Research Article

Salt stress in *Arabidopsis thaliana* seedlings: Role of indoleamines in stress alleviation

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ABSTRACT

Salinity is a major environmental stress in agriculture with significantly detrimental effects on crop productivity. The development of strategies to enhance salinity stress tolerance in plants is essential to ensure crop production in saline environments. Melatonin (Mel) and serotonin (Ser) accumulate in response to environmental stresses and are presumed to play protective roles and improve growth of tissues during recovery. In this study, the effects of Mel and Ser were investigated in *Arabidopsis* under NaCl stress. Exogenous Mel (10 µM) and Ser (10 µM) treatment significantly increased fresh weight, lateral root number, and shoot height in *A. thaliana* seedlings exposed to NaCl stress (25 mM and 50 mM) compared to the non-treated control seedlings. In order to understand the role of these indoleamines in alleviating salt stress, we investigated the effects of Mel and Ser treatments on the expression of salt stress responsive genes including, transcription factors involved in abscisic acid (ABA) signaling pathway, *ABA-INSENSITIVE 3* (*ABI3)and ABA-INSENSITIVE 5* (*ABI5);* ABA responsive gene, *RESPONSIVE TO DESSICATION 29B* (*RD29B)*, ABA-independent gene, *RESPONSIVE TO DESSICATION 29A* (*RD29A)* and Arabidopsis trithorax-like gene (*ATX1*) which function in stress responses via ABA-dependent and ABA-independent manner. Other genes included, ROS-signaling transcription factor *ZAT10* and *ZAT12*, and the genes encoding ion transporters crucial for maintaining ion homeostasis, *HIGH AFFINITY K^+ TRANSPORTER 5* (*HAK5)* and *SALT OVERLY SENSITIVE 1 (SOS1)*. Mel (10 µM) pre-treatment for 24 hrs followed by 50 mM salt treatment up-regulated *ABI3*, *RD29B*, *ZAT12* and *HAK5*. The Ser (10 µM) pre-treatment significantly up-regulated *ZAT12*.These results indicate that indoleamine pre-treatment improved plant growth under salt stress with Mel facilitating salt tolerance via upregulation of ABA responsive genes, mediation of antioxidant defense systems to counteract the salt-induced ROS overproduction as well as controlling ion homeostasis. Although Ser displayed no significant effects on ABA signaling, it was found to increase the expression of antioxidant defense gene, *ZAT12*. This study demonstrates the importance of indoleamine pathway in mediation of salt stress response and provides the first indication of the involvement of Ser in salt stress tolerance.

Key words: Salt stress, melatonin, serotonin, ABA signalling pathway, reactive oxygen species (ROS).

1. INTRODUCTION

Soil salinity is among the major environmental stresses that adversely affect productivity in crop plants (1, 2). Worldwide, salt-affected irrigated agricultural land is expected to climb from 33% to up to 50% in the next decade (3, 4). Increasing levels of soil salinity can seriously decrease food production as approximately one third of the world's food is produced on agricultural land (1, 5, 6). Despite numerous attempts in water management practices to alleviate soil salinity, the excessive salt concentration in arid and semi-arid regions around the globe has been difficult to overcome and new strategies that can enhance salt stress tolerance in crop plants must be developed (7, 8).

The indoleamines, melatonin (N-acetyl-5-methoxytryptamine; Mel) and serotonin (5-hydroxytryptophan; Ser) are derived from the aromatic amino acid tryptophan; where Ser is synthesized first serving as precursor for Mel formation (9, 10). Mel has been implicated for its role in enhancing abiotic and biotic stress tolerance in plants (11–19). In particular, Mel plays an important role in salt and drought stress tolerance in apple, soybean, cucumber, watermelon and citrus (13, 15, 18, 20, 21). Increased Mel and Ser levels have been reported in sunflower (*Helianthus annuus* L.) seedlings exposed to salinity stress. Both indoleamines promoted germination significantly and were associated with differential localization in sunflower tissues, a phenomenon which has also been implicated in thermal stress tolerance in St. John's wort (*Hypericum perforatum* L.) (22, 23). Diverse mechanisms have been demonstrated for Mel-mediated salt stress tolerance in plants, including prevention of electrolyte leakage, lipid-peroxidation and chlorophyll degradation as well as induction of antioxidant defense systems to prevent tissue damage in plants exposed to salinity stress (18, 20). In watermelon, pre-treatment with Mel on salt-stressed roots enhanced the photosynthetic rate by inhibiting the stomatal closure as well as improving light energy absorption and electron transport in photosystem II. In addition, Mel alleviated the salt-induced oxidative stress damage via enhancing redox homeostasis and activation of antioxidant enzymes (18). Similarly, in salt-stressed rapeseed (*Brassica napus* L.) seedling roots, the application of Mel together with Nitric Oxide-releasing compounds counteracted seedling growth inhibition and re-established ion homeostasis which was associated with alleviating ROS overproduction, a decrease in Na^+/K^+ ratio and modulation of the antioxidant defense genes *SODIUM HYDROGEN EXCHANGER* (*NHX1*) and *SALT OVERLY SENSITIVE 2* (19).

