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RESEARCH ARTICLE

MELATONIN ELICITED GROWTH, PHOTOSYNTHESIS AND ANTIOXIDANT RESPONSES IN PEA PLANTS: A CONCENTRATION AND MODE DEPENDENT STUDY

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ABSTRACT

The aim of this study was to dissect the response of melatonin under different mode of application with series of concentrations in pea plants for growth, photosynthesis and antioxidant system. Surface sterilized seeds of *Pisum sativum* were soaked in deionized water (control), 50, 100, and 200 µM of melatonin for 4, 8, and 12 h (shotgun approach). In another mode of application, same concentrations of ML applied through foliage of plants and were allowed to grow for 30 days. At this stage of growth, plants were sampled to assess the various growths and photosynthetic traits as well as selected biochemical characteristics. The results indicated that growth characteristics, net photosynthetic rate, antioxidants system, and proline content showed diverse response for different concentrations and mode of application. Foliar application excelled over the seed soaking and 100 µM of ML proved best in enhancing activity of antioxidant system and proline accumulation in comparison to control plants. The up-regulation of antioxidant enzymes as well as proline triggered by melatonin could have great potential for improving crop yield and act as an eco-friendly plant growth regulator for sustainable agriculture practices.

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INTRODUCTION

It was the year 1995 when two research group for the first time simultaneously identified the existence of N-acetyl-5-methoxytrytamine i.e. melatonin in vascular plants. Furthermore, various workers at the same time unravel the role of melatonin as a ROS scavengers in various animal tissues. In the starting, structural similarity between melatonin and auxin inspires various plant researcher to shed light on its biosynthetic pathway and its role in relation to growth and metabolism of plants (Arnao and Hernández-Ruiz, 2019). In 1991, (Reiter, 1991) found that tryptophan act as a precursor for melatonin biosynthetic pathway and similar pathways were identified in vascular plants by Arnao and Hernández-Ruiz (2006). Moreover, rate limiting enzyme for melatonin biosynthesis pathway in rice has been identified as N-acetylserotonin methyltransferase (ASMT; (Kang *et al*., 2011).

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It is now well established that plant melatonin plays pivotal role in wide range of cellular and physiological effects, such as alteration in intercellular calcium ion and in the membrane permeability by ion transporter (C. Li *et al*., 2016). It is also reported that melatonin facilitates changes in stomatal conductance, metabolism of carbohydrate, lipid and nitrogen along with osmoprotectant metabolites (Wei *et al*., 2015; Zhang *et al*., 2015). Moreover, melatonin affects plant growth, seed germination, rooting (N. Zhang *et al*., 2017) and also optimize photosynthetic efficiency and exchanges of gases (Li *et al*., 2017). Additionally, melatonin also regulates ripening, senescence and parthenocarpy (Liang *et al*., 2018; Liu *et al*., 2018). Plant melatonin also showed significant changes in gene expression for various physiological changes (Arnao and Hernández-Ruiz, 2019). Various transcriptomic analysis revealed that melatonin mediated responses may be due to regulation of photosynthesis, cell cycle, DNA replication and sugar metabolism and lipid biosynthesis (Wei *et al*., 2015). Some researcher believed that there is enough data to establish the fact that melatonin also functions similar to other plant hormones. This fact further strengthens by the identification and characterization of receptors in *Arabidopsis*.

It has also been established that melatonin controls the expression of numerous elements such AS enzymes, receptors, and transcription factors in the biosynthesis and catabolism of auxin, gibberellins, cytokinins, ABA, ethylene, jasmonic acid, SA, and, brassinosteroids. It also regulates polyamine metabolism (Gong *et al*., 2017). On contrary, still it has to be verified that these different plant hormones can influence plant melatonin levels. This multifunctionality response shown by melatonin in plants and the degree of complexity in its regulatory factors are the base for present study. In this study, our aim was to dissect out melatonin-mediated responses on growth biomarkers, physiological traits and biochemical attributes under different mode of application (foliar spray and seed soaking i.e. shotgun approach with different concentrations. Additionally, exploring farmer friendly application of melatonin with best-suited concentration and mode to enhance the photosynthetic efficiency of plants.

MATERIALS AND METHODS

Plant materials: Certified seeds of pea (*Pisum sativum*) were procured from a seed shop in Al Ain central market, Al Ain, UAE. Healthy and uniform sized seeds were surface sterilized with 2% aqueous solution of H_2O_2 for 15 min followed by repeated washing with deionized water.

Melatonin Preparation: Melatonin (ML) was procured from Sigma-Aldrich Chemicals, USA. The stock solution (1mM) of ML was prepared by dissolving required quantity of ML in 5 ml of ethanol, in a 100-ml volumetric flask with final volume was maintained up to 100 ml mark with deionized water. The desired concentrations (50, 100, and 200 μM) of ML were prepared by the dilution of stock solution. Surfactant "Tween-20" (0.05 %) was added to the ML solutions before foliar spray and seed soaking.

