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PHYSIOLOGICAL MECHANISM OF MELATONIN REGULATION IN SOYBEANS UNDER ALUMINUM STRESS

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Abstract

Aluminum (Al) is one of the main toxicants that affect crop growth in acidic soils. Melatonin (MT) is an important steroidal hormone that can increase the plant's stress resistance. However, the regulatory mechanism of Al stress in plants remains unclear. This study investigated the effects of MT on the growth, antioxidant system, and photosynthesis of soybean seedlings under Al stress. Results showed that, compared with the control group, Al stress resulted in lower superoxide dismutase, catalase, and peroxidase activity, increased accumulation of malondialdehyde in the leaves of soybean seedlings, increased membrane permeability, and significant decrease in the photosynthetic rate, transpiration rate, and stomatal conductance of seedlings. The fresh weight and dry weight of the seedlings decreased by 22.0% and 18.1%, respectively, and the growth was significantly inhibited. Foliar application of MT could effectively increase the anti-oxidative enzyme activity of soybean seedlings under Al stress, reduce the degree of membrane lipid peroxidation, relieve Al damage to chloroplast PSII, enhance its photosynthetic performance, and significantly increase the dry weight and fresh weight of the seedlings. Consequently, the damage caused by Al stress was effectively alleviating and the growth of seedlings was promoted.

Key words: Soybean, Aluminum stress, Melatonin, Antioxidant system, Photosynthesis.

Introduction

Aluminum (Al) is the most abundant metal element in the earth's crust, but it is not a necessary nutrient for plant growth and development (Chen *et al.*, 2010a). In the alkaline or neutral conditions, the Al in the soil exists mostly in the form of insoluble oxides or silicate precipitates. It is relatively less toxic to the plant growth and development, and generally does not cause negative effects. However, when the soil is acidic, insoluble Al can be converted into soluble Al and released into the soil solution. As a result, the content of active Al in the soil is continuously increased, thereby inhibiting the elongation of the root system of the plant, reducing its capability to absorb water and nutrients, and ultimately affecting the plant's growth, development, yield, and quality (Chen *et al.*, 2010a; Yao *et al.*, 2014). Statistics show that acid soils dominate the south of China, accounting for more than 20% of the country's land area. In addition, the application of substantial amounts of physiological acid fertilizers and the frequent occurrence of acid rain in recent years have further exacerbated the acidification of soil in China. Moreover, the content of soluble Al in the soil has continued to increase, thereby leading to an increasing inhibition of plant growth (Chen *et al.*, 2010b). As a result, Al stress has now become an extremely important obstacle to limiting the normal growth of crops in acidic soils.

Soybean (*Glycine max*) is a legume crop with a long history in China; it is rich in amino acids and vitamins. As a type of vegetable, grain, grass, and fertilizer crop, the soybean is favored by people; it plays a key role in the agricultural production in our country (Lu & Zhu, 2005; Lu & Zhu, 2006). Studies have shown that although soybean has strong adaptability and is widely cultivated and known as the pioneer crop for the improvement of acid soil in China, its tolerance to Al toxicity in acidic soils has a certain limit. Al stress causes the photosynthetic rate of soybean seedlings to decline and the plant height, weight, and dry weight to reduce by different degrees, thereby affecting their normal growth and development. Al toxicity is still an important obstacle to restricting soybean production in acidic soils (Azmat & Hasan, 2008).

MT belongs to the family of guanamines. Lerner *et al*., (1958) extracted MT from the bovine pineal gland and determined its chemical structure. The chemical name for MT is N-acetyl-5-methoxytryptamine (Jiang & Zu, 2015; Wei *et al.*, 2015). The study of MT mainly focused on the animal field at the early stage, indicating that it has the effects of enhancing animal immunity and delaying senescence. In recent years, MT has been found to promote plant seed germination and plant growth and to enhance the plant's resistance to high temperatures and salt damage. However, the effects of exogenous MT on alleviating Al stress damage are still rarely reported (Li *et al.*, 2012; Xin *et al.*, 2017; Wang *et al.*, 2016). To this end, the effects of MT on the growth, antioxidant system, and photosynthesis of soybean seedlings under Al stress were studied to confirm the protective mechanism of MT and provide reference for the reduction of Al stress damage by MT.