Salinity responses involve multiple signaling pathways integrated into a complex regulatory network which also includes ABA metabolic pathway (24–26). ABA biosynthesis is dramatically increased under high salinity and drought conditions and mediates the expression of transcription factor (TF) genes implicated in biotic and abiotic stress responses, including those responding to water dehydration stress in an ABA-dependent and independent manner (27–29). Based on the active role of Mel in alleviating salt stress, we hypothesized that Mel and Ser may effectively mitigate salt stress in *Arabidopsis* and that the pre-treatment with these compounds will lead to modifications in transcription of several drought, salinity and ABA-related genes. With the aim to understand the metabolic pathways involved in indoleamine-mediated salt stress alleviation, we conducted a targeted gene expression analysis by selecting candidate genes involved in

abiotic stress tolerance. The data from this study indicate that exogenous treatment with Mel and Ser significantly improved salt stress tolerance in *Arabidopsis* seedlings. Mel response appears to be mediated by ABA-responsive and antioxidant defense genes, whereas Ser response seems to be mediated through antioxidant defense genes in an ABA-independent manner. These protective effects with indoleamines suggested that these compounds act as inductive signals in pre-conditioning the plant to tolerate abiotic stress, a phenomenon similar to the role of Mel and Ser in plant morphogenesis and thermal stress (23).

2. MATERIALS AND METHODS

2.1. Plant material and growth conditions.

The *Arabidopsis* ecotype Columbia-0 (Col) was obtained from *Arabidopsis* Biological Resource Center (Columbus, OH, USA). Seeds were surface sterilized with 70% (v/v) ethanol for 1 min followed by 15% Clorox® bleach solution for 20 min and then washed three times with sterile water. Sterile seeds were placed in a single row in Petri dishes (70 mm diameter) containing Murashige and Skoog (MS, Phytotechnology Lab, KS, USA) modified salt mixture (30) with 1% sucrose, pH adjusted to 5.7 prior to adding 2.2 g/L phytagel (Sigma Aldrich) and autoclaved at 121°C for 20 min. Mel (0, 10, 30 μM) and Ser (0, 10, 30 μM) were filter sterilized and added to the medium cooled to 55 °C under low light conditions (5 $\mu molm^{-2}s^{-1}$). All Petri dishes were kept inclined at an angle of 65° to allow optimum light penetration for root and shoot growth and kept at 4°C for two days. This cold stratification improves subsequent seed germination and seedling development with minimum impact on plant metabolism (31). Standard growth conditions for all experiments were 25 °C with a 16 h photoperiod and a light intensity of 40 $\mu molm^{-2}s^{-1}$, provided by cool white fluorescent tubes (LI-250A, LI-COR® Biosciences, USA).

2.2. Salt stress treatment.

For salt stress treatment, five-day-old *Arabidopsis* seedlings, originally grown on Mel (0, 10, 30 μM) and Ser (0, 10, 30 μM) supplemented medium, were transferred to the same MS medium but containing various levels of NaCl (25, and 50 mM), including a control with 0 mM NaCl. Cultures were kept under 16 h photoperiod and a light intensity of 15 $\mu molm^{-2}s^{-1}$ at 25 °C. Growth parameters including shoot height, primary root length, lateral root numbers and fresh weight were measured after 14 days of growth.

For gene expression analyses, sterile *Arabidopsis* seeds were grown in 125 mL flasks containing 10 mL liquid medium (MS modified salt mixture) to get enough mass for sample analysis and rotated on an orbital shaker at 75 rpm. This liquid culture method is commonly used to grow *Arabidopsis* seedlings *in vitro*; the growth medium can be supplemented with abiotic stress treatments for efficient collection of tissues for gene expression studies (32–34). MS medium was supplemented with Mel (0 or 10 μM) and Ser (0 or 10 μM) and 11-day-old seedlings were treated with NaCl by replacing the remaining culture medium with a fresh half strength MS medium containing 50 mM NaCl. Tissues were collected from the flasks after 24 hours of NaCl treatment, immediately flash frozen in liquid nitrogen and stored at -80 °C until gene expression analyses.

2.3. RNA extraction and gene expression analysis.

Total RNA was extracted from the *Arabidopsis* seedlings using the cetyltrimethylammonium bromide (CTAB) method (35). The RNA samples were treated with DNase enzyme using the RNase-free DNase Kit (Qiagen, Toronto, ON, Canada) followed by a purification step with the RNAeasy Plant Mini Kit (Qiagen, Toronto, ON, Canada), following to the manufacturer's instructions. The DNA-free total RNA was quantified at 260 nm using a Synergy H1 Hybrid Reader spectrophotometer (BioTek, VT, USA) and the integrity was measured in agarose gel electrophoresis after the samples were mixed with 3X Ambion® NorthernMax® Formaldehyde Load Dye (Life Technologies Inc., ON, Canada). The cDNA libraries were prepared from 2.5 µg of total RNA using SuperScript™ VILO™ cDNA Synthesis Kit (Invitrogen, ON, Canada) as described in the manufacturer's instructions. Samples of cDNA were mixed with Sso-Fast EvaGreen Supermix (Bio-Rad, Mississauga, ON, Canada) and gene specific primers in triplicate prior to incubation in a CFX Connect Real-Time PCR Detection System (Bio-Rad, Mississauga, ON, Canada) for quantitative RT-PCR. Gene specific primers were designed to target the flanking coding region of each gene. Three technical replicates were assessed from each treatment. Expression of each gene was normalized to that of *Arabidopsis* β-actin gene and was calculated relative to the NaCl (50mM) - stressed samples without Mel or Ser treatment.

2.4. Statistical analysis.

For the studies conducted to determine the efficacy of Ser and Mel in improving salt stress tolerance of plants in Petri dishes, the experiment was conducted as a randomised complete block design with three replications, each treatment was applied to six Petri dishes per replication. All growth data were analyzed using JMP 10.0.0 (SAS Institute, Cary, NC) and ANOVA was conducted to determine significance of the model followed by means comparison using Student's t-test at $\alpha = 0.1$ significance level.

For gene expression study, data were compared for significant differences using two different P-values < 0.05 or ≤ 0.1. Data were presented as the mean ± the standard error of two biological replications for all genes except *ATX1* for which there was only one biological replication. Each biological replication had three technical replications.

