Melatonin priming enhances symbiotic nitrogen fixation in soybean, *Glycine max* L.

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In addition to helping plants protect against various biotic and abiotic stresses, melatonin plays important regulatory roles in plant growth, reproduction, and development. In the present study, we examined the effect of melatonin priming on soybean biological nitrogen fixation in both greenhouse and field settings. In the greenhouse experiment, melatonin treatment significantly increased number of nodules formed on soybean roots. In addition, the size of nodules was also dramatically increased in melatonin-treated group. More than 75% of nodules in melatonin-treated group were larger than 2 mm in diameter while only about 40% were larger than 2 mm in control group. The effect of melatonin on nitrogen fixation was further confirmed in field experiments with three soybean varieties being tested. Furthermore, with a three-week growth period, melatonin also significantly increased fresh shoot biomass production in the field experiment. Our results indicate the potential application of melatonin in increasing crop production and warrant a further investigation to dissect the molecular mechanism(s) regulating melatonin-mediated symbiotic N fixation in legume species.

Keywords: melatonin; soybean (*Glycine max L*); nitrogen fixation.

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Introduction

Since its first identification in the cattle pineal gland in 1958 [1], melatonin (N-acetyl-5-methoxytryptamine) was re-discovered in plant species in 1995 [2, 3], and in unicellular organisms in 2003 [4]. The biological roles of melatonin have been extensively studied in both animals and plants. In animals, melatonin influences circadian rhythms, mood, sleep, and food intake, and mediates changes in seasonal reproduction, blood glucose, as well as the immune system [5-8]. In plants, melatonin affects photosynthesis and organ development [9-10], and influences leaf senescence [11-12]. Melatonin also plays important roles in defending against both biotic and abiotic stresses in many plant species [13-19].

The chemical structure of melatonin is classified as an indolic compound and its biosynthesis in both animals and plants is through a tryptophan-dependent pathway [20-22]. Interestingly, auxin also belongs to the indole group in structure and its biosynthesis in higher plants is at least partially through tryptophan-pathway [23]. Auxin is a well-known plant hormone and plays important roles in control of plant growth and development [24]. The similarity in both chemical structure and biosynthetic pathway between melatonin and auxin triggered extensive investigations on melatonin's role in affecting plant growth and development. Many studies demonstrated that melatonin, in fact, did regulate plant growth and promote crop production. For example, melatonin was reported to promote root development in *Hypericum perforatum* L. [25], *Brassica juncea*

[26], sweet cherry [27], and corn [28]. Melatonin also promotes vegetative growth in various plant species including economically important crops such as wheat, barley, rice, soybean, corn, tomato, pepper, and cucumber [29-35] and model species *Arabidopsis thaliana* [36]. In addition, melatonin can also preserve chlorophyll content [37-39] and increase the photosynthetic efficiency [40-41].

In view of biomass and yield increase, it was also reported that melatonin can enhance crop production in certain species. For example, Tan et al. [28] reported that melatonin treated corn plants yielded 20% more grains than that from non-melatonin treated plants. In soybean, melatonin treatment improved plant growth at both vegetative and reproductive stages resulting not only an increased biomass production, but also a significant high yield [42]. The increased biomass production by melatonin was also demonstrated in tomato where the transgenic tomato plants carrying a rice IDO gene not only had lower endogenous melatonin levels but also reduced their biomass production [43]. Furthermore, a recent study reported that melatonin treatment can increase nitrogen uptake, especially in a nitrogen deficient condition in winter wheat and resulted in 16% to 23% more yields in melatonin treated plants comparing to that non-melatonin treated under either N sufficient or N deficient conditions [44].

This melatonin mediated biomass and yield increase in various crops might be due to, in general, 1) melatonin promotes lateral root development [19, 30, 45, 46], and 2) melatonin enhances nitrogen uptake [44]. However, in legume species, such as soybean, the yield and biomass increase by melatonin treatment could also be due to increase in symbiotic nitrogen fixation. Melatonin is widely known for its beneficial roles to human health [21, 47] and has less risks to negatively affect environments. In recent years, it has been extensively studied for its potential roles in improving crops production [42, 48-50]. However, no study so far is focused on investigating the role of exogenous melatonin in regulating nitrogen fixation in legume species. The objective of this study was to use soybean as a legume model to examine the effects of exogenous melatonin on symbiotic nitrogen fixation. Both greenhouse and field experiments were designed. We found that 16-hour melatonin priming did not affect seed germination rate, but significantly increased number of nodules formed on soybean root in a two-week greenhouse experiment. Furthermore, the percentage of nodules with the size larger than 2 mm was significantly increased in melatonin priming treatment. The plant heights were not affected by the priming. Similarly, in the field experiment, melatonin priming also enhanced N fixation efficiency and yielded more nitrogen nodules on soybean roots with all three soybean genotypes tested. Different soybean genotypes varied on N fixation affected by melatonin priming but the trend remained the same. Shoot biomass was also significantly affected by melatonin priming. These findings provide fundamental information for the role of melatonin in legume N fixation and warrant a further study on the molecular mechanism of melatonin function in regulating N fixation in legume species.

