

# **Exogenous melatonin induces tolerance to drought stress damage in seedlings and soybean plants**

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# ABSTRACT

Melatonin (ML) is an indolamine that regulates a wide range of physiological processes as growth of roots and shoots, antioxidant enzyme activity, seed germination, photosynthetic efficiency promoting stress tolerance in plants, mitigating the harmful effects of abiotic stress. Effects of pretreatment of soybean (Glycine max (L.) Merr.) seeds with 10, 30, 60 and 90 µM ML were evaluated in seedlings under osmotic stress measuring germination speed index, and root protrusion, growth, and DM. Under osmotic stress, ML increased germination speed index at 10 (239%), 30 (194%) and 60  $\mu$ M ML (189%); 5<sup>th</sup>-day root protrusion by 290%, 214%, 185% and 85% (10, 30, 60, and 90 µM ML respectively). The application of 10 (328%), 30 (224%), 60 (265%), 90 µM ML (129%) increased root length, followed by root DM in stressed seedlings. Drought stress reduced photosynthetic efficiency, increased ROS, and reduced antioxidant enzymatic activities in soybean. In a second experiment, well-watered (100% field capacity) (WW) and drought stressed (DS) soybean plants received 30 and 50 µM ML. Melatonin increased rate of CO<sub>2</sub> assimilation and net photosynthetic rate at 30 µM ML (WW) in 28% (2 d after application) and 50 µM ML (DS) in 48% (5 d after application). Melatonin increased antioxidant enzymes activity (catalase and peroxidase) in plants under drought stress. The ML application enhanced leaf area in WW plants, however decreased in stressed plants. An increased number of seeds, pod numbers, total seed mass, root DM were observed under WW. Drought stress increased numbers of seeds (27%), pod numbers (33%) and total seeds mass (27%) with 30 µM ML. These results demonstrated that ML mitigated negative effects produced by drought on germination and growth of soybean seedlings, and acted as a drought tolerance inducer, improving yield under stress condition.

**Key words:** Antioxidant, drought stress, gas exchange, *Glycine max*, melatonin, osmotic regulation, root growth, ROS, scavenging system, stress tolerance.

# INTRODUCTION

High temperatures and drought stress promote restrictions that affect seedling growth and crop establishment (Suliman et al., 2021). Drought stress impedes plant growth by reducing water uptake of cells and adversely influences many physiological and biochemical responses. Inhibition of photosynthesis is one of the primary physiological consequences of drought stress and occurs due to reduced intercellular  $CO_2$  concentrations (C<sub>i</sub>) due to stomatal closure, chlorophyll degradation, and impairment of photochemical apparatus. Furthermore, it causes a decrease in cell expansion, reduced absorption of  $CO_2$ , and degradation of chlorophyll *a*, which leads to a decrease in the accumulation of photoassimilates, causing less plant growth and production (Golldack et al., 2014).

Drought-induced over reduction of the electron transport chain (ETC) results in excessive accumulation of toxic reactive oxygen species (ROS) at the molecular level, causing cellular oxidative damage (Graham et al., 2014; Xu et al., 2015). The ROS accumulation increases the production of superoxide ( $O_2^{-}$ ), singlet oxygen ( $_1O^2$ ), and hydrogen peroxide ( $H_2O_2$ ), which can damage plant DNA, proteins, lipids, carbohydrates, and nucleic acids (Boogar et al., 2014). In addition, it can cause oxidative damage to cell membranes due to  $H_2O_2$  accumulation. In response to this damage, plants have developed an effective defense mechanism through their enzymatic antioxidant system, which includes the production of superoxide dismutase peroxidase (SOD), peroxidase (POD), ascorbate peroxidase (APX), and catalase (CAT) (Hasanuzzaman et al., 2013). Under drought stress, plants compensate by increasing their root systems acquisition of immobile nutrients (Muler et al., 2014).

