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Exogenous melatonin strengthens saline-alkali stress tolerance in apple rootstock M9-T337 seedlings by initiating a variety of physiological and biochemical pathways

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Abstract

Melatonin (MT) is an important plant growth regulator that significantly regulates the growth and development of plants. Previous studies confirmed the effectiveness of MT in improving plant stress tolerance. In this study, annual M9-T337 seedlings were selected as subjects, and five treatments were applied: control (CK), in which only half the concentration of Hoagland was applied; Saline-alkaline stress treatment (SA, 100 mmol·L^{-1} saline-alkaline solution); melatonin treatment (MT, CK + 200 μmol L^{-1} exogenous MT); Saline-alkaline + melatonin treatment (MS, SA + 200 μmol L^{-1} exogenous MT); and saline-alkaline stress + melatonin + inhibitor treatment (HS, additional 100 μmol L^{-1} p-CPA treatment to MS). The results showed that saline-alkaline stress negatively affected the growth of M9-T337 seedlings by reducing photosynthetic capacity, increasing Na^+, promoting reactive oxygen species such as H_2O_2, and changing the osmotic content and antioxidant system. However, the application of exogenous MT effectively alleviated saline-alkaline damage and significantly promoted the growth of M9-T337 seedlings. It significantly increased plant height, diameter, root length, root surface area, volume and activity. Furthermore, MT alleviated osmotic stress by accumulating proline, soluble sugars, soluble proteins and starch. MT improved photosynthetic capacity by delaying chlorophyll degradation and regulating gas exchange parameters as well as fluorescence parameters in leaves. Additionally, MT reduced the Na^+/K^+ ratio to reduce ion toxicity by upregulating the expression of Na^+ transporter genes (*MhCAX5*, *MhCHX15*, *MhSOS1*, and *MhALT1*) and downregulating the expression of K^+ transporter genes (*MhSKOR* and *MhNHX4*). In addition, MT can increase antioxidant enzyme activity (superoxide dismutase (SOD), peroxidase(POD), catalase (CAT), ascorbic acid oxidase (AAO), ascorbate peroxidase (APX) and monodehydroascorbate reductase (MDHAR)) in the ASA-GSH cycle and increase ascorbic acid (AsA), reduced glutathione (GSH) and oxidized glutathione (GSSG) levels to counteract the accumulation of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and Superoxide anion free radicals (O_2^-), reducing oxidative damage. Exogenous MT promotes M9-T337 seedlings growth under saline-alkaline stress by responding synergistically with auxin (IAA), gibberellin (GA_3) and zeatin (ZT) to saline-alkaline stress. Our results confirm that MT has the potential to alleviate Saline-alkaline stress by promoting root growth, increasing biomass accumulation and photosynthetic capacity, strengthening the antioxidant defense system, maintaining ionic balance, the ascorbate–glutathione cycle and the Osmoregulation facilitates and regulates endogenous hormone levels in M9-T337 seedlings.

Keywords ASA-GSH cycle, Ion homeostasis, M9-T337, Oxidative damage, Saline-alkaline stress

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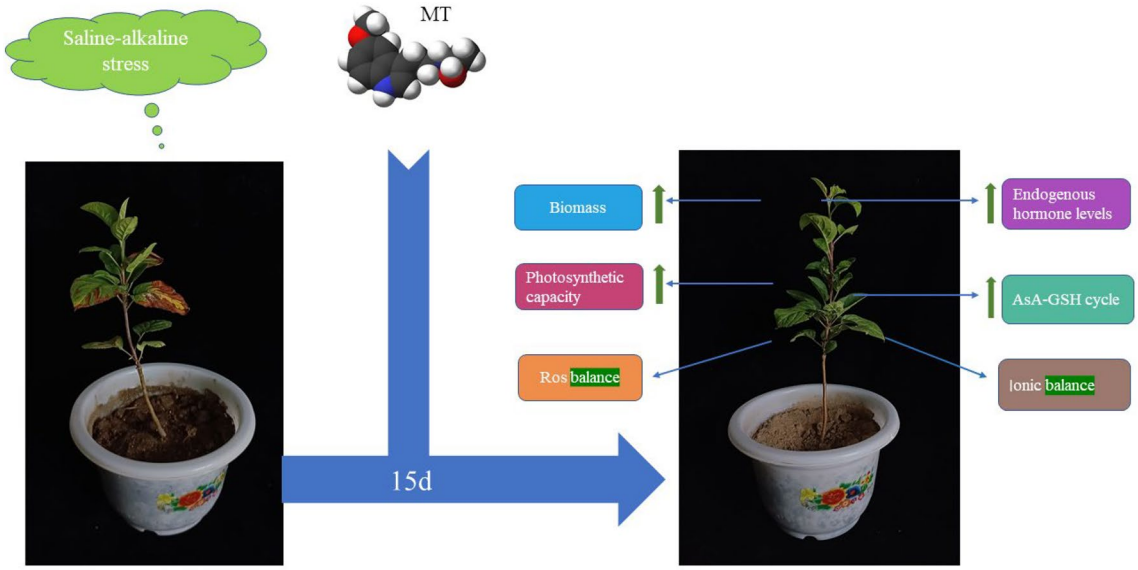
Graphical Abstract



Introduction

As a global environmental problem, soil salinization is a major factor hindering the sustainable development of agriculture [1]. With the intensification of the greenhouse effect and global warming, soil drought occurs, which leads to increased soil salinity [2]. Studies have shown that about 950 million hectares are affected by salinization worldwide, with China's saline land area being about 9.9×10^7 hectares, mainly distributing in the northwestern inland and eastern coastal areas [3]. However, apples, as an important crop in China, are subject to severe soil salinization in their main growing regions, which has a significant impact on the growth and development [4]. Therefore, it is crucial to improve the resistance of apple trees to saline-alkaline conditions to ensure sustainable development in the apple industry.

Saline-alkaline stress is one of the most important abiotic stress factors, and its damage to plants is mainly reflected in four aspects: osmotic stress, ion toxicity, high pH damage and reactive oxygen stress [5]. Osmotic stress mainly reflects that saline-alkaline stress increases the osmotic pressure of the environment of plant roots, so that the water potential of the environment of roots decreases, resulting in water leakage in plants [6]. The main manifestation of ion toxicity is that a large amount of Na^+ accumulates in plants due to soil salinization, which greatly reduces the uptake of K^+ ions by plants, resulting in a series of toxicities in plants, including destroying the cell membrane structure, inhibiting the synthesis of related enzymes, weakening photosynthesis and hindering signal transmission in vivo [7]. The harm of high pH is mainly reflected in the increase of the rhizosphere environment pH in plants, thereby damaging the root environment and reducing the root vitality. At the same time, there is also precipitation of a large amount of metal ions such as Ca^{2+} and Mg^{2+} in the soil, causing soil compaction, which ultimately leads to a reduction in plant absorption of Ca, Mg and other mineral nutrients, which affects the normal growth and development of plants impaired [6]. Active oxygen stress is mainly reflected in the accumulation of a large amount of ROS in plants under saline-alkaline stress, which aggravates the degree of lipid peroxidation of plant cell membrane and produces a large amount of the toxic substance malonaldehyde (MDA), ultimately leading to the destruction of plant cell membrane structure and protein synthesis, as well as the disruption of the electron transport chain in the photosynthetic respiratory pathway, which affects plant growth and development [8]. Under saline-alkaline stress, plants also alleviate salt-base damage through physiological and molecular regulatory mechanisms. The physiological mechanism is mainly manifested: the plant itself accelerates the synthesis of organic substances such as proline, betaine and polyols [9], increases the degree of Na^+ efflux and Na^+ compartmentalization, and promotes absorption Ca^{2+} [10], the secretion of organic acids in plants [11], the rapid response of enzyme protection systems and non-enzyme protection systems and accelerates

the synthesis of endogenous hormones such as auxin (IAA), cytokinin (ABA) and gibberellin (GA_3) in plants [12, 13]. The molecular mechanism is mainly manifested: plants first transmit salt-base stress signals through the ABA pathway, the protein kinase pathway and the SOS pathway, and then the transcription factors are accepted upstream to transmit saline-alkaline stress signals and regulate the expression of related salt-base tolerance genes downstream [14, 15], including the induction of plant osmoregulation, ion transport, antioxidants and other related genes [16–18]. Studies have found that the application of exogenous plant growth regulators under stress can improve plant resistance [19–22]. Melatonin, a hormone-like substance widely distributed in higher plants, plays an important role in alleviating abiotic stress. Studies have shown that the application of exogenous melatonin under drought conditions [23], saline-alkaline conditions [24] and low temperatures [25] is effective in promoting seed germination and dry matter accumulation in plants, alleviating leaf senescence and increasing chlorophyll content of the leaves. At the same time, it can also promote the synthesis of amino acids and osmotic regulatory substances in plants. Application of exogenous melatonin under manganese stress significantly increased MDA and hydrogen peroxide (H_2O_2) contents in tobacco seedlings, thereby increasing their antioxidant capacity [26]. Exogenous melatonin sprayed under high temperature stress can significantly promote the synthesis of endogenous hormones such as auxins, cytokinins and abscisic acid in cherries, thereby increasing their heat resistance [27]. Appropriate concentrations of melatonin can also effectively increase the content of photosynthetic pigments, osmotically regulating substances and antioxidant enzyme activity in rapeseed seedlings under salt stress, thereby alleviating the damage to seedlings caused by saline-alkaline stress [28]. In summary, the application of exogenous MT under various stresses has been reported in pepper [29], tomato [30], potato [31] and other plants. However, there are few reports on the effect of exogenous MT on the growth of apple plants under Saline-alkaline stress and its mechanism. Therefore, there is an urgent need to investigate whether it has the same regulatory mechanism as apple rootstocks.

Related studies have shown that exogenous MT treatment can reduce the adverse effects of abiotic stress on plants, indicating that these hormones are beneficial to increase agricultural production. However, under saline-alkali stress, the specific physiological mechanism by which exogenous MT enhances the saline-alkali resistance of apple dwarf rootstock M9-T337 (*Malus domestica* Borkh.) remains unclear. In this study, dwarf apple rootstock M9-T337 seedlings were utilized to investigate the effects of exogenous MT on plant growth under saline-alkaline stress. The regulatory mechanism of MT was elucidated across five key aspects: ion balance, osmotic regulation, antioxidant system, pH balance, and hormone regulation. The goal of this study was to establish a strong theoretical basis for the use of exogenous melatonin in enhancing the saline-alkaline tolerance of plants and also to provide technical support for the cultivation of apple rootstock saline-alkaline tolerance.

Materials and methods

Test materials

In May 2022, M9-T337 virus-free apple seedlings cultivated in tissue culture were procured from Shandong Huinong Horticulture Co., Ltd. The seedlings were initially nurtured for 1 week in the Laboratory of Fruit Tree Stress Physiology at the College of Horticulture, Gansu Agricultural University and then transplanted to a rain shelter at Gansu Agricultural University to facilitate standardized cultivation management procedures. Following acclimation for a period of 10 days, experimental treatments were initiated.