Materials and Methods

Soybean varieties used in the experiments

Three soybean varieties, Osage, Dillon, and breeding line VS-22 were used to examine the role of melatonin in affecting N fixation. In the greenhouse experiment, only Osage was used while in the field experiment, all three varieties were tested.

Melatonin priming

Melatonin was dissolved in 100% ethanol at the concentration of 50 mM. When priming, the storage solution was diluted to 100 µM with water. Soybean seeds were then soaked in 100 µM melatonin solution for 16 hours. As a control, the seeds were soaked in water with same amount of ethanol added. After priming, all seeds were rinsed with tap water thoroughly before germinating tests or planting in greenhouse and field.

Germination test

Thoroughly rinsed soybean Osage seeds were germinated on petri-dishes with Whatman paper for 4 days under room temperature (22°C - 23°C) with a 16h/8h light-dark cycle. The germination rate for both melatonin treated and control samples was recorded and calculated. The experiment was triplicated with about 50 seeds for each treatment.

Greenhouse experimental design

The media used for nitrogen fixation evaluation was a mixture of half Redi-Earth potting soil and half field soils from soybean growing field at the Randolph Farm of the Virginia State University. Thoroughly rinsed Osage seeds of melatonin-primed and control treatments were directly sowed in pots containing pre-watered media. The seedlings were grown in the greenhouse with natural lights and temperature of 30°C during the daytime and 25°C at the night for 14 days before recording the nodule numbers and sizes. The plant height was measured prior to pulling out plants for nodule measurement. Ten plants of each treatment were measured, and the experiment was triplicated. For statistical analysis, a Student's T test was conducted to identify significant differences between treatment and control at level of $P < 0.05$.

Field experimental design

The effect of melatonin on N fixation was further explored with three different soybean genotypes (Osage, Dillon, and VS-22) in the field experiment. All seeds were primed with 100 µM melatonin for 16 hours and thoroughly rinsed with tap water prior to sowing. Melatonin primed seeds together with the control seeds were sowed in the Randolph Farm at the Virginia State University following randomized complete block design with duplicates. To avoid possible direct root contact between neighbor plants, the spacing was set at about 20 cm apart. Eight plants from each row were harvested at the end of a three-week growing period. Fresh shoot biomass was recorded after harvesting and the roots were carefully dug out and thoroughly cleaned under running water. The number of nodules per plant were counted. T-test was conducted to detect differences between treatment and control on each genotype.

Results and Discussion

Melatonin priming does not affect soybean germination under normal condition

Several reports demonstrated that melatonin treatment improved seed germinations under different abiotic stress conditions such as water deficit [51], chilling [15], high temperature [52], and salt stress [53]. It was also reported that melatonin enhanced seed germination for hard-to-germinating species [54]. We first tested if melatonin priming affected soybean seed germination under normal growth condition without any abiotic stresses. As shown in Figure 1, both melatonin-primed and control soybean seeds were germinated at rate of about 92% within 4-day germination period. This result indicated that 100 µM melatonin treatment did not affect soybean germination under normal condition. Melatonin enhanced germination efficiency under stressed conditions through alleviating stress factors that inhibited seed germination. It is understandable that, under normal conditions when no stress occurs, the melatonin will not affect seed germination. However, Wei et al. [42] found that coating the seeds with low concentrations of melatonin (50 µM or 100 µM) promoted germination in soybean. The discrepancy between two studies may be due to 1) different soybean genotypes used; or 2) different germination methods used.