Earlier studies have been demonstrated that melatonin (N-[2-(5-methoxy-1H-indol-3-yl)ethyl]acetamide) boost antioxidant enzymes and prevent ROS-induced cell membrane damage (Zhang et al., 2013; Wang et al., 2021). Melatonin acts in the elimination of ROS and increases the activity of antioxidant enzymes, alleviating the effects of water stress (Zhang et al., 2019; Wang et al., 2021). Melatonin improves biochemical and photosynthetic mechanisms in response to water stress, with hormonal regulation, stomatal opening, sugar metabolism, and osmotic regulation (Wang et al., 2021). In seeds, melatonin improves germination performance, promotes greater primary and secondary rooting, mitigating the deleterious effects of stress (Chen et al., 2020). Melatonin regulates the growth of leaves, shoots, and explants and plays a role in leaf senescence. In addition, it can also regulate plant vegetative growth processes such as rooting, leaf aging, photosynthetic yield, and biomass yield (Arnao and Hernández-Ruiz, 2019; Jahan et al., 2020).

As a significant world cash crop, soybean (*Glycine max* (L.) Merr.) is an essential dietary source of oils and edible proteins. Soybean is sensitive to water deficit and can reduce seed yield under these conditions (Zhang et al., 2019). Drought causes a drop in water potential and a decrease in the uptake and accumulation of nutrients, reducing the production of soybean seeds. Application of melatonin is an effective strategy to mitigate the effects caused by water deficit in soybean (Zhang et al., 2019; Cao et al., 2020). Therefore, the aim of our study was to investigate whether melatonin supplementation induces tolerance to osmotic and drought stress damage in seedlings and soybean plants maintaining the antioxidant and redox systems in plants by improving production.

# MATERIALS AND METHODS

#### Germination tests

Seeds of soybean (*Glycine max* (L.) Merr.) 'Intacta' were used. The experiment with seeds was carried out in a Mangelsdorf germination chamber (ELETROLAB, São Paulo, Brazil) with a photoperiod of 12:12 h and a temperature of 28 and 25 °C day/night ( $\pm$  1° C). Two hundred seeds per treatment were used for the germination test. Treatments with different concentrations of melatonin (ML) (N-acetyl-5-methoxytryptamine) was of analytical grade, Sigma-Aldrich (St. Louis, Missouri, USA), were equivalent to 2.5 times the weight of the dry substrate of solutions with different doses of melatonin (0, 10, 30, 60 and 90 µM) directly on the Germitest sheets. Germination was carried out under two conditions, one of osmotic stress, using the mannitol (74.3 g L<sup>-1</sup>) to reach -1.0 MPa, and water treatment (control). The experiment was carried out in a 5 × 2 factorial design (five doses ML with and without osmotic conditions) with four replicates.

#### **Analyzed variables**

The germination test followed the Standards for Seed Analysis (Brasil, 2009). At 5 and 8 d after sowing, the seeds that formed seedlings were considered normal, that is, the emergence and development of the main embryonic structures were counted (Brasil, 2009). Germination speed index (GSI) was calculated according to Maguire (1962). Analysis of DM and seedling growth were performed using 4 of 10 seeds each. The following evaluations were made on the seedlings: shoot (SDM), root (RDM), and total DM (TDM = SDM + RDM), root (RL), shoot (SL) and total length (TL = RL + SL).

#### Soybean plants under drought stress

Soybean plants were cultivated under greenhouse conditions. Seeds were soaked in pots containing 8 kg eutrophic red Latosol of medium texture. Soil acidity and fertility were adjusted based on soil analysis and crop recommendation (Cantarella et al., 1998). The experiment was carried out in a complete factorial design  $(2 \times 3)$  with two conditions:

well-watered (100% field capacity) (WW) and drought stress (DS) induced by water withhold. And three forms of ML application: 0, 30, and 50  $\mu$ M ML (WW+ML and DS+ML). In all treatments, plants were watered at field capacity until the fourth leaf fully unfolded development stage (V4). Then, plant leaves were sprayed with ML doses until dripping.

