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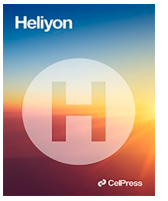

Research article

Melatonin induces proline, secondary metabolites, sugars and antioxidants activity to regulate oxidative stress and ROS scavenging in salt stressed sword lily

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ABSTRACT

Sword lily is regarded as a useful and commercially demanding cut flower crop; hence, assessing its responses to abiotic stress, particularly salt stress, is vital. Melatonin (MT) exhibits stress tolerance in crop plants and is an emerging stress relieving alternative to chemicals. Nevertheless, the possible process underlying the effects of MT under salt stress has yet to be fully elucidated in plants. Herein, the salt stress (SS) mitigation potential of MT was assessed in a commercially important cut flower, sword lily. Melatonin, expressed as MT1, MT2, MT3, and MT4, was administered at concentrations of 0.2, 0.4, 0.6, and 0.8 mM. The results revealed that SS (5 dS m^{-1}) restricted the growth and physiological aspects of sword lily. Furthermore, malondialdehyde (MDA), hydrogen peroxide (H_2O_2), membrane permeability, endogenous proline, and soluble protein contents were enhanced in SS. MT application improved morphological traits, photosynthetic pigments, and corm traits. The application of MT mitigated the effects of SS stress in *Gladiolus grandiflorus* plants by improving growth and photosynthetic pigments. MT application under SS improved the reducing and non-reducing sugar and NPK contents of the sword lily. Furthermore, MT improved the levels of secondary metabolites, such as anthocyanins, flavonoids, and ascorbic acid, in sword lily. Moreover, MT supplementation ameliorated salt-induced oxidative stress in the gladiolus, as depicted by a decrease in stress markers (EL, MDA, and H_2O_2) and an increase in defense-related enzymes (POD, CAT, and SOD) with highest increase in the MT3 treatment under salinity stress. The SOD and CAT enzyme activities were 3–3.6-fold higher in the MT3 under stress than the control. In conclusion, MT applications on cut flowers can be an effective strategy to reduce salt stress and can be used to regulate salinity stress in cut flower production. MT can be used as a safe alternative to other agrochemicals to maintain the growth and flower quality of sword lilies, with beneficial effects during vase life.

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1. Introduction

The floriculture sector plays a vital role in people's aesthetics and social well-being worldwide. Salt stress is lethal to ornamental plant production industries, particularly in arid and semiarid regions worldwide [1]. Salinity stress can compromise the ornamental value of cut flowers by reducing the growth and the size of flowers and leaves. The negative effects of the salinity depend on the duration of exposure and intensity [2]. The severity of SS and land area are increasing continuously due to climate change and are a limitation in cultivation areas along the seaside for salt infiltration in underground water and/or marine aerosols [2]. Ornamental plants, especially cut flowers, play a significant role in horticulture; however, their survival under SS conditions is limited [3]. Salt stress promotes ion imbalance and osmotic stress in plants, as well as salt-induced oxidative stress as a secondary stress that can compromise the aesthetic quality of cut flowers. Ion imbalance is due to the different rate of uptake of mineral elements. Salt stress has been found to increase the proline concentration in response to salinity exposure [4]. This non-protein amino acid has antioxidant and osmoregulation function under salinity and water stress.

Gladiolus, a monocotyledonous geophyte of the Iridaceae family, is a prominent cut flower crop grown worldwide for ornamental purposes [5]. Its gorgeous spikes, which come in a variety of attractive colours, are used for crafting bouquets and flower arrangements for interior decoration. It is regarded as a profitable crop for marginal- and small-scale farmers [5]. Sword lily contributes significantly to floriculture; however, salt-resistant sword lily is rare [6,7].

Plants reduce the Reactive Oxygen Species (ROS) accumulation by activating the detoxification enzymes [8]. These enzymes scavenge increased ROS levels in plants and provide a shield against biotic and abiotic stresses, inciting excessive ROS under oxidative stress conditions [9]. There is great interest in the use of ecologically acceptable and low-cost additives to tackle abiotic stresses that cause oxidative stress. SS induces the accumulation of ROS that can be measured through the intensity of damage to the cell membrane [1]. The main target of ROS is the phospholipid double layer of membranes.

Phytohormones are involved in regulating stress-induced processes in plants, mainly ethylene and abscisic acid (ABA). However, recently more attention has been focused on melatonin as plant hormone. Melatonin performs various functions in plants under harsh conditions such as salt stress [10,11]. Plants with high concentrations of MT have better tolerance to abiotic stresses. Treatments that upregulate genes involved in MT biosynthesis enhance tolerance to environmental stresses [12]. MT treatments have been extensively studied in several ornamental and horticultural crops, including *Gerbera jamosonii* [13], *Vicia faba* [14], sword lily [15], zinnia [16], *Chrysanthemum morifolium* [17], and other unfavourable environmental stresses [18].

Melatonin treatments (0.1 or 0.2 mM) mitigated the adverse effects of SS in ornamental Gerbera plants by inducing physiological and biochemical regulation. Treated plants increased catalase (CAT, E.C., 1.11.1.6), peroxidase (POD, E.C., 1.11.1.1), and superoxide dismutase (SOD, E.C., 1.15.1.1) in a dose-dependent manner [13 Zulfiqar et al., 2023a]. Eisa et al. [19] noted an increased SS tolerance in ornamental *Ranunculus asiaticus* L. plants in response to MT application which stimulated the proline and non-enzymatic and enzymatic antioxidants resulting in decrease in excessive release of SS induced ROS. Positive effects have been demonstrated in the application of MT for seed priming before SS in zinnia [16]. Melatonin has been studied as a stress modulator for several pollutants, such as heavy metals and industrial pollutants. In sward lily and tuberose (*Polianthes tuberosa* L.), it has been reported that MT treatment was able to mitigate, alone (tuberose) or in combination with salicylic acid (sward lily), the tolerance to arsenic stress [20].

Studies have shown that MT can be used to prevent SS damage in crop plants [21]; however, the role of preharvest MT application has rarely been explored in ornamental plants, especially cut flowers, such as sword lily.

To the best of our knowledge, little information is available regarding the effects of MT on SS-induced oxidative stress mitigation in *G. grandiforus*. The rationale of this study was to investigate if MT could mitigate the SS in the popular ornamental cut flower gladiolus. In particular, this study was focused on the effect of MT concentration on the most affect morpho-physiological traits affected by salinity such as growth, secondary metabolites, ornamental traits, ROS accumulation, and antioxidant defense systems of sword lily exposed to SS. The use of melatonin in gladiolus production is aimed at maintaining freshness, enhancing storage, and extending the vase life of the cut flowers, making them more appealing and satisfying consumer's expectation. Moreover, results from study can provide useful knowledge for the development of innovative agronomic strategies to increase the cultivation of bulbous decorative flowers in salinised areas.

2. Materials and methods

2.1. Experimental settings

Gladiolus grandiflorus cv. White Prosperity corms (uniform-sized) imported from the Netherlands were purchased from the importing firm Sunny Seeds Lahore, Pakistan. The corms were dipped in 1 % sodium hypochlorite NaClO (sodium hypochlorite) and then air dried. The uniform corms were planted in pots under natural growth conditions. Clay pots (25 cm height, 20 cm inner diameter, with a hole in the base) were filled with sandy loam soil. The physiochemical characteristics of this growing medium were sand, silt, clay, pH, and electrical conductivity [EC] of 46 %, 23 %, and 31 %, 7.3, and 2.79 dSm^{-1} respectively. Soil nutrients including nitrogen, phosphorous, and potassium were 75 g kg^{-1}, 7.11 g kg^{-1} and 145.37 g kg^{-1} soil, respectively.

After eight weeks, vigorous and uniform sword lily plants were chosen for a completely randomised trial with eight replications per treatment and four clay pots (each pot comprising one plant) per replicate. The photoperiod interval was approximately 8/16 h every day at a 22 °C/26 °C night/day average temperature, 350 μmol m^{-2} s^{-1} photon flux density of photosynthesis, and 75 % relative humidity. Each pot was irrigated at 3 d intervals with 120 mL Hoagland nutrient solution (pH 6–6.5; EC1.5 dSm^{-1}) for 40 d. Each pot was watered (120 mL) at 3 d intervals throughout the experiment.

MT was obtained from Sigma-Merck Company. The MT solute was initially dissolved in 100 % ethanol to create the MT stock solution, which was then kept at −20 °C. The required MT concentrations (0.2, 0.4, 0.6, and 0.8 mM) were obtained by diluting the stock solution to yield a combination with an ethanol-to-water ratio of 1/10,000 (v/v). NaCl was also purchased from Sigma-Aldrich, and the stock solution was prepared by dissolving the required amount of NaCl in a volumetric container and topping it with double-distilled water (DDW). The requisite NaCl concentration (5 dS m^{-1}) was obtained by diluting the stock solution. Tween-20 (0.5 mL), a surfactant, was also incorporated into the solution before foliar application of MT.

Plants were subjected to SS in the form of sodium chloride (NaCl; 5 dSm^{-1}) applied through the soil 40 days following corm sprouting (DCS), with the NaCl concentration maintained in the growing media for 20 days. For three consecutive days, foliar applications of DDW and MT (0.2, 0.4, 0.6, and 0.8 mM) were sprayed at 8:00 p.m. on both the upper and lower surfaces of the leaves at 60, 70, and 80 DCS. Each sword lily plant was sprayed with 10–20 mL of the MT solution. To prevent MT from dripping into the growing medium during the spray treatment, the top layer of the pot was covered with aluminium foil, which was removed once the spray was completed. Melatonin levels were considered during preliminary experiments based on the morphological performance of sword lily plants.

The experimental trial included four treatments:

- Control (CK): Ethanol and distilled water mixture (in a ratio of 1:10,000) application along with Tween-20 without MT and SS
- Salt stress (SS): 5 dS m^{-1} NaCl with Tween-20 without MT
- MT1: 0.2 mM MT along with 5 dS m^{-1} NaCl with Tween-20
- MT2: 0.4 mM MT along with 5 dS m^{-1} NaCl with Tween-20
- MT3: 0.6 mM MT along with 5 dS m^{-1} NaCl with Tween-20
- MT4: 0.8 mM MT along with 5 dS m^{-1} NaCl with Tween-20

Sampling was performed on the fully developed leaves in the upper half of each plant. The samples were frozen in liquid nitrogen and then stored at −80 °C.

2.2. Plant growth related measurements

The height of each gladiolus from the base to the top was measured using a centimeter scale. The total number of leaves was counted. Leaf area (cm^2) was measured using a LICOR-3000C Portable Area Meter.

2.3. Physiological measurements

Chlorophyll and carotenoid concentrations were spectrophotometrically determined according to the methodology described by Lichtenthaler [22]. Briefly, 0.1 g small pieces of leaf samples were extracted in the dark for 24 h using 8 mL of alcohol (95 %) until blanching. The blend was shaken and centrifuged (8000×*g*). Absorbance was recorded at 649, 665, and 470 nm and expressed as mg g^{-1} fresh weight (FW).