Melatonin priming increases number of nodules formed on soybean roots but not affects plant heights in greenhouse experiment

Nitrogen fixation is an important and unique trait for legume species that the rhizobia fix nitrogen from the air into ammonia nodules on legume roots, which can be used by the legume plants for their growth. To examine the effects of

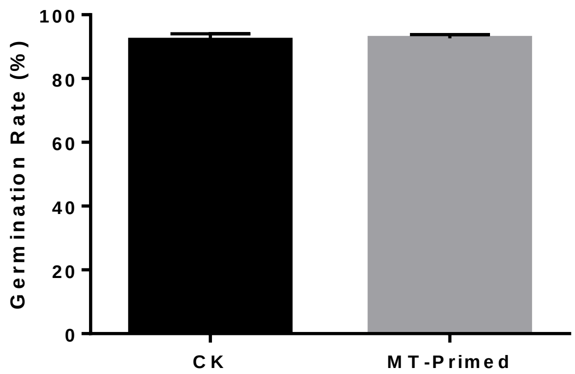

Figure 1. Effect of melatonin prime on soybean seed germination. Soybean variety Osage was used to evaluate the effect of 100μM melatonin treatment on seed germination. No significant difference between control and treatment was found.

melatonin on N fixation in soybean, 16-hour 100 μM melatonin primed seeds, together with the control, were sowed in potted soils consisting the mixture of half Redi-Earth potting media and half soils from soybean grown field. Most seeds germinated on day 3. At fourteenth day after germination, the plant heights were measured and nodules on the roots were characterized. Figure 2 is the representatives of nodules on soybean roots compared between control and melatonin primed plants. Melatonin primed soybean plants formed significantly more nodules than the control plants (Figure 2A and Figure 2B). Furthermore, the size of nodules was measured and separated into two groups as less than 2 mm in diameter and larger than 2 mm in diameter. As shown in Figure 2C, about 75% of formed nodules in melatonin-prime treatment was larger than 2 mm while only about 40% in control group was larger than 2 mm in diameter. These results demonstrate that melatonin treatment does strengthen the communication between soybean roots and rhizobia bacteria and increases both the number and size of the nodules formed on soybean roots. However, the plant heights were not significantly affected (Figure 2D) indicating that the increased N fixation efficiency was not translated into biomass increase. However, Wei et al. [42] showed that melatonin treatment did increase plant heights and biomass production. The difference between two studies is most likely due to the short growing period in our design where the fixed nitrogen does not have enough time to be used and translated into the biomass. Our results suggest that melatonin enhances nitrogen fixation of soybean plants grown in pots in greenhouse settings.

Field experiments further confirms the role of melatonin in N fixation

To further elucidate the role of melatonin in soybean N fixation, we conducted a field experiment with three different soybean genotypes: Osage, Dillon, and VS-22. As shown in Figure 3, without melatonin-prime treatment, the average nodule number per plant was 47.13, 44.88, and 39.63 for Osage, Dillon, and VS-22 respectively. Melatonin priming significantly increased the number of nodules for all soybean genotypes. After a 21-day growth, the average number of nodules per plant was 68.44, 58.31, and 60.75 for Osage, Dillon, and VS-22 respectively in melatonin-prime treatment soybeans. Comparing to the untreated controls, N fixation capability was significantly enhanced for all three soybean varieties and it increased 46.47%, 29.84%, and 53.28% for Osage, Dillon and VS-22 (Figure 3). Different soybean genotypes slightly differed for their N fixation capability, but the trend of melatonin effect on N fixation remained similar among three different soybean genotypes. These results suggest that, even though different genotypes may have different capacities for their N fixation, melatonin-priming before planting strengthen the communication between soybean and rhizobia and significantly increase the number of nodules formed on soybean roots.

Fresh biomass was also examined on different genotypes and melatonin treatment. Shown in Figure 4, after a three-week growth period, biomass accumulation was significantly increased for all three genotypes. The percentage of biomass increase was 18.64%, 27.25%, and 27.60% for Osage, Dillon, and VS-22 respectively. Several studies demonstrated that melatonin pre-treatment significantly increased biomass production in different crop species. For examples, Qiao et al. [44] reported that