Treatment pattern and experimental design: The experiment was conducted with 80 pots filled with potting soil. Eighty pots were divided into 4 sets with 20 pots in each set and 5 pots (replicates) were assigned for each treatment under randomized block design. Set I assigned to foliar application of ML in which surface sterilized seeds were sown in the pots and allowed to grow under controlled environmental conditions till 30 days stage of growth. At 15 days after sowing, foliage of plants were sprayed with ML (50, 100, and 200 μM) for 5 days. In the similar pattern, control plants were sprayed with deionized water. Each plant was sprayed thrice. Sprayer was adjusted in such a manner that it sprayed approx. 1 ml solution in each spray. Set II, III, and IV assigned to 4, 8, and 12h seed soaking (shot gun approach), respectively. Surface sterilized seeds were soaked in deionized water (control), 50, 100, and 200 μM of ML for 4, 8, and 12 h (Fig. 1) and these treated seeds were sown in the pots of their respective set and allowed to grow till 30 days stage of growth under controlled environmental conditions. The plants in all the sets were harvested at 30 days stage of growth to assess various growth biomarkers, physiological as well as biochemical traits.

Evaluation of growth biomarkers: The plants from each treatment were removed from the pots along with the soil and were dipped in a beaker filled with water. Remove the adhering soil particles and the plants were blotted with tissue towel. Length of shoot and root were measured with the help of meter scale. The same sample were weighed to record their fresh mass of root and shoot and then placed in an oven, run at 70 °C for 72 h. The samples were weighed again after allowing them to cool at room temperature to record their root and shoot dry mass. Leaf area determined by gravimetric method where the leaf area of randomly selected leaves from each treatment, was determined by tracing their outline on the graph sheet.

Determination of physiological parameters: Chlorophyll content was quantified by the method of Arnon, 1949 with slight modifications. Evaluation of net photosynthetic rate and stomatal conductance were performed by using potable photosynthesis system (LI-COR 6400; LI-COR Lincoln, NE, USA). The measurements were recorded on the uppermost fully expanded leaves of the main branch in the sampled plants.

Estimation of enzymatic activity: The activity of nitrate reductase (NR) was measured following the method adopted by (Jaworski, 1971). The fresh leaf samples were cut into small pieces and transferred to plastic vials containing phosphate buffer (pH 7.5) followed by the addition of potassium nitrate and isopropanol solutions. The reaction mixture was incubated at 30°C for 2h followed with the addition of N-1-naphthyletylene diamine dihydrochloride and sulphanilamide. The absorbance of the color was measured at 540 nm and was compared with that of the calibration curve. The activity of NR [nmole of NO2 g^{-1} (FM) s^{-1}] was computed on fresh mass basis. The activity of carbonic anhydrase (CA) was determined following the procedure described by (Dwivedi and Randhawa, 1974).

Protein quantification and antioxidant enzyme analysis: The leaf tissue was homogenized in 50 mM phosphate buffer (pH 7.8) containing 1 mM EDTA, 1 mM PMSF, 0.5% Triton X-100 (v/v), and 2% polyvinyl pyrrolidone (w/v) in a pre-chilled mortar and pestle. The homogenate was centrifuged at 12,000 × g for 20 min at 4°C, and the supernatant obtained was used for protein quantification and analysis of antioxidant enzymes (catalase, peroxidase, and superoxide dismutase). Total protein content of leaves was determined by the method followed by Bradford *et al*. (1976).

Catalase (CAT) activity was assayed by measuring the initial rate of H_2O_2 disappearance using the method of (Aebi, 1984). Peroxidase (POX) activity was assayed by the method followed by (Sánchez *et al*., 1996) with some modifications. The activity was determined by measuring the absorbance at 436 nm for 1 min at 25 °C. Control set was prepared in the same manner excluding enzyme extract. Superoxide dismutase (SOD) activity was assayed by measuring the ability of the enzyme extract to inhibit the photochemical reduction of nitrobluetetrazolium (NBT) (Kono, 1978).

Quantification of proline: The proline content in fresh leaf samples was determined by adopting the method of (Bates *et al*., 1973). Sample was extracted in sulphosalicylic acid. To the extract an equal volume of glacial acetic acid and ninhydrin solutions were added. The sample was heated at 100°C to which 5 ml of toluene was added. The absorbance of toluene layer was recorded at 528 nm on a spectrophotometer.

Statistical analysis: Data were statistically analyzed using SPSS, 17.0 for windows (SPSS, Chicago, IL, USA). Standard error was calculated and analysis of variance (ANOVA) was performed on the data to determine the least significance difference (LSD) between treatment means with the level of significance at $P \leq 0.05$.

RESULTS

Growth biomarkers: Comparing different mode (foliar or seed soaking) of ML application, foliar application excelled over seed soaking and showed significant increase in growth biomarkers (Shoot and root length, fresh and dry mass of root and shoot, and leaf area) in comparison to their control plants as well other plants grown under seed soaking treatment (Fig. 3A-F and 4 A). Moreover, out of different concentrations (0, 50, 100, or 200 µM) of ML tested either foliar spray or seed soaking, 100 µM of ML proved best and increased plant growth biomarkers in comparison to other treatments.

Chlorophyll content: Exogenous application of ML (100 µM) to the foliage of plants significantly increase the chlorophyll content in comparison to non-treated control plants. On the other hand, plants grown from the seeds given soaking (4, 8, or 12 h) treatment of ML also showed higher chlorophyll content however, 8h soaking treatment of ML (100 µM) proved best in comparison to the other soaking treatment (Fig. 4C). The order of response generated by ML (100 µM) under different (4, 8, or 12 h) seed soaking treatment was 8h > 4h > 12h.

