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Research Article

Melatonin-mediated improvement of ionic homeostasis and antioxidant defense enhances salinity tolerance in Damask Rose (*Rosa damascene*)

Roya Zomorodnia[1], Mahnaz Karimi[*1] and Mehrnaz Hatami[2]

[1] Department of Horticultural Sciences, Faculty of Agricultural Sciences, Sari Agricultural Sciences and Natural Resources University, Sari, Iran
[2] Department of Medicinal Plants, Faculty of Agriculture and Environment, Arak University, Arak, Iran

Abstract

This study investigated the role of melatonin in alleviating salinity stress in *Rosa damascena*. A factorial experiment was conducted in a completely randomized design with three replications, combining four salinity levels (0, 60, 120, and 180 mM NaCl) and three melatonin concentrations (0, 150, and 250 µM). Salinity significantly inhibited shoot and root growth, reduced chlorophyll and carotenoid contents, and decreased relative water content (RWC), while increasing electrolyte leakage, proline, malondialdehyde (MDA), and sodium accumulation. Melatonin application significantly alleviated these adverse effects. Under non-saline conditions, 250 µM melatonin enhanced chlorophyll *a* and carotenoids by 23.8% and 57.6%, respectively, compared with the control, and significantly increased antioxidant enzyme activities, particularly superoxide dismutase (SOD) and catalase (CAT). Similarly, at moderate salinity levels (60–120 mM), 250 µM melatonin maintained high enzyme activity and improved potassium retention. However, under severe salinity (180 mM NaCl), 150 µM melatonin was more effective in maintaining water balance, reducing ion leakage by up to 20%, lowering MDA by 31%, and decreasing Na^+ accumulation by up to 53%. The shift in optimal concentration from 250 µM under non-saline or mild stress to 150 µM under severe stress reflects the plant's adaptive regulation, where higher melatonin enhances growth under optimal conditions, whereas moderate levels suffice to activate antioxidant defenses and maintain ionic balance under stress. Overall, melatonin within the 150 –250 µM range effectively enhanced antioxidant activity, ionic homeostasis, and salinity tolerance in *R. damascene*.

Keywords: Antioxidant enzymes, Carotenoids, Electrolyte leakage, Lipid peroxidation, Proline

Introduction

Rosa damascene Mill., commonly known as Damask Rose, is one of the most valuable aromatic and medicinal species of the Rosaceae family. This shrub is also widely used in urban landscaping due to its beautiful and fragrant flowers (Kaddar *et al*., 2023). The high genetic diversity of this species in Iran suggests that the country may be one of the centers of its genetic variation (Kiani *et al*., 2010).

Despite its numerous benefits, sustainable production of Damask Rose faces significant challenges related to climate and soil–water conditions. One of the main challenges is the increasing salinity of water and soil resources, especially in the arid and semi-arid regions of Iran. Salinity is one of the most critical abiotic stresses that severely affects plant performance by reducing soil osmotic potential, disrupting water and nutrient uptake, accumulating toxic ions such as sodium and chloride, and increasing reactive oxygen species (ROS) (Acosta-Motos *et al*., 2017). Salt stress causes water deficit, ionic imbalance, and decreased photosynthetic activity, leading to reduced growth, leaf chlorosis, fewer flowers, and ultimately lower yields. (Omidi *et al*., 2020). Plant sensitivity to salinity varies at different developmental stages, with young or actively growing plants often being more affected.

In many regions of Iran, particularly in the northern areas, high evaporation rates from reservoirs and rising

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*Corresponding Author: karimi@sanru.ac.ir

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groundwater levels have led to increased salinity in water resources. Consequently, expanding the cultivation area of Damask rose faces serious limitations, emphasizing the urgent need to identify effective strategies to enhance plant tolerance to salinity stress (YazdaniBiuki *et al*., 2020).

One innovative biological approach that has attracted researchers' attention in recent years is the use of melatonin as a growth stimulant and protective agent against environmental stresses. Melatonin (N-acetyl-5-methoxytryptamine) is a natural indole-amine molecule known for its strong antioxidant properties. Besides its antioxidant role, melatonin is involved in various physiological processes such as seed germination, root development, aerial organ growth, leaf senescence delay, leaf expansion, chlorophyll synthesis, photosynthesis enhancement, and improved carboxylation efficiency. Therefore, it is considered a plant growth regulator and a stress-resistance biostimulant (Zhang *et al*., 2015).

Numerous studies have demonstrated the positive effects of melatonin in mitigating the adverse impacts of salinity, including ion homeostasis regulation, reduction of malondialdehyde (MDA) levels, increased proline accumulation, and stimulation of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) (Li *et al*., 2019). For instance, in a study on rosemary (*Rosmarinusofficinalis*), salinity reduced stem height, but melatonin application, particularly at 100 µM, alleviated these negative effects and promoted leaf growth. Additionally, melatonin increased the activity of antioxidant enzymes SOD and POD, prevented chlorophyll loss caused by salinity, and significantly reduced ion leakage at various salt concentrations (Mohammadi and Karimi, 2020).