The leaf gas exchange traits were evaluated between 10.00 a.m. and 11.30 a.m., on three fully expanded (3rd, 4th, and 5th leaves from the base), mature leaf blades from six gladiolus plants of an individual treatment, using a portable infrared gas analyser (Li-6400XT, LICOR, Lincoln, Nebraska, USA) set at a flow rate of 300 μmol m^{-2} s^{-1} and 400 μmol m^{-2} s^{-1} CO_2.

2.4. Ornamental traits

Spike length (cm) and floret number were measured and counted. Healthy sword lily spikes of cv. 'White Prosperity' were harvested. A total of hundred sword lily spikes were selected based on the study by Zulfiqar et al. [5]. The spikes were harvested at 8:00–9:00 a.m. Spikes were placed in a vessel containing DsW to dip the spike base. In the laboratory, the leaves were detached from the spike to lower the transpiration process. Each spike comprising 9–14 unopened florets, was recut (up to 60 cm) again under running tap water. Spikes were transferred to a vase containing 250 mL of the solution. The laboratory had 25 ± 1 °C temperature and 65 ± 4 % relative humidity, 15–20 μmol m^{-2} s^{-1} light intensity (12 h dark/12 h light). Vase life was defined as the day after flower harvest until 60 % of the florets were wilted [5].

2.5. Corm traits measurements

The corms were dug with a spade, washed, and dried, and their masses (g) and diameters (cm) were measured.

2.6. Leaf free proline and total soluble proteins determination

Leaf free proline content was screened following the method described by Bates et al. [23]. First, 500 mg of sword lily leaf material was cut and crushed into a uniform paste. This paste was then combined with sulfosalicylic acid (10 mL; 3 %) solution. The mixture was then centrifuged at 6, 000 rpm for 15 min. After this procedure, the prepared sample (2 mL) was transferred to a glass tube containing glacial acetic acid (2 mL) and acid ninhydrin (2 mL), and the test tube was transferred to a hot water bath for 30 min. Subsequently, the mixture (2 mL) was combined with toluene (4 mL) and centrifuged for 5 min (5000 rpm). After 30 min of precipitation, the upper layer

was removed using a separating funnel and the absorbance of the sample at 520 nm was measured using a spectrophotometre. Finally, the level of LFP was evaluated using the same absorbance values as those of other standard proline solutions. Total soluble proteins were determined using the method described by Bradford [24]. Briefly, a protein extract sample (100 μL was mixed in test tubes containing a bioreagent (5 mL), and the test tubes were blended instantly. After 5 min, a spectrophotometre was used to determine absorbance at 595 nm. Finally, total protein content was determined using a standard curve of bovine serum albumin.

2.7. Anthocyanin, total flavonoids, and ascorbic acid

Total anthocyanin content was evaluated spectrophotometrically using the pH-differential method described by Lee et al. [25] and Humadi and Istudor [26]. For flavonoid content, the methodology described by Elzaawely and Tawata [27] was employed. The ascorbic acid content was evaluated as described by Mukherjee and Choudhuri [28].

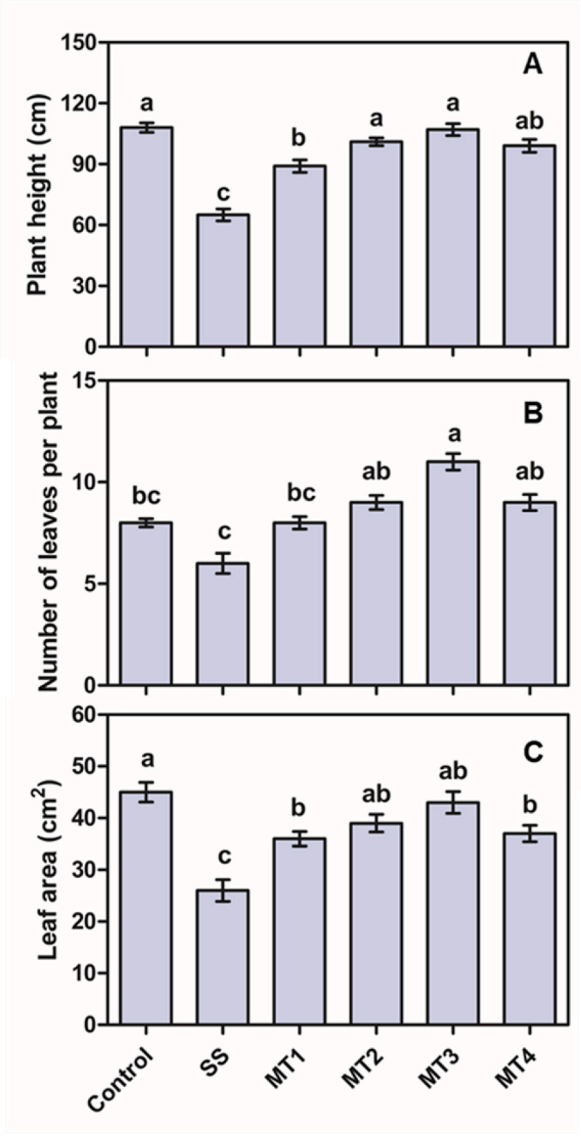

Fig. 1. Effects of exogenous applied MT (melatonin) on the plant height (**A**), number of leaves per plant (**B**), and leaf area (**C**) of ornamental sword lily plants under salt stress (SS). Different levels of MT including 0.2, 0.4, 0.6 and 0.8 mM denoted as MT1, MT2, MT3, and MT4 respectively, were tested under SS condition. Data are presented as the mean ± SE of eight replicates (n = 8). Significant differences are indicated by lowercase letters above the bars (at $P \leq 0.05$), based on the Tukey HSD test.

2.8. Reducing and non-reducing sugars

To extract sugars, the floret tissues (0.5 g) were crushed in 50 mL of boiling 80 % ethyl alcohol, filtered, and treated twice with 80 % acetone. The supernatant was then collected and evaporated in a water bath. Then, 10 mL of water was added and mixed to determine the sugars using Nelson's [29] method.

2.9. Leaf mineral contents determination

Different mineral nutrients in sword lily plants related to NPK were inspected after digesting the leaf samples. The leaf samples were oven-dried at 60 °C, ground, and prepared for analysis. Samples (approximately 0.5 g) were digested with10 mL sulfuric acid and kept overnight, after which 2 mL H_2O_2 was added. Afterwards, the samples were digested on a hot plate (300–350 °C), followed by mixing with distilled water. Filtration was performed using a Whatman filter paper. Nitrogen (N), potassium (K^+) and phosphorous (P) were measured by Kjeldahl apparatus [30], on spectrophotometer [31] and flame photometer [32].

2.10. Oxidative stress markers

The method of Premchandra et al. [33] was used to determine MSI as an electrolyte leakage (EL) or oxidative stress marker. Sword lily leaf tissue (0.2 g) was incubated for 30 min at 25 °C. The electrical conductivity (EC) of the incubated material was estimated from the EL, as read from the EC meter. After measuring the EC of the solution (C1), the sword lily leaf samples were boiled at 100 °C for 30 min before being measured again (C2). The following formula was used to quantify the MSI: The MSI was determined as follows:

MSI: C1/C2 X 100

To measure other oxidative stress markers, samples (0.5 g) from the fifth floret of the inflorescence were collected on day-4 after placing in the vase solution. Malondialdehyde (MDA) and H_2O_2 content were measured as described by Hodges et al. [34] and Patterson et al. [35], respectively.

2.11. Antioxidants enzyme activities

Fresh samples (0.5 g) were homogenised using a chilled mortar and pestle at pH 7.8, 1.0 mM EDTA, 5 mL of ice-cold 50 mM sodium

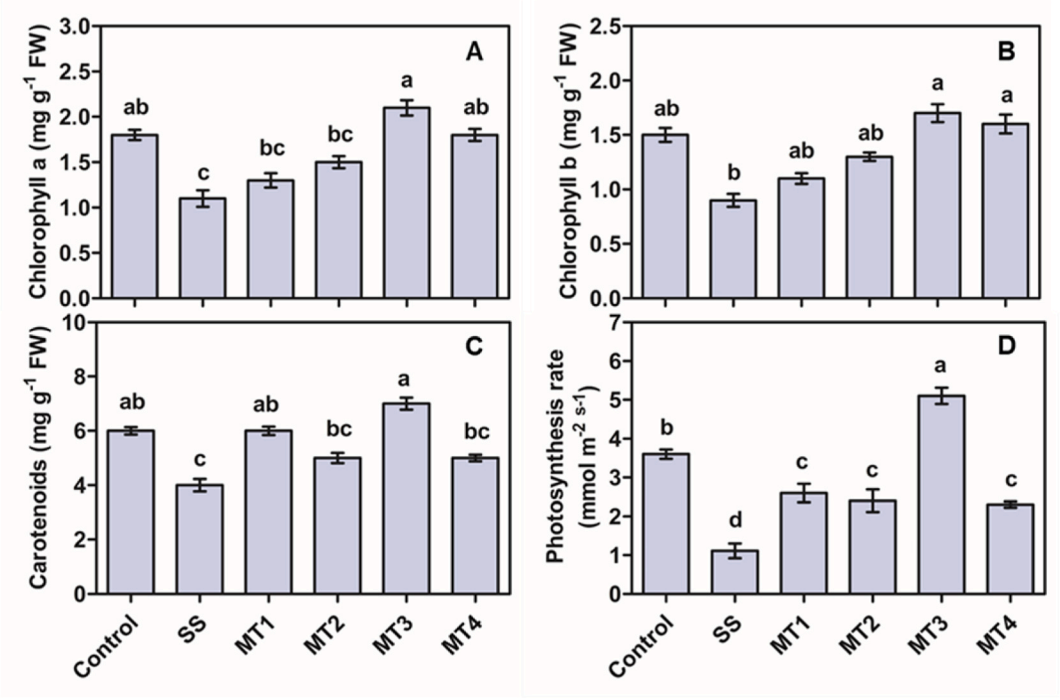

Fig. 2. Effects of exogenous applied MT (melatonin) on chlorophyll *a* (**A**), chlorophyll *b* (**B**), carotenoid content (**C**), and photosynthesis rate (**D**) in ornamental sword lily plants under SS (salt stress). Different levels of MT including 0.2, 0.4, 0.6 and 0.8 mM denoted as MT1, MT2, MT3, and MT4 respectively, were tested under SS condition. Data are presented as the mean ± SE of eight replicates (n = 8). Significant differences are indicated by lowercase letters above the bars (at $P \leq 0.05$), based on the Tukey HSD test.

phosphate buffer, and 2 % (w/v) polyvinylpyrrolidone. The homogenate was centrifuged at 4 °C for 15 min at 10,000 g. Catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) activities were measured in the stored (20 °C) supernatant following van Rossum et al. [36] and Chance and Maehly [37], respectively.

2.12. Statistical analysis

Data are reported as mean values with relative standard error. Data was subjected to one-way ANOVA analysis and differences among means were determined using Tukey's post-test.