Materials and Methods

Material cultivation and treatment: Al-sensitive soybean (BD2) was used as the material, and the single factor randomized block design was utilized in the experiment. Rounded, full, intact seeds were selected and sterilized with 0.1% mercuric chloride solution for 15 minutes and then washed four times with distilled water. Three seeds were sown in one nutrient pot filled with brand new vermiculite, and 1/2 Hoagland nutrient solution was used for cultivation. After the emergence, one plant per pot was retained. When the first ternated compound leaf emerged, soybean seedlings with comparable size and growing condition were selected and experimented on three treatments, namely, CK: control, Al: Al stress, and MT+Al: Al stress+100 mmol/L

MT. During treatment, the control group was cultured with 1/2 Hoagland nutrient solution; the Al treatment group was cultured with1/2 Hoagland nutrient solution that contained 300 μmol/L Al chloride; the MT+Al treatment group was treated with a certained amount of corresponding MT, and the CK and Al groups were sprayed with the corresponding amount of fresh water on the first day and once every three days afterwards. The spray was performed three times, and the nutrient solution in each treatment was irrigated once every two days. The required indicators were measured after nine days of treatment. Thirty seedlings were treated each time and repeated three times.

Experimental measurement: The study referred to the previous method (Lu & Zhu, 2005) to measure the growth indices, the previous method to measure membrane permeability, MDA content, superoxide dismutase (SOD), and peroxidase (POD) activity (Li, 2000), and the Dhindsa's method to measure CAT enzyme activity (Dhindsa *et al*., 1982). The generation rate of the superoxide anion and hydrogen peroxide content is presented in the references (Lu *et al.*, 2012a).

CIRAS-3F portable photosynthesis instrument of the PP-SYTEMS company was used to measure photosynthetic parameters and determine the net photosynthetic rate (P_n), transpiration rate (T_r), stomatal conductance (G_s), and intercellular CO_2 concentration (C_i) of the third functional leaf of the seedlings (Lu *et al.*, 2012b). An open gas circuit was used for measurement, the light intensity was controlled at 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and the chamber temperature, reference chamber CO_2 concentration, and relative humidity were controlled at 25°C, 80 $\mu mol \cdot L^{-1}$, and 60%-70%, respectively. The fluorescence-induced kinetic curve was measured with a continuous excitation fluorometer (Handy PEA, Hansatech, UK).

Data analysis: The data were analyzed using SPSS 17.0 software. Multiple comparisons were performed using Duncan's new complex-difference method. Data statistics and mapping were performed using Microsoft Excel 2003.

Results

Effect of MT on the growth of soybean seedlings under Al stress: Table 1 shows that under Al stress, the growth of soybean was significantly inhibited, and the total fresh weight, upper dry weight, lower dry weight, and total dry weight of the seedlings were decreased by 22.0%, 37.3%, 26.9%, and 18.1%, respectively, compared with the soybean seedlings that grow normally. However, under Al stress, after spraying with MT, the seedlings showed better growing conditions compared with those under Al stress alone, and the total fresh weight, upper dry weight, lower dry weight, and total dry weight increased significantly by 14.3%, 24.0%, 11.8%, and 22.1%, respectively.

Effect of MT on the antioxidant enzyme activity of soybean seedlings under Al stress: Compared with the control group, SOD, POD, and CTA activities in soybean seedlings under Al stress were significantly decreased by 37.1%, 43.0%, and 37.2%, respectively. However, compared with seedlings under Al stress alone, SOD, POD, and CAT activities in the leaves of grafted soybean seedlings after foliar spraying of MT were significantly increased by 22.9%, 33.9%, and 29.6%, respectively. Evidently, the foliar spray of MT on the soybean seedlings under Al stress can regulate the antioxidant enzyme activity, thereby increasing the resistance of soybean seedling plants to Al stress, and effectively protecting the growth of soybean seedlings (Fig. 1).