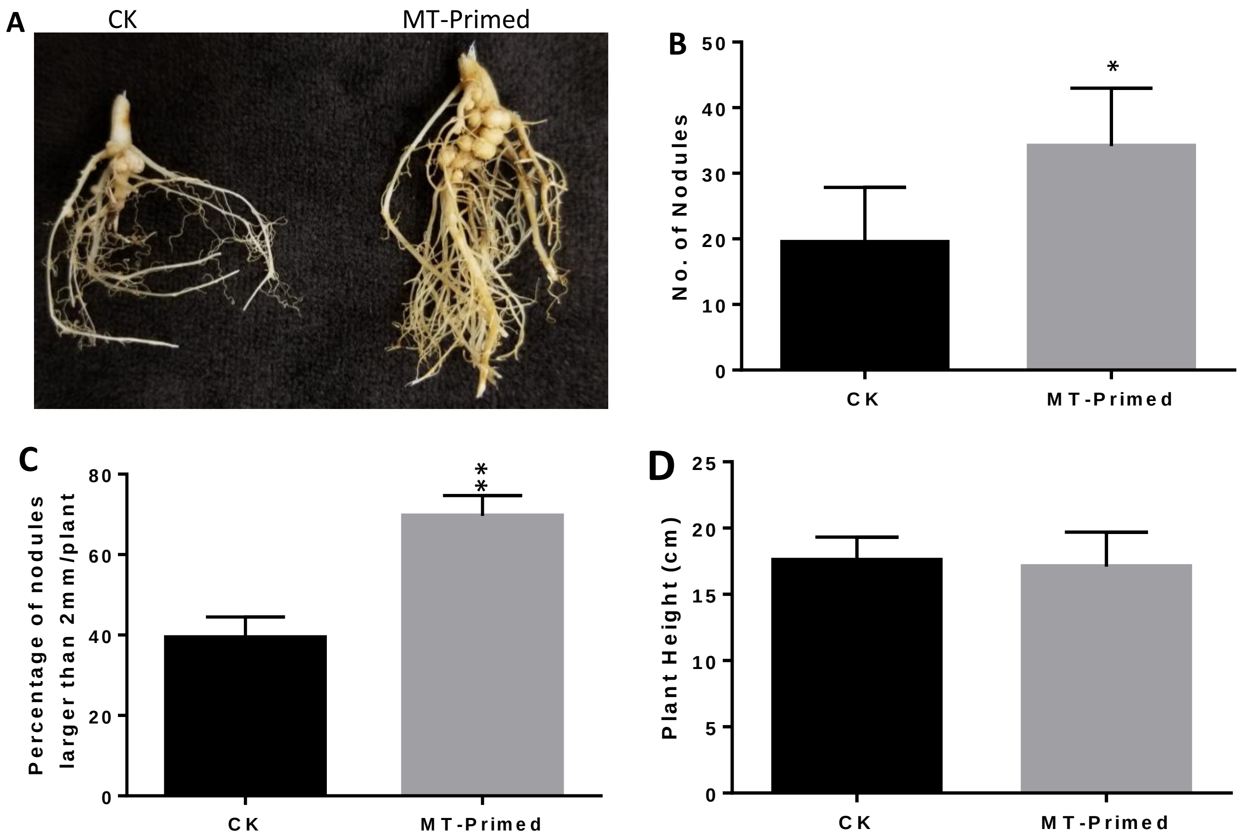

Figure 2. Melatonin prime affects nitrogen fixation in greenhouse experiment. **A.** Representatives of root nitrogen fixation phenotypes between control and MT-primed soybean variety of Osage; **B.** Number of nodules formed on soybean roots; **C.** Percentage of nodules formed that are larger than 2 mm in size per plant; **D.** Effect of melatonin treatment on plant heights. Experiment was triplicated with 10 plants per repeat for statistical analysis. * represents a significant difference at $P < 0.05$, and ** represents a significant difference at $P < 0.01$.

melatonin treatment significantly increased shoot biomass production in winter wheat under both normal and N deficient conditions with a 15-day growth period. In addition, Wei et al. [42] showed that melatonin-treated soybean plants grew bigger than that of control plants after a three-week growth. However, they focused on only one variety in their studies. In our current study, we used three different soybean genotypes to examine the effect of melatonin on shoot biomass production. In consistent withthe literatures' reports, all three genotypes demonstrated that melatonin did enhance biomass production in soybean.

N fixation is one of the important features for legume species, which is more environmental-friendly than industrialized nitrogen fertilizer synthesis. Increasing symbiotic N fixation efficiency would significantly reduce the cost of N fertilizer synthesis. Our current findings demonstrated that melatonin could improve soybean's N fixation efficiency. This discovery not only has application potential but also warrants a further investigation to understand the molecular mechanism(s) on this melatonin-mediated N fixation in soybean specifically, and other legume species in general.