Treatment and experimental design

The experiment was carried out in the rain shelter of Gansu Agricultural University (N 36° 1′–37° 9′, E 106° 21′–107° 44′). Average temperature: 26 °C, relative humidity: 50%. The M9-T337 seedlings were transferred into flowerpots with the same size and nutrient soil weight (11.2 cm × 16.8 cm, containing 1 kg substrate), one seedling per pot. Unified management, regular weeding watering. Based on previous research conducted by the research group [32, 33], preliminary experiments were carried out with corresponding concentrations of MT before the formal experiment. Finally, the exogenous MT concentration were determined by verifying changes in plant phenotype, growth parameters, REC, MDA, and SPAD content (Additional file 4: Table S1). This experiment consisted of five treatments: (1) Control (CK): irrigation with half concentration of Hoagland nutrient solution; (2) Saline-alkaline stress (SA): application of a compound saline-alkaline solution with a concentration of 100 mmol·L^{-1} based on the control; (3) Melatonin treatment (MT): application of 200 μmol L^{-1} exogenous MT on a control basis; (4) saline-alkaline stress + melatonin treatment (MS): application of 200 μmol L^{-1} exogenous MT based on SA; (5) saline-alkaline stress + melatonin + p-CPA treatment (HS): application of 100 μmol L^{-1} p-CPA based on MS. Three replicates were used for each treatment and six plants were used for each replicate. Seedlings were evenly spaced for each treatment to ensure consistent light exposure. Melatonin was applied every 3 days from 7:00–8:00 p.m until the

leaves dripped, for a total of 3 applications. Saline-alkaline stress was carried out by irrigating saline-alkaline solution (100 mmol · L^{-1}NaCl + 100 mmol · $L^{-1}$$NaHCO_3$) once a day, 500 mL each time, for 3 days. On day 15, the functional leaves of M9-T337 seedlings were harvested for relevant measurements.

Growth parameter

Three seedlings of each treatment were randomly selected for the determination of plant height, stem diameter, leaf area, leaf circumference, fresh weight and dry weight.

Each plant was selected for processing and placed in the root system scanner (STD-4800 company, Canada) to capture images of the root system. Subsequently, the obtained root images were analyzed using WinRHIZO5.0 software (Regent Instruments Inc., Hydro Quebec, Canada) to determine various indices, including length, total root surface area, average root diameter, total root volume, and number of root tips.

The root tip tissue weighing 0.5 g was assessed for root activity using the triphenyltetrazolium chloride (TTC) method by Chu et al. [34]. Each treatment was repeated 3 times.

Photosynthetic measurements

The chlorophyll a (Chl a), chlorophyll b (Chl b), chlorophyll a + b (Chl a + b), chlorophyll a/b (Chl a/b), and carotenoids contents of the leaves were measured according to the method described by Arnon et al. [35].

As described by Lin et al. [36], the net photosynthetic rate (*Pn*), transpiration rate (*Tr*), intercellular CO_2 concentration (*Ci*), and stomatal conductance (*Gs*) of the third leaf from the top to bottom of each plant were quantified using a portable photofluorometric apparatus (CIRAS-2, PP-system, UK). Each treatment was repeated 3 times.

As described by Yuan et al. [37], M9-T337 seedlings were subjected to a 30-min period of darkness using a modulated leaf green fluorescence imager (image-PAM, WALZ, Germany). The third fully developed leaf was selected from the bottom of the plant for determining initial fluorescence (F0), maximum fluorescence (Fm), maximum photochemical quantum yield (Fv/Fm), and photochemical quenching coefficient (qP) following dark adaptation. Each treatment was repeated 3 times.

Antioxidant measurements

Fresh leaves (0.1 g) were homogenized with 1 mL of PBS solution with pH 7.8 on ice and centrifuged for 10 min at 4 °C and 12,000 rpm. The resulting supernatant was used to determine the activity levels of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) using a kit from Beijing Solarbio Technology Co., Ltd. used. Each treatment was repeated three times.

Lipid peroxidation

Relative electrical conductivity (REC) was measured using a DDS-307 conductivity meter, as described by Yuan et al. [38]. Malondialdehyde (MDA) content was determined using the thiobarbituric acid (TBA) colorimetric method [39]. Each treatment was repeated 3 times.

ROS

Superoxide anion free radicals (O_2^-) were measured using the hydroxylamine oxidation method [40]. Fresh leaves (1 g) were weighed and placed in a mortar. Phosphate buffer (65 mmol·L^{-1}, 3 mL) was added. Homogenization was carried out by grinding, and the resulting homogenate was transferred to a centrifuge tube. The homogenate was centrifuged at 10,000 r/min for 15 min, and the supernatant was used for measurement. The hydrogen peroxide (H_2O_2) content was determined using the xylenol orange method [41].

ASA-GSH cycle

Fresh leaves (0.1 g) were homogenized with 1 mL of extract on ice, and the resulting homogenate was placed into a centrifuge tube. The homogenate was centrifuged at 12,000 rpm for 10 min at 4 °C, and the supernatant was used to determine the levels of reduced glutathione (GSH), reduced ascorbic acid (AsA), oxidized glutathione (GSSG), ascorbic acid oxidase (AAO), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), and glutathione reductase (GR) using assay kits from Beijing Soleibao Technology Co., Ltd. Each treatment was repeated 3 times.

Osmolytes

Soluble sugars (SS) was measured using the 3,5-dinitrosalicylic acid method, as described by Cut et al. [42]. Soluble proteins (SP) was measured using the Coomassie brilliant blue G-250 staining method, as described by Sevket et al. [43]. Free proline (Pro) was measured using the acid ninhydrin method, as described by Wang et al. [44], and starch (St) was measured using the anthrone colorimetric method, as described by Eros et al. [45].

Endogenous hormone

Endogenous hormone contents were determined according to method of Zheng et al. [46]. The endogenous hormone levels of auxin (IAA), gibberellin (GA_3), abscisic acid (ABA), and zeatin (ZT) were determined in 0.5 g leaves using ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC–MS/MS). The

chromatographic column was used a Symmetry C18 column (4.6 mm 250 mm, 5 μm) at a temperature of 30 °C, with a mobile phase consisting of a 1:9 ratio of methanol to 0.1% phosphoric acid at a flow rate of 1.0 mL/min. An injection volume of 10 μL was used.

Na^+, K^+, and Ca^{2+} contents

The leaf powder samples (5 g) were precisely weighed and dried, followed by digestion with H_2SO_4-H_2O_2. The concentrations of Na^+, K^+, and Ca^{2+} were determined using an inductively coupled plasma-optical emission spectrometer (Perkin Elmer, Waltham, Massachusetts, USA), following the methodology described by Kuang et al. [47]. Each treatment was repeated 3 times.

qRT–PCR

Total RNA was extracted from leaves using the Plant RNA Extraction Kit and reverse transcribed with the RT Master Mix Reverse Transcription Kit, both provided by Takara Clontech Biochemicals in Shanghai. The apple actin gene (Accession No. MDPO000774288), was used as an internal reference gene. Nine apple saline-alkaline-responsive genes were selected and detected via fluorescence quantification with qRT-PCR. The reaction system, consisting of 20 μL, included 2 μL each of upstream and downstream primers, 2 μL of cDNA template, 10 μL of Trans Start Top Green qPCR Super Mix, and 6 μL of ddH_2O. The qPCR primers were designed and synthesized by Shanghai Sangon Biotech, which were checked for specificity using BLAST in the Apple genome database (https://www.rosaceae.org). See Additional file 4: Table S2 for a list of the primers used. Each treatment was repeated 3 times.

Statistical analysis

Data processing was performed using Microsoft Office Excel 2019, and the graphs were plotted using Origin 2022 software. Statistical analysis was carried out using the IBM SPSS Statistics 25 program (SPSS Inc., Chicago, IL, USA). An analysis of the variance (ANOVA) was used to compare mean values between samplings. The Duncan post-hoc test was used to determine differences between treatments. Significance levels of 95% ($P<0.05$) are indicated in figure legends.

Results

Effects of exogenous MT on leaves and roots of M9-T337 seedlings under saline-alkaline stress

After 15 days of saline-alkaline stress treatment, the phenotypic responses of M9-T337 seedlings to each treatment are illustrated in Fig. 1A. Compared to the control treatment (CK), the growth of seedlings subjected to the MT treatment did not exhibit significant advantages. Conversely, under saline-alkaline stress (SA), the leaves of seedlings displayed conspicuous chlorosis and wilting. Upon application of melatonin, the leaves of seedlings in MS treatment regreened and exhibited robust growth. However, the combined treatment of melatonin and p-CPA (HS) did not effectively alleviate the chlorosis and wilting of seedling leaves under saline-alkaline stress, nor did it demonstrate a discernible advantage over SA treatment. The root systems of seedlings under different treatments also exhibited marked differences (Fig. 1B). The total length, average diameter, volume, surface area, tip number, and overall activity of the roots under SA treatment were notably lower. Upon exogenous MT treatment, the damage to the root length, diameter, volume, surface area, tip number, and activity under saline-alkali stress was mitigated. In comparison with SA treatment, the increases were 43.22% in length, 10.71% in diameter, 96.06% in volume, 73.75% in surface area, and 9.70% in tip number, as shown in Additional file 4: Table S3. Nevertheless, the combined treatment of melatonin and p-CPA (HS) could not alleviate the saline-alkaline damage to the root growth of M9-T337 seedlings under saline-alkaline stress, as depicted in Fig. 1B.

Effects of exogenous MT on growth parameters of M9-T337 seedlings under saline-alkaline stress

The growth rate of M9-T337 seedlings was significantly inhibited under saline-alkaline stress (Fig. 2). The plant height, stem diameter, leaf area, and leaf perimeter were notably lower than those of the control treatment (CK). However, after spraying exogenous MT (MS), these parameters showed significant increases compared to the SA treatment, with increments of 1.11 times for plant height, 1.59 times for stem diameter, 1.43 times for leaf area, and 1.33 times for leaf perimeter, respectively. Conversely, when both MT and p-CPA (HS) were simultaneously applied under saline-alkaline stress conditions, these mentioned parameters were significantly reduced without any advantage over the SA treatment. When exogenous MT was sprayed on top of the CK treatment as a supplement, there was a slight increase in plant height, stem diameter, leaf area and leaf perimeter of M9-T337 seedlings compared to the CK treatment; however, the difference between the two treatments was not statistically significant.