Enzymatic activities of NR and CA: The Fig. 4B and D revealed that enzymatic activities of NR and CA increased in the presence of ML either through foliar spray or through seed soaking. However, maximum significantly increase showed by the 100 µM of ML treatment through foliar spray. However, on comparing the different duration (4, 8, or 12 h) of seed soaking for different concentrations (0, 50, 100, or 200 µM) of ML, 8 h soaking of seeds in 100 µM proved best and increases chlorophyll content but the foliar spray excelled in their response.

Net photosynthetic rate and stomatal conductance: Treatment with ML (50, 100, or 200 µM) as pre-sowing seed soaking (4, 8, or 12 h) increased the net photosynthetic rate and stomatal conductance in comparison to their respective control plants (Fig. 4E and F). However, this increase was further elevated when treatment of ML was changed to another mode of application i.e. foliar application with 100 µM of ML. Moreover, foliar spray excelled over the seed soaking and the order of response for 100 µM under foliar spray and seed soaking was foliar spay > 8h seed soaking > 4 h seed soaking > 12 seed soaking.

Protein content: Two different mode (seed soaking and foliar spray) of application of ML (50, 100, or 200 µM) showed promising response in increasing the total protein content in comparison to their controls. However, comparing both mode of application, foliar spray of 100 µM proved more effective and significantly increased the protein content over all other treatments (Fig. 5A). Moreover, when different durations were compared, 8 h proved best for the protein content.

Antioxidant enzymes: The Fig. 5B–D showed that 100 µM of ML under both mode of application (foliar spray and seed soaking) increased the activities of antioxidant enzymes (CAT, POX, and SOD) however, on comparing the modes of application, foliar spray showed maximum increase for the CAT, POX and SOD over their control as well as other treated plants. On the other hand, different duration (4, 8, or 12h) of seed soaking respond differentially, 8 h of seed soaked in 100 µM of ML proved best over the other seed soaking treatments. Overall, foliar spray of ML (100 µM) excelled over all the other treatments and showed maximum increase in the activities of antioxidant enzymes.

Proline accumulation: Proline content in the leaves increased in response to various concentrations of ML (Fig. 6). Out of various concentrations (50, 100, or 200 µM) of ML tested, 100 µM of ML showed significant increase in proline content in comparison to their control plants under both mode of application. Moreover, foliar application of ML showed maximum increase in the proline content over the other treatments. On the other hand, various duration (4, 8, or 12h) of seed soaked in ML were tested, 8h soaking duration proved best in comparison to other soaking duration.

DISCUSSION

Availability of enough report revealed that plant melatonin could be considered as a new phytohormone that regulates plant growth and development under natural as well stress conditions. In the present study, different mode (foliar and seed soaking) and concentrations (50, 100, or 200 µM) of melatonin were tested to explore the better mode and best suited concentration. Application of 100 µM of ML significantly increased the growth traits such as root and shoot length; fresh and dry mass of shoot and root and leaf area over the non-treated control plants through both mode of application (Fig. 3A-F and 4A). However, foliage application excelled in comparison to seed soaking. It is believed that growth and developmental process of plants are mediated by several plant hormones especially auxin. Melatonin is a kind of indole-amine and shared same precursor, tryptophan with IAA, therefore researchers assumed that melatonin should play role in the regulation of plant growth and development same as auxin (Arnao and Hernández-Ruiz, 2007; Hernández-Ruiz *et al*., 2005). It is auxinic hormone in plants. This thought of researchers were strengthen by the findings of various workers, Hernández-Ruiz *et al*. (2004) reported that coating of soybean seed with ML significantly improved growth and yield characteristics. In *Lupinus alba*, melatonin found to be responsible for advancement of vegetative growth and redevelopment of lateral and adventitious root (Arnao and Hernández-Ruiz, 2007). In cucumber, melatonin improved the nutrient uptake efficiency and nitrogen metabolism (R. Zhang *et al*., 2017). Our findings also corroborate these findings. Moreover, exogenous application of melatonin enhanced the level of auxin and resulted in improved root activity (Chen *et al*., 2009). Therefore, it is recognized that melatonin act as a significant plant growth regulator that enhance the production capacity of plants. Overall amendments in growth capacity of plants directly or indirectly associated with effective photosynthetic machinery, increased chlorophyll content, activity of carbonic anhydrase and nitrate reductase. In the present study, ML (100 µM) applied either way showed

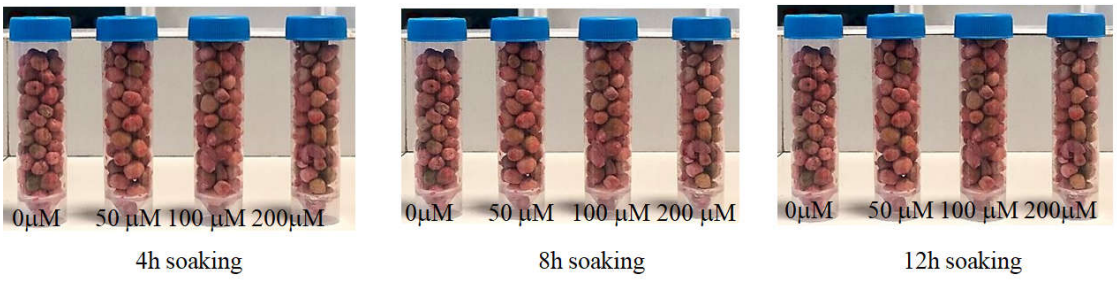

Fig 1. Seeds of *Pisum sativum* (Pea) were soaked in different concentrations of melatonin for different duration prior to sowing in pots