3. RESULTS

3.1. Mel and Ser independently induced salt stress tolerance in *Arabidopsis* seedlings.

To compare the effects between exogenous Mel and Ser treatment on plant growth and development under salt stress, *Arabidopsis* seedlings were grown in different salt concentrations with and without these indoleamines. Overall, better root and shoot growth was observed in the *Arabidopsis* seedlings treated with Mel or Ser compared to the non-treated seedlings subjected to salt stress, indicating that Mel and Ser treatments reduced the growth inhibition caused by salt stress (Figures 1 and 2).

Fig. 1. Effects of different concentrations of melatonin on the growth of *Arabidopsis* seedlings.

Arabidopsis seedlings were cultured for 5 days on MS medium supplemented with Melatonin (Mel) (0, 10, and 30 µM) for pre-treatment. The pre-treated seedlings were grown for additional 10 days on fresh MS medium without Mel but supplemented with 25 mM NaCl (A) and 50 mM NaCl (B).

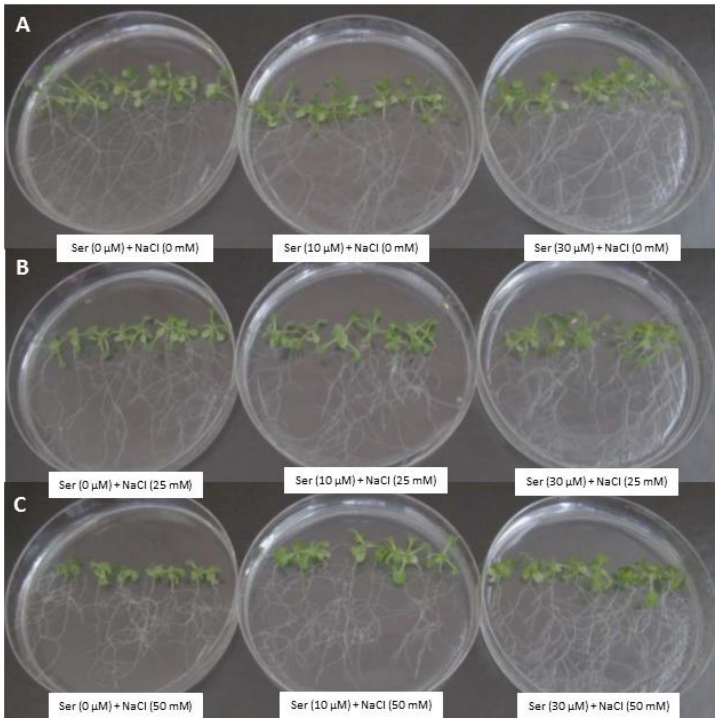

Fig. 2. Effects of different concentrations of serotonin on the growth of *Arabidopsis* seedlings.

Arabidopsis seedlings were cultured for 5 days on MS medium supplemented with Serotonin (Ser) (0, 10, and 30 µM) for pre-treatment. The pre-treated seedlings were grown for additional 10 days on fresh MS medium without Ser but supplemented with 0 mM NaCl (A), 25 mM NaCl (B) and 50 mM NaCl (C).

However, at high salt concentration (50 mM NaCl) Ser appeared to be a better stress modulating molecule than Mel as demonstrated with higher fresh weight, root number and root length in 10 µM Ser treatment compared to 10 µM Mel (Figure 3). During moderate salt stress treatment (25 mM NaCl), both Mel and Ser regardless of their concentration showed higher indicators of plant growth and development than the control plants without indoleamine treatment (Figure 3). Increased fresh weight (67% and 100) was observed in plants under Ser treatments (10 and 30 µM) relative to the control treatment at high salt concentration of 50 mM. Similarly, Mel treatments at 10 and 30 µM showed increased fresh weight (31 and 25%) compared to the control treatment under salt stress concentration of 50 mM. In particular, the number of lateral roots was significantly higher in both Mel (55.29%) and Ser (45.88 %) treatments relative to the control with a salt concentration of 25 mM (Figure 3C). For both indoleamines, 10 µM treatment seems to be the ideal concentration to alleviate salt stress in *Arabidopsis* seedlings as opposed to the treatment with 30 µM. Under no stress condition (0 mM NaCl), *Arabidopsis* seedlings treated with Ser showed higher fresh weight than the seedlings treated with Mel. Although both indoleamines helped the seedlings escape from the adverse conditions imposed by NaCl treatment, exogenous application of Ser appeared to have a better ameliorating effect than Mel.

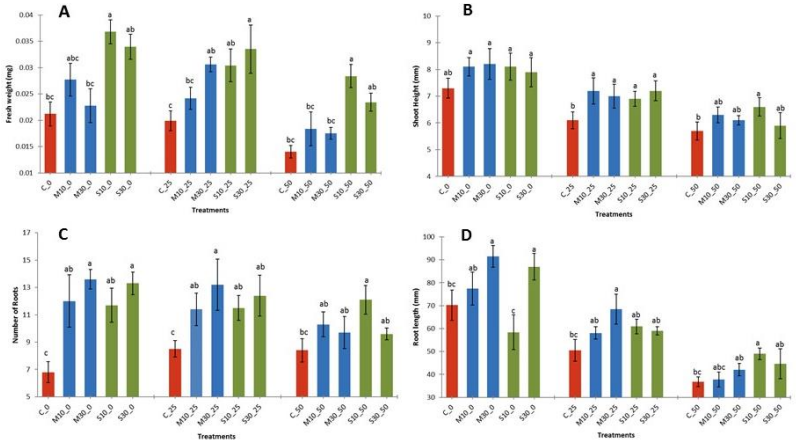

Fig. 3. Effect of Melatonin (Mel: 0, 10, and 30 µM) and Serotonin (Ser: 0, 10, and 30 µM) on *Arabidopsis* seedlings.