Effect of melatonin on the superoxide anion production rate and hydrogen peroxide content in soybean seedlings under Al stress: Under Al stress, the production rate of superoxide anion ($O_2^{-\cdot}$) in soybean seedlings was increased to 81.6% higher than that of the control group. At the same time, the hydrogen peroxide (H_2O_2) content was also increased by 78.7% compared with the control group, thereby indicating that the active oxygen metabolism was unbalanced. The production rate of O_2^- and H_2O_2 content after melatonin treatment increased significantly compared with the control group. However, the increases were significantly less than those under Al stress alone, at only 69.9% and 66.7%. Evidently, MT can significantly reduce the production rate of H_2O_2 and $O_2^{-\cdot}$in soybean seedlings under Al stress and the damage caused by active oxygen (Fig. 2).

Effect of MT on MDA content and membrane permeability of soybean seedlings under Al stress: Compared with the control group, the MDA content and relative electrical conductivity (REC) of soybean seedlings under Al stress increased by 87.7% and 95.6%, respectively, and the difference was significant (Fig. 3). However, after MT treatment, the MDA content and REC of soybean seedlings were decreased by 33.2% and 22.6%, respectively, compared with those under Al stress alone. The results showed that MT could reduce the membrane lipid peroxidation and cell membrane permeability of soybean seedlings under Al stress, thereby increasing their tolerance to Al stress.

Table 1. Effects of MT on growth of soybean seedlings under Al stress.

Treatment	Fresh weight/g	Dry weight/g	Dry weight of shoot/g	Dry weight of root/g
CK	14.89 ± 0.52a	2.66 ± 0.09a	2.31 ± 0.09a	0.35 ± 0.02a
Al	11.62 ± 0.93c	1.86 ± 0.18c	1.61 ± 0.15c	0.25 ± 0.02c
Al+MT	13.28 ± 1.52b	2.28 ± 0.17b	2.00 ± 0.15b	0.28 ± 0.02b

Note: Small letters mean significant difference among treatment at 0.05 level. The same below

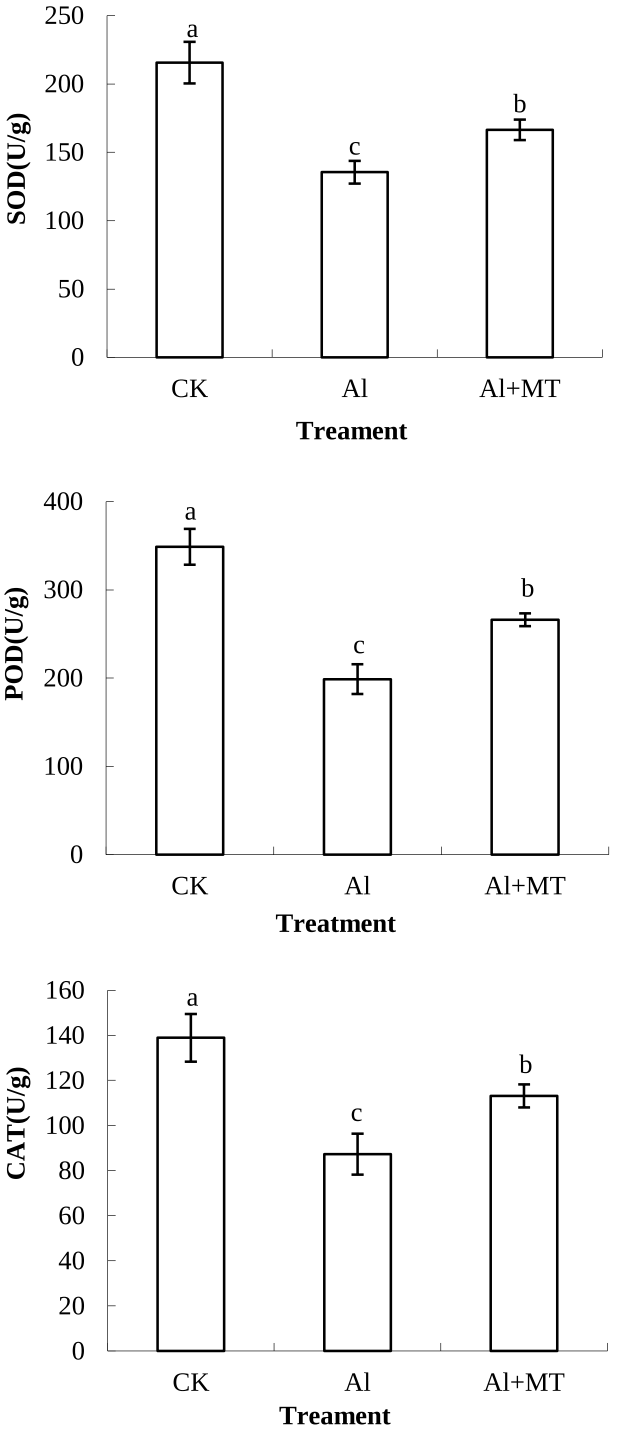

Fig. 1. Effects of MT on SOD，POD and CAT activities in leaves of soybean seedlings under Al stress .