In the same way, distilled water was also applied to the control plants by spraying until dripping, followed by irrigation suspension. The irrigated control plants were kept at 100% field capacity. Two days after ML application, plants were monitored until leaf water potential ( $\Psi$ f) of the treatment without ML application reached -1.0 MPa at 5 d; then, after 6 d were rehydrated until reaching  $\Psi$ f values close to -0.3 MPa (similar to those observed in plants maintained at field capacity). At this point, new measurements were carried out. The  $\Psi$ f measurements were performed at midday with a Scholander pressure chamber (model 1000, PMS Instruments, Albany, Oregon, USA).

#### Leaf gas exchange measurements

Instantaneous leaf gas exchange measurements were performed in six biological replicates per treatment using an open system infra-red gas analyzer (LI-6400XTR, Li-COR, Lincoln, Nebraska, USA). All measurements were performed on healthy and fully expanded leaves under saturating irradiance of 1200  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>, air CO<sub>2</sub> concentration of 400  $\mu$ mol mol<sup>-1</sup>, and 28 °C. Under these conditions, the following gas exchange parameters were measured: net photosynthetic rate (P<sub>n</sub>), stomatal conductance (g<sub>s</sub>), intercellular CO<sub>2</sub> concentration (C<sub>i</sub>), and transpiration rate (E).

#### Antioxidant enzymatic activity

The analyses were carried out when the plant on drought stress reached -1.0 MPa. Three sample points were collected during the experiment, and then, they were immediately frozen in liquid nitrogen and stored at -80 °C until analysis.

Spectrophotometric analyses of the antioxidant enzymes superoxide dismutase (SOD) activity were estimated based on the method by Giannopolitis and Ries (1977) using the photochemical nitroblue tetrazolium salt (NBT) method. Catalase (CAT) was determined using the hydrogen peroxide ( $H_2O_2$ ) and peroxidase (POD) method described by Peixoto et al. (1999).

#### **Biometric and yield analyses**

The total leaf area (LA) was evaluated at the end of the experiment 119 d after sowing with a portable leaf area meter (model LI-300A, Li-COR). After drying plants at 65 °C until constant weight, roots DM (RDM) and total seed mass were measured. Also, number of pods (Np) and number of seeds (Ns) with six replicates per treatment were determined.

#### Statistical analysis

The results were processed using ANOVA (p < 0.05). The averages of the values were compared by the Tukey test (P < 0.05), with the statistical program Sisvar 5.1 (Universidade Federal de Lavras, Departamento de Estatística, Lavras, Minas Gerais, Brazil). The graphs were elaborated with the Origin 8 software (OriginLab Corporation, Northampton, Massachusetts, USA). Principal component analysis (PCA) was performed to verify the grouping of the different seedling's responses to ML doses, considering the entire set of parameters measured. Considering that measurement units differed doses, data were log-transformed to reduce the effect of the numeric scale using the PCord program PC-ORD software (Wild Blueberry Media, Corvallis, Oregon, USA).

# RESULTS

#### Soybean seed germination

The results showed that under stress soybean seedlings germination speed index (GSI) and root protrusion were significantly different between drought stress (DS) and ML application (Figure 1). The doses 10, 30, and 60  $\mu$ M ML showed better performance in the GSI, with an increase of 239%, 194% and 189%, respectively. There were significant differences in all doses compared to control for the 5<sup>th</sup>-day root protrusion, 290%, 214%, 185% and 85%, respectively for 10, 30, 60, and 90  $\mu$ M ML. On the 8<sup>th</sup> day, root protrusion increased with ML application on DS. However, on the 8<sup>th</sup>-day root protrusion (60 and 90  $\mu$ M ML) increased to 76% and 114% and at 10  $\mu$ M ML was 33% lower compared with WW. Germination (%G) did not show significance between well-watered and stressed conditions.

Figure 1. Germination rate (A), germination speed (B), 5<sup>th</sup>-day root protrusion (C), 8<sup>th</sup>-day root protrusion (D). Treatments used different melatonin (ML) concentrations (0-90  $\mu$ M) in soybean seeds cultivated in a well-watered (WW) and drought stress (DS).