Conclusion

Nitrogen is one of the most essential elements for all living organisms. Without it, plants will no longer grow and produce foods for human consumption. Production of synthetic nitrogen fertilizer costs significantly and the use of it leads to environmental pollution. Symbiotic nitrogen

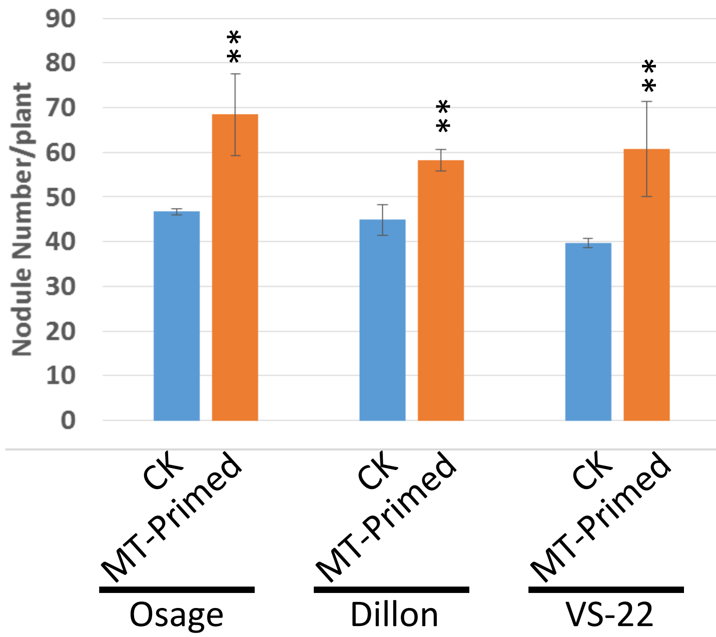

Figure 3. Effect of MT-prime on nitrogen fixation in field experiment. Three different soybean varieties were used for investigation. MT-prime significantly increased number of nodules formed on each plant for all varieties tested. ** represents a significant difference between control and MT primed samples at P < 0.01.

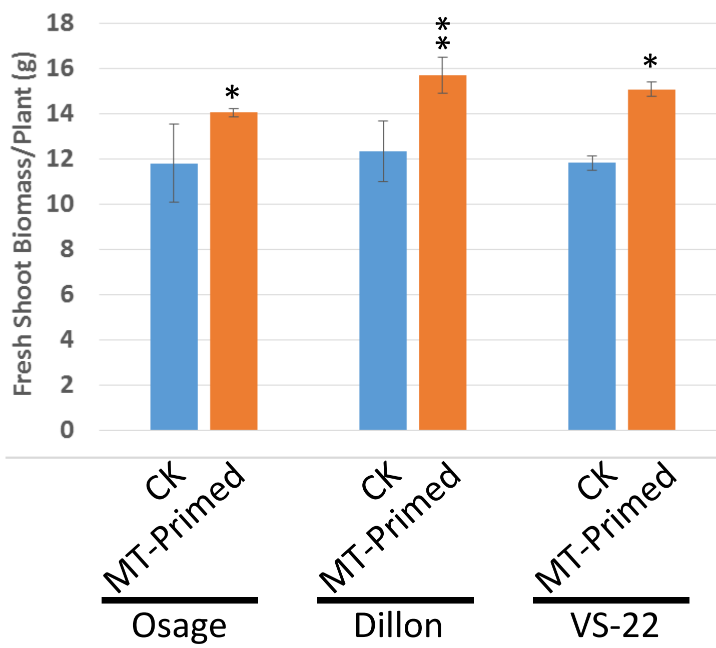

Figure 4. Effect of MT-prime on shoot biomass production in field experiment. Three different soybean varieties were used for investigation. MT-prime significantly increased shoot biomass production in all three variety. * represent a significant difference between control and MT primed at P < 0.05; ** represents a significant difference between control and MT primed samples at P < 0.01.

fixation in legume species is another important approach in nature to fix atmospheric nitrogen into a bioavailable form. Many legume species are economically important crops or are used as cover crops to improve soil nitrogen contents. Increasing the efficiency of this bioprocess will significantly reduce the need of synthetic N fertilizer therefore reducing both the cost and potential environmental pollution. In the present study, the role of melatonin on symbiotic nitrogen fixation in soybean was investigated. Our data indicated that significant more nodules were formed on soybean roots when primed with melatonin in both greenhouse and field experiments. Furthermore, the percentage of formed nodules with large size was also dramatically increased by melatonin priming. Our results suggest that melatonin can be potentially used to increase the efficiency of symbiotic N fixation in soybean specifically, and maybe other legume species in general, and has great potential to reduce N fertilizer application in crop production. Furthermore, the field experiment with three different genotypes also demonstrates that melatonin pre-treatment leads to shoot biomass increase. These discoveries provide a basis for the potential application of melatonin in increasing crop production and warrant a further study to dissect the molecular mechanism(s) regulating melatonin-mediated symbiotic N fixation in legume species.