Effect of melatonin on photosynthetic exchange parameters of soybean seedlings under Al stress: The net photosynthetic rate (P_n), stomatal conductance (g_s), and transpiration rate (T_r) of soybean seedlings under Al stress were significantly decreased by 19.8%, 16.8%, and 17.1%, respectively, compared with the control group. Although the intercellular CO_2 concentration (C_i) was decreased by 5.31% compared with the control group, the difference was not significant. After foliar spraying of MT, P_n, g_s, and T_r of soybean seedlings were significantly increased by 11.3%, 18.5%, and 13.7%, respectively, compared with those under Al stress alone. The intercellular CO_2 concentration (C_i) was lower than that under Al stress alone and was significantly different from that of the control group (Table 2).

Effect of MT on the chlorophyll fluorescence curve of soybean seedling leaves under Al stress: Although the initial phase fluorescence Fo (point O) increased to varying degrees after the Al stress treatment and the MT treatment, the increase after the MT treatment was lower than that after the Al stress treatment alone, thereby indicating that the MT treatment would reduce the number of active reaction centers on soybean leaves and thus mitigate the damage of Al stress to the leaf reaction center. The value of Fm, that is, the fluorescence intensity at the maximum fluorescence point (point P) represents the photochemical activity of PSII and reflects the electron transport status of PSII. Al stress and MT treatment reduced the Fm value of the leaves by 23.1% and 8.6%, respectively, compared with that of the control group. Therefore, Al stress significantly reduced the photochemical activity of chloroplast PSII, thereby reducing the light energy absorbed per unit area of the leaf. Moreover, MT treatment mitigated the effect of Al stress on the photochemical activity of the chloroplast photosynthetic system PSII (Fig. 4).

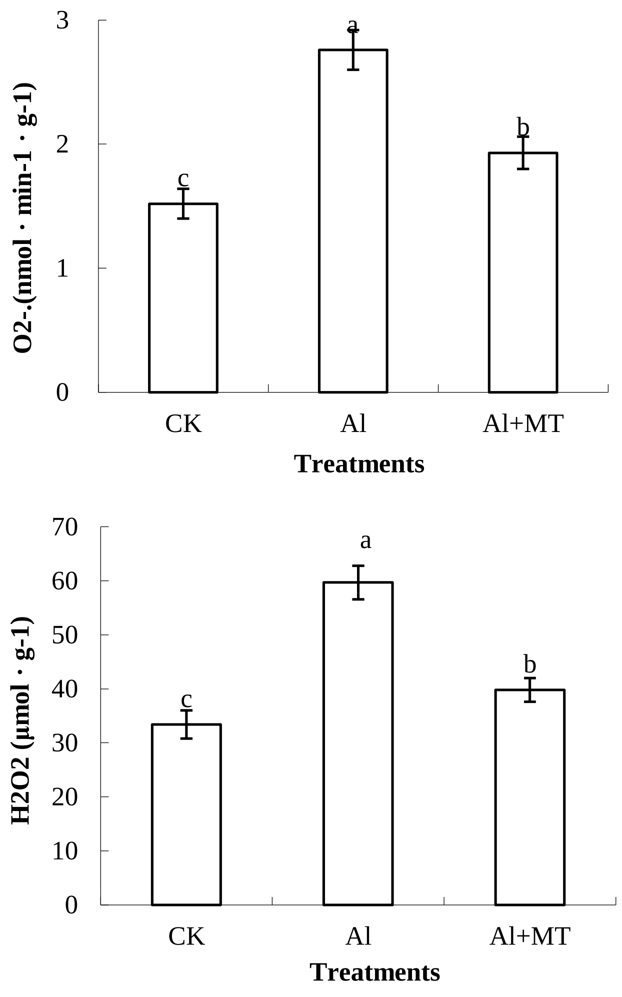

Fig. 2. Effects of MT on the $O_2^{\cdot-}$ production rate, H_2O_2 contents of soybean seedlings under Al stress.