Considering the species-specific responses and the woody perennial nature of Damask Rose, higher melatonin concentrations (150 and 250 µM) were selected to ensure a pronounced effect. The present study aims to investigate the effects of melatonin on the morphological, physiological, and biochemical traits of *Rosa damascena*under different levels of salinity stress, including ion homeostasis and antioxidant responses.

Materials and methods

Experimental site, plant material, and experimental design: The experiment was conducted at Sari Agricultural Sciences and Natural Resources University (36°39′45″ N, 53°4′4″ E) from February 2024 to August 2025 in a greenhouse. Greenhouse conditions were maintained at 22–25 °C during the day and 18–20 °C at night, with a relative humidity of 55–65% and natural sunlight supplemented with artificial lighting to provide a 16 h photoperiod suitable for *R. damascene.*The study was arranged as a factorial experiment based on a completely randomized design with three replications. The first factor was melatonin at three levels (0, 150, and 250 µM), and the second factor was sodium chloride at four levels (0, 60, 120, and 180 mM).Two-year-old healthy plants of *R. damascena* (Kashan genotype) were obtained from a medicinal plant nursery located in Amol (Mazandaran). Plants were grown in 20 cm diameter pots filled with a mixture of loam soil, cocopeat, and perlite in a 1:1:1 ratio (v/v).

Melatonin and salinity treatments: After one month of acclimatization, foliar application of melatonin (Sigma brand) was performed three times at 10-day intervals. Approximately 8 mL of the melatonin solution was applied per plant during each application, ensuring thorough wetting of the leaves without runoff. The desired concentrations were first dissolved in 70% ethanol and then diluted with distilled water. A control treatment sprayed with distilled water alone was included to account for any potential solvent effects. Following melatonin application, salinity treatments were applied using NaCl solution. To avoid sudden osmotic shock, the initial salinity was started at 60 mM and gradually increased at four-day intervals for eight weeks. The EC of the leachate was monitored with an EC meter to control stress intensity.

Measured traits: The evaluated traits included new shoot length, number of leaves, biomass, photosynthetic pigments, proline content, electrolyte leakage, antioxidant enzyme activities (CAT and SOD), malondialdehyde (MDA) content, relative water content (RWC), and leaf sodium and potassium concentrations.

For fresh and dry weight measurement, aerial parts and roots were separated, weighed immediately using a digital balance (0.001 g accuracy), and dried in an oven at 72°C for 24 h before reweighing. The shoot-to-root (S:R) ratio was calculated by dividing shoot dry weight by root dry weight for each plant.

Photosynthetic pigments were determined according to Carter and Knapp (2001). Leaf discs were incubated in pure methanol in complete darkness at 30°C, and absorbance was measured using a UV-VIS spectrophotometer. Pigment concentrations were calculated according to Lichtenthaler and Buschmann (1983). Relative water content was measured using uniform leaves. Fresh weight was recorded, then leaves were immersed in distilled water at 4°C for 24 h to obtain saturated weight, followed by oven-drying at 75°C for 48 h to determine dry weight (Sanchez *et al*., 1998). Proline content was determined following Bates *et al*. (1973). Fresh leaf tissue was homogenized in sulfosalicylic acid, filtered, reacted with ninhydrin and acetic acid, and heated in a water bath. After cooling, the organic phase was extracted with toluene, and absorbance was read at 520 nm.

Catalase activity was determined by the method of Aebi (1984) through monitoring the decomposition of H_2O_2.Superoxide dismutase activity was assayed according to Beauchamp and Fridovich (1971) based on the inhibition of NBT reduction under light. Malondialdehyde content, as an index of lipid peroxidation, was estimated according to Ohkawa *et al*. (1979) using the reaction of thiobarbituric acid (TBA) with tissue extracts, followed by spectrophotometric

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measurement.Sodium and potassium concentrations were measured after dilution of samples with cesium chloride (1:9). Concentrations were determined using a flame photometer (AME model, Thermo Electron, USA) at wavelengths of 589 nm (Na) and 766.5 nm (K) according to Wahing *et al*. (1989). Electrolyte leakage was determined following Lutts *et al*. (1996). Leaf discs were incubated in distilled water for 24 h at room temperature, and initial EC was recorded. After boiling the samples for 30 min and cooling, the final EC was measured.

Statistical analysis: Statistical analysis was performed using SAS software (version 9.4). Treatment means were compared using the LSD test at a 5% probability level (P< 0.05).