Bars represent the standard error of the mean of three replicates. Upper case letters compare within the same treatments (WW or DS), and lowercase letters compare the same doses for the different treatments. Different letters indicate significant differences, according to the test of Tukey ( $p \le 0.05$ ).

### Soybean seedling growth parameters

Drought stress had a strong inhibitory effect on overall plant growth. However, exogenous ML significantly alleviated those declines compared with DS plants that had received no supplementation. Those increases were then 328% for RL (10  $\mu$ M ML), followed by the doses of 30, 60, and 90  $\mu$ M ML, with an increase of 224%, 265%, and 129%, compared to treatment without ML. For SL had an increase on plants with 10 and 30  $\mu$ M ML. In this way, TL of seedlings obtained a significant difference between the treatments with ML and control (Figure 2). On DS+ML, all doses increased RDM, with 95%, 68%, 54%, and 40%, respectively. However, 90  $\mu$ M ML in the WW+ML decreased 32%. Melatonin only increased SDM with 10  $\mu$ M under osmotic stress. On the other hand, in the plants WW+ML, only 30  $\mu$ M ML promoted increases, 60 and 90  $\mu$ M ML decreased 13% and 40% compared with control values (Figure 3).

Figure 2. Shoot length (A) and root length (B). Treatments used different melatonin (ML) concentrations (0-90  $\mu$ M) in soybean seeds cultivated in a well-watered (WW) and drought stress (DS).



Bars represent the standard error of the mean of three replicates. Upper case letters compare within the same treatments (WW or DS), and lowercase letters compare the same doses for the different treatments. Different letters indicate significant differences, according to the test of Tukey ( $p \le 0.05$ ).

Figure 3. Root dry matter (A) and shoot dry matter (B). Treatments used different melatonin (ML) concentrations (0-90  $\mu$ M) in soybean seeds cultivated in a well-irrigated (WW) and drought stress (DS).



Bars represent the standard error of the mean of three replicates. Upper case letters compare within the same treatments (WW or DS), and lowercase letters compare the same doses for the different treatments. Different letters indicate significant differences, according to the test of Tukey ( $p \le 0.05$ ).

As well as the observations in growth parameters, ML treatment (DS+ML) also induced a significant increase in TDM (SDM + RDM). These results showed that the application of 10  $\mu$ M ML was more effective than 30, 60, and 90  $\mu$ M ML (109%, 68%, 54%, and 40%, respectively). Compared to the control plants, WW+ML had nonsignificant effects at 10 and 30  $\mu$ M ML. However, 60 and 90  $\mu$ M ML were 13% and 33% lower than untreated plants (Figure 4). These data suggest that ML treatment induce root growth under drought stress.

The results of the PCA analysis show that all groups were distinct considering the different ML doses and different water conditions. The PCA performed with all the data (Figure 5) represented 78.18% of the total variation of the original data by principal component 1, with principal component 2 being nonsignificant. The most critical parameters for discrimination in main component 1 were TDM, TL, RL, 5<sup>th</sup>-day root protrusion, RDM, SDM, SL and GSI.



Figure 4. Total dry matter. Treatments used different melatonin (ML) concentrations (0-90  $\mu$ M) in soybean seeds cultivated in a well-watered (WW) and drought stress (DS).

Bars represent the standard error of the mean of three replicates. Upper case letters compare within the same treatments (WW or DS), and lowercase letters compare the same doses for the different treatment. Different letters indicate significant differences, according to the test of Tukey ( $p \le 0.05$ ).

Figure 5. Principal component analysis (PCA) for soybean.



ML: Melatonin; WW: well-watered treatments; DS: drought stress.

#### Physiological performance of soybean plants under water stress

The selection of the best ML concentration on seedlings to understand the particular effect on plant growth and development is essential, though it is highly variable across different crops. The stomatal conductance  $(g_s)$  showed that the WW and DS groups were separated (Figure 6). After 24 h rehydration, we observed that water stressed plants treated with ML showed an increase in the  $g_s$  parameter.