(A) fresh weight, (B) shoot height (C) number of lateral roots and (D) primary root length of Arabidopsis seedlings after 15 days of salt treatment (0, 25 and 50 mM of NaCl). The data are presented as the mean ± standard error of three replications. Means with the same letter are not significantly different at P<0.1

3.2. Mel and Ser modulated expression of salt tolerance genes in *Arabidopsis.*

To understand the possible molecular mechanisms through which Mel and Ser mediate salt stress tolerance in plants, the expression of salt and drought stress responsive genes, *ABI3*, *ABI5*, *ATX1*, *RD29A*, *RD29B*, *ZAT10*, *ZAT12*, *SOS1* and *HAK5* was investigated in *Arabidopsis* seedlings pre-treated with Mel (10 µM) and Ser (10 µM) and exposed to 50 mM NaCl relative to control seedlings without any indoleamine pre-treatment but exposed to 50 mM NaCl. The relative expression of *ABI3* was up-regulated by three-fold in plants pre-treated with Mel after 1-day (1d) of salt treatment compared to the control plants (Figure 4). Conversely, *ABI5* expression

remained unaltered in response to Mel pre-treatments after salt stress, suggesting that only *ABI3*, which is a central ABA-induced TF, is capable of responding to Mel treatment in salt-stressed plants. As opposed to Mel pre-treatment, *ABI3* and *ABI5* appear to be unresponsive to Ser pre-treatment which was reflected in similar expression levels of these genes after NaCl treatment relative to the control plants (Figure 4B).

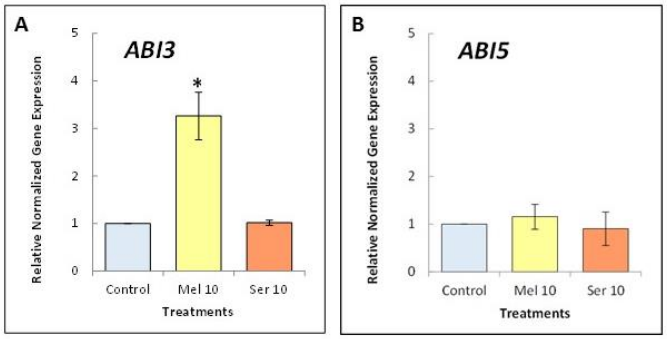

Fig. 4. Relative normalized expression of ABA-dependant transcription factors (*ABI3* and *ABI5*).

*ABI3 and ABI5implicated in seed maturation and drought stress adaptation after exposure of the seedlings to salt stress (50 mM) for one day. The expression of ABI3 and ABI5 was normalized to that of β-actin and calculated relative to the control treatment (0 μM, Mel or Ser) at the same salt-stress level. Values represent the mean ± standard error of two biological replicates for both ABI3 and ABI5. Student's t-test was utilized to determine the statistically significant difference between treatments and control at *p≤0.05.*

ATX1 expression remained unchanged in Mel and Ser pre-treated plants (Figure 5A). The downstream genes RD29A and RD29B, which are regulated by ATX1 gene product, showed negligible expression changes in Ser pre-treated plants after salt stress exposure (Figure 5B and 5C). However, RD29B expression was significantly upregulated by Mel pre-treatment after 1d of salt stress (Figure 5 C).

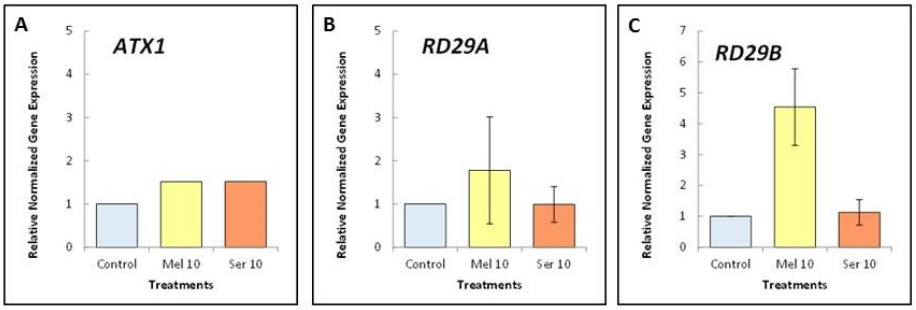

Fig. 5. Relative normalized expression of transcription factors *ATX1*, *RD29A* and *RD29B*.

*ATX1 modulating downstream genes related to water deficit and salt stress RD29A and RD29B, after exposure of the seedlings to salt stress (50 mM) for one day. The expression of ATX1, RD29A and RD29B was normalized to that of β-actin and calculated relative to the control treatment (0 μM, Mel or Ser) at the same salt-stress level. Values represent the mean ± standard error of two biological replicates for RD29A and RD29B and one biological replicate for ATX1. Student's t-test was utilized to determine the statistical significant difference between treatments and control at *p≤0.1.*

Genes encoding *ZAT10* and *ZAT12* TF, which mediate salt stress tolerance in plants, were upregulated after 1d of exposure to salt stress treatment in both Ser and Mel pre-treated plants (Figure 6). Although both *ZAT10* and *ZAT12* transcript levels were upregulated, only ZAT12 shows statistically significant difference in expression with Mel and Ser treatments compared to the control (Figure 6B). Two stress related transcripts, *SOS1* and *HAK5*, required to maintain Na^+ and K^+ homeostasis in *Arabidopsis* were also investigated in this study. The expression of *HAK5* was upregulated in Mel pre-treated plants relative to the control after exposure to salt stress (Figure 7B). In contrast, the expression levels of *HAK5* and *SOS1* remained unchanged in Ser pre-treated plants exposed to salt (Figure 7).