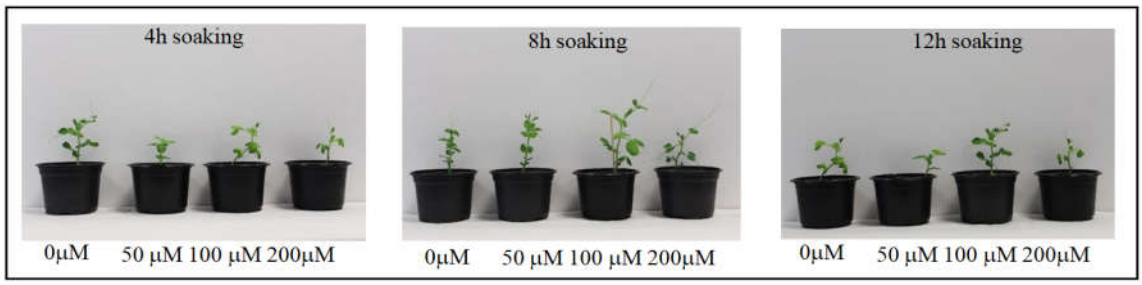

Fig. 2A. Growth pattern of pea plants under seed soaking mode of application at 30 days after sowing

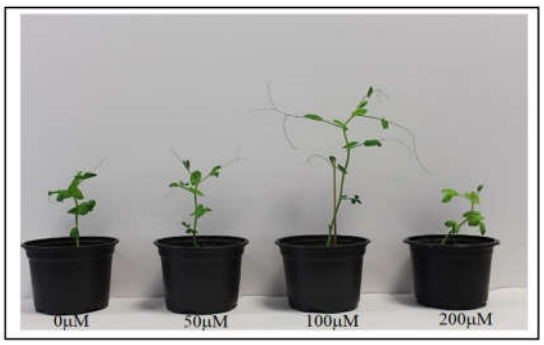

Fig. 2B Growth pattern of pea plants under foliar spray mode of application at 30 days after sowing

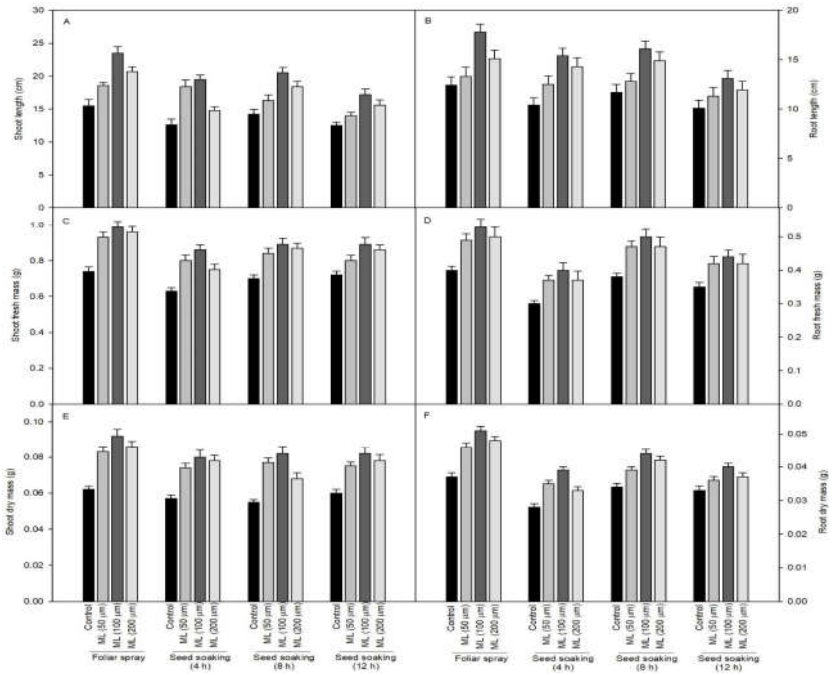

Fig 3. Effect of melatonin (ML; 0, 50, 100, or 200 μM) as foliar spray and seed soaking (4, 8, or 12 h) on (A) shoot length, (B) root length, (C) shoot fresh mass, (D) root fresh mass, (E) shoot dry mass, and (F) root dry mass of *Pisum sativum* (pea) plants at 30-day stage of growth. All the data are the mean of five replicates (n=5), vertical bars show standard error (±SE) and the least significance difference (LSD) between treatment means that were calculated at the level of significance ($P \leq 0.05$)