Bars represent the standard error of the average of three replicates according to the test of Tukey ( $p \le 0.05$ ).

In the first moment,  $g_s$  followed the  $C_i$  (2 d after ML application). However,  $C_i$  after 5 d, 30  $\mu$ M ML (DS) increased compared to other treatments. After rehydration, no difference between WW and DS was found. After 2 d of ML application, net photosynthetic rate ( $P_n$ ) on 30  $\mu$ M ML (DS) plants showed higher values than other plants. Nevertheless, the  $P_n$  on 30  $\mu$ M ML (DS) was decreased 5 d after DS, and the highest values were observed in 50  $\mu$ M ML plants (DS). After rehydration, plants with 30  $\mu$ M ML (WW) decreased compared to other plants WW and DS.

Exogenous 50  $\mu$ M melatonin (DS) decreased transpiration (Figure 6) 2 d after application, followed by a behavior similar to the other days (5 and rehydration) and both groups (WW and DS). We evaluated gas exchanges (Figure 6) and found that after 5 d, plants subjected to drought stress, treated or not with ML, showed lower P<sub>n</sub>, g<sub>s</sub>, and E. Nevertheless, 30  $\mu$ M (WW) and 50  $\mu$ M ML (DS) plants showed higher P<sub>n</sub>, even when C<sub>i</sub> and g<sub>s</sub> did not differ from the other treatments. After rehydration, a behavior similar to all treatments was observed in plants subjected to DS and controls (WW). Moreover, all plants subjected to DS also exhibited higher rates of gas exchanges.

#### **Biochemical performance**

The analysis of the antioxidant enzyme superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) was carried out when leaf water potential ( $\Psi$ f) was maximal -1.0 MPa. Superoxide dismutase showed a reduction with WW+ML; 30  $\mu$ M ML decreased 48% compared to the control (0WW). The plants under DS increased 46% (30  $\mu$ M ML) SOD activity compared to plants under DS and without ML application (Figure 7).

The treatment 50  $\mu$ M ML did not show a difference to control (0DS). The CAT activity decreased in all treatments with ML and WW (59% and 10% with 30 and 50  $\mu$ M ML) compared to the irrigated control (Figure 7). The CAT activity increased significantly for all treatments supplied with ML + DS compared to the control. The treatments 30 and 50  $\mu$ M ML showed increases of 62% and 120%, respectively. Peroxidase activities decreased significantly in all treatments supplied with ML (WW and DS).

#### **Biometric and yield analyses**

Melatonin application acted positively on the leaf area (Figure 8) with 30 (40%) and 50  $\mu$ M ML (200%); number of seeds 50  $\mu$ M ML (38%), pod numbers 30 (87%) and 50  $\mu$ M ML (112%), total seed mass 30 (63%) and 50  $\mu$ M ML (25%), roots DM 30 (60%) and 50  $\mu$ M ML (72%), under WW (Figure 9). In contrast, ML pretreatment under drought stress reduced leaf area, and most characteristics (e.g., numbers of seed 27%, pod numbers 33%), and total seeds mass 27% increased with 30  $\mu$ M ML.





Bars represent the standard error of the mean of three replicates. Upper case letters compare within the same treatments (WW or DS), and lowercase letters compare the same doses for the different treatment. Different letters indicate significant differences, according to the test of Tukey ( $p \le 0.05$ ).

Drought stress had a strong inhibitory effect on overall plant growth. However, exogenous ML (30  $\mu$ M) significantly alleviated those declines compared with DS plants that had received no such supplementation. We also observed that there was a positive effect with WW treatment with ML.



Figure 8. Leaf area in soybean cultivated in well-watered (WW) and drought stress (DS), and treated with 30 (M30) and 50  $\mu$ M (M50) melatonin (ML).