3. Results

3.1. Morphological traits

Plant height, leaf number, and leaf area were measured at harvest, and all of these parameters decreased remarkably in response to

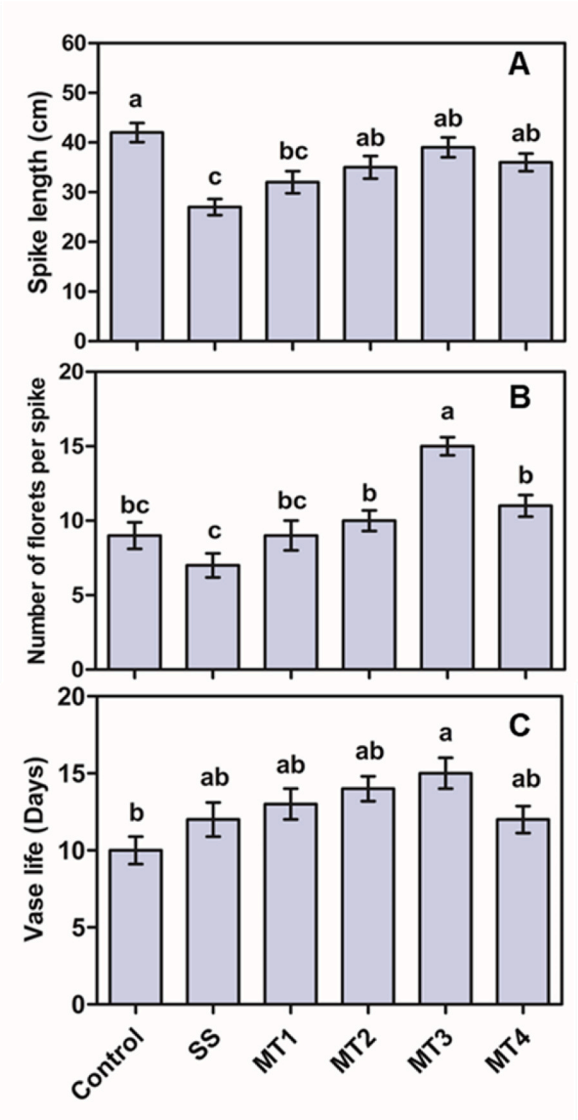

Fig. 3. Effects of exogenous applied MT (melatonin) on spike length (**A**), number of florets per spike (**B**), and vase life (**C**) of ornamental sword lily cut flowers obtained from plants grown under salt stress (SS). Different levels of MT including 0.2, 0.4, 0.6 and 0.8 mM denoted as MT1, MT2, MT3, and MT4 respectively, were tested under SS condition. Data are presented as the mean ± SE of eight replicates (n = 8). Significant differences are indicated by lowercase letters above the bars (at $P \leq 0.05$), based on the Tukey HSD test.

SS (Fig. 1ABC). Salt stress decreased plant height by 37 % compared with that of the control (Fig. 1A). The maximum plant height was obtained under MT3 (65 %), followed by MT2 (55 %), compared to salt-stressed plants without any treatment (SS) (Fig. 1A). MT4 and MT1 had plant height level greater by 52 % and 37 % than SS (Fig. 1A). Salt stress also decreased the number of leaves by 25 % than the control (Fig. 1B). Maximum number of leaves was obtained under MT3 (83 %) followed by MT2 and MT4 in which there was about 50 % more leaves than salt stressed plants without any treatment (SS) (Fig. 1B). MT1 had a 33 % greater number of leaves than SS (Fig. 1B). Salt stress decreased leaf area by 42 % compared to that of the control (Fig. 1C). The maximum leaf area was obtained under MT3 (65 %), followed by MT2 (39 %), compared to salt-stressed plants without any treatment (SS) (Fig. 1C). MT4 and MT1 had leaf area levels greater by 42 % than those of SS (Fig. 1C).

3.2. Physiological traits

Salt stress decreased chlorophyll *a* by 39 % compared with the control. Maximum chlorophyll *a* levels were obtained under MT3 (91 %) compared with salt-stressed plants without any treatment (SS) (Fig. 2A). Chlorophyll *a* in response to MT4 and MT2 was 64 % and 36 % greater, respectively, than that of salt-stressed plants without any treatment (SS). MT1 had a chlorophyll *a* level 24 % greater than that of SS (Fig. 2A).

Salt stress decreased chlorophyll *b* by 40 % compared with that in the control. The maximum chlorophyll *b* level was obtained under MT3 (89 %) compared with salt-stressed plants without any treatment (SS) (Fig. 2B). Chlorophyll *b* in response to MT4 and MT2 was 78 % and 44 % greater, respectively, than in salt-stressed plants without any treatment (SS). MT1 had a chlorophyll *b* level 22 % greater than that of SS (Fig. 2B).

Salt stress decreased carotenoid content by 33 % compared with the control. The maximum carotenoid content was obtained in MT3 (75 %) compared to salt-stressed plants without any treatment (SS) (Fig. 2C). The carotenoid content in response to MT1 treatment was 50 % greater than that in salt-stressed plants without any treatment (SS). MT2 and MT4 had carotenoid content levels greater by 25 % than that of SS (Fig. 2C).

Salt stress decreased the photosynthetic rate by 69 % compared to that of the control. The maximum photosynthesis rate was obtained under MT3 (359 %) compared with salt-stressed plants without any treatment (SS) (Fig. 2D). The photosynthetic rate in response to MT1 was 134 % greater than that in salt-stressed plants without any treatment (SS). MT2 and MT4 had photosynthesis rate levels greater by 116 % and 112 %, respectively, than that of SS (Fig. 2D).

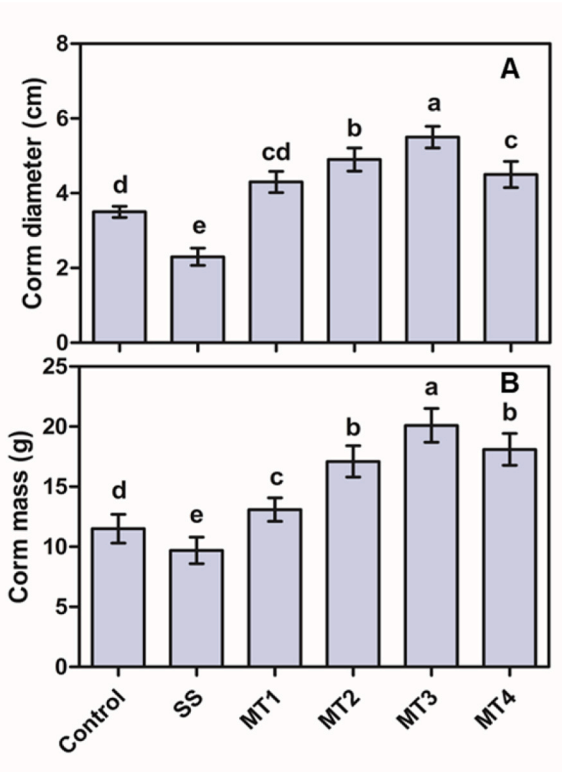

Fig. 4. Effects of exogenous applied MT (melatonin) on corm diameter (**A**) and mass (**B**) of ornamental sword lily plants under salt stress (SS). Different levels of MT including 0.2, 0.4, 0.6 and 0.8 mM denoted as MT1, MT2, MT3, and MT4 respectively, were tested under SS condition. Data are presented as the mean ± SE of eight replicates (n = 8). Significant differences are indicated by lowercase letters above the bars (at $P \leq 0.05$), based on the Tukey HSD test.

3.3. Floral traits

Salt stress (NaCl) decreased the spike length of the gladiolus plants by approximately 55 %. The application of MT2, MT3, and MT4 prevented spike length reduction compared to non-treated salt-stressed plants (Fig. 3A). The number of florets per spike was reduced by 22 % under salt-stress conditions. Plants treated with MT3 showed a significant increase in the number of florets per spike compared to those treated with SS (Fig. 3 B). The life cycle (vase life) of the cut gladiolus ranged from 11 to 14 days. The MT3 treatment significantly extended the vase life of cut sword lily cut spikes compared to that of the control and SS (Fig. 3C).

3.4. Corm traits

Corm diameter and biomass decreased by 34 % and 18 %, respectively, in response to the SS treatment (Fig. 4AB). However, under SS conditions, the application of MT1, MT2, MT3, and MT4 significantly increased the corm diameter by 87 %, 113 %, 139 %, and 96 %, respectively, whereas the corm mass increased by 33 %, 88 %, 133 %, and 100 %, respectively, compared to non-treated SS treatment plants (Fig. 4AB).

3.5. Leaf free proline and total soluble proteins

Salt stress improved proline levels by 56 % compared to the control. Maximum proline levels were obtained in MT3 (40 %) compared to salt-stressed plants without any treatment (SS) (Fig. 5A). Proline contents in response to MT4 and MT2 were 28 % and 24 % greater, respectively, than those in salt-stressed plants without any treatment (SS). MT1 had a proline level that was 16 % higher than that of SS (Fig. 5A).

Salt stress decreased the total soluble protein content by 14 % compared with the control. Maximum total soluble protein levels were obtained under MT3 (51 %) compared to salt-stressed plants without any treatment (SS) (Fig. 5B). Total soluble proteins in response to MT2 were 31 % higher than those in salt-stressed plants without any treatment (SS). MT4 and MT1 had total soluble protein levels greater by 22 % and 9 %, respectively, than SS (Fig. 5B).

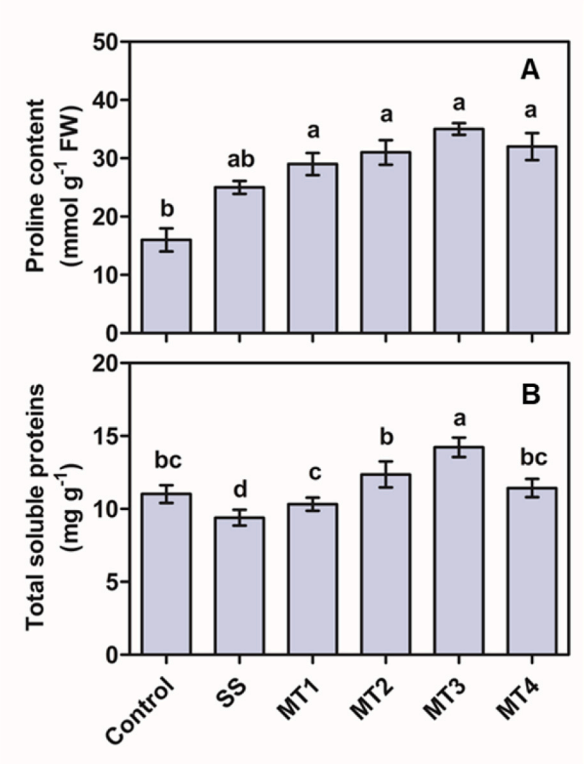

Fig. 5. Effects of exogenously applied MT (melatonin) on leaf free proline (**A**) and total soluble proteins (**B**) of ornamental sword lily plants under salt stress (SS). Different levels of MT including 0.2, 0.4, 0.6 and 0.8 mM denoted as MT1, MT2, MT3, and MT4 respectively, were tested under SS condition. Data are presented as the mean ± SE of eight replicates (n = 8). Significant differences are indicated by lowercase letters above the bars (at $P \leq 0.05$), based on the Tukey HSD test.