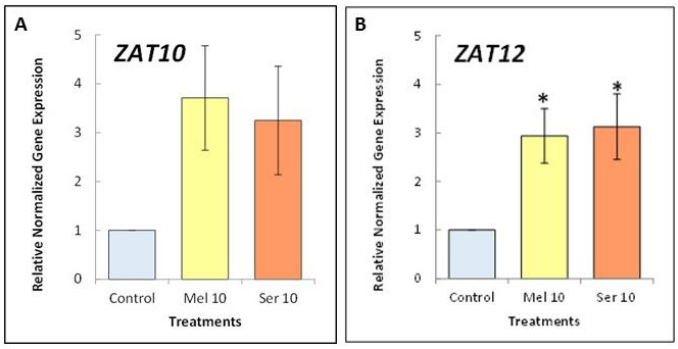

Fig. 6. Relative normalized expression of transcription factors *ZAT10* and *ZAT12*.

*ZAT10 and ZAT12 implicated in activation of ROS-related antioxidant genes, after exposure of the seedlings to salt stress (50 mM) for one day. The expression of ZAT10 and ZAT12 was normalized to that of β-actin and calculated relative to the control treatment (0 μM, Mel or Ser) at the same salt-stress level. Values represent the mean ± standard error of two biological replicates for both ZAT10 and ZAT12. Student's t-test was utilized to determine the statistical significant difference between treatments and control at *p≤0.1.*

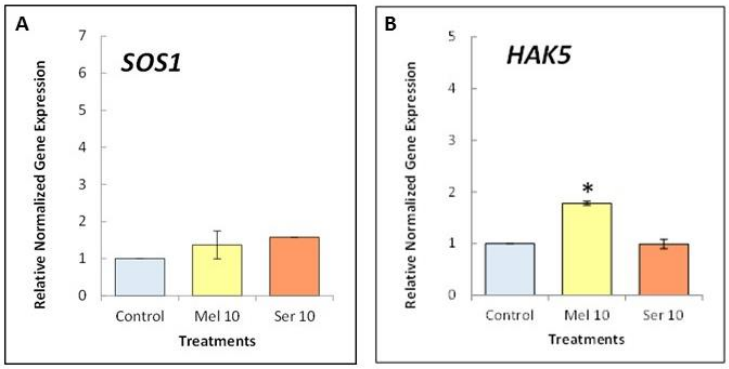

Fig. 7. Relative normalized expression of *SOS1* and *HAK5* genes.

*SOS1 and HAK5 genes implicated in salt tolerance via ion homeostasis, after exposure of the seedlings to salt stress (50 mM) for one day. The expression of SOS1 and HAK5 was normalized to that of β-actin and calculated relative to the control treatment (0 μM, Mel or Ser) at the same salt-stress level. Values represent the mean ± standard error of two biological replicates for both SOS1 and HAK5. Student's t-test was utilized to determine the statistical significant difference between treatments and control at *p≤0.1.*

4. DISCUSSION

Water resources, environmental pollution and increased salinization of soils represent three major threats for agricultural sustainability. In particular, soil salinity is one of the most devastating environmental stresses causing serious reductions in cultivated land (29, 36). The accumulation of soluble salts in the soil is known to suppress plant growth and development, ultimately leading to loss in crop productivity (37). Increased soil salinization in arable land is positively correlated with the world population, which is expected to increase by 1.5 billion in the next two decades (1, 38). If soil salinity is not overcome within this period, the increasing demand of major crops such as rice, wheat and maize required to feed the world's growing population will not be met (1). Different strategies to overcome salinity problems have been developed since the early 90s, including reclamation of salt-affected soils and development of salt-tolerant cultivars (36). However, these strategies have been unable to increase crop production at the pace required to feed the world's growing population.

Pre-treatment with Mel has shown to effectively rescue plant growth and development from salt stress in crops such as sunflower, wheat, watermelon and rice and could represent an important mitigation strategy to enhance crop productivity in saline soils (13, 15, 18, 20, 22, 39). However, molecular mechanisms in the role of these indoleamines with respect to inducing salt stress tolerance in plants remains largely unknown. We investigated the effects of Mel as well as its precursor Ser in promoting salt-stress tolerance. Mel-mediated salt stress mitigation has been described in diverse species including apple, grape, fava bean, cucumber, soybean, rice and bitter orange (13, 15, 20, 40–44). The role of Mel in alleviating salt stress has been attributed to its antioxidant effects such as prevention of electrolyte leakage, lipid peroxidation, pigment degradation as well as regulation of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APX) (13, 18, 20, 45). In soybean seedlings, Mel alleviated the inhibitory effects of salt stress by up-regulating the expressions of genes that are otherwise inhibited by high salt concentration (44). In contrast, the effects of Ser in salt stress mitigation have not been extensively investigated. Mukherjee et al. (2014) reported that Ser as well as Mel improved survival of sunflower (*H. annuus*) seedlings under salt stress, which may be associated with tissue specific increases in endogenous indoleamines production (22). Therefore, the investigation of the role of Ser in salt stress mediation together with the expression of associated genes is important to determine if Ser modulated salt stress tolerance is via a Mel independent mechanism.