Bars represent the standard error of the mean of three replicates. Upper case letters compare within the same treatments (WW or DS), and lowercase letters compare the same doses for the different treatment. Different letters indicate significant differences, according to the test of Tukey ( $p \le 0.05$ ).

Figure 9. Numbers of seeds, total seeds mass, pod numbers and root dry mass in soybean cultivated in well-watered (WW) and drought stress (DS), and treated with 30 (M30) and 50  $\mu$ M (M50) melatonin (ML).



Bars represent the standard error of the mean of three replicates. Upper case letters compare within the same treatments (WW or DS), and lowercase letters compare the same doses for the different treatment. Different letters indicate statistically significant differences, according to the test of Tukey ( $p \le 0.05$ ).

#### Principal component analysis (PCA)

The PCA for each water condition showed a separation of treatments considering the analyzed variants. The PCA (Figure 10) performed with all the data represented 50.3% of the total variation of the original data by principal component, with PC1 being 27.2% and PC2 23.1%. The parameters for discrimination in main component 1(PC1) were  $P_n$  5 d after application (eigenvector = 0.9284), E 5 d after application (eigenvector = 0.8904), and  $g_s$  5 d after application (eigenvector = -0.8730) and PC2 were  $g_s$  2 d after application (eigenvector = 0.8979),  $P_n$  2 d after application (eigenvector = 0.8605) and E 2 d after application (eigenvector = 0.8282).

It was possible to verify the separation of control and another groups, 50  $\mu$ M ML on WW and DS at the ends of the bottom right and left of Figure 10, while in the center of the graph, there are an approximation between 30  $\mu$ M ML on WW and DS.

## Figure 10. Principal component analysis (PCA) for soybean.



WW: Well-watered treatments; DS: drought stress. Square, circle, and triangles represent concentrations of melatonin at 0 (M0), 30 (M30), and 50  $\mu$ M (M50) melatonin, respectively.

## DISCUSSION

Water restriction critically limits plant growth, reduces leaf water potential ( $\Psi$ f), and may reduce crop production. In contrast, pre-treatment with ML promotes germination and improves significantly the growth of roots, and the accumulation of nutrients can effectively alleviate the impact of drought stress (Zhang et al., 2013; Sharma et al., 2020).

The germination of seeds begins with water absorption, a precondition for seed germination significantly affects these stages of plant metabolism. Seeds can only germinate after absorbing a certain amount of water, which is essential for the growth of the resulting seedlings (Xiao et al., 2019). Previous reports have also shown the regulatory function of ML in promoting growth activity in seedlings plants (Zhang et al., 2013). These results show that ML treatment promote soybean seedling's growth exposed to drought stress. Melatonin induces the catabolism of abscisic acid, upregulates the gibberellin acid biosynthesis, and other vital hormones for seed germination (Hai-Jun et al., 2015), acts as auxin promoting the growth of the seedling under drought stress (Li et al., 2018; Huang et al., 2019).

In the present study, we reported the role of ML in resistance to drought stress, promoting increases in drought tolerance in seedlings, since the plants that received this treatment maintained shoot growth similar to that of the control (wellwatered) plants and had a significant increase in root growth. Under drought and salt stresses during seed germination, starch hydrolysis is inhibited, making it difficult to supply large amounts of nutrients to the seeds, thus inhibiting seed germination. In cotton seeds germination, ML promoted  $\alpha$ -amylase and  $\beta$ -amylase activity under osmotic stress, promoting the germination (Chen et al., 2021), and exogenous ML increased biomass and leaf area of maize seedlings under drought stress (Wei et al., 2014), demonstrating alternative mechanisms of action for the seed to germinate under stressful conditions.