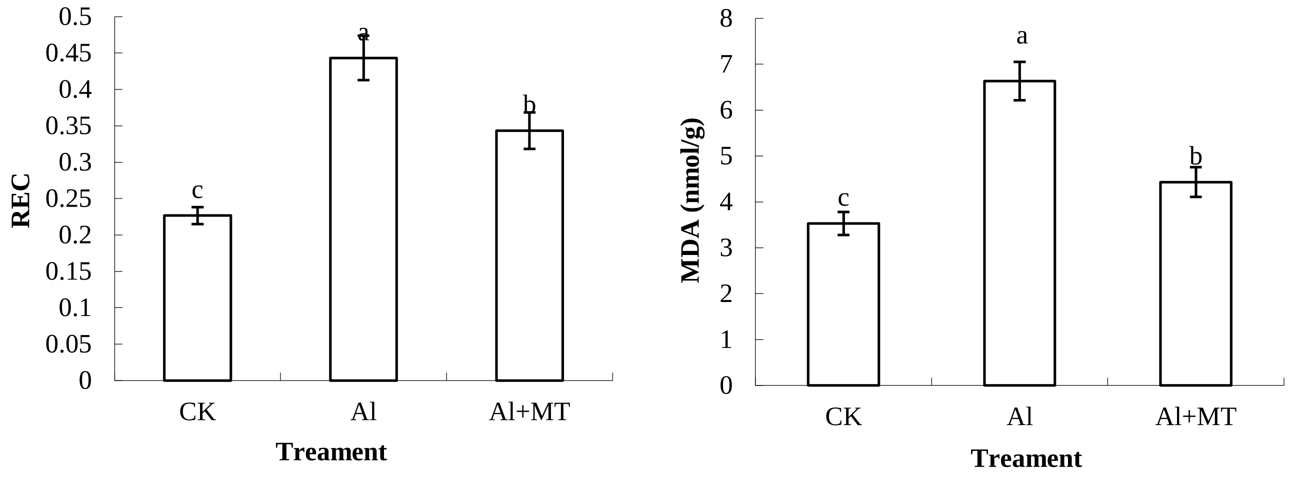

Fig. 3. Effects of MT on MDA and cell membrane permeability of soybean seedlings under Al stress.

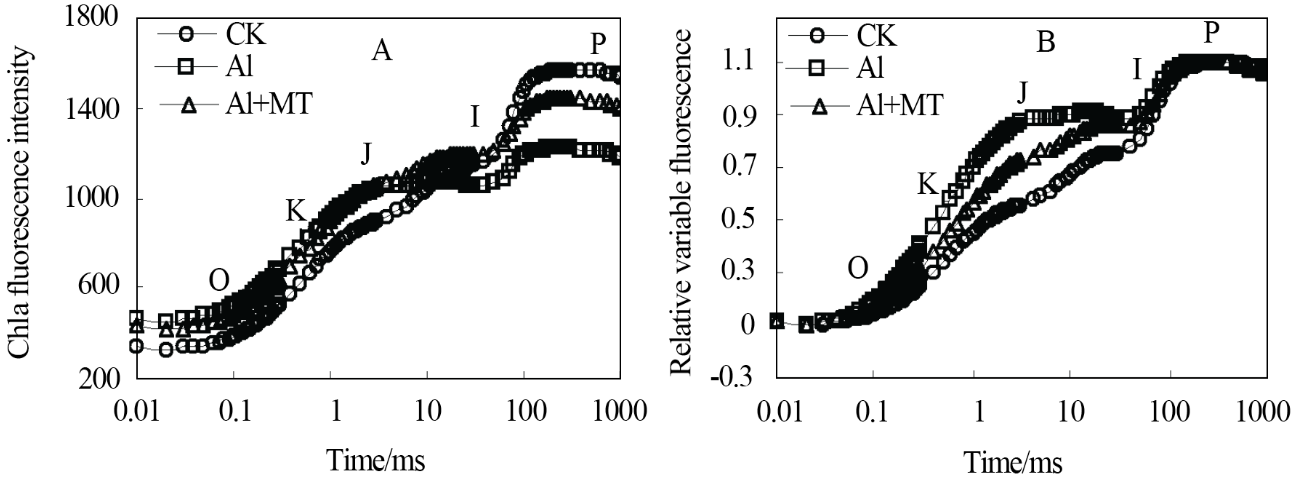

Fig. 4. Effect of MT on the chlorophyll fluorescence curve of soybean seedling leaves under Al stress.