ABA is well-known for its role in abiotic stresses and acts through the activation of ABA-responsive genes to induce stress tolerance in *planta*. Among these genes, *ABI3* and *ABI5* have been shown to enhance drought stress adaptation in cotton plants (46). In addition, ectopic expression of *ABI3* increased the ABA-induced accumulation of cold- and drought- responsive transcripts in *Arabidopsis* seedlings (47). The gene-expression data from our study indicated that Mel pre-treatment up-regulated *ABI3*, but not *ABI5* in *Arabidopsis* seedlings exposed to salt-stress (Figure 4). In addition, Mel up-regulated the expression of *RD29B* after salt stress; however, the expression of its counterpart, *RD29A*, remained unaffected (Figure 5). The *RD 29A* is known to be mainly induced via an ABA-independent pathway whereas *RD 29B* induction depends upon ABA availability (48, 49). *ABI3* is a central TF with a hierarchical role in activating downstream genes via signal transduction cascades (50, 51). In Mel pre-treated *Arabidopsis* seedlings, one of these *ABI3*-regulated genes could be encoded by *RD29B*. In this case, Mel could have activated *ABI3* expression that would trigger a signaling cascade involving

RD29B. Interestingly, Nakashima et al (2006) showed that *ABI3* can strongly up-regulate the expression of *RD29B* in *Arabidopsis*, but not that of *RD29A*, which remained unaltered in our study after salt stress exposure (52). Moreover, *RD29B* has been shown to have transcriptional stress memory by increasing its rate of transcription upon additional stress treatments in drought-stressed *Arabidopsis* plants, whereas no signs of transcriptional memory have thus far been observed for *RD29A* (53). Taken together, these results indicate that Mel pre-treatment induces stress response in ABA-dependent manner and there might be a cross-talk between ABA and Mel. The results also indicate that Mel treatment might induce transcriptional stress memory in salt-stressed plants through the activation of a TF cascade involving *ABI3* and *RD29B*. Mel pre-treatment has previously been shown to stimulate the biosynthesis of ABA in *Arabidopsis* and grape plants (54, 55). Thus, the possibility remains that Mel pre-treatment could increase ABA biosynthetic and signalling pathways prior to salt stress exposure.

ATX1, which is involved in dehydration stress responses in *Arabidopsis* is not up-regulated by Mel or Ser pre-treatment. Although *ATX1* can activate the downstream genes *RD29A* and *RD29B* in the presence of ABA, this plant hormone can also induce the expression of *RD29A* and *RD29B* in *atx1* mutants as well as in wild type plants (27), suggesting that *RD29A* and *RD29B* activation could also occur independent of *ATX1*. Taken together, it appears that up-regulation of *RB29B* in salt exposed *Arabidopsis* plants pre-treated with Mel could be independent of *ATX1*.
Mel and Ser pre-treatment also enhanced the expression of *ZAT10* and *ZAT12* genes which are TFs related to abiotic stress responses in *Arabidopsis*. Induction of *ZAT10* and *ZAT12* transcripts by Mel has also been described in *Arabidopsis* plants subjected to cold stress (17). In other stress-related studies, *ZAT10* and *ZAT12* stimulated the expression of reactive oxygen-defense transcripts, and enhanced the tolerance of plants to salinity, heat and osmotic stresses (13, 56). *HAK5*, a structural gene encoding an ion transporter implicated in stress tolerance was upregulated by Mel after 1d of salt stress. Previously, Mel had been related to ion homeostasis for inducing the expression of ion transporter genes in apple plants under salt stress (57). *HAK5* is required to maintain ion homeostasis in plants, which is severely impaired after exposure to high salt concentration (45, 58).

Overall, the results from this study suggest that Mel and Ser can effectively mitigate salinity stress with both indoleamines acting via modulation of stress-related genes in metabolic pathways of ABA, ROS, and ion homeostasis. It is noteworthy that this study also highlights the role of Ser in salt stress amelioration. This effect may involve shared pathways that are both similar and different than those described for melatonin. Moreover, Ser showed strong antioxidant effect but less influence on ABA related gene upregulation. Both Mel and Ser can act as antioxidants, exhibit wide ranging effects in plant development under normal and stress conditions, and are known to improve survival of plants in response to biotic and abiotic stresses (21, 59–63). In addition to antioxidation, these stress survival mechanisms may include altered biosynthesis, redistribution, interconversions and feedback mechanisms in the indoleamines pathway (23). It is plausible that Mel and Ser act in coordination via interactive pathways to mediate salinity stress in *Arabidopsis* with Ser balancing Mel-mediated growth and survival response under normal and stress conditions.

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AUTHORSHIP

MRS, VSB, and PKS were involved in the development of the hypotheses and experimental design. MRS and VSB conducted the majority of the experiments and the manuscript was developed by MRS, VSB, and JAFC. All authors participated in the final revision of the manuscript.