In addition, we also analyzed the biomass accumulation of M9-T337 seedlings. As shown in Additional file 1: Fig. S1, the fresh weight and dry weight of both aboveground and underground parts of the seedlings treated with CK and MT were significantly higher than those of the other treatments, but there was no significant difference between them. Saline-alkaline (SA) stress significantly reduced the fresh weight and dry weight of each part of

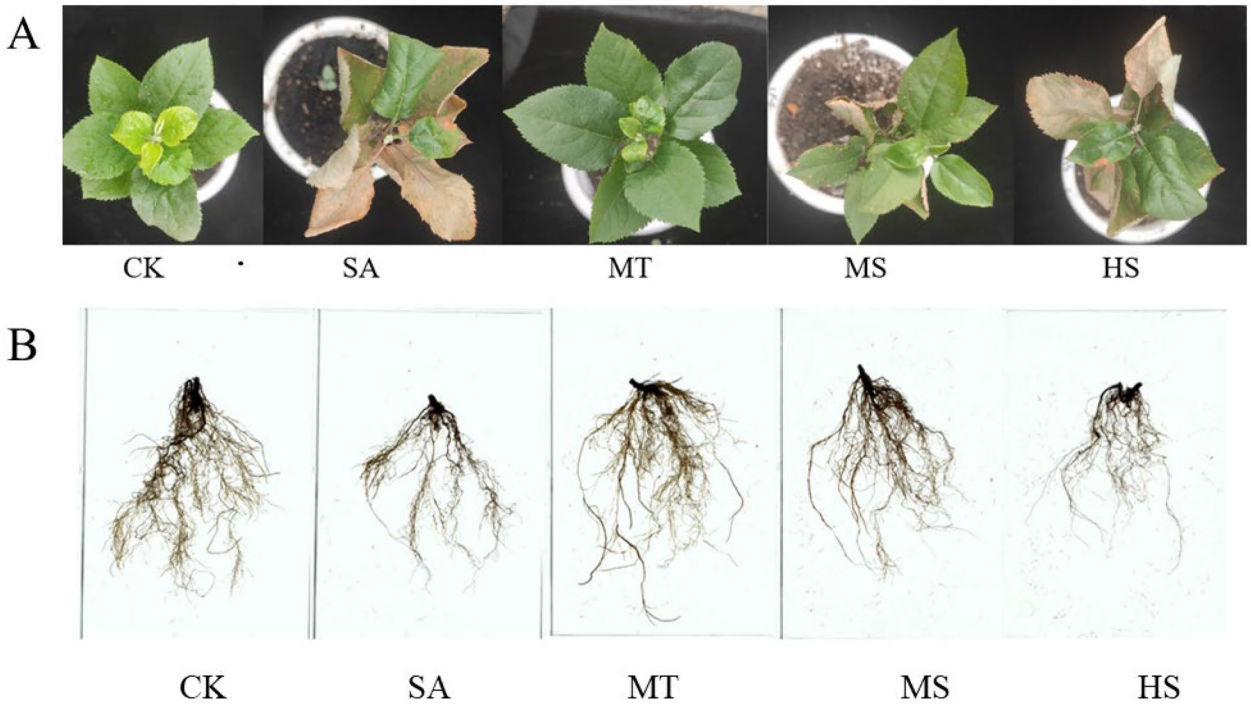


Fig. 1 Effects of exogenous MT on leaves and roots of M9-T337 seedlings under saline-alkaline stress. CK represents watering with a 1/2 concentration of Hoagland nutrient solution only; SA represents the application of 100 mmol·L^{-1} composite saline-alkaline solution based on the control; MT represents the spraying of 200 μmol L^{-1} exogenous MT based on the control; MS represents the spraying of 200 μmol L^{-1} exogenous MT based on SA; HS represents the spraying of 100 μmol L^{-1} p-CPA on the basis of MS. **A** Plant phenotype; **B** root phenotype. Vertical bars represent the standard errors of the means (three replicates. Data show the mean ± SE ($n = 3$). Different lowercase letters indicate significant differences between treatments with $P < 0.05$

the plant, but exogenous MT application mitigated this reduction caused by saline-alkaline stress. Compared to SA treatment alone, SA + MT (MS) treatment significantly increased the fresh weight and dry weight of each part of the seedlings (16.92% and 15.69% for aboveground; 80.56% and 70.97% for underground). When MT and p-CPA (HS) were simultaneously applied under saline-alkaline stress, the fresh weight and dry weight of both aboveground and underground parts were significantly lower than those in MS treatment, measuring only 1.45 g, 2.11 g, 0.53 g, and 0.35 g, respectively.

Effects of exogenous MT on photosynthesis in leaves of M9-T337 seedlings under saline-alkaline stress

The levels of Chl a (Fig. 3A), Chl b (Fig. 3B), total chlorophyll (Chl a + b) (Fig. 3C), and carotenoids (Car) (Fig. 3D) in the leaves of CK and MT treatments were significantly higher than those of the other treatments, with no significant difference between the two treatments. However, under SA treatment, the photosynthetic pigment content of leaves notably decreased, showing reductions of 36.25%, 53.19%, 41.09%, and 46.27% compared to CK treatment, respectively. On the contrary, under SA + MT (MS) treatment, the photosynthetic pigment content of leaves was significantly higher than that of SA treatment. Conversely, concurrent application of MT and p-CPA (HS) under saline-alkaline stress resulted in a significant decrease in the photosynthetic pigment content in leaves, without any significant difference from the SA treatment.

Additionally, after 15 days of treatment, the *P*n, *T*r, *G*s, and *C*i of leaves under different treatments were measured. There were no significant differences in photosynthetic parameters between the MT and CK treatments. The *Pn*, *T*r, and *G*s of leaves under SA treatment were the lowest, with reductions of 30.67%, 45.19%, and 33.07% compared to CK. Conversely, *C*i significantly increased, reaching 1.67 times that of CK. However, when exogenous MT was applied under saline-alkaline stress conditions, the *P*n, *T*r, and *G*s values of leaves in the MS treatment were higher by 30.66%, 39.93%, and 49.91% than those in the SA treatment, respectively (Fig. 4ACD). In contrast, the *C*i value for leaves was lower by 23.52% compared to that in the SA treatment (Fig. 4B). Furthermore, it was observed that simultaneous application of MT and p-CPA (HS) did not result in significant changes in *P*n, *T*r, *G*s, and *C*i values for leaves under

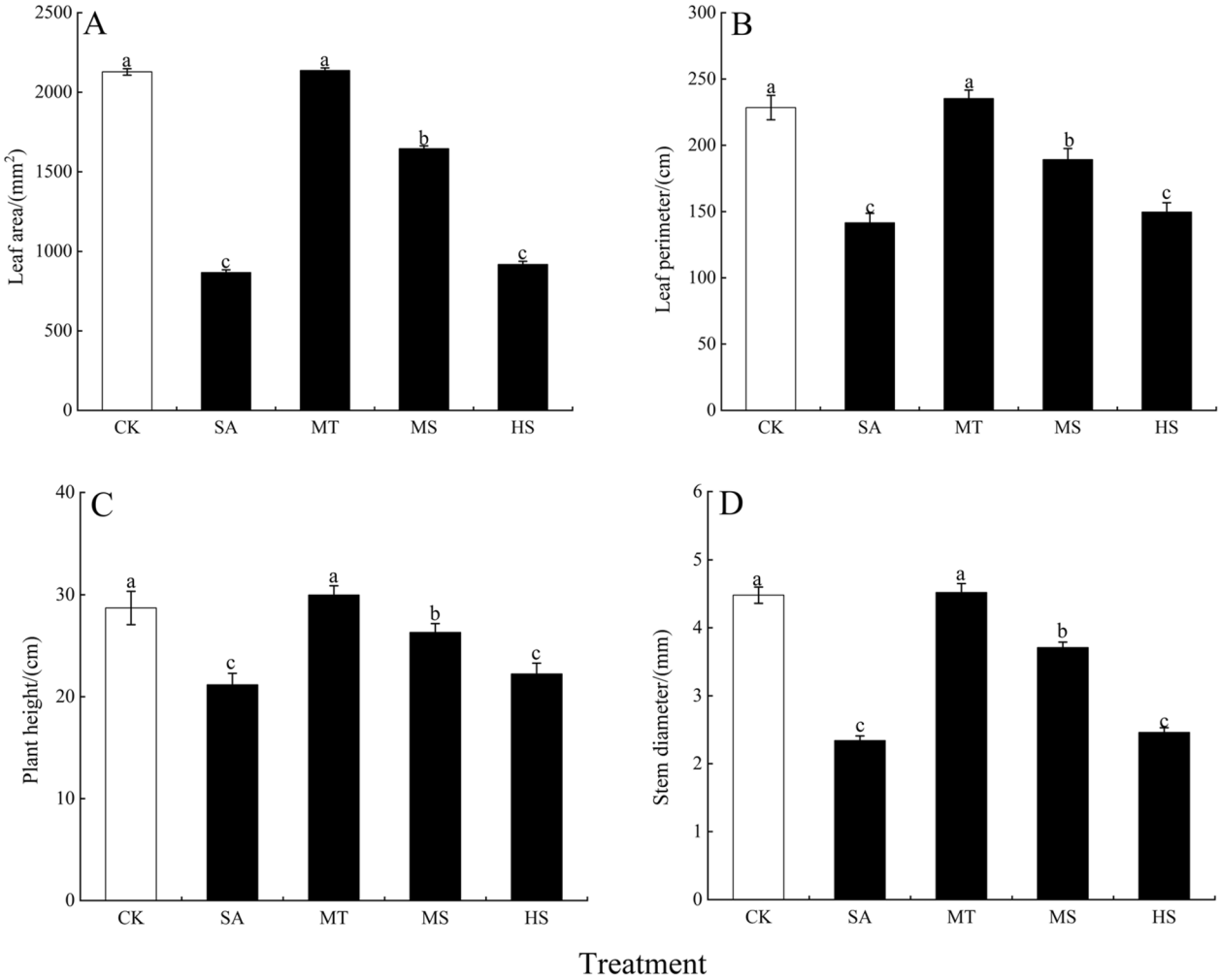


Fig. 2 Effects of exogenous MT on growth parameters and biomass accumulation parameters of M9-T337 seedlings under saline-alkaline stress. CK represents watering with a 1/2 concentration of Hoagland nutrient solution only; SA represents the application of 100 mmol·L^{-1} composite saline-alkaline solution based on the control; MT represents the spraying of 200 μmol L^{-1} exogenous MT based on the control; MS represents the spraying of 200 μmol L^{-1} exogenous MT based on SA; HS represents the spraying of 100 μmol L^{-1} p-CPA on the basis of MS. **A** Leaf area; **B** Leaf perimeter; **C** Plant height; **D** Stem diameter. Vertical bars represent the standard errors of the means (three replicates. Data show the mean ± SE ($n = 3$). Different lowercase letters indicate significant differences between treatments with $P < 0.05$

saline-alkaline stress conditions when compared with the SA treatment.

Finally, the chlorophyll fluorescence parameters (F0, Fm, Fv/Fm, and qP) of leaves subjected to different treatments were also measured. In comparison with CK treatment, the F0, Fm, Fv/Fm, and qP of the MT treatment did not show significant changes, while the SA treatment notably reduced these parameters in the leaves. However, the F0, Fm, Fv/Fm, and qP of seedling leaves in the MS treatment were significantly higher than those in the SA treatment, albeit still lower than those in the CK treatment (Additional file 2: Fig. S2). Furthermore, there were no significant changes in leaf F0, Fm, Fv/Fm, and qP, when MT and p-CPA (HS) were simultaneously applied under saline-alkaline stress in comparison with the SA treatment.