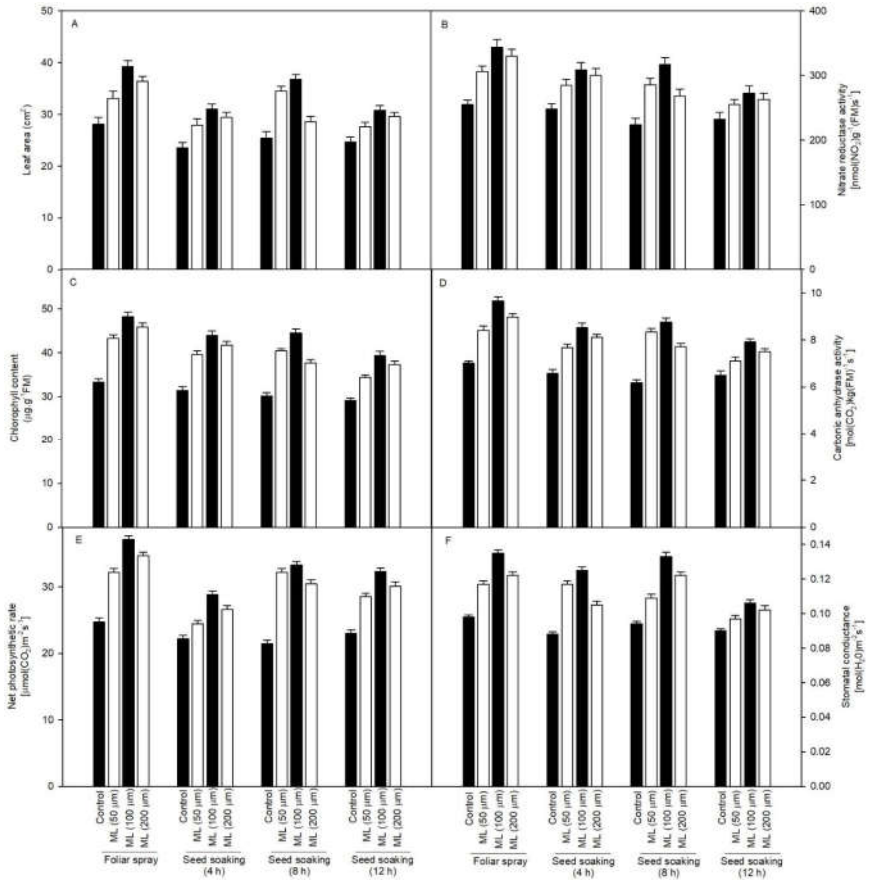

Fig 4. Effect of melatonin (ML; 0, 50, 100, or 200 µM) as foliar spray and seed soaking (4, 8, or 12 h) on (A) leaf area, (B) nitrate reductase activity, (C) chlorophyll content, (D) carbonic anhydrase activity, (E) net photosynthetic rate, and (F) stomatal conductance of *Pisum sativum* (pea) plants at 30-day stage of growth. All the data are the mean of five replicates (n=5), vertical bars show standard error (±SE) and the least significance difference (LSD) between treatment means that were calculated at the level of significance ($P \leq 0.05$)

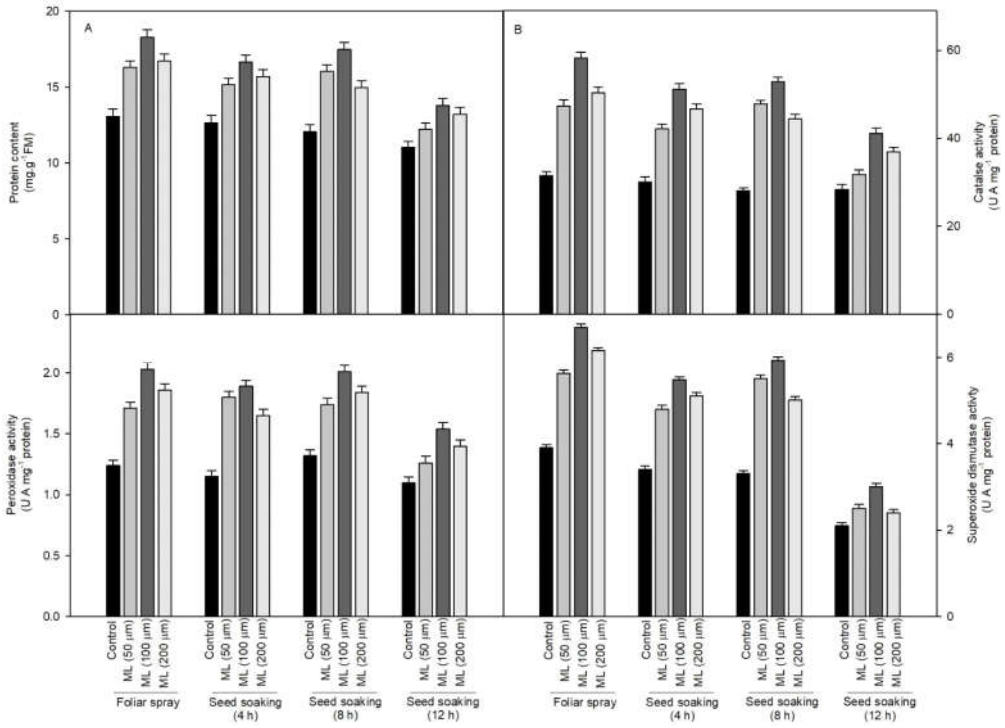

Fig 5. Effect of melatonin (ML; 0, 50, 100, or 200 µM) as foliar spray and seed soaking (4, 8, or 12 h) on (A) protein content, (B) catalase activity, (C) peroxidase, and (D) superoxide dismutase activity of *Pisum sativum* (pea) plants at 30-day stage of growth. All the data are the mean of five replicates (n=5), vertical bars show standard error (±SE) and the least significance difference (LSD) between treatment means that were calculated at the level of significance ($P \leq 0.05$)