CONFLICT OF INTEREST

The authors have no conflict of interest

REFERENCES

1. Godfray HCJ, *et al.* (2010) Food security: The challenge of feeding 9 billion people. *Science* **327**: 812–818.
2. Zörb C, Geilfus CM, Dietz KJ (2019) Salinity and crop yield. *Plant Biol.* **21**: 31–38.
3. Jamil A, Riaz S, Ashraf M, Foolad MR (2011) Gene expression profiling of plants under salt stress. *CRC. Crit. Rev. Plant Sci.* **30**: 435–458.
4. Hasanuzzaman M, Nahar K, Fujita M (2013) "Plant Response to salt stress and role of exogenous protectants to mitigate salt-induced damages" in *Ecophysiology and Responses of Plants under salt stress*, Ahmad P, Azooz M, Prasad M, Eds. (Springer New York, 2013), pp. 28–87.
5. Tilman D, *et al.* (2001) Forecasting agriculturally driven global environmental change. *Science* **292**: 281–284.
6. Foley JA, DeFries R, Asner GP, Barford C, Bonan G (2005) Global consequences of land use. *Science* **309**: 570–574.
7. Hernández JA (2019) Salinity tolerance in plants: trends and perspectives. *Int. J. Mol. Sci.* **20**: 2408.
8. van Zelm E. Zhang Y, Testerink C (2020) Salt tolerance mechanisms of plants. *Annu. Rev. Plant Biol.* **71**: 403–433.
9. Reiter RJ (1991) Pineal melatonin: cell biology of its synthesis and of its physiological interactions. *Endocr. Rev.* **12**: 151–180.
10. Murch SJ, Campbell SS, Saxena PK (2001) The role of serotonin and melatonin in plant morphogenesis: regulation of auxin-induced root organogenesis in in vitro-cultured explants of St. John's wort (*Hypericum perforatum* L.). *In. Vitr. Cell. Dev. Biol.* **37**: 786–793.
11. Afreen F, Zobayed S, Kozai T (2006) Melatonin in *Glycyrrhiza uralensis*: response of plant roots to spectral quality of light and UV-B radiation. *J. Pineal Res.* **41**: 108–115.
12. Tan D-X, Manchester LC, Terron MP, Flores LJ, Reiter RJ (2007) One molecule, many derivatives: A never-ending interaction of melatonin with reactive oxygen and nitrogen species? *J. Pineal Res.* **42**: 28–42.
13. Li C, *et al.* (2012) The mitigation effects of exogenous melatonin on salinity-induced stress in *Malus hupehensis*. *J. Pineal Res.* **53**: 298–306.
14. 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.
15. Wang P, *et al.* (2012) Delayed senescence of apple leaves by exogenous melatonin treatment: toward regulating the ascorbate-glutathione cycle. *J. Pineal Res.* **53**: 11–20.

16. Yin L, *et al.* (2013) Exogenous melatonin improves *Malus* resistance to Marssonina apple blotch. *J. Pineal Res.* **54**: 426–434.
17. Bajwa VS, Shukla MR, Sherif SM, Murch SJ, Saxena PK (2014) Role of melatonin in alleviating cold stress in *Arabidopsis thaliana*. *J. Pineal Res.* **56**: 238–245.
18. Li H, *et al.* (2017) Exogenous melatonin confers salt stress tolerance to watermelon by improving photosynthesis and redox homeostasis. *Front. Plant Sci.* **8**: 295.
19. Zhao G, *et al.* (2018) Nitric oxide is required for melatonin-enhanced tolerance against salinity stress in rapeseed (*Brassica napus* L.) seedlings. *Int. J. Mol. Sci.* **19:** 1912.
20. 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 Physiol. Biochem.* **86:** 155–165.
21. Lee H-J, Back K (2019) 2-Hydroxymelatonin confers tolerance against combined cold and drought stress in tobacco, tomato, and cucumber as a potent anti-stress compound in the evolution of land plants. *Melatonin Res.* **2:** 35–46.
22. Mukherjee S, David A, Yadav S, Baluška F, Bhatla SC (2014) Salt stress-induced seedling growth inhibition coincides with differential distribution of serotonin and melatonin in sunflower seedling roots and cotyledons. *Physiol. Plant.* **152:** 714–728.
23. Erland LAE, Shukla MR, Singh AS, Murch SJ, Saxena PK (2018) Melatonin and serotonin: Mediators in the symphony of plant morphogenesis. *J. Pineal Res.* **64:** e12452.
24. Tuteja N (2007) Abscisic acid and abiotic atress aignaling. *Plant Signal. Behav.* **2:** 135–138.
25. Sah SK, Reddy KR, Li J (2016) Abscisic acid and abiotic stress tolerance in crop plants. *Front. Plant Sci.* **7**:571.
26. Zhang L, *et al.* (2020) Wheat TabZIP8, 9, 13 participate in ABA biosynthesis in NaCl-stressed roots regulated by TaCDPK9-1. *Plant Physiol. Biochem.* **151:** 650–658.
27. Ding Y, Avramova Z, Fromm M (2011) The Arabidopsis trithorax-like factor ATX1 functions in dehydration stress responses via ABA-dependent and ABA-independent pathways. *Plant J.* **66:** 735–744.
28. Rock CD (2000) Pathways to abscisic acid-regulated gene expression. *New Phytol.* **148:** 357–396.
29. Yamaguchi T, Blumwald E (2005) Developing salt-tolerant crop plants: challenges and opportunities. *Trends Plant Sci.* **10:** 615–620.
30. Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **15:** 473–497.
31. Lindsey BE, Rivero L, Calhoun CS, Grotewold E, Brkljacic J (2017) Standardized method for high-throughput sterilization of *Arabidopsis* seeds. *J. Vis. Exp.* **128**: e56587.
32. Clouse SD, Hall AF, Langford M, McMorris TC, Baker ME (1993) Physiological and molecular effects of brassinosteroids on *Arabidopsis thaliana*. *J. Plant Growth Regul.* **12:** 61–66.
33. Malboobi MA, Lefebvre DD (1997) A phosphate-starvation inducible β-glucosidase gene (psr3.2) isolated from Arabidopsis thaliana is a member of a distinct subfamily of the BGA family. *Plant Mol. Biol.* **34:** 57–68.
34. Hétu M-F, Tremblay LJ, Lefebvre DD (2005) High root biomass production in anchored *Arabidopsis* plants grown in axenic sucrose supplemented liquid culture. *Biotechniques* **39:** 345–349.
35. Logemann J, Schell J, Willmitzer L (1987) Improved method for the isolation of RNA from plant tissues. *Anal. Biochem.* **163:** 16–20.