Effect of exogenous MT on membrane lipid peroxidation degree of M9-T337 seedlings under saline-alkali stress

As depicted in Fig. 5A–D, no significant difference in H_2O_2 content, O_2^-, REC, and MDA content was observed in leaves treated with MT compared to CK. However, the H_2O_2 content, O_2^-, REC, and MDA content in leaves treated with SA significantly increased, reaching 1.38 times, 2.01 times, 1.61 times, and 2.02 times that of CK, respectively. Conversely, the increase

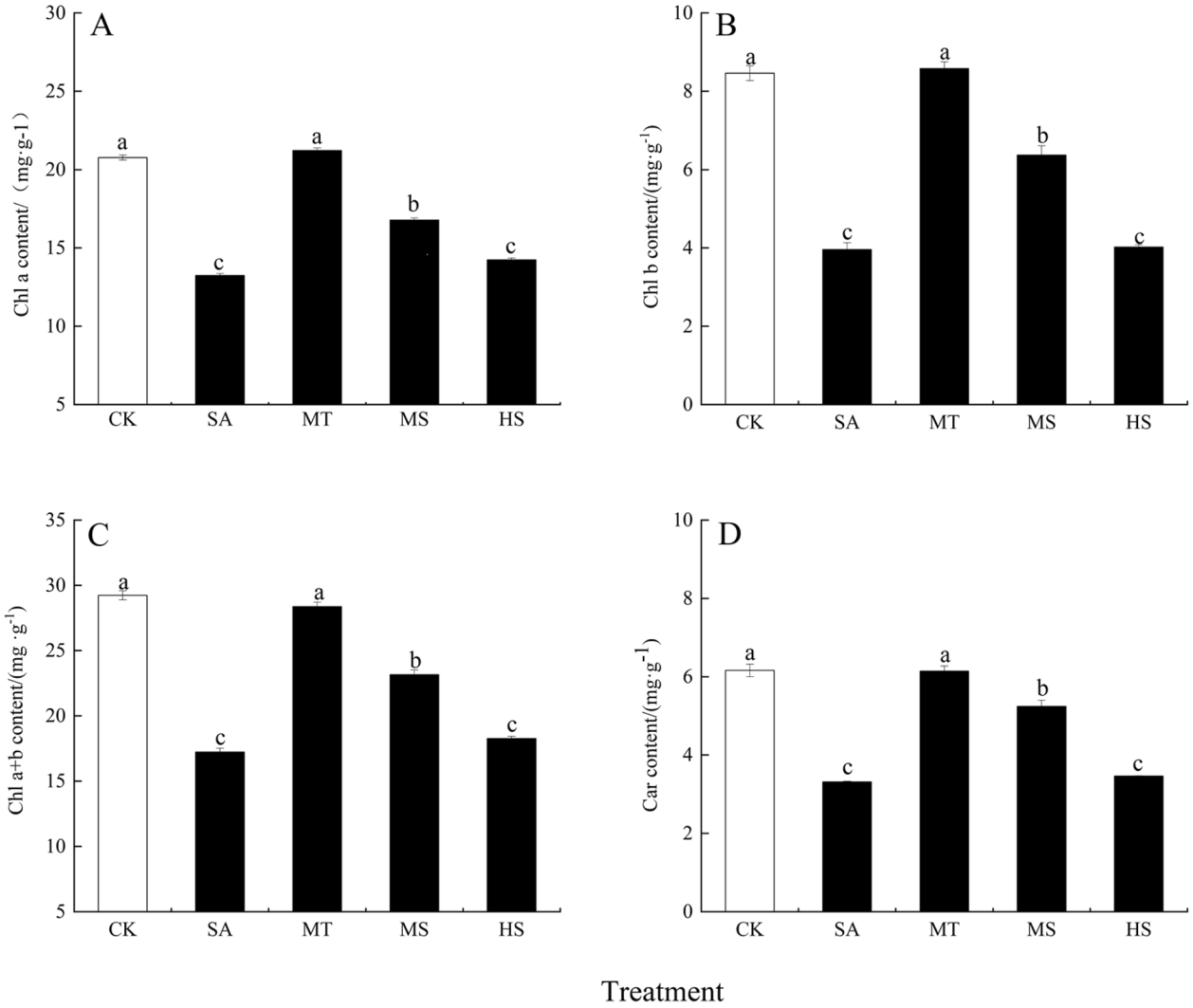


Fig. 3 Effects of exogenous MT on photosynthetic pigment in leaves of M9-T337 seedlings under saline-alkaline stress. CK represents watering with a 1/2 concentration of Hoagland nutrient solution only; SA represents the application of 100 mmol·L^{-1} composite saline-alkaline solution based on the control; MT represents the spraying of 200 μmol L^{-1} exogenous MT based on the control; MS represents the spraying of 200 μmol L^{-1} exogenous MT based on SA; HS represents the spraying of 100 μmol L^{-1} p-CPA on the basis of MS. **A** Chl a; **B** Chl b; **C** Chl a + b; **D** Car. Vertical bars represent the standard errors of the means (three replicates. Data show the mean ± SE ($n = 3$). Different lowercase letters indicate significant differences between treatments with $P < 0.05$

in H_2O_2 content, O_2^-, REC, and MDA content in leaves was mitigated with the application of exogenous MT under saline-alkaline stress, which showed a reduction of 13.72%, 41.24%, 14.55%, and 26.99% compared to the SA treatment, respectively. However, concurrently applying MT and p-CPA (HS) under saline-alkaline stress did not inhibit the upward trend of H_2O_2 content, O_2^-, REC, and MDA content in the leaves and showed no significant difference as compared to the SA treatment.

The enzyme activities of SOD, POD, and CAT in the leaves of M9-T337 seedlings were notably increased under SA treatment, being 2.13 times, 1.36 times, and 1.42 times higher than those in the control group (CK), as demonstrated in Fig. 5E–G. Moreover, when MT was applied under saline-alkali stress, the activities of SOD, POD, and CAT in the leaves of M9-T337 seedlings exhibited further increases of 36.76%, 12.45%, and 34.04% compared to the SA treatment. However, concurrent application of MT and p-CPA (HS) under

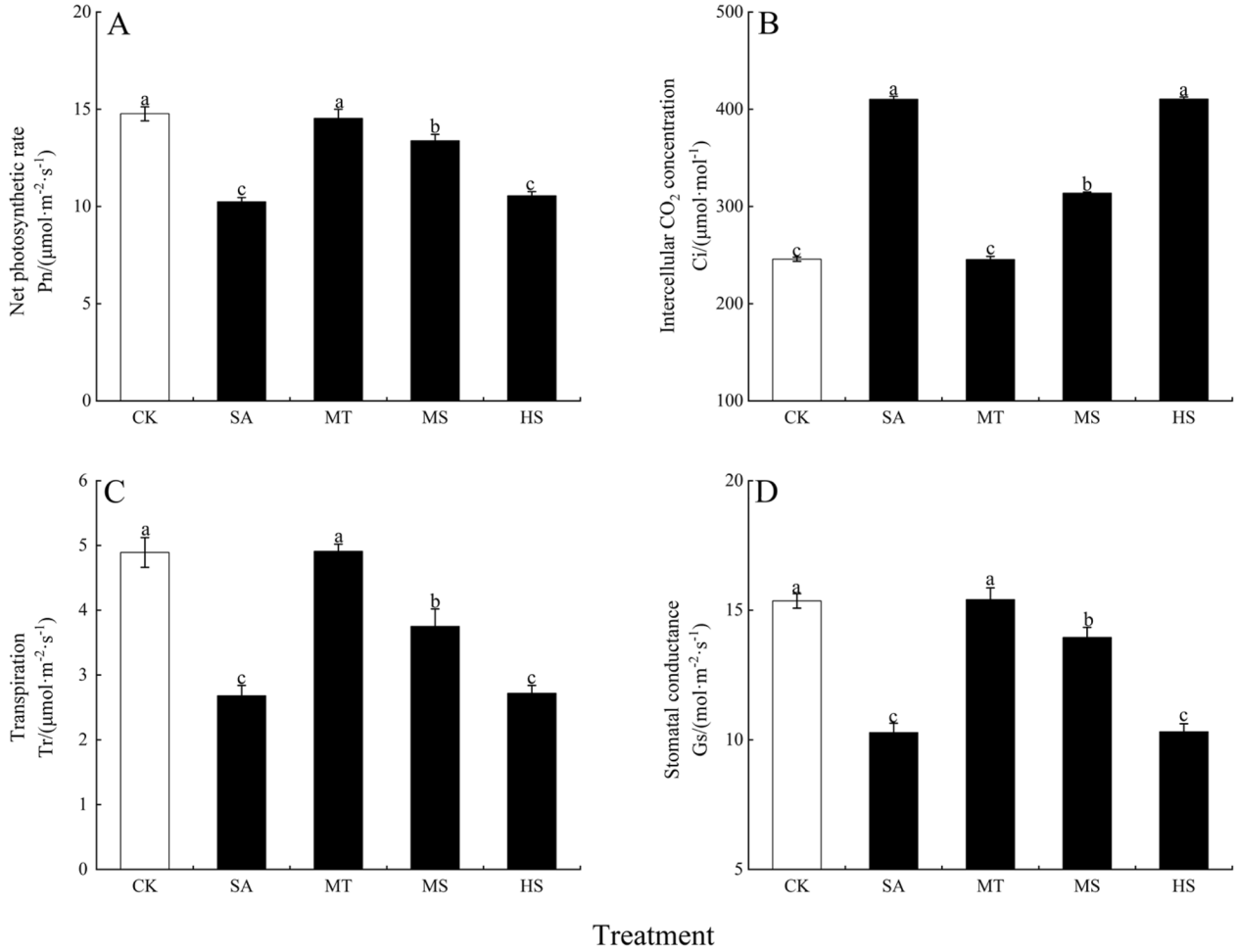


Fig. 4 Effects of exogenous MT on photosynthetic gas parameters in leaves of M9-T337 seedlings under saline-alkaline stress. CK represents watering with a 1/2 concentration of Hoagland nutrient solution only; SA represents the application of 100 mmol·L^{-1} composite saline-alkaline solution based on the control; MT represents the spraying of 200 μmol L^{-1} exogenous MT based on the control; MS represents the spraying of 200 μmol L^{-1} exogenous MT based on SA; HS represents the spraying of 100 μmol L^{-1} p-CPA on the basis of MS. **A** Net photosynthetic rate (*Pn*); **B** Intercellular CO_2 concentration (*Ci*); **C** Transpiration (*Tr*); **D** Stomatal conductance (*Gs*). Vertical bars represent the standard errors of the means (three replicates. Data show the mean ± SE ($n = 3$). Different lowercase letters indicate significant differences between treatments with $P < 0.05$

saline-alkali stress resulted in the inhibition of the increasing trend of SOD, POD, and CAT activities in M9-T337 seedling leaves, showing reductions of 25.05%, 7.69%, and 22.46% compared to the SA treatment, respectively. Additionally, it was also observed that the application of MT on the basis of CK treatment did not lead to significant changes in SOD, POD, and CAT activities in M9-T337 seedling leaves.

(See figure on next page.)