3.6. Anthocyanin, total flavonoids, and ascorbic acid

Salt stress increased anthocyanin content by 22 % compared with that in the control (Fig. 6A). Application of MT further increased the anthocyanin content, and the maximum increase was noticed in MT3 which was 90 % greater than that of SS (Fig. 6A). Total flavonoid content and ascorbic acid were also improved in SS by 56 % and 100 %, respectively, compared to the control (Fig. 6AB). The total flavonoid and ascorbic acid contents showed a maximum increase of 135 % and 100 %, respectively, in response to MT3 compared to SS (Fig. 6BC).

3.7. Reducing and non-reducing sugars

Salt stress reduced reducing and non-reducing sugars by 58 % and 55 %, respectively, compared with the control (Fig. 7AB). However, the application of MT enhanced the levels of sugars compared with SS. The maximum reducing and non-reducing sugar contents increased by 280 % and 185 %, respectively, in MT3 plants compared to non-sprayed salt-affected plants (Fig. 7AB).

3.8. Leaf mineral nutrients

The leaf mineral content, including N, P, and K, decreased in response to SS (Fig. 8ABC). However, the application of MT significantly improved the N, P, and K content in the leaves of salt-stressed sword lily plants. (Fig. 8ABC). Leaf N content was enhanced

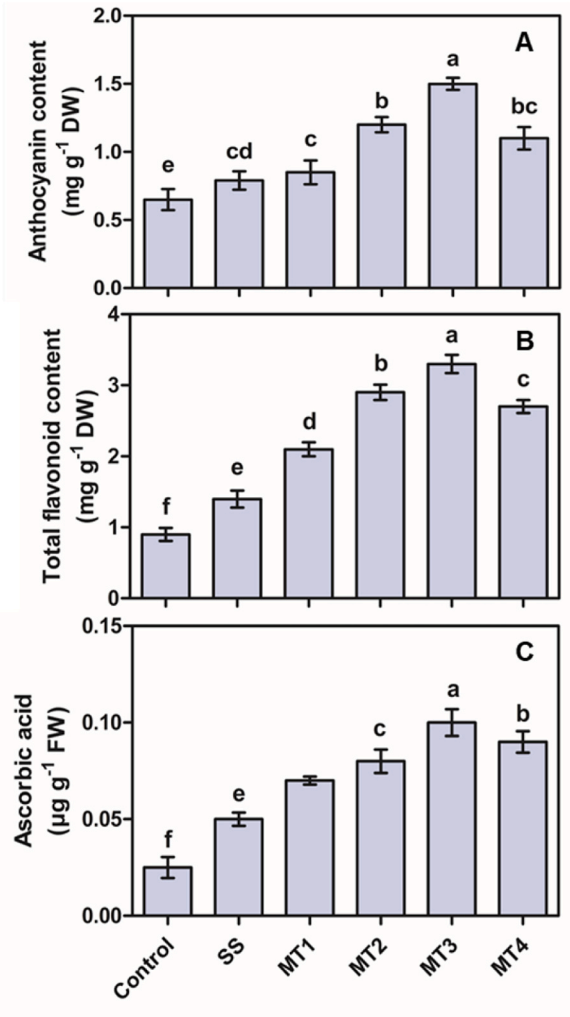

Fig. 6. Effects of exogenously applied MT (melatonin) on anthocyanin (**A**), total flavonoid (**B**), and ascorbic acid (**C**) contents of ornamental sword lily plants under salt stress (SS). Different levels of MT including 0.2, 0.4, 0.6 and 0.8 mM denoted as MT1, MT2, MT3, and MT4 respectively, were tested under SS condition. Data are presented as the mean ± SE of eight replicates (n = 8). Significant differences are indicated by lowercase letters above the bars (at $P \leq 0.05$), based on the Tukey HSD test.

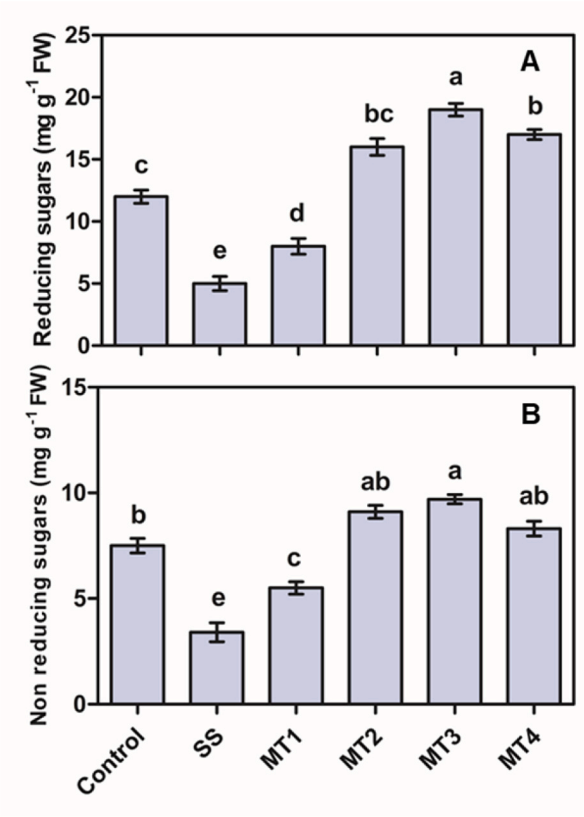

Fig. 7. Effects of exogenously applied MT (melatonin) on reducing sugars (**A**) and non-reducing sugars (**B**) in ornamental sword lily plants under salt stress (SS). Different levels of MT including 0.2, 0.4, 0.6 and 0.8 mM denoted as MT1, MT2, MT3, and MT4 respectively, were tested under SS condition. Data are presented as the mean ± SE of eight replicates (n = 8). Significant differences are indicated by lowercase letters above the bars (at $P \leq 0.05$), based on the Tukey HSD test.

differently under different MT concentrations. Salt stress decreased leaf N content by 49 % compared with the control. The maximum leaf N content was obtained under MT3 (160 %) compared to salt-stressed plants without any treatment (SS) (Fig. 8A). Leaf N content in response to MT2 and MT4 was greater by approximately 142 % than that in salt-stressed plants without any treatment (SS). MT1 had a leaf N content level 124 % greater than that of SS (Fig. 8A).

Salt stress decreased leaf P content by 31 % compared with the control. The maximum leaf P content was obtained under MT3 (388 %) compared to salt-stressed plants without any treatment (SS) (Fig. 8B). The leaf P content in response to MT2 was approximately 222 % greater than that in salt-stressed plants without any treatment (SS). MT1 and MT4 had leaf P content levels approximately 166 % greater than SS (Fig. 8B). Salt stress decreased leaf K content by 65 % compared with the control. The maximum leaf K content was obtained under MT3 (427 %) compared to salt-stressed plants without any treatment (SS) (Fig. 8C). Leaf K contents in response to MT2 were greater by about 318 % than salt stressed plants without any treatment (SS). MT1 and MT4 had leaf K content levels greater by 272 % and 218 %, respectively, than those of the SS treatment (Fig. 8C).

3.9. Lipid peroxidation and oxidative stress markers

Salt stress boosted the leaf membrane permeability level by 133 % compared with the control. The maximum decrease (−52 %) in leaf membrane permeability was obtained under MT3 compared to salt-stressed plants without any treatment (SS) (Fig. 9A). Leaf membrane permeability in response to MT4 and MT2 were lowered by 38 % and 28 % than salt stressed plants without any treatment (SS). MT1 had a leaf membrane permeability that was 13 % lower than that of SS (Fig. 9A). SS stress increased H_2O_2 levels by 200 % compared with the control (Fig. 9B). The application of MT1, MT2, MT3, and MT4 decreased the H_2O_2 level by 22 %, 34 %, 55 %, and 43 %, respectively, compared to SS (Fig. 9B). MDA content also increased by 85 % compared to that in the control (Fig. 9C). The application of MT1, MT2, MT3, and MT4 decreased MDA levels by 16 %, 13 %, 41 %, and 33 %, respectively, compared to SS (Fig. 9C).

3.10. Antioxidative defense enzymes activity

Salt stress improved CAT activity by 83 % compared with that of the control. The maximum CAT activity level was obtained in MT3

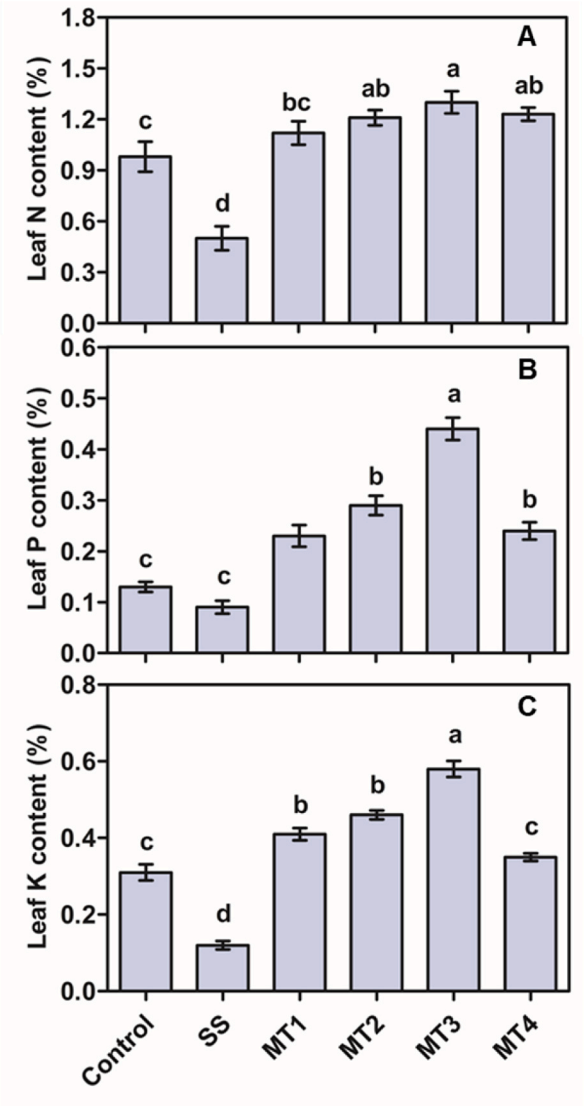

Fig. 8. Effects of exogenously applied melatonin (MT) on leaf nitrogen (N; **A**), leaf phosphorous (P; **B**), and leaf potassium (K; **C**) in ornamental sword lily plants under SS (salt stress). Different levels of MT including 0.2, 0.4, 0.6 and 0.8 mM denoted as MT1, MT2, MT3, and MT4 respectively, were tested under SS condition. Data are presented as the mean ± SE of eight replicates (n = 8). Significant differences are indicated by lowercase letters above the bars (at $P \leq 0.05$), based on the Tukey HSD test.