Results

New shoot length: The effects of salinity and melatonin on new shoot length were significant (P< 0.01). The greatest shoot length (7.33 cm) was recorded in plants treated with 250 µM melatonin under non-saline conditions, representing an increase of about 29.5% compared with the corresponding control (5.66 cm). The lowest stem length (1.66 cm) occurred in the untreated control exposed to 180 mM NaCl. Moreover, under 120 mM salinity, the application of 150 µM melatonin increased new shoot length to 4.66 cm, which was 40% higher than that of the corresponding control (3.33 cm) (Table 1).

Number of new leaves: The number of new leaves was also significantly affected by the treatments (P< 0.01). The maximum number of leaves (46.66) was observed in plants treated with 250 µM melatonin under non-saline conditions, which was about 40% higher than the corresponding control (33.33). The minimum value was recorded in the untreated control under 180 mM salinity. At all salinity levels, the application of 150 µM melatonin resulted in an increase in leaf number compared with the respective controls (Table 1). A representative image of leaf morphology under salinity stress and melatonin treatments is illustrated in Figure 1.

Plant biomass (shoot and root fresh and dry weight): Salinity and melatonin treatments significantly affected both shoot and root biomass. For shoot fresh weight (P< 0.01), the highest value (22.62 g) was obtained with 150 µM melatonin under non-saline conditions, which was 16.9% higher than the corresponding control (19.35 g). The lowest shoot fresh weight was found in the untreated control under 180 mM NaCl. Under 120 and 180 mM salinity, the application of 150 µM melatonin increased shoot fresh weight by 37% and 33%, respectively, compared with the corresponding controls.

Shoot dry weight was also significantly influenced (P< 0.01). The maximum value (13.67 g) was observed with 150 µM melatonin under non-saline conditions, which represented a 26.7% increase compared with the control (10.79 g). The lowest shoot dry weight (6.47 g) occurred in the untreated control at 180 mM NaCl. At 60 mM salinity, application of 150 µM melatonin enhanced shoot dry weight by 16.5% compared with the respective control.

Similarly, root fresh weight was significantly affected (P< 0.01). The greatest value (17.90 g) was obtained with 250 µM melatonin under non-saline conditions, representing a 20.6% increase over the control (14.84 g). The lowest value was recorded in the untreated control under 180 mM NaCl. At 120 and 180 mM salinity, 150 µM melatonin increased root fresh weight by 17% and 37%, respectively, compared to the controls.

Root dry weight was also significantly influenced (P< 0.01). The highest value (8.38 g) was recorded in plants treated with 250 µM melatonin under non-saline conditions, which was 20.7% higher than the corresponding control (6.94 g). In contrast, the lowest root dry weight (3.12 g) occurred in the untreated control under 180 mM NaCl. At moderate salinity levels (60 and 120 mM), the application of 150 µM melatonin increased root dry weight by 15.6% and 32.5%, respectively, compared with the corresponding controls. Overall, melatonin application partially alleviated the negative effects of salinity on root biomass (Table 1).

The shoot-to-root (S:R) ratio was calculated based on mean shoot and root dry weight values (Table 1). Salinity generally increased the S:R ratio, indicating a reduction in root biomass relative to shoots. Application of melatonin (150 and 250 µM) under high salinity (180 mM NaCl) partially mitigated this increase, whereas at lower salinity levels (60 and 120 mM) no notable reduction was observed.

Carotenoids: The effects of salinity and melatonin on carotenoid content were significant (P< 0.01). The highest carotenoid concentration (1.04 mg g^{-1} FW) was observed in plants treated with 250 µM melatonin under non-saline conditions, representing a 57.6% increase compared with the respective control (0.66 mg g^{-1} FW). The lowest carotenoid content was recorded in the untreated control under 180 mM salinity. At all salinity levels, application of 150 and 250 µM melatonin increased carotenoid content relative to the corresponding controls (Table 2).

Chlorophyll *a*: Chlorophyll *a* content was significantly affected by the treatments (P< 0.01). The maximum level was obtained in plants treated with 250 µM melatonin under non-saline conditions, showing a 23.8% increase compared with the corresponding control. The minimum value was recorded in the untreated control under 180 mM salinity. Overall, melatonin application contributed to maintaining chlorophyll *a* levels under all salinity conditions compared with the untreated controls (Table 2).

Relative water content: Leaf RWC was also significantly influenced by salinity and melatonin (P< 0.01). The highest RWC was found in plants treated with 150 µM melatonin under 60 mM salinity, although this treatment was not significantly different from those receiving 250 µM melatonin under 60 mM salinity or

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Table 1. Interactive effects of salinity and melatonin on growth traits and biomass components of *Rosa damascena*