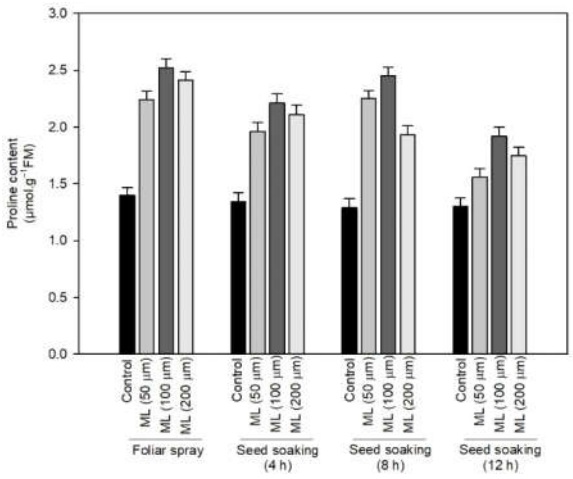

Fig 6. Effect of melatonin (ML; 0, 50, 100, or 200 µM) as foliar spray and seed soaking (4, 8, or 12 h) on proline content of *Pisum sativum* (pea) plants at 30-day stage of growth. All the data are the mean of five replicates (n=5), vertical bars show standard error (±SE) and the least significance difference (LSD) between treatment means that were calculated at the level of significance ($P \leq 0.05$)

increased net photosynthetic rate, stomatal conductance, chlorophyll content and carbonic anhydrase activity (Fig. 4). It is reported that treatment of ML enhanced the carbon assimilation (H. Li *et al*., 2016; Xu *et al*., 2016) that are reflected in our study by increased activity of carbonic anhydrase in the ML treated leaves (Fig. 4D). Meng *et al*. (2014) showed that ML also increased the stomatal conductance and this is in line with our finding where ML treated plants showed increased stomatal conductance (Fig. 4F). Photochemical efficiency of PSII is also stimulated by melatonin, enhancing the overall photosynthesis (Ye *et al*., 2016; Zuo *et al*., 2017). Additionally, melatonin also boosts the activity of RuBisCO along with enhanced total nitrogen and protein content (Zhao *et al*., 2018; Fig. 5A). The cumulative effect of all these altered processes resulted in increased activity of CA and finally net photosynthetic rate (Fig. 4E). Furthermore, transcriptome analysis revealed that melatonin might exert its functions through regulation of photosynthesis, the cell cycle, DNA replication, starch/ sucrose metabolism, and lipid biosynthesis (Wei *et al*., 2015). Naturally plants undergo oxidative stress where excess free oxygen radicals suppresses the radical scavenging tactics that lead to an disparity between ROS and antioxidant systems (Foyer and Harbinson, 2011). All organisms possess intrinsic cellular defense mechanism to combat oxidative stress; termed as antioxidant system. Plants have efficient complex enzymatic and non-enzymatic antioxidant defense systems. Antioxidant systems include SOD, CAT, and POX, while non-enzymatic systems consist of low molecular weight antioxidants (ascorbic acid, glutathione, proline, and carotenoids) and high molecular weight secondary metabolites such as tannins. In the present study, exogenous application of melatonin through either seed or foliage modifies the activity of antioxidants system i.e. CAT, POX and SOD (Fig. 5B-D). Moreover, the exogenous application of melatonin to roots of grape cuttings increased their activity of antioxidant enzymes and the activities of non enzymatic antioxidants; melatonin treatment also kept the internal lamellar system of chloroplasts well preserved and reduced its ultrastructural destruction (Meng *et al*., 2014).

This antioxidative effect of melatonin has been reported in several plant species (apple, rice, and grape; (Park *et al*., 2013; Vitalini *et al*., 2013; Wang *et al*., 2012; Yin *et al*., 2013). Using high-throughput sequencing technology, the important roles of melatonin in plant defence have also been revealed. Melatonin up-regulates transcript levels of many defence-related factors, including stress receptors, kinases, and transcription factors (Weeda *et al*., 2014). Additionally, melatonin may have the ability to regulate plant growth and its productivity. Proline is a well-known proteogenic amino acid and accumulates under both stress and non stress conditions as a beneficial solute in plants. Recently, it is established fact that proline plays a pivotal role in plant growth and differentiation across its life cycle. It is one of the key factor for many cell wall proteins that showed involvement in plant development (Kishor *et al*., 2015). The role of extensins, arabinogalactan proteins and hydroxyproline- and proline-rich proteins as important components of cell wall proteins that play pivotal roles in cell wall signal transduction cascades, and plant development. Molecular insights are also provide plausible roles of proline transporters modulating key events in plant development.