(60 %) compared to salt-stressed plants without any treatment (SS) (Fig. 10A). The CAT activity in response to MT4 and MT2 was 30 % and 25 % greater than that in salt-stressed plants without any treatment (SS). MT1 had a CAT activity level that was 10 % greater than that of SS (Fig. 10A). Salt stress enhanced the SOD activity by 86 % compared with that in the control. Maximum SOD activity level was obtained under MT3 (84 %) than salt stressed plants without any treatment (SS) (Fig. 10B). SOD activity in response to MT4 and MT2 were greater by 50 % than salt stressed plants without any treatment (SS). MT1 had SOD activity level 48 % greater than SS (Fig. 10B). Salt stress improved the POD activity by 18 % than the control. The maximum POD activity level was obtained under MT3 (13 %) compared with salt-stressed plants without any treatment (SS) (Fig. 10C). POD activity in response to MT2 and MT1 was 8 % and 6 % greater than that in salt-stressed plants without any treatment (SS), respectively. MT4 had a POD activity level 5 % greater than that of SS (Fig. 10C).

4. Discussion

Salt stress mostly impacts agricultural yield in dry or semi-arid climatic zones, notably in coastal areas. Melatonin is a pleiotropic chemical that enters the cell compartment smoothly. Melatonin offers low-cost usage advantages, environmental conservation, and exceptional impact. Exogenous MT in agricultural settings can mitigate climate change-induced stress and improve crop quality.

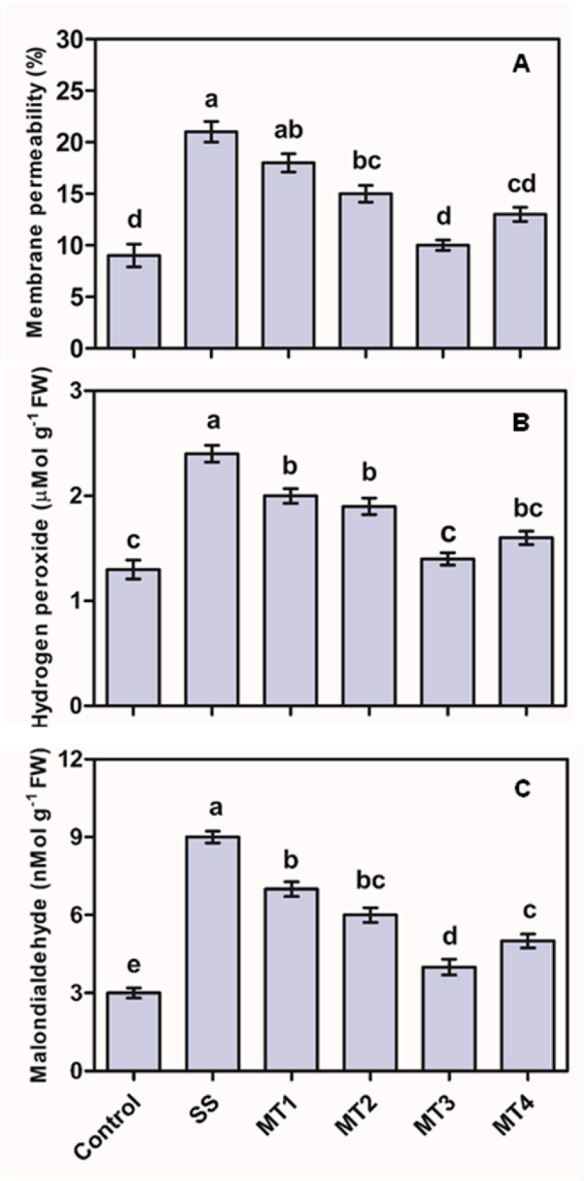

Fig. 9. Effects of exogenous applied MT (melatonin) on membrane permeability (**A**), hydrogen peroxide (**B**), and malondialdehyde content (**C**) of ornamental sword lily plants under salt stress (SS). Different levels of MT including 0.2, 0.4, 0.6 and 0.8 mM denoted as MT1, MT2, MT3, and MT4 respectively, were tested under SS condition. Data are presented as the mean ± SE of eight replicates (n = 8). Significant differences are indicated by lowercase letters above the bars (at $P \leq 0.05$), based on the Tukey HSD test.

Melatonin is a natural compound hypothesised to modulate plant metabolic activity under diverse environmental conditions [13,15]. Melatonin, either exogenously or endogenously, can improve plant resilience to abiotic stimuli, such as salt, cold, and drought, while also delaying leaf senescence [38].

Sword lily plants were adversely affected in terms of growth and physiological biomarkers, including photosynthetic pigments and net photosynthesis rate, when exposed to SS (Figs. 1 and 2). However, the foliar application of MT, especially MT3, not only retrieved the ill effects of SS but also promoted growth traits, including plant height, leaf number and area, and physiological aspects such as pigments and net photosynthesis level of sword lily plants under SS (Figs. 1 and 2). These negative effects of SS could be attributed to a reduction in root water absorption, induced osmotic stress, ion toxicity, hindered mineral nutrient uptake, secondary metabolites, oxidative stress, and stomatal pore closure, all of which upset the optimum morpho-physiological and metabolic functions of plants. Furthermore, SS triggers chlorophyll destruction, adversely affects electron transport from PSII to PSI, lowers the activities of Calvin cycle enzymes, and induces stomatal closure, which in turn decreases the availability of CO_2 and decreases the activity of the two primary photosynthetic enzymes, Rubisco activity and Carbonic Anhydrase [39], thereby limiting the growth of sword lily. In the

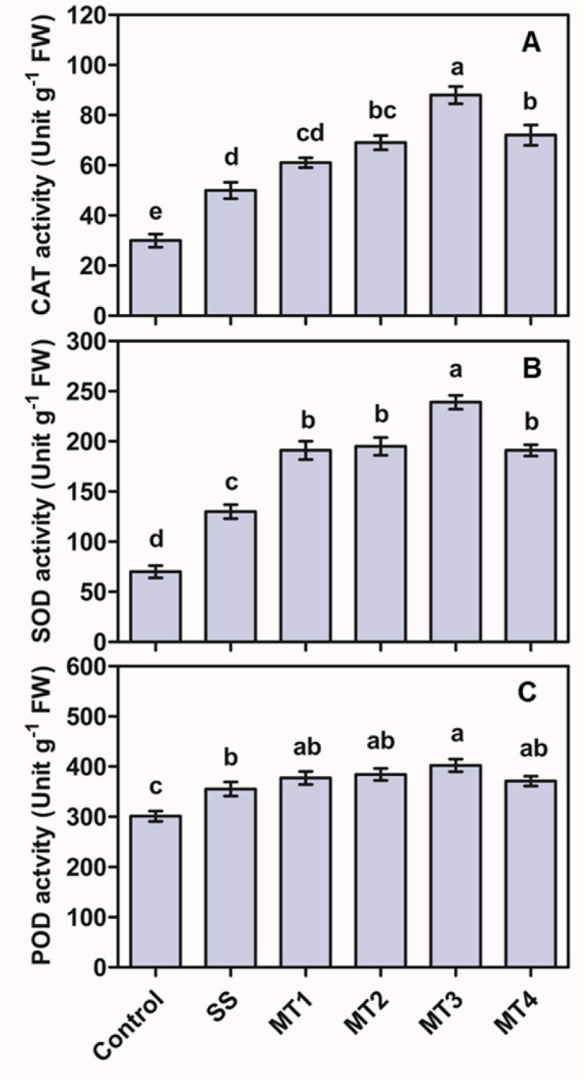

Fig. 10. Effects of exogenously applied melatonin (MT) on catalase (CAT; **A**), superoxide dismutase (SOD; **B**), and peroxidase (POD; **C**) in ornamental sword lily plants under SS (salt stress). Different levels of MT including 0.2, 0.4, 0.6 and 0.8 mM denoted as MT1, MT2, MT3, and MT4 respectively, were tested under SS condition. Data are presented as the mean ± SE of eight replicates (n = 8). Significant differences are indicated by lowercase letters above the bars (at $P \leq 0.05$), based on the Tukey HSD test.

present study, the protective role of MT against the SS has been revealed, and MT improved growth and ornamental attributes in sword lily plants under SS conditions (Figs. 1 and 3). MT directly interacts with various ROS, neutralizing them before they can cause cellular damage. It can enhance the activity of endogenous antioxidant enzymes, thereby boosting the cell's intrinsic defense mechanisms. Plants raised under SS followed by exogenous MT application had reduced impairment of the growth biomarkers and ornamental parameters and significantly lower inhibition of photosynthetic processes as well as the accumulation of proline, ascorbic acid, flavonoids, antioxidant enzyme activities, and lower levels of oxidative injury markers compared to the plants raised under only SS (Figs. 1–3 and 6 and 9 and 10). Recent studies have focused on how plants under salt stress respond to MT, but few studies have been conducted on the influence of MT on ornamental plants. In white beans, the application of MT increased tolerance to SS, with an increase in plant growth, leaf pigments, proline, and POD enzyme activity [40]. These results were also confirmed in our previous studies on gerberas and zinnia [13,16]. This study examined the efficiency of MT to gain a better understanding of how it might increase growth performance in salt-stressed sword lily plants, which is a vital cut flower crop worldwide, and attempted to determine the effect of MT under SS by morpho-physiological and biochemical investigations. Melatonin-mediated increase in SS tolerance could be due to the fact that MT regulates stomatal behaviour and expands the stomatal pore under stress conditions, boosting the accessibility of internal CO_2 levels while improving the carboxylation procedure, the activity of Carbonic Anhydrase activity, chlorophyll content, and photosynthetic efficiency [39,41]. These findings explain the higher biomass accumulation and better growth observed in gladiolus plants treated with MT.

Photosynthesis is a vital physiological mechanism that promotes plant growth and dry matter generation. Salt stress has a deleterious effect on photosynthetic and other physiological functions of plants. In our study, higher photosynthetic rate in salt-stressed sword lily plants was defined as plant metabolic stimulus through MT (Fig. 2). These results could be explained by the retention of chlorophyll concentration, which allowed the preservation of light-use efficiency (Fig. 2). This could be mediated by the delayed degradation of chlorophyll accompanied by the production of porphyrins through the correct activity of D-aminolevulinate synthase [42]. Analogous results were observed in cucumber (*Cucumis sativum* L.) exposed to 200 mM SS and treated with various MT concentrations. The most efficient treatment was observed in stressed plants treated with 100 μM MT [43]. The application of MT in salt stressed cucumber reduced the chlorophyll degradation and preserved the photosynthetic activity (Fig. 2). The protection of photosynthetic machinery can explain the higher biomass accumulation as corm diameter and biomass (Fig. 4). At the yield level, MT prevented flower spike reduction and all ornamental parameters associated with flower quality (spike length, florets per spike) and vase life (Fig. 3). The application of MT preserved plant performance and efficiently allowed plants to function better in stressful environments by influencing detoxification enzymes related to avoiding ROS accumulation and boosting photosynthetic efficiency. Furthermore, an overall improvement in plant growth and a decline in oxidative stress metabolism and osmoprotectant parameters, as evidenced by proline and other biochemical traits, depict a reduction in stress conditions. Modulation of osmotic equilibrium is an important stress-relieving method used by plants to combat SS [44].