Fig. 5 Effects of exogenous MT on membrane lipid peroxidation degree in leaves of M9-T337 seedlings under saline-alkaline stress. CK represents watering with a 1/2 concentration of Hoagland nutrient solution only; SA represents the application of 100 mmol·L^{-1} composite saline-alkaline solution based on the control; MT represents the spraying of 200 μmol L^{-1} exogenous MT based on the control; MS represents the spraying of 200 μmol L^{-1} exogenous MT based on SA; HS represents the spraying of 100 μmol L^{-1} p-CPA on the basis of MS. **A** H_20_2; **B** O_2^-; **C** Relative conductivity (REC); **D** Malonaldehyde (MDA); **E** SOD; **F** POD; **G** CAT. Vertical bars represent the standard errors of the means (three replicates. Data show the mean ± SE ($n = 3$). Different lowercase letters indicate significant differences between treatments with $P < 0.05$

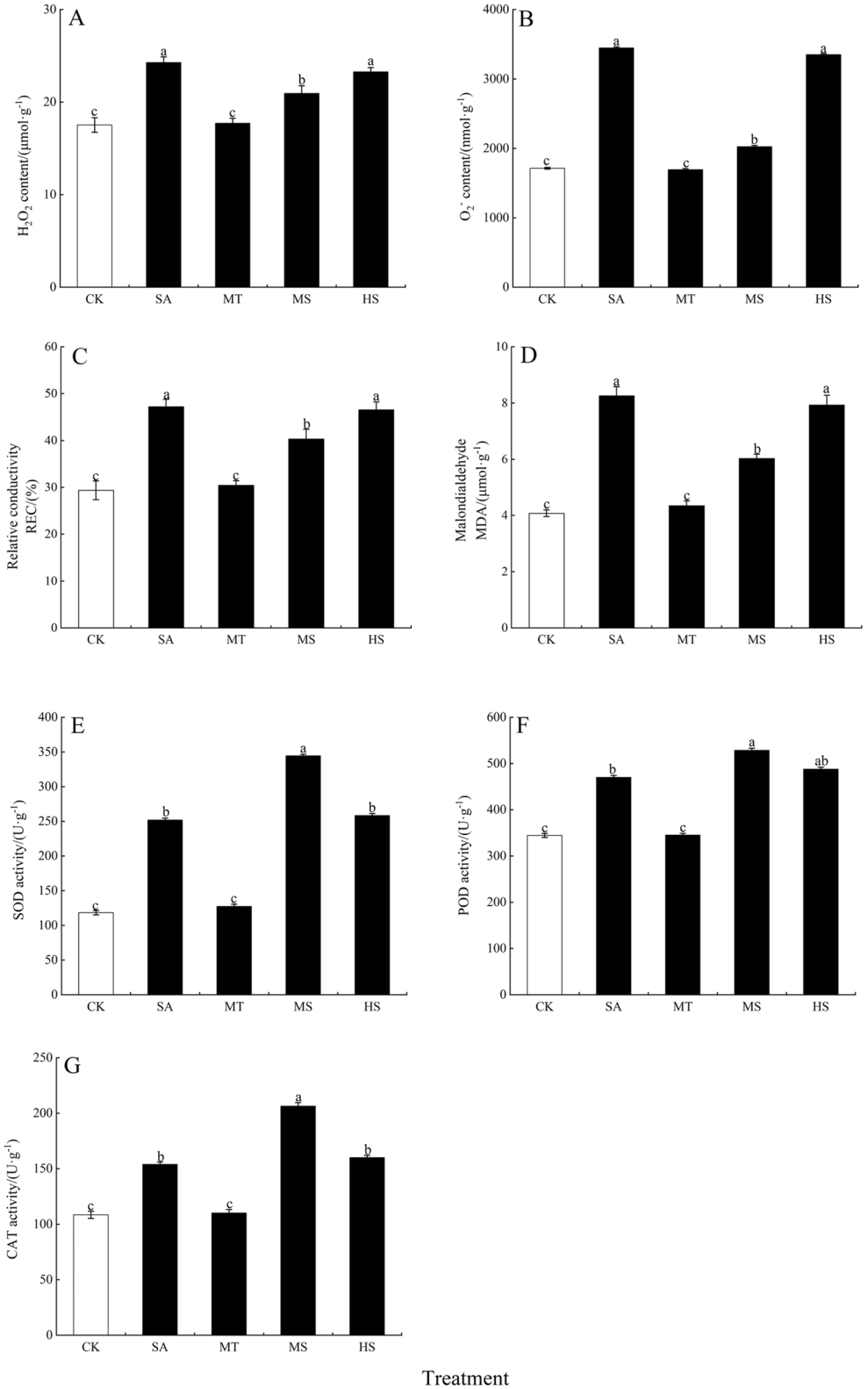


Fig. 5 (See legend on previous page.)

Effects of exogenous MT on ASA-GSH cycle of M9-T337 seedlings under saline-alkaline stress

The activities of AAO, APX, GR, and MDHAR were measured to investigate the impact of exogenous MT on the activities of AsA-GSH cycle-related enzymes in seedling leaves, as shown in Fig. 6A–D. After 15 days of saline-alkaline stress, there was a significant decrease in AAO activity in the leaves. In contrast, the activities of APX, GR, and MDHAR were notably increased, showing significant differences compared to CK treatment. Upon the exclusive application of exogenous MT, the activities of AAO, APX, GR, and MDHAR in MT-treated leaves exhibited a marginal increase, with only AAO activity being significantly different from CK. However, following the spraying of exogenous MT under saline-alkali stress, the activities of AAO, APX, GR, and MDHAR in the leaves of MS-treated plants were 2.6 times, 1.89 times, 2.31 times, and 1.65 times compared to those in SA treatment. However, when MT and p-CPA (HS) were simultaneously applied under saline-alkaline stress, the activities of AAO, APX, GR, and MDHAR in the leaves of M9-T337 seedlings notably decreased by 56.41%, 44.07%, 53.73%, and 37.79%, respectively, compared to the SA treatment. These findings suggest that exogenous MT plays a crucial role in enhancing the activity of AsA-GSH cycle-related enzymes in M9-T337 seedlings under saline-alkaline stress.

Furthermore, the contents of AsA-GSH cycle-related substances (AsA, GSH, and GSSG) in the leaves of different treatments were also assessed. As shown in Fig. 6E–G, there were no significant differences in the contents of AsA, GSH, and GSSG in the leaves of seedlings treated with MT and CK. However, under saline-alkaline stress (SA) treatment, the contents of AsA and GSH in the leaves of M9-T337 seedlings notably decreased by 57.02% and 34.77%, respectively, compared to those treated with CK. In contrast, the content of GSSG significantly increased in the leaves reaching 2.69 times that of CK treatment. Upon the application of MT under saline-alkaline stress, the contents of AsA and GSH in leaves of MS treatment increased to different degrees, which were 2.98 times and 1.21 times that of SA treatment, respectively, while the GSSG content decreased significantly, being 47.23% lower than that of the SA treatment. However, when MT and p-CPA (HS) were simultaneously applied under saline-alkaline stress, there was an impediment to changes in AsA, GSH, and GSSG contents in the leaves of seedlings as indicated by a significant decrease in AsA and GSH contents and a significant increase in GSSG content.

Effects of exogenous MT on Osmotic adjustment of M9-T337 seedlings under saline-alkaline stress

Figure 7 illustrates that only after applying exogenous MT, the levels of Pro (Fig. 7A), SS (Fig. 7B), SP (Fig. 7C), and St (Fig. 7D) in the leaves of the MT treatment did not significantly differ from those of the CK treatment. Conversely, under saline-alkaline stress (SA) treatment, the contents of Pro, SS, SP, and St in the leaves of M9-T337 seedlings significantly increased to reach 2.15 times, 1.19 times, 1.26 times, and 0.48 times than those of the CK treatment, respectively. Subsequently, after the application of exogenous MT under saline-alkaline stress, the levels of Pro, SS, SP, and St in the leaves of M9-T337 seedlings further increased. The content achieved was 1.99 times higher for Pro, 1.37 times higher for SS, 1.14 times higher for SP, and 1.43 times higher for St compared to the SA treatment. However, when MT and p-CPA were simultaneously applied under saline-alkaline stress, the levels of Pro, SS, SP, and St in the leaves of M9-T337 seedlings were significantly lower than those in the MS treatment but showed no significant difference from the SA treatment.

Effects of exogenous MT on endogenous hormone content in leaves of M9-T337 seedlings under saline-alkaline stress

The impact of exogenous MT on the endogenous hormone content in the leaves of M9-T337 seedlings is demonstrated in Fig. 8. After 15 days of saline-alkaline stress, the levels of ZT, GA_3, IAA, and ABA notably increased in the leaves of M9-T337 seedlings compared to the CK treatment. However, there were no discernible differences in the ZT, GA3, IAA, and ABA contents between the CK and MT treatments. Upon the application of exogenous MT under saline-alkaline stress, the ZT, GA_3, and IAA contents in the leaves of the MS treatment increased by 64.85%, 46.69%, and 42.90%, respectively, compared to the SA treatment (Fig. 8A–C), while the

(See figure on next page.)

Fig. 6 Effects of exogenous MT on ASA-GSH cycle of M9-T337 seedlings under saline-alkaline stress. CK represents watering with a 1/2 concentration of Hoagland nutrient solution only; SA represents the application of 100 mmol·L^{-1} composite saline-alkaline solution based on the control; MT represents the spraying of 200 μmol L^{-1} exogenous MT based on the control; MS represents the spraying of 200 μmol L^{-1} exogenous MT based on SA; HS represents the spraying of 100 μmol L^{-1} p-CPA on the basis of MS. **A** APX; **B** AAO; **C** MDHAR; **D** GR; E: AsA; **F** GSH; **G** GSSG. Vertical bars represent the standard errors of the means (three replicates. Data show the mean ± SE ($n = 3$). Different lowercase letters indicate significant differences between treatments with $P < 0.05$

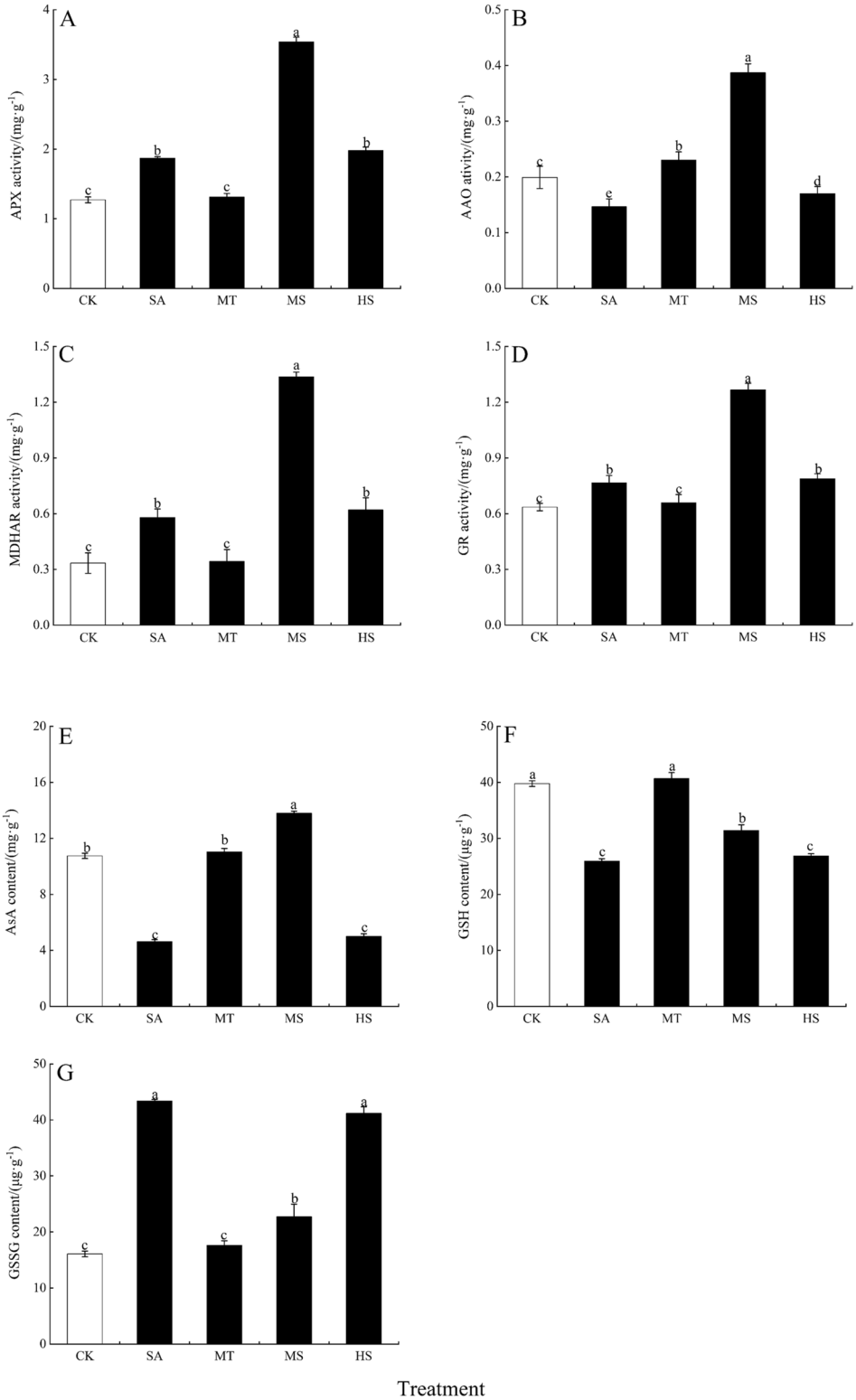


Fig. 6 (See legend on previous page.)