NaCl (mM)	Melatonin (µM)	New shoot length (cm)	Number of new leaves	shoot FW (g)	Shoot DW (g)	Root FW (g)	Root DW (g)	S DW:R DW ration
0	0	5.66 ^{c}	33.33 ^{bc}	19.35 ^{c}	10.79 ^{c}	14.84 ^{c}	6.94 ^{c}	1.55^{g}
	150	6.66 ^{ab}	38.33 ^{b}	22.62 ^{a}	13.67 ^{a}	16.77 ^{b}	7.87 ^{b}	1.74^{d}
	250	7.33 ^{a}	46.66 ^{a}	21.68 ^{b}	12.49 ^{b}	17.90 ^{a}	8.38 ^{a}	1.49^{h}
60	0	4.66 ^{d}	28.33 ^{cd}	16.20 ^{e}	9.20 ^{f}	13.22 ^{ef}	5.62 ^{ef}	1.64^{f}
	150	6.00 ^{bc}	33.33 ^{bc}	17.53 ^{d}	10.57 ^{cd}	14.39 ^{cd}	6.50 ^{cd}	1.63^{f}
	250	4.66 ^{d}	26.66 ^{d}	17.07 ^{d}	10.11 ^{de}	13.96 ^{de}	6.04 ^{de}	1.67^{e}
120	0	3.33 ^{e}	16.66 ^{ef}	11.55 ^{i}	6.86 ^{h}	10.71 ^{h}	3.94 ^{h}	1.74^{d}
	150	4.66 ^{d}	28.33 ^{cd}	15.83 ^{ef}	9.55 ^{ef}	12.54 ^{fg}	5.22 ^{fg}	1.83^{c}
	250	3.23 ^{e}	16.66 ^{ef}	15.21 ^{f}	9.26 ^{f}	12.10 ^{g}	4.87 ^{g}	1.90^{b}
180	0	1.66 ^{f}	8.33 ^{g}	10.33 ^{j}	6.47 ^{h}	8.08 ^{j}	3.12 ^{i}	2.07^{a}
	150	3.66 ^{e}	18.33 ^{e}	13.48 ^{g}	7.81 ^{g}	9.80 ^{i}	4.28 ^{h}	1.83^{c}
	250	2.33 ^{f}	11.66 ^{fg}	12.38 ^{h}	7.53 ^{g}	9.10 ^{i}	3.92 ^{h}	1.92^{b}
Significance	**	**	**	**	**	**	**	**
C.V. (%)		12.28	12.62	2.97	4.12	3.47	5.11	4.52

Note: Values followed by different letters within each column are significantly different at P< 0.05 according to the LSD test. Significance levels: P< 0.05 (*), P< 0.01 (**). Abbreviations: FW: fresh weight; DW: dry weight; S:R ration: Shoot: Root ration.

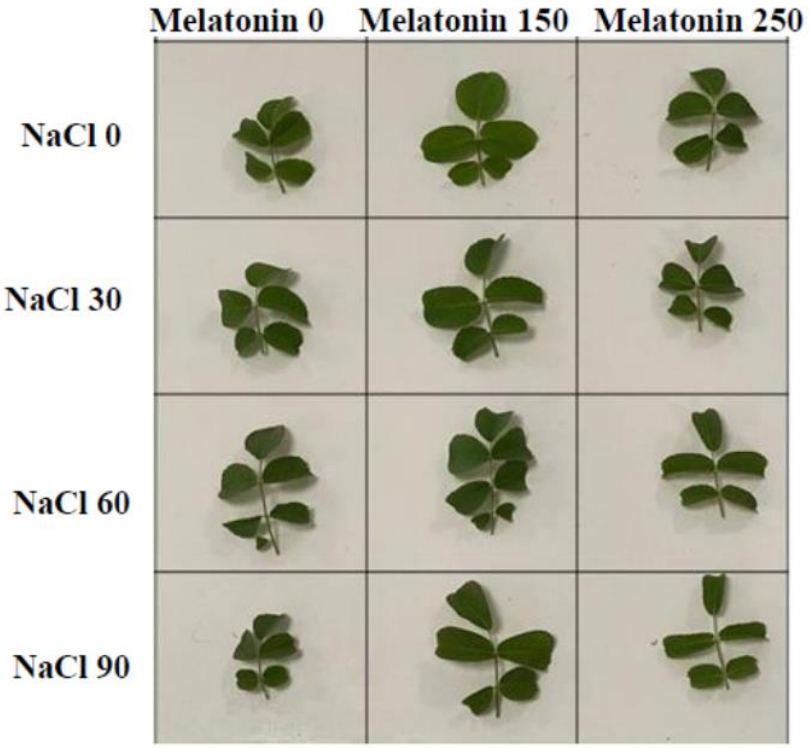


Figure 1. The effects of salinity (NaCl) and melatonin on leaf morphology in *Rosa damascena*

the control under the same level. In contrast, the lowest RWC (49.07%) was recorded in the untreated control under 180 mM NaCl. Under non-saline conditions, the control plants (0 µM melatonin) showed an RWC of 76.42%, whereas increasing salinity to 120 and 180 mM reduced RWC to 50.45% and 49.07%, respectively. These results indicate that melatonin partially mitigated the decline in RWC caused by salinity stress (Table 2).