Ornamental traits such as spike length, floret numbers, and vase life of cut flowers are important traits that define the overall quality of cut flower crops. In our study, SS reduced ornamental traits while improving vase life (Fig. 3ABC) owing to enhanced antioxidant defense activity in plants. Moreover, the size and mass of corms decreased under SS conditions (Fig. 4AB). Application of MT boosted ornamental traits, vase life, and corm characteristics These results are consistent with the previous studies. Wang et al. [45] reported improved vase life and ornamental traits in response to MT application during postharvest condition. The improvement in corm features could be linked with improved photosynthesis and secondary metabolites in our study. Safaei et al. [46] also reported an improved vase life of cut carnations in response to MT application.

Accumulation of proline is a crucial strategy for the survival of plants under SS. To tackle osmotic stress during salt stress, plants produce osmoprotectants such as proline, which regulate the osmotic conditions in plant cells. Melatonin enhances proline levels in tissues under saline conditions to tackle oxidative stress [47]. Proline levels in salt-stressed sword lily plants increased considerably when MT was exogenously applied (Fig. 5A). Melatonin treatment improved osmolyte acquisition in NaCl-treated plants by improving proline biosynthesis genes and Δ 1-pyrroline-5-carboxylate synthase activity [48]. Consequently, an increase in proline levels in salt-stressed plants with the application of MT was observed, which might boost the leaf osmotic potential, resulting in an increase in water uptake, boosting the processes of photosynthesis, and eventually yielding traits in salt-stressed plants [48].

In the current study, SS on sword lily plants resulted in lower TPC levels (Fig. 5B). This could be related to ROS overproduction and membrane structural disruption, which cause nutritional imbalance, protein oxidation, and reduced photosynthetic activity [49]. However, foliar MT treatment enhanced TPC in salt-stressed sword lily plants. These results are consistent with recent studies on cotton and tomato seedlings, revealing that exogenous MT increases protein content under SS [39,50]. This could be because MT protects protein biomolecules against disorganization and oxidation by scavenging the excess release of ROS and upregulating the expression of defence-associated genes [50,51]. Furthermore, MT treatment increased the activity of Nitrate Reductase, which is the limiting enzyme in the nitrogen assimilation process and is responsible for converting nitrate reduction (nitrate to nitrite), regulating unnecessary nitrate accumulation, and coordinating nitrogen and carbon metabolic processes.

Anthocyanin, an essential pigment, helps plants adapt to SS by preventing oxidative stress [52]. In the present study, SS induced anthocyanin accumulation in sword lily (Fig. 6A). This observation emphasises the significance of anthocyanins in the tolerance of plants to SS. These findings are consistent with earlier research showing that exogenous MT application increased anthocyanin levels [53,54]. Application of MT further boosted the level of anthocyanin in sword lily, and maximum accumulation was noted in response to MT3 (Fig. 6A). Plants use flavonoids, a significant secondary metabolite, to protect themselves against abiotic stressors by scavenging already-generated ROS or blocking the production of new ROS [55]. In the present study, SS induced flavonoid accumulation in sword lily (Fig. 6A). Similar results have been observed in *Brassica napus* [56], pea [57], and peanut [52], where the contents of total flavonoid and anthocyanin compounds showed a remarkable increase in tolerance to SS. Application of MT further boosted the level of flavonoids in sword lily, and maximum accumulation was noted in response to MT3 (Fig. 6B). Sheikhalipour et al. [58] also observed similar findings in which application of MT boosted the level of flavonoids in sage plant under SS. In the present study, SS induced ascorbic acid accumulation in sword lily (Fig. 6A). Ascorbic acid acts as a powerful nonenzymatic antioxidant in plants to combat oxidative stress. The level of ascorbic acid was reported to be enhanced under SS conditions [16].

Application of MT further boosted the level of ascorbic acid in sword lily, and maximum accumulation was noted in response to MT3 (Fig. 6C). Zhang et al. [54] also noted improved levels of secondary metabolites such as anthocyanin, flavonoids, and ascorbic acid in salt-stressed eggplants under SS conditions. In summary, MT application has the potential to boost SS tolerance by improving the production of secondary metabolites.

Sugars are the principal form of energy for plant metabolism and are involved in osmotic regulation. They also operate as strong free-radical scavengers [59]. In our study, SS exposure led to a decrease in the accumulation of sugars (reducing and non-reducing sugars) in sword lily (Fig. 7AB). These results are contradictory to those of previous studies in which stress conditions enhanced the accumulation of sugars because under stress conditions, additional carbohydrates and energy are needed to increase plant respiratory efficiency [59 Samanta et al., 2020]. However, exogenous MT supplementation increased reducing and non-reducing sugars, particularly in response to MT3 (Fig. 7AB). This indicates that MT preserves the constantly changing equilibrium in sugar metabolism, which helps plants adapt to environmental stressors. The MT-induced excess sugar accumulation served as a substrate for sword lily plant metabolic pathways, meeting the extra needs for ATP, NADPH, and other metabolites to successfully reduce SS. Shi et al. [60]

also found that exogenous MT controlled the metabolism of carbohydrates and greatly increased soluble sugar and sucrose content in Arabidopsis during SS, osmotic stress, and cold stress as well as under biotic stress. Zhong et al. [61] complemented our observations by demonstrating that exogenous MT increased biomass, photosynthetic performance, and pigment production in grape plants by stimulating sucrose metabolism.

Mineral uptake is a key adaptation for SS tolerance [62]. In our study, leaf NPK decreased in response to SS (Fig. 8ABC). The SS condition has been reported to decrease mineral content, particularly macronutrients. Interestingly, the application of MT enhanced leaf mineral content in sword lily leaves (Fig. 8ABC). These results are consistent with those of previous studies, in which MT application improved leaf mineral nutrition. Jahan et al. [62] noted an improved N and K^+ contents in response to MT application under SS conditions in tomato plants. Talaat and Shawky [63] also reported improved NPK under SS in response to MT and salicylic acid treatments in wheat. Nitrogen is a vital macronutrient responsible for the normal functioning of plants. It is also important for boosting the defense enzyme activity under stress conditions such as SS [64]. Salt stress hinders the uptake of N and its assimilation thereby affecting the growth [63] In a recent study by Xu et al. [64] the authors observed that under low nitrogen applications MT can boost the uptake of N and hence under SS MT can boost the N uptake in plant tissues. Phosphorous play crucial role in plant development and SS reduces the uptake of P. Application of MT enhanced the uptake of P under SS. Similar findings were found observed improved P uptake in response to MT under SS [65,66].

Potassium is also an important component of plant normal metabolism. However, SS reduces it uptake by accumulating Na^+ ions in the roots. In our study application of MT improved the K^+ uptake. Yan et al. [66] reported an improved K^+ uptake in response to MT under SS by regulating nitric oxide signaling in rice.

In this study, SS elevated the oxidative stress of sword lily plants, whereas exogenous MT reduced the OS of sword lily caused by SS and increased OS resistance, implying that exogenous MT treatment may mitigate the damage related to the growth of sword lily plants under SS, thereby improving sword lily SS tolerance (Fig. 9). In this study, the effects of SS on the growth, physiology, biochemistry, secondary metabolites, antioxidant activity, inorganic mineral ions, and vase life of sword lily were evaluated. Moreover, the countereffect of foliar treatment with exogenous MT at different concentrations to relieve SS was analysed. These results are supported by previous research indicating that excessive salt stress causes the formation of ROS, which affects carbon metabolism and the buildup of NADPH oxidase [66,67]. Lipid peroxidation is widely used as a marker of membrane integrity and its measurement can be used to determine malondialdehyde (MDA). This biochemical marker is frequently used to measure oxidative lipid damage induced by environmental stress. That is, the greater the MDA readings, the more damage that occurred [66]. Melatonin treatments have been demonstrated to reduce ROS accumulation such as H_2O_2. In tomatoes, MT reduced ROS accumulation, such as H_2O_2 and anion superoxide, under SS [48]. This effect protects the membrane integrity and reduces electrolyte leakage. These findings confirm the role of melatonin in mitigating salinity stress, and are consistent with our results in the gladiolus. Furthermore, MT metabolites exhibit antioxidant capabilities, and MT may easily traverse cellular membranes, making it a viable molecule for detoxification of excess ROS [68].

The key components of nitro-oxidative reactions were also evaluated to determine the role of MT in reducing the negative effects of SS. Under stressful conditions, the exogenous application of MT reduced the generation of H_2O_2. Foyer and Noctor [69] theoretically described the ability of photosynthesis to create superoxide, hydrogen peroxide, and singlet oxygen, all of which are buffered by the plant's antioxidant system. Plants skip oxidative stress via MT application, which improves the activity of detoxification enzymes and prevents ROS accumulation [70]. These findings indicated that an increase in oxidative stress markers due to SS caused membrane damage. MT spraying could overcome this negative effect on sword lily plants. Similar reports on MT-based ROS detoxification have been published in different studies related to agriculture [11–14].

Plants use antioxidant enzyme strategies to regulate oxidative [11,14]. Importantly, the negative effects of salt stress were mitigated by the use of MT, which increased the enzyme activity. MT is a vital antioxidant that protects against ROS [13]. In the present study, MT application boosted the antioxidant levels in sword lily (Fig. 10ABC). Previous reports on MT have also shown enhanced antioxidant activity in SS [13]. Moreover, spraying MT decreased ROS levels in salt-stressed sword lily seedlings, as indicated by a reduction in oxidative stress markers. Therefore, MT treatment reduced the salt-mediated harmful effects of ROS in sword lily plants and boosted plant resistance to salt stress. Consistent with previous studies, SOD and POD activities increased in maize under SS conditions [70,71].

Overall, our findings suggest that MT recued stress, which led to a better plant physiological state and, as a result, improved SS tolerance in sword lily. However, it must be highlighted that high concentrations of MT can nullify its positive performance and may have negative effects. Therefore, an adequate dose-response experiments must be performed for crops where MT has not been tested. Further works should be performed to understand the crosstalk with other plant hormones. Mainly, it will be interesting to study the interaction with ethylene and ABA, since both are also important hormones in the senescence regulation.

Results obtained demonstrated that the correct MT concentrations and applications may expand the cultivation in geographical area suffering of high soil salinity or where the salty water is used for irrigation.

5. Conclusions

MT significantly enhanced the growth, physiology, and biochemical traits of sword lily, a popular ornamental cut flower, by mitigating oxidative and osmotic stress. This study is the first to explore how MT confers stress tolerance in sword lilies. MT treatment, particularly at the MT3 concentration, increased proline and antioxidant activities in leaves, promoting better crop development and performance. MT3 treatment also improved floret numbers and diameter, extended vase life, and increased corm mass and diameter. These benefits are likely due to higher photosynthesis and sugars. Moreover, MT3 increased secondary metabolite production under

stress, which could explain the mode of action of MT. Subsequently, MT reduced levels of malondialdehyde (MDA) and hydrogen peroxide. These findings highlight MT's potential as a cost-effective, sustainable method for improving the quality and yield of sword lilies and other ornamental plants in stress-prone areas. Future research should focus on the molecular responses of ornamental plants to salt stress to further develop stress mitigation strategies in horticulture.