Plants have a complex system of antioxidant defenses, represented in enzymatic and nonenzymatic components that protect them from the harmful ROS action. Antioxidant enzymes play the dominant role in the defense mechanisms. In this sense, ML increased the activity of the antioxidant enzymes, reducing the oxidative damages caused by the stresses. Accordingly, the present study showed that drought-stressed plants promoted the most significant stimulus for the CAT and SOD activity. The antioxidative enzyme activity links to the regulation of key genes encoding these enzymes, maintaining cell redox balance under stressful conditions (Sharma et al., 2020). Exogenous ML application significantly reduces the drought-induced ROS by activating antioxidant defense mechanisms in most of the crops, such as observed for apple (Wang et al., 2012), maize (Ahmad et al., 2019), tomato (Ding et al., 2018), and soybean (Zou et al., 2019). The ROS are able to upregulate ML biosynthesis pathway genes and, therefore, enhance the plant's endogenous levels of ML (Zhan et al., 2019). Suggesting that, improvement in the enzymatic activities possibly decreased ROS levels.

There are several studies showing that exogenous ML can alleviate water stress photosynthesis inhibition (Wei et al., 2014; Zou et al., 2019). Photosynthetic activity depends on Rubisco content and its activity as well as the photosynthetic pigments chlorophylls and carotenoids. Melatonin is a biomolecule in ameliorating abiotic and biotic stresses of crop plants emerging as a multifunctional phytoprotectant (Tiwari et al., 2020).

This study demonstrated that treatment with 30 (WW) and 50  $\mu$ M ML (DS) increased photosynthetic rate and no difference for transpiratory rates, stomatal conductance, allowing the more excellent supply of assimilates for growing tissues (seeds weight). Photosynthesis is a vital energy conversion metabolic system for crop growth and development, and is the primary determinant of crop yields (Long et al., 2015).

Under drought stress conditions, plants limited their  $g_s$ ,  $P_n$ , and E (Figure 6), which caused stomatal closure, insufficient  $CO_2$  absorption, and restricted photosynthesis. However, 50  $\mu$ M ML increased  $P_n$ , despite  $C_i$  and  $g_s$  was no difference with other doses. This increase in  $P_n$  demonstrates up-regulation of Calvin cycle enzymes (Sadak et al., 2020).

Maintenance of photosynthetic efficiency, reduction of transpiration, and stomatal conductance, were promoted by the application of ML in apple and tomato plants, increasing their tolerance under drought stress (Ding et al., 2018; Liang et al., 2018). Water deficit has an adverse effect on the physiological production characteristics of soybean. However, ML maintained a high photosynthetic rate, significantly increased dry mass of roots, pod numbers, total seeds mass, and root biomass.

Treatment with ML reduces the inhibition of light energy absorption and utilization caused by water stress, increases the sucrose content through C assimilation, and contributes to increased growth and productivity (Zou et al., 2021). Melatonin is a ubiquitous and physiological compound proposed to be an important regulator controlling root development (Zhang et al., 2013). Auxin is induced by ML, acting on root growth, affecting water absorption, and causing an irreversible process of cell wall extension (Ahmad et al., 2019). Melatonin applications in both treatments (WW and DS) promoted a positive effect on root growth.

The present study showed that ML supplementation during water stress had the most significant impact on yield. Numerous studies showed that ML applications significantly alleviated the decline of indices and modulators of plant growth under different abiotic stress conditions (Xu et al., 2015; Zou et al., 2019). Collectively in the PCA, our experimental results indicate that ML is effective in connection to ML30WW and ML30DS. Plants subjected to stressful environments or aggressive conditions, such as severe water stress, showed the action of ML in combating challenging physiological situations (Arnao and Hernández-Ruiz, 2015). Thus, ML acts as a mitigation of the drought effect on soybean, enhancing the activity of antioxidant enzymes in soybean leaves, maintaining the photosynthetic rate and promoting increased production, maintaining its physiological and biochemical metabolism under water stress conditions, similar to well-watered plants.

# CONCLUSIONS

In summary, the present study demonstrated that treatment with exogenous melatonin reduces water stress damage to soybean seeds and promotes seedling growth. Exogenous melatonin significantly improved functioning of the antioxidant enzymes and the yield of soybean plants. This could be a determining factor to melatonin application at field levels.

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