Table 2. Effects of MT on photosynthetic gas exchange parameters of soybean seedlings under Al stress.

Treatments	Pn/(μmol·m^{-2}·s^{-1})	Gs/(mmolH_2O·m^{-2}·s^{-1})	Tr /(mmolH_2O·m^{-2}·s^{-1})	Ci/(μmolCO_2 mol^{-1})
CK	19.96 ± 0.91a	441.7 ± 25.7a	8.8 ± 0.30a	314.0 ± 8.7a
Al	16.00 ± 1.29c	367.3 ± 12.7b	7.3 ± 0.20 b	297.3 ± 12.6ab
Al+MT	18.13 ± 0.15b	435.3 ± 20.0a	8.3 ± 0.10 a	281.3 ± 3.5b

Discussion

Under normal environmental conditions, the production and elimination of reactive oxygen species (ROS) are in a dynamic equilibrium. When plants are subjected to stress, the self-regulating homeostasis in cells is disrupted, leading to the production of large amounts of ROS, such as O_2^- and H_2O_2, which cause increased membrane lipid peroxidation and inhibit plant growth or even cause death of the plant. MDA, as a product of membrane lipid peroxidation, can cause damage to the membrane structure, resulting in increased membrane permeability that impedes normal metabolism and growth. The relative electrical conductivity of MDA and cell membranes, to a certain extent, reflect the degree of damage of the cell membrane (Rosenbaugh *et al.*, 2012). Studies have shown that SOD, POD, and CAT are the most important antioxidant enzymes in plants. When plants are under stress, SOD, POD, and CAT activities also change. SOD is the first barrier to eliminate ROS. When stress occurs, the level of superoxide anion in plants is increased. When SOD eliminates superoxide anion, it also produces singlet molecular oxygen with strong oxidizing capability and singlet molecular oxygen H_2O_2. POD and CAT can effectively prevent the accumulation of hydrogen peroxide in plants, thereby eliminating its potential damage to the plant cell membrane structure, and the elimination of H_2O_2 in plants depends on the interaction of two protective enzymes CAT and POD (Fariduddin & Chalkoo, 2011).

Gao *et al.,* (2014) found that the stem diameter, plant height, and dry fresh weight of cucumber seedlings significantly increased after spraying with exogenous MT under low temperature and low light. Moreover, the activities of SOD, POD, CAT, and APX were significantly increased; the electrolyte permeability of seedling leaves were increased, and the MDA accumulation was decreased. These findings proved that exogenous MT could improve cucumber seedlings' resistance to low temperature. Xu *et al.,* (2011) found that MT could significantly increase the activity of antioxidant enzymes in cucumber seedlings, reduce H_2O_2 content, decrease MDA and membrane permeability, inhibit ROS production in cucumber seedlings, improve the antioxidant system, protect the integrity of lipid membrane, enable normal physiological metabolism and enhance photosynthetic capacity of seedlings, and improve cucumber seedlings' capability to withstand high temperatures. Zhang *et al.,* (2014) found that exogenous MT could increase SOD, POD, CAT, and other antioxidant enzymes in *Pennisetum alopecuroides* under salt stress, reduce MDA content, reduce the damage caused by ROS, promote dry matter accumulation, and enhance the anti-NaCl stress capability of *P. alopecuroides*. In the present study, compared with the control group, Al stress resulted in a decrease in superoxide dismutase and catalase activity in leaves of soybean seedlings, an increase in peroxidase activity and MDA accumulation, and an enhancement of membrane permeability. Foliar spraying of MT can effectively increase the antioxidant enzyme activities of soybean seedlings under Al stress, enhance the antioxidant system of seedlings, reduce the MDA toxicity of seedlings, reduce the degree of membrane lipid peroxidation, and improve the resistance of seedlings, thereby effectively mitigating the damage caused by Al stress.