Ethical approval statement

No human or animal was included in the experiments.

Data availability statement

The data are reported in the manuscript; however, raw data can be provided by the authors upon request.

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CRediT authorship contribution statement

Faisal Zulfiqar: Conceptualization, Formal analysis, Methodology, Writing - original draft, Writing – review & editing. **Muhammad Nafees:** Supervision. **Anam Moosa:** Formal analysis, Writing – review & editing. **Antonio Ferrante:** Writing – review & editing. **Anastasios Darras:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] S. Toscano, D. Romano, A. Ferrante, Molecular responses of vegetable, ornamental crops, and model plants to salinity stress, Int. J. Mol. Sci. 24 (4) (2023) 3190, https://doi.org/10.3390/ijms24043190.
[2] S. Toscano, F. Branca, D. Romano, A. Ferrante, An evaluation of different parameters to screen ornamental shrubs for salt spray tolerance, Biology 9 (9) (2020) 250, https://doi.org/10.3390/biology9090250.
[3] P. García-Caparrós, M.T. Lao, M. The effects of salt stress on ornamental plants and integrative cultivation practices, Sci. Hortic. 240 (2018) 430–439, https://doi.org/10.1016/j.scienta.2018.06.022.
[4] A. Trivellini, G. Carmassi, G. Scatena, P. Vernieri, A. Ferrante, Molecular and physiological responses to salt stress in salinity-sensitive and tolerant Hibiscus rosa-sinensis cultivars, Molecular Horticulture 3 (1) (2023) 28, https://doi.org/10.1186/s43897-023-00075-y.
[5] I. Mittal, S. Jhanji, K.K. Dhatt, Efficacy of sodium nitroprusside, a nitric oxide donor, on vase life and postharvest attributes of gladiolus spikes, Acta Physiol. Plant. 43 (7) (2021) 108.
[6] R. Qian, X. Ma, X. Zhang, Q. Hu, H. Liu, J. Zheng, Effect of exogenous spermidine on osmotic adjustment, antioxidant enzymes activity, and gene expression of Gladiolus gandavensis seedlings under salt stress, J. Plant Growth Regul. 40 (4) (2021) 1353–1367, https://doi.org/10.1007/s00344-020-10198-x.
[7] M.F.H. Ferdosi, A. Shoaib, S. Habib, K.A. Khan, Modulation of salt-induced stress impact in Gladiolus grandiflorus L. by exogenous application of salicylic acid, Sci. Rep. 11 (1) (2021) 15597, https://doi.org/10.1038/s41598-021-95243-9.
[8] P. Wang, W.C. Liu, C. Han, S. Wang, M.Y. Bai, C.P. Song, Reactive oxygen species: multidimensional regulators of plant adaptation to abiotic stress and development, J. Integr. Plant Biol. 66 (3) (2024) 330–367.
[9] M. Asad, M. A. U., Zhang, Y., Zhou, L., X. Guan, F. Cheng, How abiotic stresses trigger sugar signaling to modulate leaf senescence?. Plant Physiol. Biochem., (20249 108650.
[10] M.B. Arnao, J. Hernández-Ruiz, Melatonin and its relationship to plant hormones, Ann. Bot. (Lond.) 121 (2) (2018) 195–207, https://doi.org/10.1093/aob/mcx114.
[11] M.A. Altaf, Y. Hao H. Shu, M.A. Mumtaz, S. Cheng, M.N. Alyemeni, P. Ahmad, Z. Wang, Melatonin enhanced the heavy metal-stress tolerance of pepper by mitigating the oxidative damage and reducing the heavy metal accumulation, J. Hazard Mater. 454 (2023) 1–12, https://doi.org/10.1016/J.JHAZMAT.2023.131468.
[12] N. Zhang, Q. Sun, H. Zhang, Y. Cao, S. Weeda, S. Ren, Y.D. Guo, Roles of melatonin in abiotic stress resistance in plants, J. Exp. Bot. 66 (3) (2015) 647–656, https://doi.org/10.1093/jxb/eru336.
[13] F. Zulfiqar, A. Moosa, A. Ferrante, M. Nafees, A. Darras, M.M. Nazir, T.M. Soliman, Exogenous foliar application of melatonin mitigates salt induced oxidative stress and promotes growth in Gerbera jamosonii, South Afr. J. Bot. 161 (2023) 678–684, https://doi.org/10.1016/j.sajb.2023.08.055.
[14] M.H. Siddiqui, S. Alamri, M.N. Khan, F.J. Corpas, A.A. Al-Amri, Q.D.P. Alsubaie, Q. Ahmad, Melatonin and calcium function synergistically to promote the resilience through ROS metabolism under arsenic-induced stress, J. Hazard Mater. 398 (2020) 122882, https://doi.org/10.1016/j.jhazmat.2020.122882.
[15] F. Zulfiqar, A. Moosa, A. Ferrante, M. Nafees, A. Darras, M.M. Nazir, F.G. Elsaid, Melatonin and salicylic acid synergistically improve arsenic induced oxidative stress tolerance in ornamental sword lily, Sci. Hortic. 322 (2023) 112389, https://doi.org/10.1016/j.scienta.2023.112389.
[16] F. Zulfiqar, A. Moosa, A. Ferrante, A. Darras, T. Ahmed, S. Jalil, A. El Sabagh, Melatonin seed priming improves growth and physio-biochemical aspects of Zinnia elegans under salt stress, Sci. Hortic. 323 (2024) 112495, https://doi.org/10.1016/j.scienta.2023.112495.

[17] Y. Luo, T. Hu, Y. Huo, L. Wang, L. Zhang, R. Yan, Effects of exogenous melatonin on chrysanthemum physiological characteristics and photosynthesis under drought stress, Horticulturae 9 (1) (2023) 106, https://doi.org/10.3390/horticulturae9010106.

[18] C. Qin, H. Lian, F.M. Alqahtani, M.A. Ahanger, Chromium mediated damaging effects on growth, nitrogen metabolism and chlorophyll synthesis in tomato can be alleviated by foliar application of melatonin and jasmonic acid priming, Sci. Hortic. 323 (2024) 112494, https://doi.org/10.1016/j.scienta.2023.112494.

[19] E.A. Eisa, P. Honfi, A. Tilly-Mándy, I. Mirmazloum, Exogenous melatonin application induced morpho-physiological and biochemical regulations conferring salt tolerance in *Ranunculus asiaticus* L, Horticulturae 9 (2) (2023) 228, https://doi.org/10.3390/horticulturae9020228.

[20] F. Zulfiqar, A. Moosa, A. Darras, M. Nafees, A. Ferrante, K.H. Siddique, Preharvest melatonin foliar treatments enhance postharvest longevity of cut tuberose via altering physio-biochemical traits, Front. Plant Sci. 14 (2023) 1151722, https://doi.org/10.3389/fpls.2023.1151722.

[21] T. Gao, X. Liu, K. Tan, D. Zhang, B. Zhu, F. Ma, C. Li, Introducing melatonin to the horticultural industry: physiological roles, potential applications, and challenges, Hortic. Res. 9 (2022), https://doi.org/10.1093/hr/uhac094.

[22] H.K. Lichtenthaler, Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes Methods in Enzymology, vol. 148, Acad. Press, 1987, pp. 350–382, https://doi.org/10.1016/0076-6879(87)48036-1.

[23] L.S. Bates, R.A. Waldren, I.D. Teare, Rapid determination of free proline for water-stress studies, Plant Soil 39 (1973) 205–207, https://doi.org/10.1007/BF00018060.

[24] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254, https://doi.org/10.1016/0003-2697(76)90527-3.

[25] J. Lee, R.W. Durst, R.E. Wrolstad, M.M. Giusti, J. Hach, H. Hofsommer, S. Koswig, D.A. Krueger, S. Kupina, S.K. Martin, B.K. Martinsen, T.C. Miller, F. Paquette, A. Ryabkova, G. Skrede, U. Trenn, J.D. Wightman, Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study, J. AOAC Int. 88 (5) (2005) 1269–1278, https://doi.org/10.1093/jaoac/88.5.1269.

[26] S.S. Humadi, V. Istudor, *Lythrum salicaria* (purple loosestrife). Medicinal use, extraction and identification of its total phenolic compounds, FARMACIA 57 (2) (2009) 192–200.

[27] A.A. Elzaawely, S. Tawata, Antioxidant activity of phenolic rich fraction obtained from Convolvulus arvensis L. leaves grown in Egypt, Asian Journal of Crop Science 4 (1) (2012) 32–40, https://doi.org/10.3923/ajcs.2012.32.40.

[28] S.P. Mukherjee, M.A. Choudhuri, Implication of hydrogen peroxide–ascorbate system on membrane permeability of water stressed vigna seedlings, New Phytol. 99 (3) (1985) 355–360, https://doi.org/10.1111/j.1469-8137.1985.tb03663.x.

[29] N. Nelson, A photometric adaptation of the Somogyis method for the determination of reducing sugar, Anal. Chem. 31 (1944) 426–428.

[30] M.L. Jackson, Chemical composition of soil, in: F.E. Bean (Ed.), Chemistry of Soil, Van Nostrand Reinheld Co, New York, NY, USA, 1962, pp. 71–144.

[31] S.R. Olsen, C.V. Cole, F.S. Watanabe, L.A. Dean, Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate; Circular No. 939, USDA, US Government Printing Office, Washington, DC, USA, 1954.

[32] H.D. Chapman, P.F. Pratt, Method of Analysis for Soil, Plants and Waters, University of California Division Agriculture Science, Berkeley, CA, USA, 1961.

[33] G.S. Premachandra, H. Saneoka, S. Ogata, Cell membrane stability, an indicator of drought tolerance, as affected by applied nitrogen in soyabean, J. Agric. Sci. 115 (1) (1990) 63–66, https://doi.org/10.1017/S0021859600073925.

[34] D.M. Hodges, J.M. DeLong, C.F. Forney, R.K. Prange, Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds, Planta 207 (1999) 604–611, https://doi.org/10.1007/s004250050524.

[35] B.D. Patterson, E.A. MacRae, I.B. Ferguson Estimation of hydrogen peroxide in plant extracts using titanium (IV), Anal. Biochem. 139 (1984) 487–492, https://doi.org/10.1016/0003-2697(84)90039-3.

[36] M.W. van Rossum, M. Alberda, L.H. van der Plas, Role of oxidative damage in tulip bulb scale micropropagation, Plant Sci. 130 (2) (1997) 207–216, https://doi.org/10.1016/S0168-9452(97)00215-X.

[37] A. Maehly, B. Chance, Catalases and peroxidases, Methods Biochem. Anal. 1 (1954) 357–424, https://doi.org/10.1002/9780470110171.ch14.