Proline content: Leaf proline content was significantly affected by the treatments (P< 0.01). The maximum concentration (8.57 µmol g^{-1} FW) was obtained in plants treated with 250 µM melatonin under 120 mM salinity. The lowest proline level (3.10 µmol g^{-1} FW) was recorded in the control plants under non-saline conditions without melatonin. Moreover, under 180 mM salinity, the control (0 µM melatonin) showed a proline concentration of 5.55 µmol g^{-1} FW, which was 79% higher than that of the non-saline control (Table 2).

Ion leakage: Salinity and melatonin also had a significant effect on ion leakage percentage (P< 0.05). The highest ion leakage (82.19%) occurred in the untreated control under 180 mM NaCl, whereas the lowest value (65.64%) was found in plants treated with 150 µM melatonin under non-saline conditions. At all salinity levels, melatonin application reduced ion leakage compared with the corresponding controls

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Table 2. Effects of salinity and melatonin on growth and biomass of *Rosa damascena*

NaCl (mM)	Melatonin (µM)	Carotenoids (mg/gFW)	Chlorophyll *a* (mg/gFW)	RWC (%)	Proline (µmol/gFW)	Ion Leakage (%)
0	0	0.66 ^{ef}	1.3^{bc*}	76.42 ^{ab}	3.10 ^{f}	72.20 ^{de}
	150	0.71 ^{e}	1.37^{b}	74.13 ^{bc}	3.32 ^{ef}	65.64 ^{f}
	250	1.04 ^{a}	1.61^{a}	73.59 ^{bc}	3.61 ^{def}	65.73 ^{f}
60	0	0.63 ^{fg}	1.09^{def}	71.87 ^{c}	4.08 ^{cde}	74.55 ^{bcd}
	150	0.92 ^{b}	1.14^{de}	79.46 ^{a}	3.82 ^{def}	71.23 ^{e}
	250	0.86 ^{bcd}	1.19^{cd}	78.82 ^{a}	4.74 ^{c}	67.28 ^{f}
120	0	0.64 ^{fg}	0.98^{fg}	50.45 ^{f}	4.37 ^{cd}	76.06 ^{bc}
	150	0.84 ^{cd}	1.006^{efg}	62.09 ^{e}	3.87 ^{def}	72.98 ^{de}
	250	0.82 ^{cd}	1.04^{defg}	66.97 ^{d}	8.57 ^{a}	73.43 ^{cde}
180	0	0.58 ^{g}	0.59^{h}	49.07 ^{f}	5.55 ^{b}	82.19 ^{a}
	150	0.80 ^{d}	0.93^{g}	71.76 ^{c}	3.82 ^{def}	76.82 ^{b}
	250	0.87 ^{bc}	0.95^{fg}	73.83 ^{bc}	4.24 ^{cd}	73.80 ^{cde}
Significance		**	**	**	**	*
C.V. (%)		5.02	12.62	3.36	10.62	2.24

Note: Values followed by different letters within each column are significantly different at P< 0.05 according to the LSD test. Significance levels: P< 0.05 (*), P< 0.01 (**). Abbreviations: RWC: Relative Water Content.

Table 3. Effects of salinity and melatonin on lipid peroxidation, antioxidant enzyme activities, and mineral nutrients in *Rosa damascena*

NaCl (mM)	Melatonin (µM)	MDA (nmol/g FW)	SOD (U/g FW min)	CAT (U/g FW min)	Sodium (mg/g DW)	Potassium (mg/g DW)
0	0	3.47^{d}	14.96^{fg}	0.16^{f}	0.27 ^{de}	1.165 ^{d}
	150	3.17^{de}	16.30^{ef}	1.51^{de}	0.21 ^{g}	1.171 ^{cd}
	250	2.94^{e}	17.95^{de}	2.05^{d}	0.25 ^{f}	1.173 ^{c}
60	0	4.19^{c}	16.12^{ef}	0.30^{f}	0.28 ^{d}	1.122 ^{e}
	150	3.06^{de}	21.81^{c}	3.35^{c}	0.17 ^{h}	1.231 ^{b}
	250	2.88^{e}	33.66^{a}	6.55^{a}	0.28 ^{d}	1.256 ^{a}
120	0	4.73^{b}	18.62^{de}	0.82^{ef}	0.33 ^{b}	1.107 ^{f}
	150	3.54^{d}	29.91^{b}	2.98^{c}	0.21 ^{g}	1.094 ^{h}
	250	3.11^{de}	33.67^{a}	5.00^{b}	0.26 ^{ef}	1.100 ^{gh}
180	0	6.88^{a}	12.32^{g}	0.13^{f}	0.36 ^{a}	1.105 ^{fg}
	150	4.76^{b}	17.42^{def}	0.63^{ef}	0.30 ^{c}	1.106 ^{fg}
	250	4.08^{c}	20.14^{cd}	0.96^{ef}	0.20 ^{g}	1.116 ^{e}
Significance	**	**	**	**	**	*
C.V. (%)		7.62	7.76	25.77	2.65	0.32

Note: Values followed by different letters within each column are significantly different at P< 0.05 according to the LSD test. Significance levels: P< 0.05 (*), P< 0.01 (**). Abbreviations: MDA: Malondialdehyde; CAT: Catalase; SOD:Superoxide Dismutase

(Table 2).