Photosynthesis is the largest material energy conversion on earth. Its strength plays a crucial role in crop yield. Photosynthetic rate can directly reflect the strength of photosynthesis of plants, and this rate is an important indicator of whether the photosynthetic system is operating normally (Anuradha & Rao, 2009). Xu *et al.,* (2016) found that exogenous MT could increase the content of chlorophyll in seedlings, and the increase in P_n could alleviate the damage of high temperature stress to the photosynthetic system of cucumber; thus, the damage caused by high temperature to cucumber seedlings is mitigated. Yu *et al.,* (2015) used the red long eggplant as the experimental material and found that the appropriate concentration of MT could promote the increase of chlorophyll content and P_n to enhance the photosynthetic capacity of the eggplant seedlings and to improve the resistance of eggplant seedlings to high temperature. Ye *et al.,* (2015) confirmed through experiment that by improving the antioxidant capacity of wheat seedlings, MT could inhibit oxidative damage, maintain a relatively high chlorophyll content, enhance the P_n of wheat seedlings, enhance their photosynthetic performance, and significantly increase the dry weight and fresh weight of seedlings, thereby promoting the growth of seedlings and enhancing their photosynthetic capacity. In the present study, compared with the control group, Al stress led to a significant decrease in the photosynthetic rate, stomatal conductance, and transpiration rate of soybean seedlings. MT could slow down the decline of the photosynthetic rate, stomatal conductance, and transpiration rate of soybean seedlings under Al stress, enhance their photosynthetic performance, and thus promote seedling growth and effectively mitigate the Al damage.

Chlorophyll fluorescence technology is an important method to measure the stability of photosystem during the photosynthesis of leaves under stress. The heat dissipation of the PSII antenna pigment often leads to a decrease in F_0, whereas the destruction or reversible deactivation of the PSII reaction center can cause an increase in F_0, resulting in photoinhibition. This study found that Al stress significantly reduced the photochemical activity of chloroplast PSII, resulting in a decrease in effective light energy absorption and photosynthetic efficiency. A treatment with appropriate concentration of MT could effectively mitigate the damage to PSII caused by Al stress and maintain the operation of PSII electron transfer.

Conclusion

In summary, Al stress led to increased SOD, CAT, and POD activities, as well as increased MDA accumulation and membrane permeability. Moreover, Al stress significantly decreased photosynthetic and transpiration rate, decreased dry weight and fresh weight, and significant inhibition of the growth of soybean seedlings. Foliar application of MT can effectively increase the anti-oxidative enzyme activity of soybean seedlings under Al stress, reduce their membrane lipid peroxidation, mitigate the damage to chloroplast PSII caused by Al, enhance their photosynthetic performance, and significantly increase the dry weight and fresh weight of seedlings. Such change led to effective mitigationof the damage caused by Al stress and promotion of seedling growth.

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References

Anuradha, S. and S.S.R. Rao. 2009. Effect of 24-epibrassinolide on the photosynthetic activity of radish plants under cadmium stress. *Photosynthetica*, 47: 317-320.

Azmat, R. and S. Hasan, 2008. Photochemistry of light harvesting pigments and some biochemical changes under aluminium stress. *Pak. J. Bot.*, 40(2):779-784.

Chen, L.S., Y.P. Qi and H.X. Jiang. 2010. Photosynthesis and photoprotective systems of plants in response to aluminum toxicity. *Afri. J. Biotech.*, 9(54): 9237-9247.

Chen, N., X.R. Wang and X.L. Yan. 2010. Interactive effects of P deficiency and Al toxicity on soybean growth: a pot experiment with acid soil. *Chin. J. Appl. Ecol.*, 21(5):1301-1307.

Dhindsa, R.S., P. Plumb-Dhindsa and T.A. Thorpe. 1982. Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot.*, 32: 93-101.

Fariduddin, Q.M.Y. and S. Chalkoo. 2011. 28-homobrassinolide improves growth and photosynthesis in *Cucumis sativus* through an enhanced antioxidant system in the presence of chilling stress. *Photosynthetica*, 49: 55-64.