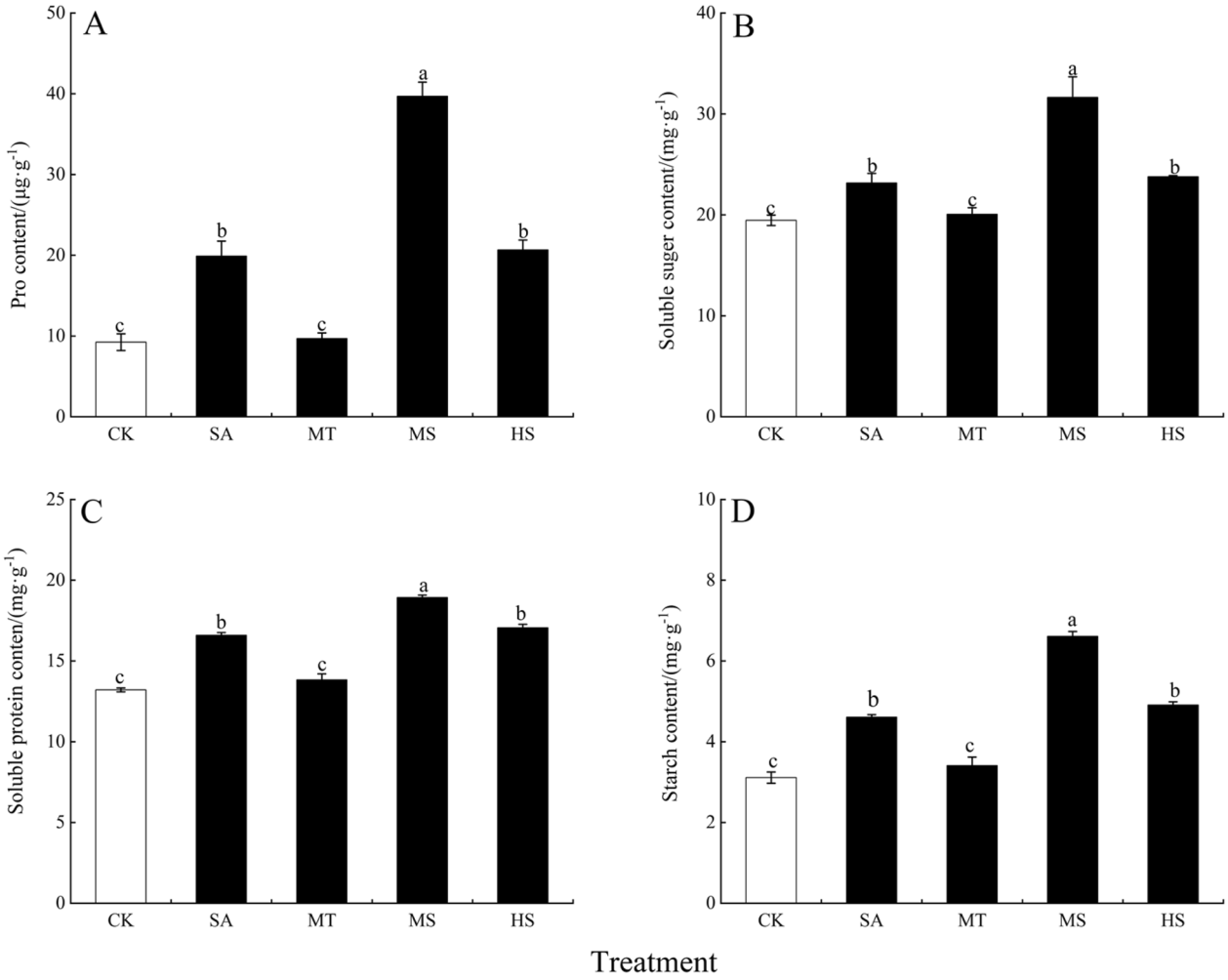


Fig. 7 Effects of exogenous MT on Osmotic adjustment of M9-T337 seedlings under saline-alkaline stress. CK represents watering with a 1/2 concentration of Hoagland nutrient solution only; SA represents the application of 100 mmol·L^{-1} composite saline-alkaline solution based on the control; MT represents the spraying of 200 μmol L^{-1} exogenous MT based on the control; MS represents the spraying of 200 μmol L^{-1} exogenous MT based on SA; HS represents the spraying of 100 μmol L^{-1} p-CPA on the basis of MS. **A** Pro; **B** Soluble sugar; **C** Soluble protein; **D** Starch. Vertical bars represent the standard errors of the means (three replicates. Data show the mean ± SE ($n = 3$). Different lowercase letters indicate significant differences between treatments with $P < 0.05$

ABA content decreased by 20.66% (Fig. 8D). Conversely, simultaneous application of MT and p-CPA under saline-alkaline stress resulted in a reversal of changes in ZT, GA_3, IAA, and ABA contents in the leaves. Specifically, there was a decrease in ZT, GA_3, and IAA contents while the ABA content increased.

Effects of exogenous melatonin on Na^+, K^+, and Ca^{2+} contents in leaves of M9-T337 seedlings under saline-alkaline stress

Figure 9A illustrates that there were no significant differences in the levels of Na^+, K^+, Ca^{2+}, and Na^+/K^+ in the leaves between the MT and CK treatments. However, under saline-alkaline stress (SA) treatment, the Ca^{2+} content in the leaves of M9-T337 seedlings was notably lower than that of the CK treatment. Conversely, compared to the CK treatment, there was a significant increase in the content of Na^+ and K^+, as well as an increase in the Na^+/K^+ ratio. When exogenous MT was applied under saline-alkaline stress, the contents of Ca^{2+} and K^+ in the leaves of the MS treatment significantly increased to 1.18 times and 1.67 times compared with those of the SA treatment, respectively. Meanwhile, there was a significant decrease in Na^+ content and Na^+/K^+ ratio by 20.45% and 53.33%, respectively, compared with the SA treatment.

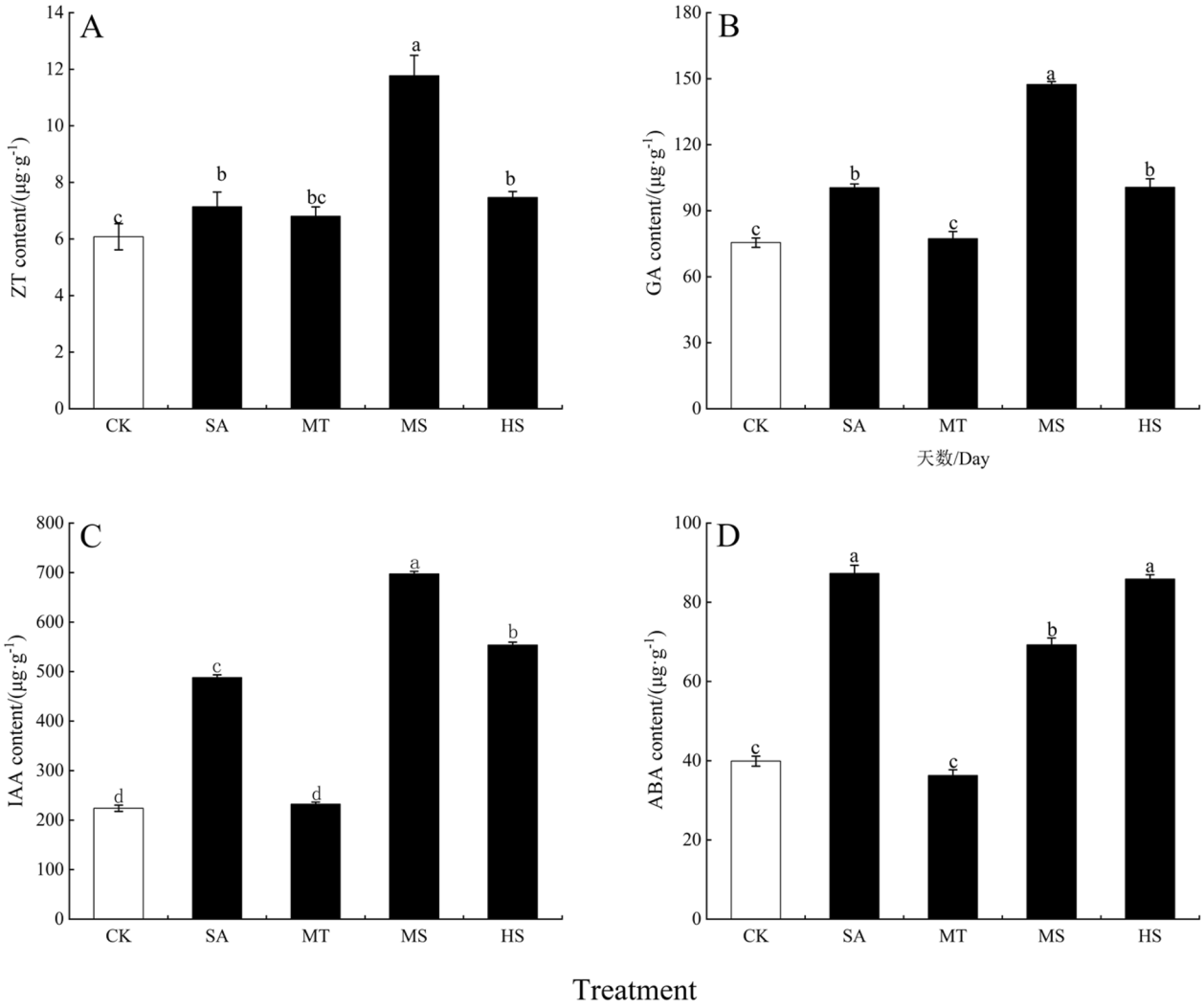


Fig. 8 Effects of exogenous MT on endogenous hormone content in leaves of M9-T337 seedlings under saline-alkaline stress. CK represents watering with a 1/2 concentration of Hoagland nutrient solution only; SA represents the application of 100 mmol·L^{-1} composite saline-alkaline solution based on the control; MT represents the spraying of 200 μmol L^{-1} exogenous MT based on the control; MS represents the spraying of 200 μmol L^{-1} exogenous MT based on SA; HS represents the spraying of 100 μmol L^{-1} p-CPA on the basis of MS. **A** ZT; **B** GA; **C** IAA; **D** ABA. Vertical bars represent the standard errors of the means (three replicates. Data show the mean ± SE ($n = 3$). Different lowercase letters indicate significant differences between treatments with $P < 0.05$

Effects of exogenous melatonin on saline-alkaline response gene expression in M9-T337 seedlings

As shown in Additional file 3: Fig. S3, the expression levels of leaf Na^+ transport genes (*MhCAX5*, *MhSOS*, *MhALT1*, and *MhCHX15*), K^+ transport genes (*MhSKOR*, *MhNHX4*), and antioxidant enzyme regulation genes (*MhPOD*, *MhCAT*, and *MhSOD*) in the MT treatment showed no significant change compared to CK. However, under saline-alkaline stress (SA) treatment, the expression levels of the four Na^+ transporter genes and *MhSKOR* were notably down-regulated, while the expression levels of *MhNHX4* and three antioxidant enzyme regulatory genes were significantly up-regulated. Remarkably, this phenomenon was reversed by MS treatment. However, the co-application of exogenous MT and p-PCA under saline-alkaline stress led to a state that the ability of MT to regulate the above genes was impeded.