Malondialdehyde: The MDA content in the control plants without melatonin was 3.47 nmol/g FW, which increased under salinity stress and reached the highest level at 180 mM NaCl. Application of melatonin significantly reduced MDA levels at all salinity concentrations compared with the corresponding non-melatonin treatments. At 180 mM NaCl, melatonin treatment decreased MDA by 31% compared with the respective control (Table 3).

Antioxidant enzyme activities: Superoxide dismutase and catalase activities were significantly affected by the interaction between salinity and melatonin ($P < 0.01$). The lowest SOD activity (12.32 $U \cdot g^{-1}$ $FW \cdot min^{-1}$) was observed in the untreated control under 180 mM NaCl, whereas the highest SOD activity (33.66–33.67 $U \cdot g^{-1}$ $FW \cdot min^{-1}$) was recorded at 250 µM melatonin under both 60 and 120 mMNaCl (Table 3). Compared with the respective controls at each salinity level, SOD activity at 250 µM increased by 2.09-fold at 60 mM (33.66 vs. 16.12 $U \cdot g^{-1}$) and by ~1.81-fold at 120 mM (33.67 vs. 18.62 $U \cdot g^{-1}$). Catalase activity was very low in the non-saline control (0.16 $U \cdot g^{-1}$ $FW \cdot min^{-1}$), but rose sharply when melatonin was applied under salinity. The maximum CAT activity (6.55 $U \cdot g^{-1}$ $FW \cdot min^{-1}$) was observed at 250 µM melatonin with 60 mMNaCl, corresponding to an increase of 6.55 / 0.16 (≈41-fold) relative to the non-saline control (0 µM melatonin). These data indicate that melatonin substantially enhanced antioxidant defenses under salinity stress (Table 3).

Mineral nutrients: In the absence of salinity, sodium (Na) concentration was low (0.27 mg/g DW), but it significantly increased with rising salinity, reaching 0.36 mg/g DW at 180 mM NaCl in the control plants. Application of melatonin, especially at 150 µM under 60 mM NaCl, markedly reduced Na accumulation to 0.17 mg/g DW, which was the lowest among treatments. potassium (K) concentration in control plants without salinity was 1.165 mg/g DW and

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decreased with increasing salinity. However, the highest K concentration (1.256 mg/g DW) was recorded with 250 μM melatonin under 60 mM NaCl, representing a 7.8% increase compared with the non-saline control.

Discussion

Salt stress is one of the most critical environmental constraints affecting plant growth and productivity, as it severely reduces growth and development by disrupting water uptake, disturbing ionic balance, and altering metabolic processes (Garcia-Caparros and Lao, 2018; Younessi-Hamzekhanlu *et al*., 2021). Stomatal closure under saline conditions limits gas exchange and photosynthesis, thereby restricting root growth and nutrient absorption capacity. Additionally, salt accumulation in tissues leads to increased respiration and decreased photosynthetic efficiency, which negatively impacts overall plant performance (Boorboori, 2023; Hawrylak-Nowak *et al*., 2019).

In the present study, exogenous melatonin application mitigated some of the detrimental effects of salinity. The observed enhancement in vegetative growth, including stem elongation, leaf number, and biomass, indicates that melatonin modulates salinity stress through multiple mechanisms, such as improving water status, enhancing the antioxidant defense system, and promoting photosynthesis. Similar protective effects of melatonin under salt stress have been reported in other species, such as rosemary (Mohammadi and Karimi, 2020) and cotton (Zhang *et al*., 2021), demonstrating its role in maintaining plant growth under saline conditions. Arnao and Hernandez-Ruiz (2015) also reported that melatonin application enhances plant height, leaf number, and root growth, which aligns with the findings of this study.

