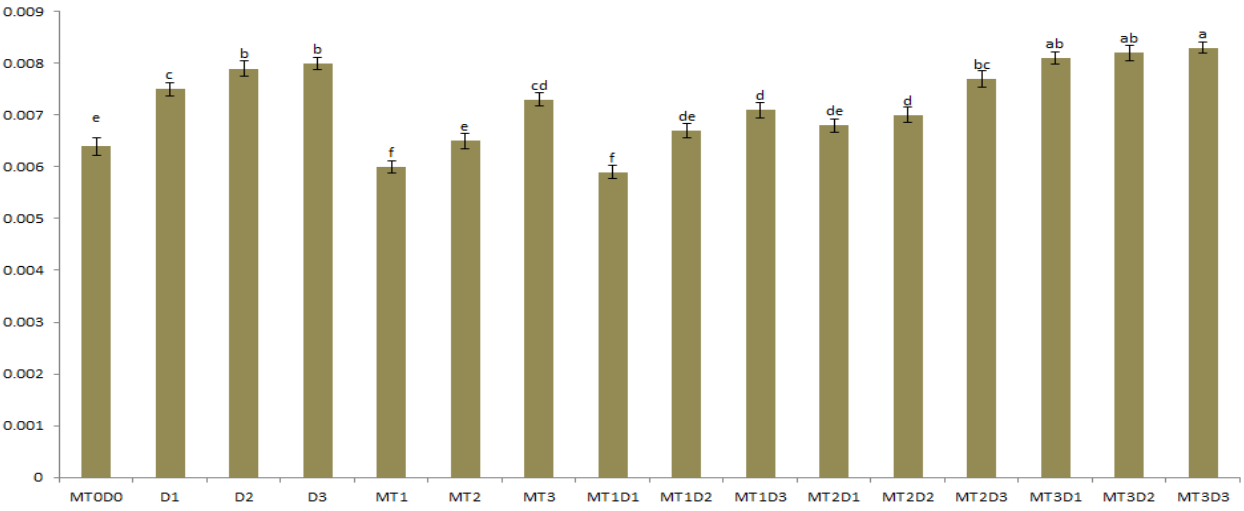

Fig. 4. Effect of melatonin on the malondialdehyde content in tobacco leaves under drought stress

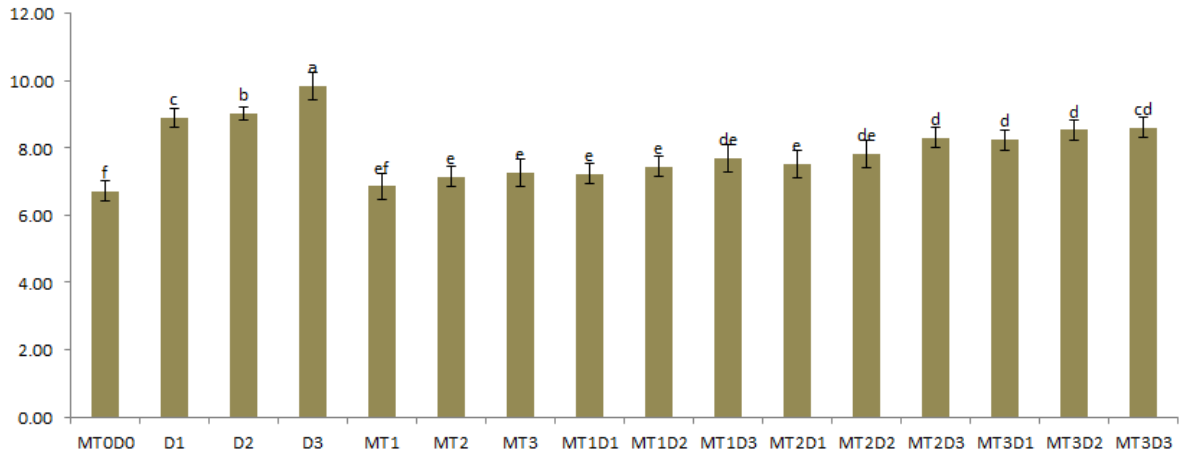

Fig. 5. Effect of melatonin on the malondialdehyde content in tobacco leaves under drought stress

"MDA and H_2O_2 are well-known oxidative damage metrics and both were elevated under drought in the current study, indicating increased oxidative stress in drought-treated plants" [31].

"MT pre-treatments alleviated oxidative stress in drought-treated plants, as indicated by lower H_2O_2 and MDA levels. Additional elevations in GST were achieved with the pre-treatment of MT under drought, showing that GST plays a positive function in detoxifying H_2O_2" [32].

"This confirms the role of MT as an inducer of antioxidant enzymatic activity and eliminating ROS. Melatonin is a multifunctional molecule that is known as an all-purpose antioxidant per se" [33].

Effect of melatonin and drought stress on sugar content in leaves: Data in Fig. 5 indicate that there are significant differences ($P<0.05$) between the studied treatments in terms of the sugar content in leaves.

drought stress led to an increase in sugar content in leaves, While treatment with melatonin decreased sugar content in leaves compared to the control.

Treatment with melatonin under drought conditions at low concentration also led to an decrease in the sugar compared to the remaining treatments and the control.

"Osmolytes, such as proline and soluble sugars, are produced by plants due to drought stress to maintain high osmotic pressure and water potential within the cells, maintaining the integrity of the cell membranes and enhancing drought resistance in crops" [34].

The accumulation and synthesis of soluble compounds, such as amino acids, particularly proline and soluble sugars, control osmoregulation during drought [35-37].

4. CONCLUSIONS

The study concluded that the application of melatonin can stimulate the antioxidant defense system, lower ROS levels. According to the results, using exogenous melatonin can be a viable and affordable way to lower crop losses and raise potential yields in water-limited environments. In order to increase plant tolerance and drought adaptation, our study also emphasizes the necessity of more research into the molecular mechanisms underpinning melatonin action and its effect on nutrient absorption. In light of global warming, the development of low-cost, environmentally friendly technologies, such as melatonin application, is critical. Our research offers fresh perspectives on the potential uses of melatonin in crop production.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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