In the present study, foliar application of melatonin (100 µM) significantly increased its accumulation in comparison to non-stressed control plants (Fig. 6). Arnao and Hernández-Ruiz, (2015) also reported that melatonin promotes the high osmotic metabolites levels (proline), low cell osmotic potential, high cell turgor, and optimal stomata opening, all of which increase CO_2 availability, optimizing the photosynthetic process. The mechanisms of action of melatonin is not clearly understood in plants; however, it modifies plant growth and development by acting as an antioxidant, membrane stabilizer, and by up and down regulating gene expression. Some of melatonin actions in plants may be receptor-mediated while others are receptor-independent. Recently, (Arnao and Hernández-Ruiz, 2019) suggested that melatonin performs some of its functions in plants by actions similar to those of indole-3-acetic acid (IAA). Overall observations of this study revealed that the concentration dependent variation appeared almost in all the parameters investigated. Moreover, pre-sowing seed soaking and foliar applied 100 µM of ML optimized their effects than other concentrations. Interestingly, the question arises that how and/or why foliar spray excelled over the seed soaking treatment at the same concentration of ML. The most possible reason behind this may be that these aqueous solution of ML appeared to be absorbed more effectively on the expanded surface i.e. foliar surface where not only they are perceived by the receptors and expressed in the young, rapidly growing leaves but also transported through the leaf vascular strands to other actively growing tissues (apical meristems, axillary buds, differentiating vasculature, flower buds their pollens, and root apical meristem etc.). The sensitivity and hence biosynthesis of ML have been reported to be optimal in rapidly growing meristems, where the former is strictly dependent on the density of ML-receptors expressed on the membranes of these tissues.

Conclusion

The present study concluded that melatonin showed concentration and mode dependent responses that were conspicuous in terms of increased growth and photosynthetic efficiency. Foliar application of melatonin excelled over

shotgun approach for all the parameters studied and 100 µM ML recognized as most suited concentration for enhancing the activities of enzyme associated with antioxidant system of plant and showed increased accumulation of proline. Application of ML (100 µM) through foliage of plants improved photosynthetic efficiency and antioxidant system.

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Conflict of interest: The authors declare that there is no conflict of interest.

REFERENCES

Aebi H 1984. Catalase in Vitro. Methods Enzymol. https://doi.org/10.1016/S00766879(84)05016-3

Arnao MB, Hernández-Ruiz J. 2019. Melatonin: A New Plant Hormone and/or a Plant Master Regulator? Trends Plant Sci. 24: 38–48. https://doi.org/ 10.1016/ j.tplants. 2018. 10. 010

Arna MB, Hernández-Ruiz J 2015. Functions of melatonin in plants: a review. J. Pineal Res. 59: 133-50. https://doi.org/10.1111/jpi.12253

Arnao MB, Hernández-Ruiz J 2007. Melatonin promotes adventitious and lateral root regeneration in etiolated hypocotyls of Lupinus albus L. J. Pineal Res. 42: 147-152. https://doi.org/10.1111/j.1600-079X.2006.00396.x

Arnao MB, Hernández-Ruiz J 2006. The physiological function of melatonin in plants. Plant Signal. Behav. 1: 89-15. https://doi.org/10.4161/psb.1.3.2640

Arnon DI. 1949. Copper enzymes in isolated chloroplasts. polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24: 1-15. https://doi.org/10.1104/pp.24.1.1

Bates LS, Waldren RP, Teare ID. 1973. Rapid determination of free proline for water-stress studies. Plant Soil. 39: 205-207. https://doi.org/10.1007/BF00018060

Bradford MM 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 1-2. https://doi.org/10.1016/0003-2697(76)90527-3

Chen Q, Qi WB, Reiter RJ, Wei W, Wang BM. 2009. Exogenously applied melatonin stimulates root growth and raises endogenous indoleacetic acid in roots of etiolated seedlings *Brassica juncea*. J. Plant Physiol. 166: 324-8. https://doi.org/10.1016/j.jplph.2008.06.002

Dwivedi RS, Randhawa NS. 1974. Evaluation of a rapid test for the hidden hunger of zinc in plants. Plant Soil. 40: 445-451. https://doi.org/10.1007/BF00011531

Foyer C.H., Harbinson, J., 2011. Photosynthetic regulation, in: Terrestrial Photosynthesis in a Changing Environment a Molecular, Physiological and Ecological Approach. https://doi.org/10.1017/CBO9781139051477.005

Gong X, Shi S, Dou F, Song Y, Ma F. 2017. Exogenous melatonin alleviates alkaline stress in Malus hupehensis Rehd. by regulating the biosynthesis of polyamines. Molecules 22: 1542. https://doi.org/10.3390/ molecules 22091542

Hernández-Ruiz J, Cano A, Arnao MB. 2005. Melatonin acts as a growth-stimulating compound in some monocot species. J. Pineal Res. 39: 137-142. https://doi.org/10.1111/j.1600-079X.2005.00226.x

Hernández-Ruiz J, Cano A, Arnao, MB 2004. Melatonin: A growth-stimulating compound present in lupin tissues. Planta. 220: 140-144. https://doi.org/10.1007/s00425-004-1317-3

Jaworski EG. 1971. Nitrate reductase assay in intact plant tissues. Biochem. Biophys. Res. Commun. 43: 1274-1279. https://doi.org/10.1016/S0006-291X(71)80010-4

Kang K, Kong K, Park S, Natsagdorj U, Kim YS, Back K 2011. Molecular cloning of a plant N-acetylserotonin methyltransferase and its expression characteristics in rice. J. Pineal Res. 50: 304-9. https://doi.org/10.1111/j.1600-079X.2010.00841.x

Kishor PBK, Hima Kumari P, Sunita MSL, Sreenivasulu N (2015). Role of proline in cell wall synthesis and plant development and its implications in plant ontogeny. Front. Plant Sci. 6: 544 https://doi.org/10.3389/fpls.2015.00544

Kono Y (1978) Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. Arch. Biochem. Biophys. 186: 189-195. https://doi.org/10.1016/0003-9861(78)90479-4