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References

1. Lerner AB, Case JD, Takahashi Y, Lee TH, Mori W. 1958. Isolation of melatonin, the pineal gland factor that lightens melanocytes. J Am Chem Soc. 80:2587.
2. Dubbels R, Reiter RJ, Klenke E, Goebel A, Schnakenberg E, Ehlers C, Schiwara HW, Schloot W. 1995. Melatonin in edible plants identified by radioimmunoassay and by high-performance liquid chromatography-mass spectrometry. J Pineal Res. 18:28-31.
3. Hattori A, Migitaka H, Iigo M, Itoh M, Yamamoto K, Ohtanikaneko R, Hara M, Suzuki T, Reiter RJ. 1995. Identification of melatonin in plants and its effects on plasma melatonin levels and binding to melatonin receptors in vertebrates. Biochem Mol Bio Int. 35:627-634.
4. Hardeland R, Poeggeler B. 2003. Non-vertebrate melatonin. J Pineal Res. 34:233-241.
5. Carrillo-Vico A, Lardone PJ, Alvarez-Sanchez N, Rodriguez-Rodriguez A, Guerrero PJ. 2013. Melatonin: buffering the immune system. Int J Mol Sci. 14:8638-8683.
6. Hardeland R, Madrid A, Tan DX, Reiter RJ. 2012. Melatonin, the circadian multioscillator system and health: the R.J.eed for detailed analyses of peripheral melatonin signaling. J Pineal Res. 52:139-166.
7. Pandi-Perumal SR, Trakht I, Spence D, Srinivasan V, Dagan Y, Cardinali D. 2008. The roles of melatonin and light in the pathophysiology and treatment of circadian rhythm sleep disorders. Nat Clin Pract Neurol. 4:436-447.
8. Tuomi T, Nagorny CL, Singh P, Bennet H, Yu Q, Alenkvist I, Isomaa B, Ostman B, Soderstrom J, Pesonen A, Martikainen S, Raikkonen K, Forsen T, Hakaste L, Almgren P, Storm P, Asplund O, Shcherbina L, Fex M, Fadista J, Tengholm A, Wierup N, Groop L, Mulder H. 2016. Increased melatonin signaling is a risk factor for type 2 diabetes. Cell Metabolism. 23:1067-1077.
9. Arnao MB and Hernandez-Ruiz J. 2014. Melatonin: plant growth regulator and/or biostimulator during stress? Trends in Plant Science. 19:789-797.
10. Arnao MB and Hernandez-Ruiz J. 2015. Functions of melatonin in plants: a review. J Pineal Res. 59:133-150.
11. Byeon Y, Park S, Kim YS, Park DH, Lee S, Back K. 2012. Light-regulated melatonin biosynthesis in rice during the senescence process in detached leaves. J Pineal Res. 53:107-111.
12. Shi HT, Reiter RJ, Tan DX, Chan ZL. 2015. Indole-3-acetic acid inducible 17 positively modulates natural leaf senescence through melatonin-mediated pathway in *Arabidopsis*. J Pineal Res. 58:26-33.
13. Weeda S, Zhang N, Zhao X, Ndip G, Guo Y, Buck G, Fu C, Ren S. 2014. *Arabidopsis* transcriptome analysis reveals key roles of melatonin in plant defense systems. Plos One. 9(3): e93462.
14. Pape C and Luning K. 2006. Quantification of melatonin in phototrophic organisms. J Pineal Res. 41:157-165.
15. Posmyk MM, Balabusta M, Wieczorek M, Sliwinska E, Janas K. 2009. Melatonin applied to cucumber (*Cucumis sativus* L.) seeds improves germination during chilling stress. J Pineal Res. 46:214-223.
16. Byeon Y and Back K. 2014. Melatonin synthesis in rice seedlings in vivo is enhanced at high temperatures and under dark conditions due to increased serotonin N-acetyltransferase and N-acetylserotonin methyltransferase activities. J Pineal Res. 56:189-195.
17. Kostopoulou Z, Therios I, Roumeliotis E, Kanellis AK, Molassiotis A. 2015. Melatonin combined with ascorbic acid provides salt adaptation in *Citrus aurantium* L. seedlings. Plant Physiology and Biochemistry. 86:155-165.
18. Liu N, Jin Z, Wang S, Gong B, Wen D, Wang X, Wei M, Shi Q. 2015. Sodic alkaline stress mitigation with exogenous