Gao, Q., Y. Wang, X.M. Lu and Y. Miao. 2014. Effects of exogenous melatonin on growth and antioxidant system of leaves in cucumber seedlings under low temperature and weak light stress. *Acta Bot. Boreali-Occidentalia Sin*., 34(8): 1608-1613.

Huang, S.C., Q.H. Gao and Y.J. Shu. 2016. Effects of $LaCl_3$ on the photo-synthesis and anti-oxidant capacity of soybean seedlings under aluminum stress. *J. Anhui Sci. & Tech. Univ.*, 30(2):10-15.

Jiang, C. and C. Zu. 2015. Advances in melatonin and its roles in abiotic stress resistance in plants. *Biotech. Bull.*, 31(4): 47-55.

Li, C., P. Wang and Z. Wei. 2012. The mitigation effects of exogenous melatonin on salinity-induced stress in Malushupehensis. *J. Pineal Res.*, 53(3): 298-306.

Li, H.S. 2000. Principles and techniques of plant physiological and biochemical experiments. Higher Education Press, Beijing.

Lu, X. and S. Zhu. 2005. Effects of several drugs on seeding growth and resistance physiology of early--maturing maodou under water stress. *J. Soil Water Conserv.*, 19(2):195-198.

Lu, X.M. and S.D. Zhu. 2006. Effects of some drugs on resistance of early-maturing Glycine max under water stress. *ActaPrataculturae Sin.*, 15(3):86-92.

Lu, X.M., J. Sun and S.R. Guo. 2012. Effects of brassinolide on the mitochondria antioxidant system and cellular ultrastructure of cucumber seedling roots under hypoxic stress. *Acta Hort. Sin.*, 39(5): 888-896.

Lu, X.M., J.Sun, S.R. Guo and B. Li. 2012. Effects of exogenous 2,4-epibrassinolide on the leaf photosynthetic characteristics and polyamines content of cucumber seedlings under hypoxia stress. *Chin. J. Appl. Ecol.*, 23(1): 140-146.

Rosenbaugh, E.G., D.S. Manickam and E.V. Batrakova. 2012. Neuronal uptake and subcellular localization of functional nanoformulated copper/zinc superoxide dismutase(SOD nano). *FASEB J.*, 26: 893.

Wang, L.Y., J.L. Liu and W.X. Wang. 2016. Exogenous melatonin improves growth and photosynthetic capacity of cucumber under salinity-induced stress. *Photosynthetica*, 54(1): 19-27.

Wei, W., Q.T. Li, Y.N.Chu, R.J.Reiter, X.M.Yu and D.H. Zhu. 2015. Melatonin enhances plant growth and abiotic stress tolerance in soybean plants. *J. Exp. Bot.*, 66(3): 695-707.

Xin, D., J.J. Si and L.P. Kou. 2017. Postharvest exogenous melatonin enhances quality and delays the senescence of cucumber. *Acta Hort. Sin.*, 44(5): 891-901.

Xu, X.D., Y. Sun and X.Q. Guo. 2011. Effect of exogenous melatonin on photosynthesis and chlorophyll fluorescence parameters in leaves of cucumber seedlings under high temperature stress. *J. Nucl. Agri. Sci.*, 25(1): 179-184.

Yao, X., J. Li, X. He, H. Liu and X.F. Shen. 2014. Effect of silicon on the morphological and physiological characteristics of peanut seedling under aluminum stress. *Chin. J. Oil Crop Sci.*, 36(6): 815-818.

Ye, J., X.P. Deng, S.W. Wang and L.N. Yin. 2015. Effects of melatonin on growth, photosynthetic characteristics and antioxidant system in seedling of wheat under drought stress. *J. Triticeae Crop*, 35(9): 1275-1283.

Yu, X., J. Li, Y. Xie and Y. Tang. 2016. Effects of exogenous melatonin on photosynthesis and antioxidant activity of eggplant seedlings under high temperature stress. *J. Hunan Agri. Uni.*, 42(5): 496-499.

Zhang, N., Q. Jiang, D.B. Li, L.T. Cai, H.J. Zhang and W.J. Si. 2014. Effect of exogenous melatonin on germination of *Pennisetum alopecuroides* under nacl stress. *J. China Agri. Uni.*, 19(4): 54-60.

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