Li C, Liang B, Chang C, Wei Z, Zhou S, Ma F 2016. Exogenous melatonin improved potassium content in Malus under different stress conditions. J. Pineal Res. 61:218-229 https://doi.org/10.1111/jpi.12342

Li H, Chang J, Chen H, Wang Z, Gu X, Wei C, Zhang Y, Ma J, Yang J, Zhang X (2017). Exogenous melatonin confers salt stress tolerance to watermelon by improving photosynthesis and redox homeostasis. Front. Plant Sci. 8: 295. https://doi.org/10.3389/fpls.2017.00295

Li H, He J, Yang X, Li X, Luo D, Wei C, Ma J, Zhang Y, Yang J, Zhang X 2016. Glutathione-dependent induction of local and systemic defense against oxidative stress by exogenous melatonin in cucumber (Cucumis sativus L.). J. Pineal Res. 60: 206-216. https://doi.org/10.1111/jpi.12304

Liang D, Shen Y, Ni Z, Wang Q, Lei Z, Xu N, Deng Q, Lin L, Wang J, Lv X, Xia H 2018. Exogenous melatonin application delays senescence of kiwifruit leaves by regulating the antioxidant capacity and biosynthesis of flavonoids. Front. Plant Sci. 9: 426. https://doi.org/10.3389/fpls.2018.00426

Liu J, Zhai R, Liu F, Zhao Y, Wang H, Liu L, Yang C, Wang Z, Ma F, Xu L 2018. Melatonin induces parthenocarpy by regulating genes in gibberellin pathways of 'starkrimson' pear (*Pyrus communis* L.). Front. Plant Sci. 9: 946. https://doi.org/10.3389/fpls.2018.00946

Meng JF, Xu TF, Wang ZZ, Fang YL, Xi ZM, Zhang ZW 2014. The ameliorative effects of exogenous melatonin on grape cuttings under water-deficient stress: Antioxidant metabolites, leaf anatomy, and chloroplast morphology. J. Pineal Res. 57: 200-212. https://doi.org/10.1111/jpi.12159

Park S, Lee DE, Jang H, Byeon Y, Kim YS, Back K. 2013. Melatonin-rich transgenic rice plants exhibit resistance to herbicide-induced oxidative stress. J. Pineal Res. 54: 258-263. https://doi.org/10.1111/j.1600-079X.2012.01029.x

Reiter RJ. 1991. Pineal melatonin: Cell biology of its synthesis and of its physiological interactions. Endocr. Rev. 12: 151-180. https://doi.org/10.1210/edrv-12-2-151

Sánchez M, Peña MJ, Revilla G, Zarra I. 1996. Changes in Dehydrodiferulic Acids and Peroxidase Activity against Ferulic Acid Associated with Cell Walls during Growth of

Pinus pinaster Hypocotyl. Plant Physiol. 111: 941-946. https://doi.org/10.1104/pp.111.3.941

Vitalini S, Gardana C, Simonetti P, Fico G, Iriti M (2013). Melatonin, melatonin isomers and stilbenes in Italian traditional grape products and their antiradical capacity. J. Pineal Res. 54: 322-333. https://doi.org/10.1111/jpi.12028

Wang P, Yin L, Liang D, Li C, Ma F, Yue Z. 2012. Delayed senescence of apple leaves by exogenous melatonin treatment: Toward regulating the ascorbate-glutathione cycle. J. Pineal Res. 53: 11-20. https://doi.org/10.1111/j.1600-079X.2011.00966.x

Weeda S, Zhang N, Zhao X, Ndip G, Guo Y, Buck GA, Fu C, Ren S. 2014. Arabidopsis transcriptome analysis reveals key roles of melatonin in plant defense systems. PLoS One.9: e93462. https://doi.org/10.1371/journal. pone.009 3462

Wei W, Li QT, Chu YN, Reiter RJ, Yu XM, Zhu DH, Zhang WK, Ma B, Lin Q, Zhang JS, Chen SY. 2015. Melatonin enhances plant growth and abiotic stress tolerance in soybean plants. J. Exp. Bot. 66: 695-707. https://doi.org/10.1093/jxb/eru392

Xu W, Cai SY, Zhang Y, Wang Y, Ahammed GJ, Xia XJ, Shi K, Zhou YH, Yu JQ, Reiter RJ, Zhou J. 2016. Melatonin enhances thermotolerance by promoting cellular protein protection in tomato plants. J. Pineal Res.61: 457-469. https://doi.org/10.1111/jpi.12359

Ye J, Wang S, Deng X, Yin L, Xiong B, Wang X 2016. Melatonin increased maize (*Zea mays* L.) seedling drought tolerance by alleviating drought-induced photosynthetic inhibition and oxidative damage. Acta Physiol. Plant. 38: 48 https://doi.org/10.1007/s11738-015-2045-y

Yin L, Wang P, Li M, Ke X, Li Cuiying Liang D, Wu S, Ma X, Li Chao Zou Y, Ma F 2013. Exogenous melatonin improves Malus resistance to Marssonina apple blotch. J. Pineal Res.54: 426-434. https://doi.org/10.1111/jpi.12038

Zhang N, Sun Q, Zhang H, Cao Y, Weeda S, Ren S, Guo YD 2015. Roles of melatonin in abiotic stress resistance in plants. J. Exp. Bot. 66: 647-656.

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