Correlation and principal component analysis of various indexes of M9-T337 seedlings under different treatments

Figure 10A shows the results of a correlation analysis performed on 35 physiological indicators of M9-T337 seedlings after treatment. The results show that the *Pn* of M9-T337 seedlings has a highly significant positive

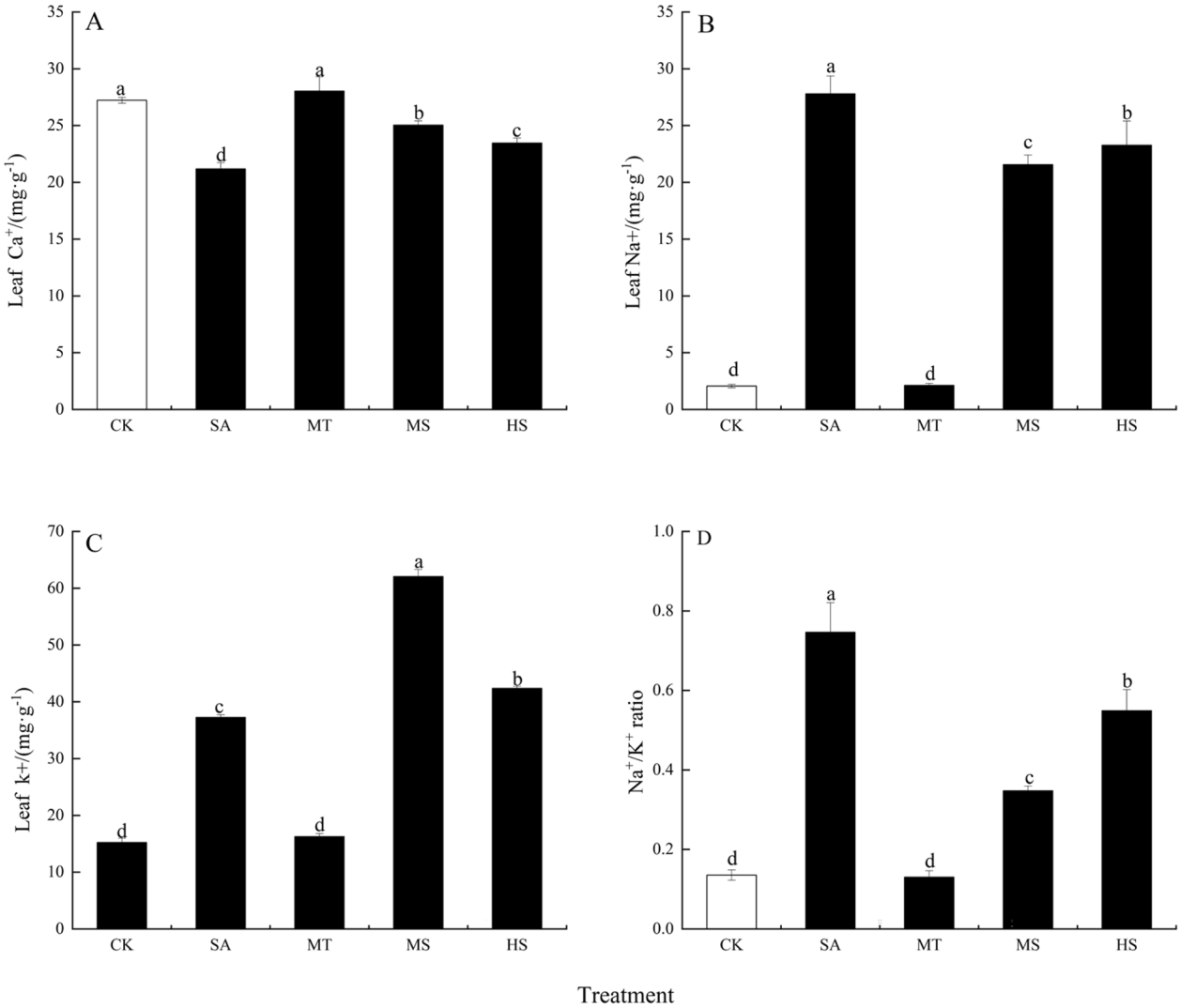


Fig. 9 Effects of exogenous melatonin on Na^+, K^+ and Ca^{2+} contents in leaves of M9-T337 seedlings under saline-alkaline stress. CK represents watering with a 1/2 concentration of Hoagland nutrient solution only; SA represents the application of 100 mmol·L^{-1} composite saline-alkaline solution based on the control; MT represents the spraying of 200 μmol L^{-1} exogenous MT based on the control; MS represents the spraying of 200 μmol L^{-1} exogenous MT based on SA; HS represents the spraying of 100 μmol L^{-1} p-CPA on the basis of MS. **A** Leaf Ca^{2+}; **B** Leaf Na^+; **D** Leaf K^+; **C** Na^+/K^+. Vertical bars represent the standard errors of the means (three replicates. Data show the mean ± SE ($n = 3$). Different lowercase letters indicate significant differences between treatments with $P < 0.05$

correlation with *Tr*, *Gs*, F0, Fm, Fv/Fm, qP, Chl a, Chl b, Chl a + b, Cal, SP, ST, AsA, GSH and root activities ($P < 0.01$). Furthermore, it was significantly positively correlated with AAO ($P < 0.05$), but showed extremely negative correlation with *Ci*, MDA, REC, H_2O_2, O_2^-, GSSG and ABA ($P < 0.01$). Furthermore, it was negatively correlated with SOD, POD and IAA ($P < 0.05$).

To comprehensively evaluate the physiological response of M9-T337 seedlings to exogenous MT under saline-alkaline stress, we performed principal component analysis for 48 indices after treatment. Two principal components with eigenvalues greater than 1 were extracted. The differential contribution rates of the first and second principal components were 75.1% and 20.7%, respectively, and the cumulative variance contribution rate was 95.8%. Comprehensive ranking found that MS treatment was the most effective (Fig. 10B).

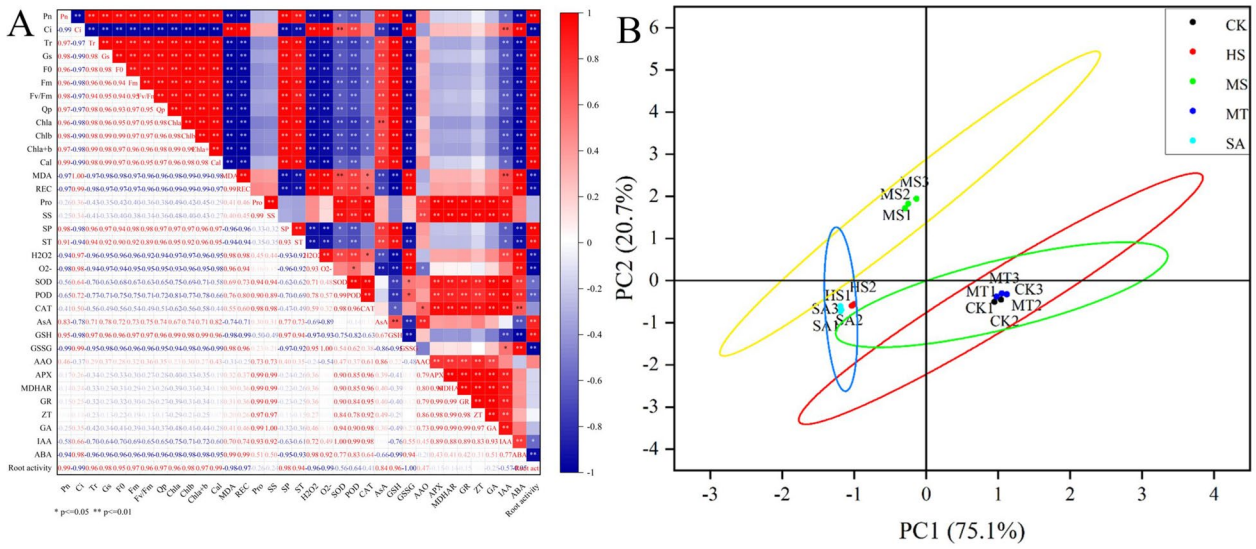


Fig. 10 Correlation and principal component analysis of various indexes of M9-T337 seedlings under different treatments

Discussion

Upon exposure to saline-alkaline stress, plant roots initially generate stress signals. Through self-regulation, these signals are transmitted to the aboveground sections of the plants, subsequently affecting their regular growth and development [48]. Such stress provokes the degradation of photosynthetic pigments within plant leaves, leading in turn to leaf senescence, shrinkage, and a reduction in photosynthetic capacity [49]. Research indicates that the application of exogenous melatonin (MT) can effectively decelerate the decline in chlorophyll content within plant leaves, thereby mitigating the inhibitory effect of saline-alkaline stress on plant photosynthetic capacity [50–52]. In this experiment, the content of Chl a, Chl b, Chl a + b and Car in the leaves of M9-T337 seedlings significantly decreased under saline-alkaline stress, with plant height, stem diameter, dry weight, and fresh weight all significant reductions. This aligns with the findings of Ashraf et al. [53] and He et al. [54], which may be because saline-alkaline stress may induce the increase of soil osmotic pressure, disrupt ion balance in plant cells, impede chlorophyll synthesis, and trigger oxidative stress, impacting photosynthetic pigment content, growth indices, and ultimately leading to diminished photosynthesis, growth, and development of M9-T337 seedlings [55]. Following treatment with exogenous MT, there was a significant increase in biomass accumulation, Chl a, Chl b, Chl a + b, and Car contents in the leaves, along with a considerable rise in root activity and promotion of root development. This enhancement is likely attributable to the ability of exogenous MT to regulate the expression levels of chlorophyll-related genes and promote chlorophyll synthesis, thereby boosting photosynthetic capacity, augmenting biomass accumulation, and stimulating root development, ultimately mitigating the damage to plants caused by saline-alkaline stress [56]. The stability of photosynthetic gas exchange parameters significantly influences plant photosynthesis [57]. Correlation analysis in this experiment revealed a significant positive correlation between *Pn* and Chl a, Chl b, Chl a + b, and Car, indicating that the decline in photosynthetic pigment content may be the key factor leading to reduced photosynthesis. Previous studies have indicated that both stomatal and non-stomatal constraints may contribute to a decrease in *Pn* in plant leaves [58]. A reduction in *Gs* of leaves with a stable or increased *Ci* in stomata indicates that non-stomatal factors, particularly the diminished photosynthetic assimilation capacity of mesophyll cells, are the primary causes of the decline in photosynthetic rate [59]. The results of this experiment indicate that the reduction in Pn of the leaves is caused by non-stomatal limiting factors, signifying decreased photosynthetic activity of mesophyll cells. This decrease could be attributed to saline-alkaline stress hindering photosynthetic electron transport rate and thylakoid protein synthesis, resulting in decreased stomatal conductance, slow CO_2 assimilation, reduced leaf transpiration rate, and photosynthetic rate [60]. Following the application of exogenous MT, *Pn*, *Tr*, and *Gs* of M9-T337 seedlings increased significantly, while *Ci* decreased markedly, which may be because exogenous MT can inhibit the excessive decline in photosynthetic activity of mesophyll cells, maintain the stability of photosynthetic gas exchange parameters in plants, and enhance the photosynthetic efficiency of