Reduction in both aboveground and belowground biomass is a major consequence of salinity stress, primarily resulting from decreased photosynthetic activity and limited nutrient uptake (Farsaraei *et al*., 2020). In the present study, salinity significantly reduced fresh and dry weights of plant organs; however, melatonin treatment largely mitigated this reduction. The observed increase in biomass in the presence of melatonin can be attributed to improved photosynthetic efficiency, maintenance of photosynthetic pigments, and protection of chloroplast membranes. Previous studies have similarly reported that melatonin plays a crucial role in enhancing growth and biomass accumulation by preventing chlorophyll oxidation and improving energy transfer within the photosynthetic apparatus (Li *et al*., 2019; Arnao and Hernandez-Ruiz, 2015). The observed increase in the shoot-to-root (S:R) ratio under salinity indicates that root growth is more severely affected than shoot growth, which is a common response to osmotic and ionic stress. This shift in biomass allocation can compromise water and nutrient uptake due to reduced root size. The partial mitigation of this effect by melatonin at high salinity (180 mM NaCl) suggests that melatonin supports root growth under stress, promoting a more balanced allocation between shoots and roots. Such an effect may enhance the plant's ability to cope with osmotic and ionic stress by maintaining water and nutrient uptake capacity. Similar protective effects of melatonin on root development under salinity have been reported in other plant species, where it alleviated oxidative stress and enhanced antioxidant enzyme activities, contributing to improved biomass allocation (Arnao and Hernandez-Ruiz, 2015).

From a physiological perspective, the improvement in growth and biomass in melatonin-treated plants is likely related to the ability of this molecule to enhance water and nutrient uptake in roots, as well as protect cells against oxidative damage. By reducing reactive oxygen species (ROS) and lipid peroxidation, melatonin stabilizes cellular membranes and prevents ion leakage (Cui *et al*., 2017; Zulfiqar *et al*., 2024 a). Consequently, plants receiving melatonin exhibited better growth and higher biomass production across varying salinity levels.

Salinity stress typically causes a marked reduction in chlorophyll and carotenoid contents in plants, which can be attributed to chloroplast degradation, lipid peroxidation, disruption of ionic balance, and the accumulation of reactive oxygen species (Sharma *et al*., 2019). In the present study, chlorophyll *a* and carotenoid contents decreased under salinity stress, reaching their lowest levels in the control plants without melatonin, consistent with previous reports highlighting the sensitivity of photosynthetic pigments to salt stress.

However, melatonin treatment effectively mitigated the adverse effects of salinity on these pigments. Application of melatonin across all salinity levels helped maintain or even increase chlorophyll a and carotenoid contents relative to the untreated controls. Under non-stress conditions, pigment levels in melatonin-treated plants were higher than those in the control. These findings align with previous studies; for instance, Yan *et al*. (2023) reported in wheat that melatonin upregulates genes involved in chlorophyll and carotenoid biosynthesis, thereby protecting photosynthetic structures from degradation. Similarly, research on cotton demonstrated that melatonin reduces oxidative damage, preserves chloroplast membrane integrity, and maintains photosynthetic performance (Zhang *et al*., 2021).

One of the potential mechanisms underlying the protective effect of melatonin on photosynthetic pigments is the inhibition of ROS generation and the reduction of lipid peroxidation, which prevents chloroplast membrane degradation. Other studies have shown that melatonin enhances the activity of antioxidant enzymes, reduces ion leakage, and improves membrane stability (Ke *et al*., 2018). By mitigating oxidative damage, melatonin helps maintain the internal structure of chloroplasts.

Additionally, melatonin may facilitate the uptake and distribution of essential mineral elements required for chlorophyll and carotenoid biosynthesis, such as

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magnesium and iron. Several studies have demonstrated that under stress conditions, melatonin helps maintain ionic balance and reduces sodium accumulation, which can support the availability of critical components for pigment synthesis (Zhang *et al*., 2021).

Salinity stress disrupts cellular membrane stability and increases its permeability, leading to enhanced ion leakage and a reduction in relative water content. These effects serve as critical physiological indicators for assessing stress severity, with decreased RWC reflecting a diminished capacity of the plant to maintain water balance and osmotic adjustment (Farouk *et al*., 2020; EL-Bauome *et al*., 2024). Our findings indicated that under the highest salinity level (180 mM NaCl), control plants without melatonin exhibited the lowest RWC (49.07%) and the highest ion leakage (82.19%), suggesting severe membrane damage and impaired cellular water status.

The application of melatonin significantly mitigated the negative effects of salinity. In melatonin-treated plants, RWC increased while ion leakage decreased compared to the control, indicating improved membrane integrity and enhanced cellular water retention. The likely mechanisms underlying this protective effect include the antioxidant activity of melatonin and its ability to reduce ROS production, thereby preventing lipid peroxidation and membrane degradation (EL-Bauome *et al*., 2024). Furthermore, melatonin enhances root performance and increases root membrane permeability, promoting water and nutrient uptake and improving cellular hydration. Similar observations in *Ranunculus asiaticus* L. demonstrated that exogenous melatonin application increases leaf RWC and enhances growth parameters under salinity stress (Abdelhakim Eisa *et al*., 2023).

Salinity-induced osmotic imbalance and ROS overproduction also lead to lipid peroxidation and membrane damage, as reflected by elevated malondialdehyde levels. This process reduces photosynthetic capacity, increases ion leakage, and lowers leaf RWC, thereby limiting the plant's ability to maintain hydration and nutrient acquisition (Farouk *et al*., 2020; EL-Bauome *et al*., 2024). In the present study, MDA levels significantly increased with rising salinity, reaching a maximum under 180 mM NaCl, indicating severe oxidative stress.