36. Shahbaz M, Ashraf M (2013) Improving salinity tolerance in cereals. *CRC. Crit. Rev. Plant Sci.* **32:** 237–249.
37. Paul D (2013) Osmotic stress adaptations in rhizobacteria. *J. Basic Microbiol.* **53:** 101–110.
38. FAOSTAT database (2013) Food and Agriculture Organization of the United Nations, Statistics Division, Rome. 2013. http://fenix.fao.org/faostat/beta/en/#home. Accessed 11 Jan 2019.
39. Ke Q *et al.* (2018) Melatonin mitigates salt stress in wheat seedlings by modulating polyamine metabolism. *Front. Plant Sci.* **9:** 914.
40. Meng J-F, *et al.* (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.
41. Zhang H-J, *et al.* (2014) Melatonin promotes seed germination under high salinity by regulating antioxidant systems, ABA and GA $_4$ interaction in cucumber (*Cucumis sativus* L.). *J. Pineal Res.* **57**: 269–279.
42. Dawood MG, El-Awadi ME (2015) Alleviation of salinity stress on *Vicia faba* L. plants via seed priming with melatonin. *Acta Biológica Colomb.* **20:** 223–235.
43. Liang C *et al.* (2015) Melatonin delays leaf senescence and enhances salt stress tolerance in rice. *J. Pineal Res.* **59:** 91–101.
44. Wei W *et al.*, (2015) Melatonin enhances plant growth and abiotic stress tolerance in soybean plants. *J. Exp. Bot.* **66:** 695–707.
45. Jiang C *et al.*, (2016) Melatonin improves antioxidant capacity and ion homeostasis and enhances salt tolerance in maize seedlings. *Acta Physiol. Plant.* **38**: 82.
46. Mittal A *et al.*, (2014) Related to ABA-Insensitive3(ABI3)/Viviparous1 and AtABI5 transcription factor coexpression in cotton enhances drought stress adaptation. *Plant Biotechnol. J.* **12**: 578–589.
47. Tamminen I, Mäkelä P, Heino P, Palva ET (2001) Ectopic expression of *ABI3* gene enhances freezing tolerance in response to abscisic acid and low temperature in *Arabidopsis thaliana*. *Plant J.* **25**: 8.
48. Msanne J, Lin J, Stone JM, Awada T (2011) Characterization of abiotic stress-responsive *Arabidopsis thaliana* RD29A and RD29B genes and evaluation of transgenes. *Planta* **234:** 97–107.
49. Fujita Y, *et al.* (2005) AREB1 is a transcription activator of novel ABRE-dependent ABA signaling that enhances drought stress tolerance in *Arabidopsis*. *Plant Cell* **17:** 3470–3488.
50. McCarty DR (1995) Genetic control and integration of maturation and germination pathways in seed development. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **46:** 71–93.
51. Lopez-Molina L, Mongrand S, McLachlin DT, Chait BT, Chua N-H (2002) ABI5 acts downstream of ABI3 to execute an ABA-dependent growth arrest during germination. *Plant J.* **32:** 317–328.
52. Kazuo N *et al.* (2006) Transcriptional regulation of ABI3-and ABA-responsive genes including RD29B and RD29A in seeds, germinating embryos, and seedlings of *Arabidopsis*. *Plant Mol. Biol.* **60**: 51–68.
53. Ding Y, Fromm M, Avramova Z (2012) Multiple exposures to drought "train" transcriptional responses in *Arabidopsis*. *Nat. Commun.* **3:** 740.
54. Weeda S *et al.*, (2014) Arabidopsis transcriptome analysis reveals key roles of melatonin in plant defense systems. *PLoS One* **9:** e93462.
55. Xu L, Yue Q, Xiang G, Bian F, Yao Y (2018) Melatonin promotes ripening of grape berry

via increasing the levels of ABA, H_2O_2, and particularly ethylene. *Hortic. Res.* **5**: 41.
56. Mittler R *et al.* (2006) Gain- and loss-of-function mutations in *Zat10* enhance the tolerance of plants to abiotic stress. *FEBS Lett.* **580:** 6537–6542.
57. Li Y *et al.* (2010) Overexpression of a Malus vacuolar Na+/H+ antiporter gene (MdNHX1) in apple rootstock M.26 and its influence on salt tolerance. *Plant Cell Tissue Organ Cult.* **102:** 337–345.
58. Nieves-Cordones M, Alemán F, Martínez V, Rubio F (2010) The *Arabidopsis thaliana* HAK5 K+ transporter is required for plant growth and K+ acquisition from low K+ solutions under saline conditions. *Mol. Plant* **3**: 326–333.
59. Arnao M, Hernández-Ruiz J (2014) Melatonin: plant growth regulator and/or biostimulator during stress? *Trends Plant Sci.* **19:** 789–797.
60. Erland LAE, Murch SJ, Reiter RJ, Saxena PK (2015) A new balancing act: The many roles of melatonin and serotonin in plant growth and development. *Plant Signal. Behav.* **10:** e1096469.
61. Reiter R, *et al.* (2015) Phytomelatonin: assisting plants to survive and thrive. *Molecules* **20**: 7396–7437.
62. Erland LAE, Yasunaga A, Li ITS, Murch SJ, P. K. Saxena (2019) Direct visualization of location and uptake of applied melatonin and serotonin in living tissues and their redistribution in plants in response to thermal stress. *J. Pineal Res.* **66**: e12527.
63. Forsyth JA, Erland LAE, Shipley PR, Murch SJ (2020) Plant Perception of Light: The role of indoleamines in *Scutellaria* species. *Melatonin Res.* **3**: 161–176.

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