plants under stress, which is consistent with the findings of Zhang et al. [61] in cotton. Chlorophyll fluorescence parameters in plants serve as internal indicators of photosynthesis, offering insights into the absorption, transformation, and physiological changes of photosynthetic products in plants. These parameters not only impact the dynamic balance of the carbon cycle, but also play a crucial role in the growth and development of plants [62, 63]. This study uncovered a significant reduction in Fo, Fm, Fv / Fm, and qP in the leaves of M9-T337 seedlings under saline-alkaline stress, suggesting that the potential active sites of PS II in these seedlings were affected, leading to the degradation of PS II receptor-related electron transport proteins, ultimately resulting in decreased light energy utilization and light response inhibition [64–66]. Following exogenous MT treatment, this trend has been reversed, which aligns with the findings of Yan et al. [67]. This enhancement may be attributed to the ability of exogenous MT to diminish the relative efficiency of electron transfer and dissipate a significant amount of light intensity as heat, effectively alleviating the damage of saline-alkaline stress to PS II.

Saline-alkaline stress disrupts the equilibrium between photosynthetic electron transport and the Calvin cycle in plants, leading to the transfer of electrons from chloroplasts and mitochondria to oxygen molecules, consequently generating a significant amount of ROS, especially H_2O_2 and O_2^-. The accumulation of ROS triggers membrane lipid peroxidation, resulting in cell membrane damage, ultimately leading to cellular injury and possible death [68, 69]. REC and MDA content is used to assess the extent of cell membrane damage [70]. Under saline-alkaline stress, there was a noteworthy increase in H_2O_2 and O_2^- levels in the leaves of M9-T337 seedlings, along with a significant rise in REC and MDA levels, mirroring the findings of Weisany et al. [71] and High et al. [72]. Following exogenous MT treatment, this trend was reversed, which was consistent with the outcomes of Liang et al. [73]. This could be attributed to the induction of ROS scavenging genes by exogenous MT to participate in the ROS scavenging process in M9-T337 seedlings under saline-alkaline stress, thereby suppressing membrane lipid peroxidation and preserving the balance of photosynthetic electron transfer and the Calvin cycle [26].

When the concentration of reactive ROS in plants surpasses the normal threshold, the plant's antioxidant system eliminates the excess ROS, with SOD, POD, and CAT playing a crucial role in this process [74, 75]. The findings of this experiment revealed a significant increase in the activities of SOD, POD, and CAT in M9-T337 seedlings under saline-alkaline stress. This elevation may be attributed to the stimulation of the plant's internal defense system (enzymes and non-enzymes) in response to saline-alkaline stress, resulting in heightened Antioxidant enzyme activity in the leaves [76]. Subsequent to exogenous MT treatment, the activities of SOD, POD, and CAT in the leaves of M9-T337 seedlings were further elevated, surpassing those under saline-alkaline stress. This aligns with the results of the study by Chen et al. [77] in maize. This increase is likely due to the exogenous MT regulating the high expression of genes encoding antioxidant enzymes, thereby enhancing the activity of antioxidant enzymes in the leaves, and ultimately timely removing excess ROS [78–80].

The H_2O_2 in plants is predominantly eliminated in the AsA-GSH cycle, which is completed through the combined action of APX, GR, MDHAR, DHAR, AsA, and GSH, repairing the damage caused by free radicals [81]. APX reduces H_2O_2 to H_2O, generating an unstable initial product MDHA, which is subsequently reduced to AsA by MDHAR. Additionally, GSH is oxidized to GSSG, then converted back to GSH by GR through the reduction of coenzyme II, thereby preserving the cellular redox balance [82, 83]. This study observed a significant reduction in AsA and GSH contents in the leaves of M9-T337 seedlings under saline-alkaline stress, alongside a notable increase in GSSG content. After exogenous MT treatment, the contents of AsA, GSH, and GSSG in leaves showed the opposite trend, aligning with the findings of Wu et al. [84]. This could be attributed to the promotion of the conversion of AsA to DHA by exogenous MT and the regulation of the exchange between GSH and GSSG, thus maintaining redox homeostasis and enhancing the saline-alkaline tolerance of M9-T337 seedlings. Furthermore, AAO and APX catalyze the conversion of AsA with H_2O_2 to form MDHA, which is then reduced to AsA by MDHAR. Simultaneously, GSSG is reduced to GSH by GR [85]. The results of this study demonstrated varying degrees of increase in the activities of APX, MDHAR, and GR in the leaves of M9-T337 seedlings under saline-alkaline stress, while AAO activity decreased significantly. Similar to the findings of Xu et al. [86]. Post exogenous MT treatment, the changes of APX, MDHAR, GR and AAO activities in leaves were reversed, consistent with the results of Wu et al. [84]. This may be due to the regulatory effect of exogenous MT on the expression of AsA-GSH cycle-related genes, thereby modulating the corresponding enzyme activity, fortifying antioxidant defense, and enhancing the antioxidant stress resistance of plants.

Osmotic regulators play a crucial role in alleviating the harmful effects of saline-alkaline stress. Soluble sugar, soluble protein, and proline can enhance the stability of the cell membrane by increasing the relative water content and cytoplasmic osmotic pressure in plants, thus

enabling them to resist saline-alkaline stress [87–89]. The results of this study showed that under saline-alkaline stress, the levels of proline, soluble sugar, and soluble protein in the leaves of M9-T337 seedlings significantly increased. After exogenous MT treatment, the content of osmotic regulatory substances in the leaves further increased, which is consistent with the findings of Zhang et al. [90] in sugar beet. This could be attributed to the MT regulate the activities of proline synthase, glycolytic enzyme, and glycosidase, providing antioxidant protection. Additionally, it could modulate the equilibrium of plant hormones and bolster cell membrane stability. These alterations consequently facilitate the synthesis and accumulation of osmotic adjustment substances, thus enabling plants to more proficiently resist saline-alkaline stress.

Plant hormones, as important small-molecule osmotic substances in plants, play a crucial role in plant growth, development, and stress response, despite their low concentration [91]. Complex signaling pathways involve interactions between hormones, regulating plant development and stress responses [92, 93]. Studies have demonstrated that salt stress inhibits rice seed germination, and the application of exogenous gibberellin can mitigate this effect, thereby enhancing the salt tolerance of rice [24]. Additionally, research has indicated an increase in the expression of auxin-related genes in *Arabidopsis thaliana* under salt stress [94]. Furthermore, the results of a specific study revealed a significant increase in the contents of ZT, GA_3, IAA, and ABA in M9-T337 seedling leaves under saline-alkaline stress. Following exogenous MT treatment, there was further increased in the contents of ZT, GA_3, and IAA in the leaves, whereas ABA content decreased. These findings align with the research outcomes of Jia et al. [95] in cherry. It is suggested that exogenous melatonin could influence plant growth, development, cell division, extension, and response to environmental stress by regulating endogenous hormone levels, thus enhancing plant resistance to saline-alkaline stress. This process likely involves a range of physiological, biochemical, and signal transduction mechanisms, warranting further detailed investigation.

Under saline-alkaline stress, ion balance is of paramount importance for plants in maintaining membrane integrity [96]. Numerous studies have demonstrated that an excess of sodium ions infiltrates cells under this stress, leading to a cation imbalance that can cause cell damage and potentially result in cell death [97, 98]. This study's findings revealed a significant down-regulation of the Na + transporter gene and a considerable reduction in the expression of the K^+ transporter gene in plant leaves under saline-alkaline stress. These changes led to a notable increase in Na^+ and Ca^{2+} content, coupled with a significant decrease in K^+ content in the leaves, culminating in an ion imbalance [99]. Nevertheless, the application of exogenous melatonin reversed this trend, reducing Na^+ content and significantly increasing K^+ content, which aligns with the findings reported by Wei et al. [100]. This effect may result from exogenous MT promoting the high expression of Na^+ transporter genes and concurrently down-regulating K^+ transporter genes. Consequently, this facilitates the expulsion of Na^+ from vacuoles, reduces the discharge of K^+ and ultimately maintains the Na^+/K^+ balance within plant cells.

Conclusions

In this study, 200 μmol L^{-1} exogenous MT was used to test the effect of MT on the saline-alkaline tolerance of M9-T337 seedlings. The results demonstrated that exogenous MT treatment significantly enhanced the saline-alkaline tolerance of M9-T337 seedlings, which was evident in the promotion of growth, improvement in osmotic regulation ability, enhancement of antioxidant capacity, facilitation of endogenous reactive oxygen species scavenging, reduction in cell membrane damage, enhancement of photosynthetic rate, mitigation of ion toxicity, and elevation of endogenous hormone content. Furthermore, melatonin induced the biosynthesis of GSH, GSSG, and AsA in the AsA-GSH cycle, and significantly enhancing the activities of APX, DHAR, and MDHAR. Although recent studies have established the positive influence of exogenous MT on enhancing the salinity tolerance of M9-T337 seedlings, further research is required to elucidate the specific mechanism of MT and its synergistic effects with other compounds such as ZT, IAA, GA_3, and ABA. In summary, understanding the intervention pathways and potential biochemical reactions of MT is essential for its improved utilization in boosting the tolerance of M9-T337 seedlings to saline-alkaline stress.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-024-00577-x.

Additional file 1. Effects of exogenous MT on biomass of M9-T337 seedlings

Additional file 2. Effects of exogenous MT on chlorophyll fluorescence parameters of M9-T337 seedlings

Additional file 3. Effects of exogenous MT on the expression of key genes in M9-T337 seedlings

Additional file 4: Table S1. MT concentration screening. **Table S2.** Primer sequences for qRT–PCR. **Table S3.** Effects of exogenous MT on root morphological parameters and root activity of M9-T337 seedlings under saline-alkali stress.

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Author contributions
Xulin Xian and Yanxiu Wang designed the research. Xulin Xian, Zhongxing Zhang, Jiao Cheng and Shuangcheng Wang performed the experiments. Yanlong Gao, Naiying Ma and Cailong Li performed the data analysis and inter-pretation. Xulin Xian and Yanxiu Wang prepared the figures and tables. Xulin Xian wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials
All the data is present inside the manuscript. There is no supplementary file.

Declarations

Ethics approval and consent to participate
We all declare that manuscript reporting studies do not involve any human participants, human data, or human tissue. So, it is not applicable. Experimental research and field studies on plants (either cultivated or wild), including the collection of plant material, must comply with relevant institutional, national, and international guidelines and legislation. No permission is required. Plants material was purchased.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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