The application of melatonin, as a natural signaling molecule and antioxidant, effectively mitigated the adverse effects of salinity stress. Melatonin prevents membrane degradation and maintains cellular membrane stability by inhibiting ROS production and lipid peroxidation. Furthermore, the activities of key antioxidant enzymes, such as superoxide dismutase and catalase, were enhanced by melatonin, improving the plant's capacity to cope with oxidative stress and protecting cells from damage (Nawaz *et al*., 2020). In the present study, the highest CAT and SOD activities were observed in melatonin-treated plants under salinity stress, confirming the protective role of this hormone.

Another crucial mechanism for salinity tolerance is the accumulation of osmolytes, particularly proline. Proline maintains cellular osmotic potential, preserves water content, and protects proteins and membranes, thereby preventing photosynthetic inhibition caused by excessive stomatal closure (Hardeland, 2016). In this study, melatonin significantly increased proline content, highlighting its role in improving cellular water status and enhancing plant tolerance to environmental stress (Chen *et al*., 2018b; Zulfiqar *et al*., 2024b). Notably, the highest proline accumulation was observed under 120 mM NaCl with 250 µM melatonin, rather than at the highest salinity level. This non-linear response suggests that melatonin-mediated osmotic adjustment operates optimally within a moderate stress range. At this salinity level, melatonin not only enhanced proline synthesis but also significantly increased the activities of key antioxidant enzymes, catalase (CAT) and superoxide dismutase (SOD), thereby providing coordinated protection against oxidative stress. Under more severe salinity (180 mM NaCl), the accumulation of proline and enzyme activities did not further increase, indicating that melatonin may shift its protective strategy toward other mechanisms, such as stabilization of membranes, enhancement of non-enzymatic antioxidants, or regulation of hormonal signaling, to maintain cellular homeostasis (Chen *et al*., 2018b; Zulfiqar *et al*., 2024b). This integrated regulation of osmolytes and antioxidants allows plants to efficiently cope with varying intensities of salt stress.

Salinity stress typically increases sodium accumulation and reduces potassium uptake in plant tissues, disrupting ionic balance and metabolic processes, ultimately reducing growth and productivity (Deinlein *et al*., 2014; Chen *et al*., 2018a). High intracellular Na^+, especially under elevated NaCl concentrations, promotes K^+ efflux and decreases the cytosolic K^+/Na^+ ratio (Himabindu *et al*., 2016). Since potassium is critical for turgor regulation, electrical balance, and enzymatic activities, its depletion can exacerbate Na^+ toxicity and impair physiological functions (Benito *et al*., 2014)

Melatonin plays a protective role under salinity by reducing Na^+ accumulation and enhancing K^+ retention in leaves. In this study, melatonin application significantly decreased Na^+ levels while increasing K^+ content. Interestingly, 150 µM melatonin under 60 mM NaCl resulted in the lowest Na^+ concentration observed, suggesting a particularly strong role of melatonin in preventing Na^+ uptake under moderate salinity conditions. This effect may result from improved root function, increased ion selectivity, and regulation of ion transporters such as H^+-ATPase and Na^+/H^+antiporters, which have been previously reported to maintain Na^+/K^+ homeostasis under salinity stress (Zhang *et al*., 2021).

From a practical perspective, this finding highlights the potential application of melatonin in irrigation management. In areas where water salinity is moderate,

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foliar or root application of melatonin at optimal concentrations could reduce sodium accumulation in plant tissues, maintain favorable K^+/Na^+ ratios, and thus sustain plant growth and productivity without requiring drastic reductions in water salinity. By mitigating ionic stress, melatonin helps preserve cellular water balance, turgor pressure, and photosynthetic activity, ultimately enhancing plant resilience and yield under suboptimal conditions.

Conclusion

The results of this study indicate that melatonin can effectively mitigate the adverse effects of salinity in *Rosa damascena*. Salinity reduced shoot and root growth, chlorophyll and carotenoid contents, and relative water content, while increasing ion leakage, proline, MDA, and sodium accumulation. Application of melatonin at the tested concentrations (150 and 250 μM) alleviated these negative effects and enhanced antioxidant enzyme activities and ionic balance. The effects of melatonin were dependent on both salinity level and trait type: under low to moderate salinity, 250 μM generally showed the greatest positive effects on growth, chlorophyll, and antioxidant enzyme activities. Under severe salinity, 150 μM was more effective in reducing ion leakage, MDA, and sodium accumulation, whereas 250 μM maintained higher activities of antioxidant enzymes and certain pigments. These results suggest that melatonin application should consider the stress intensity and target traits, and further studies are needed to optimize its concentration and timing under different salinity conditions.

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