melatonin involves reactive oxygen metabolism and ion homeostasis in tomato. Scientia Horticulturae. 181:18-25.
19. Zhang N, Sun Q, Zhang H, Cao Y, Weeda S, Ren S, Guo Y. 2015. Roles of melatonin in abiotic stress resistance in plants. J Exp Botany. 66:647-656.
20. Arnao MB and Hernandez-Ruiz J. 2006. The physiological function of melatonin in plants. Plant Signal Behav. 1:89-95.
21. Tan DX, Manchester LC, Esteban-Zubero E, Zhou Z, Reiter RJ. 2015. Melatonin as a potent and inducible endogenous antioxidant: synthesis and metabolism. Molecules. 20:18886-18906.
22. Back K, Tan DX, Reiter RJ. 2016. Melatonin biosynthesis in plants: multiple pathways catalyze tryptophan to melatonin in the cytoplasm or chloroplasts. J Pineal Res. 61:426-437.
23. Zhao YD. 2012. Auxin biosynthesis: a simple two-step pathway converts tryptophan to indole-3-acetic acid in plants. Molecular Plant. 5:334-338.
24. Zhao Y. 2010. Auxin biosynthesis and its role in plant development. Annual review of plant biology. 61:49-64.
25. Murch SJ, Simmons CB, Saxena PK. 1997. Melatonin in feverfew and other medicinal plants. The Lancet. 350:1598-1599.
26. Chen Q, Qi W, Reiter RJ, Wei W, Wang B. 2009. Exogenously applied melatonin stimulates root growth and raises endogenous indoleacetic acid in roots of etiolated seedlings of *Brassica juncea*. J Plant Physiology. 166(3):324-328.
27. Sarropoulou VN, Therios I, Dimassi-Theriou K. 2012. Melatonin promotes adventitious root regeneration in in vitro shoot tip explants of the commercial sweet cherry rootstocks CAB-6P (*Prunus cerasus* L.), Gisela 6 (*P. cerasus* x *P. canescens*), and MxM 60 (*P. avium* x *P. mahaleb*). J Pineal Res. 52:38-46.
28. Tan DX, Hardeland R, Manchester LC, Korkmaz A, Ma S, Rosales-Corral S, Reiter RJ. 2012. Functional roles of melatonin in plants, and perspectives in nutritional and agricultural science. J Exp Botany. 63(2):577-597.
29. Hernandez-Ruit J, Cano A, Arnao MB. 2005. Melatonin acts as a growth-stimulating compound in some monocot species. J Pineal Res. 39:137-142.
30. Arnao MB, Hernandez-Ruiz J. 2007. Melatonin promotes adventitious- and lateral root regeneration in etiolated hypocotyls of *Lupinus albus* L. J Pineal Res. 42:147-152.
31. Arnao MB, Hernandez-Ruiz J. 2017. Growth activity, rooting capacity, and tropism: three auxinic precepts fulfilled by melatonin. Acta Physiol Plant. 39:127.
32. Han QH, Huang B, Ding CB, Zhang ZW, Chen YE, Hu BC, Zhou LJ, Huang Y, Liao JQ, Yuan S, Yuan M. 2017. Effects of melatonin on anti-oxidative systems and photosystem ii in cold-stressed rice seedlings. Front. Plant Sci. 8:785.
33. Wen D, Gong B, Sun S, Liu S, Wang X, Wei M, Yang F, Li YH, Shi Q. 2016. Promoting roles of melatonin in adventitous root development of *solanum lycopersicum* l. by regulating auxin and nitric oxide signaling. Front. Plant Sci. 7:718.
34. Korkmaz A, Karaca A, Kocacinar F, Cuci Y. 2017. The effects of seed treatment with melatonin on germination and emergence performance of pepper seeds under chilling stress. Tarim Bilimleri Dergisi-J Agricultural Sciences. 23:167-176.
35. Zhang RM, Sun Y, Liu Z, Jin W, Sun Y. 2017. Effects of melatonin on seedling growth, mineral nutrition, and nitrogen metabolism in cucumber under nitrate stress. J Pineal Res. 62(4).
36. Wang Q, An B, Wei Y, Reiter RJ, Shi H, Luo H, He C. 2016. Melatonin regulates root meristem by repressing auxin synthesis and polar auxin transport in *Arabidopsis*. Front Plant Sci. 7:1882.