[38] J. Ahmad, F. Hayat, U. Khan, N. Ahmed, J. Li, S. Ercisli, J. Chen Melatonin, A promising approach to enhance abiotic stress tolerance in horticultural plants, South Afr. J. Bot. 164 (2024) 66–76, https://doi.org/10.3389/fpls.2023.1140803.

[39] T.A. Khan, M. Saleem, Q. Fariduddin, Melatonin influences stomatal behavior, root morphology, cell viability, photosynthetic responses, fruit yield, and fruit quality of tomato plants exposed to salt stress, J. Plant Growth Regul. 42 (2023) 2408–2432, https://doi.org/10.1007/s00344-022-10713-2.

[40] M. Askari, N. Hamid, Z. Abideen, F. Zulfiqar, A. Moosa, M. Nafees, A. El-Keblawy, Exogenous melatonin application stimulates growth, photosynthetic pigments and antioxidant potential of white beans under salinity stress, South Afr. J. Bot. 160 (2023) 219–228, https://doi.org/10.1016/j.sajb.2023.07.014.

[41] L. Dai, J. Li, H. Harmens, X. Zheng, C. Zhang, Melatonin enhances drought resistance by regulating leaf stomatal behaviour, root growth and catalase activity in two contrasting rapeseed (Brassica napus L.) genotypes, Plant Physiol. Biochem. 149 (2020) 86–95, https://doi.org/10.1016/j.plaphy.2020.01.039.

[42] M.H.P.J.C. Ashraf, P.J. Harris, Photosynthesis under stressful environments: an overview, Photosynthetica 51 (2013) 163–190, https://doi.org/10.1007/s11099-013-0021-6.

[43] L.Y. Wang, J.L. Liu, W.X. Wang, Y. Sun, Exogenous melatonin improves growth and photosynthetic capacity of cucumber under salinity-induced stress, Photosynthetica 54 (2016) 19–27, https://doi.org/10.1007/s11099-015-0140-3.

[44] Y. Yin, J. Xu, X. He, Z. Yang, W. Fang, J. Tao, Role of exogenous melatonin involved in phenolic acid metabolism of germinated hulless barley under NaCl stress, Plant Physiol. Biochem. 170 (2022) 14–22, https://doi.org/10.1016/j.plaphy.2021.11.036.

[45] Y. Wang, X. Liu, M. Sun, W. Zhu, Y. Zheng, S. Zhu, X. Yu, Melatonin enhances vase life and alters physiological responses in peony (*Paeonia lactiflora* Pall.) cut flowers, Postharvest Biol. Technol. 212 (2024) 112896, https://doi.org/10.1016/j.postharvbio.2024.112896.

[46] A. Safaei Far, S. Mousavi-Fard, A. Rezaei Nejad, F. Shahbazi, M. Ahmadi-Majd, D. Fanourakis, Nano Silver and melatonin effectively delay the senescence of cut carnation flowers under simulated vibrational stress, J. Hortic. Sci. Biotechnol. (2024) 1–12, https://doi.org/10.1080/14620316.2024.2334306.

[47] S. Spormann, P. Nadais, F. Sousa, M. Pinto, M. Martins, B. Sousa, C. Soares, Accumulation of proline in plants under contaminated soils—are we on the same page? Antioxidants 12 (3) (2023) 666.

[48] M.H. Siddiqui, S. Alamri, M.Y. Al-Khaishany, M.N. Khan, A. Al-Amri, H.M. Ali, I.A. Alaraidh, A.A. Alsahli, Exogenous melatonin counteracts NaCl-induced damage by regulating the antioxidant system, proline and carbohydrates metabolism in tomato seedlings, Int. J. Mol. Sci. 20 (2019) 353, https://doi.org/10.3390/ijms20020353.

[49] B. Gupta, B. Huang, Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization, Int. J. Genomics. (2014), https://doi.org/10.1155/2014/701596.

[50] L. Chen, B. Lu, L. Liu, W. Duan, D. Jiang, J. Li, Z. Bai, Melatonin promotes seed germination under salt stress by regulating ABA and GA_3 in cotton (*Gossypium hirsutum* L.), Plant Physiol. Biochem. 162 (2021) 506–516, https://doi.org/10.1016/j.plaphy.2021.03.029.

[51] S. Sun, D. Wen, W. Yang, Q. Meng, Q. Shi, B. Gong, Overexpression of caffeic acid O-methyltransferase 1 (COMT1) increases melatonin level and salt stress tolerance in tomato plant, J. Plant Growth Regul. 39 (2020) 1221–1235, https://doi.org/10.1007/s00344-019-10058-3.

[52] G. Li, X. Guo, Y. Sun, S.S. Gangurde, K. Zhang, F. Weng, C. Zhao, Physiological and biochemical mechanisms underlying the role of anthocyanin in acquired tolerance to salt stress in peanut (*Arachis hypogaea* L.), Front. Plant Sci. 15 (2024) 1368260, https://doi.org/10.3389/fpls.2024.1368260.

[53] Y. Sandoval, R. Tighe-Neira, C. Inostroza-Blancheteau, B. Soto-Cerda, J. González-Villagra, Melatonin improves plant water status, photosynthetic performance, and antioxidant defense system in highbush blueberry (*Vaccinium corymbosum* L.) plants subjected to drought stress, Sci. Hortic. 323 (2024) 112528, https://doi.org/10.1016/j.scienta.2023.112528.

[54] Y. Zhang, C. Yang, S. Liu, Z. Xie, J. Zhang, F. Ji, Alleviation of salt toxicity by melatonin supplementation in eggplant involves the up-regulation of ascorbate–glutathione and glyoxalase system, Sci. Hortic. 326 (2024) 112720, https://doi.org/10.1016/j.sajb.2023.07.014.

[55] I. Hernández, L. Alegre, F. Van Breusegem, S. Munné-Bosch, How relevant are flavonoids as antioxidants in plants? Trends Plant Sci. 14 (3) (2009) 125–132, https://doi.org/10.1016/j.tplants.2008.12.003.

[56] J. Kim, W.J. Lee, T.T. Vu, C.Y. Jeong, S.W. Hong, H. Lee, High accumulation of anthocyanins via the ectopic expression of AtDFR confers significant salt stress tolerance in *Brassica napus* L, Plant Cell Rep. 36 (2017) 1215–1224, https://doi.org/10.1007/s00299-017-2147-7.

[57] M. Farooq, R. Ahmad, M. Shahzad, Y. Sajjad, A. Hassan, M.M. Shah, S.A. Khan, Differential variations in total flavonoid content and antioxidant enzymes activities in pea under different salt and drought stresses, Sci. Hortic. 287 (2021) 110258, https://doi.org/10.1016/j.scienta.2021.110258.
[58] M. Sheikhalipour, M. Kulak, S.A. Mohammadi, B. Esmaielpour, M. Nouraein, M.Z. Kocak, F. Vita, Foliar application of either melatonin or sodium nitpoprusside regulates the antioxidant status, and the morpho-physiological attributes and essential oil production in sage (*Salvia officinalis* L.) under salinity stress, Sci. Hortic. 323 (2024) 112526, https://doi.org/10.1016/j.scienta.2023.112526.
[59] S. Samanta, A. Singh, A. Banerjee, A. Roychoudhury, Exogenous supplementation of melatonin alters representative organic acids and enzymes of respiratory cycle as well as sugar metabolism during arsenic stress in two contrasting indica rice cultivars, J. Biotechnol. 324 (2020) 220–232, https://doi.org/10.1016/j.jbiotec.2020.10.013.
[60] H. Shi, Y. Qian, D.X. Tan, R.J. Reiter, C. He, Melatonin induces the transcripts of CBF/DREB1s and their involvement in both abiotic and biotic stresses in Arabidopsis, J. Pineal Res. 59 (3) (2015) 334–342, https://doi.org/10.1111/jpi.12262.
[61] L. Zhong, L. Lin, L. Yang, M.A. Liao, X. Wang, J. Wang, Y. Tang, Exogenous melatonin promotes growth and sucrose metabolism of grape seedlings, PLoS One 15 (4) (2020) e0232033, https://doi.org/10.1371/journal.pone.0232033.
[62] M.S. Jahan, G. Li, D. Xie, et al., Melatonin mitigates salt-induced growth inhibition through the regulation of carbohydrate and nitrogen metabolism in tomato seedlings, J. Soil Sci. Plant Nutr. 23 (2023) 4290–4308, https://doi.org/10.1007/s42729-023-01348-7.
[63] N.B. Talaat, B.T. Shawky, Synergistic effects of salicylic acid and melatonin on modulating ion homeostasis in salt-stressed wheat (Triticum aestivum L.) plants by enhancing root H+-pump activity, Plants 11 (3) (2022) 416, https://doi.org/10.3390/plants11030416.
[64] Y. Xu, R. Xu, S. Li, S. Ran, J. Wang, Y. Zhou, F. Zhong, The mechanism of melatonin promotion on cucumber seedling growth at different nitrogen levels, Plant Physiol. Biochem. 206 (2024) 108263.
[65] M. Ali, Z. Malik, G.B. Abbasi, M. Irfan, S. Ahmad, M. Ameen, S. Ali, Potential of melatonin in enhancing antioxidant defense system and yield of maize (*Zea mays* L.) hybrids under saline condition, Sci. Hortic. 325 (2024) 112665.
[66] F. Yan, H. Wei, W. Li, Z. Liu, L. Chen, G. Li, Melatonin improves K+ and Na+ homeostasis in rice under salt stress by mediated nitric oxide, Ecotoxicol. Environ. Saf. 206 (2020) 111358.
[67] X. Wu, J. Li, L.Y. Song, L.L. Zeng, Z.J. Guo, D.N. Ma, H.L. Zheng, NADPH oxidase-dependent H2O2 production mediates salicylic acid-induced salt tolerance in mangrove plant Kandelia obovata by regulating Na+/K+ and redox homeostasis, Plant J. (2024), https://doi.org/10.1111/tpj.16660.
[68] T.A. Khan, Q. Fariduddin, F. Nazir, M. Saleem, Melatonin in business with abiotic stresses in plants, Physiol. Mol. Biol. Plants 26 (2020) 1931–1944, https://doi.org/10.1007/s12298-020-00878-z.
[69] W. Kong, F. Liu, C. Zhang, J. Zhang, H. Feng, Non-destructive determination of Malondialdehyde (MDA) distribution in oilseed rape leaves by laboratory scale NIR hyperspectral imaging, Sci. Rep. 6 (2016) 35393, https://doi.org/10.1038/srep35393.
[70] L.C. Manchester, A. Coto-Montes, J.A. Boga, L.P.H. Andersen, Z. Zhou, A. Galano, R.J. Reiter, Melatonin: an ancient molecule that makes oxygen metabolically tolerable, J. Pineal Res. 59 (4) (2015) 403–419, https://doi.org/10.1111/jpi.12267.
[71] S. Ahmad, W. Cui, M. Kamran, et al., Exogenous application of melatonin induces tolerance to salt stress by improving the photosynthetic efficiency and antioxidant defense system of maize seedling, J. Plant Growth Regul. 40 (2021) 1270–1283, https://doi.org/10.1007/s00344-020-10187-0.