37. Arnao MB, Hernández-Ruiz J. 2008. Protective effect of melatonin against chlorophyll degradation during the senescence of barley leaves. J Pineal Res. 46:58-63.
38. Tal O, Haim A, Harel O, Gerchman Y. 2011. Melatonin as an antioxidant and its semi-lunar rhythm in green macroalga Ulva sp. J Exp Botany. 62(6):1903-1910.
39. Kang K, Lee K, Park S, Kim YS, Back K. 2010. Enhanced production of melatonin by ectopic overexpression of human serotonin N-acetyltransferase plays a role in cold resistance in transgenic rice seedlings. J Pineal Res. 49:176-182.
40. Wang P, Yin L, Liang D, Li C, Ma F, Yue Z. 2011. Delayed senescence of apple leaves by exogenous melatonin treatment: toward regulating the ascorbate–glutathione cycle. J Pineal Res. 53:11-20.
41. Yang XL, Xu H, Li D, Gao X, Li TL, Wang P. 2018. Effect of melatonin priming on photosynthetic capacity of tomato leaves under low-temperature stress. Photosynthetica. 56(3): 884-892.
42. Wei W, Li QT, Chu YN, Reiter RJ, Yu XM, Zhu DH, Zhang WK, Lin MQ, Zhang JS, Chen SY. 2015. Melatonin enhances plant growth and abiotic stress tolerance in soybean plants. J Exp Botany. 66(3):695-707.
43. Okazaki M, Higuchi K, Aouini A. 2010. Lowering intercellular melatonin levels by transgenic analysis of indoleamine 2, 3-dioxygenase from rice in tomato plants. J Pineal Res. 49:239-247.
44. Qiao Y, Yin L, Wang B, Ke QB, Den XP, Wang SW. 2019. Melatonin promotes plant growth by increasing nitrogen uptake and assimilation under nitrogen deficient condition in winter wheat. Plant Physiology and Biochemistry. 139:342-349.
45. Chen Z, Gu Q, Yu X, Huang L, Xu S, Wang R, Shen W, Shen WB. 2018. Hydrogen peroxide acts downstream of melatonin to induce lateral root formation. Annals of Botany. 121(6):1127-1136.
46. Ren S, Rutto L, Katuuramu D. 2019. Melatonin acts synergistically with auxin to produce lateral root development through fine tuning auxin transport in *Arabidopsis thaliana*. Submitted.
47. Nawaz MA, Huang Y, Bie Z, Ahmed W, Reiter GJ, Niu M, Hameed S. 2015. Melatonin: current status and future perspectives in plant science. Front. Plant Sci. 6:1230.
48. Janas KM, Posmyk MM. 2013. Melatonin, an underestimated natural substance with great potential for agricultural application. Acta Physiologiae Plantarum. 35:3285-3292.
49. Reiter RJ, Tan DX, Cruz M, Broto LF. 2015. Phytomelatonin: assisting plants to survive and thrive. Molecules. 20:7396-7437.
50. Zuo Z, Sun L, Wang T, Miao P, Zhu X, Liu S, Song F. 2017. Melatonin improves the photosynthetic carbon assimilation

and antioxidant capacity in wheat exposed to nano-ZnO stress. Molecules. 22:e1727.

51. Zhang N, Zhao B, Zhang H, Weeda S, Yang C, Yang Z, Ren S, Guo Y. 2013. Melatonin promotes water-stress tolerance, lateral root formation, and seed germination in cucumber (*Cucumis sativus* L.). J Pineal Res. 54:15–23.
52. Tiryaki I, Keles H. 2012. Reversal of the inhibitory effect of light and high temperature on germination of *Phacelia tanacetifolia* seeds by melatonin. J Pineal Res. 52:332-339.
53. Castanares JL, Bouzo CA. 2019. Effect of exogenous melatonin on seed germination and seedling growth in melon (*cucumis melo* l.) under salt stress. Horticultural Plant J. 5:79-87.
54. Simlat M, Ptak A, Skrzypek E, Warchoł M, Morańska E. 2018. Melatonin significantly influences seed germination and seedling growth of *Stevia rebaudiana* Bertoni. PeerJ. 6:E5009.