Effects of exogenous melatonin on plant growth, root hormones and photosynthetic characteristics of trifoliate orange subjected to salt stress

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Abstract

Soil salinity has negative effects on crop growth and production, and melatonin (MT) plays an important role in regulating plant salt stress. However, it is not clear whether exogenous melatonin mitigates the negative effect on citrus plants subjected to salt stress. This study aimed to explore the response of exogenous melatonin (0, 50, 100, 150 µmol/L) on plant growth, root hormone levels and the photosynthetic system of trifoliate orange (Citrus (Poncirus) trifoliata L.) seedlings exposed to 0 or 150 mmol/L NaCl for 4 weeks. The 150 mmol/L NaCl treatment significantly increased root zeatin riboside, gibberellin and brassinolide levels, while dramatically reducing plant growth, root auxin levels, leaf photosynthesis and fluorescence indexes of seedlings. However, melatonin treatment partially ameliorated reductions in plant height and dry matter accumulation caused by salt stress. Melatonin (100 µmol/L) appears to interact with IAA but not the other hormones studied. Furthermore, the effects of NaCl stress on the net photosynthetic rate, stomatal conductance, maximum photochemical efficiency, PSII effective photon yield, photochemical quenching and other indicators of seedlings leaves were also partially alleviated and the damage of NaCl stress was also reduced when seedlings were treated with melatonin. This suggests that 100 µmol/L melatonin may be an effective treatment.

Keywords Hormones · Melatonin · Photosynthetic · Salt stress · Trifoliate orange

Introduction

Salt stress, one of the important abiotic stresses in the plant kingdom, considerably restricts the normal growth of crops (Stassinos et al. 2021). Cl− and CO32− ions are the main factors associated with soil salinization, which seriously restricts agricultural development in arid areas (Wang et al. 2019). Saline-alkali soils are not conducive to the growth and development of crops, as they can damage crop tissues and result in physiological drought of crops (Zhou et al. 2021). The salt in the soil can make the seeds of crops fail to germinate in the soil and become mouldy (Zhou et al. 2021). Too much salt will also cause the roots of crops to dehydrate and die (Sowmyalakshmi et al. 2015). Salt stress significantly inhibits the growth and dry matter accumulation of plants, resulting in slow plant growth, decreased leaf areas and photosynthetic parameters (He et al. 2005a, b). There are two reasons salt stress inhibits the photosynthesis of plants. Firstly, the toxic effect of inorganic salt ions inhibits the activity of photosynthetic pigments; secondly, excessive inorganic salt ions affect the osmotic potential of plant cells and further affect the photosynthetic rate (Lu et al. 2003). Salt stress can cause a decrease of net photosynthetic rate and reduces the synthesis of organic compounds in plants, eventually affecting normal growth (Lu et al. 2003). Preliminary studies have shown that increased stomatal resistance
results in the decreased photosynthetic rate of plants under salt stress (Ennahli and Earl 2005). In addition, another important factor in response to salt damage is changes in hormone levels (Mahmud et al. 2016). Hormones regulating salt responses can be divided into two groups, namely, growth-related hormones (e.g., auxin-IAA, gibberellins-GAs, brassinosteroids-BRs and cytokinins-CKs) and stress hormones (e.g., salicylic acid-SA, jasmonic acid-JA and abscisic acid-ABA) (Yu et al. 2020).

Melatonin (MT) was first discovered in 1995 as an indole compound, and is a natural phytohormone that occurs in most plants (Dubbels et al. 1995; Sun et al. 2020). In plants, melatonin can increase leaf chlorophyll, regulate the photoperiod and improve tolerance to abiotic and biotic stresses which are similar effects to those of auxin (Tan et al. 2012; Janas and Posmyk 2013; Sun et al. 2020). Melatonin alleviates the damage caused by stress by regulating the transcription level of genes encoding antioxidant enzymes (Tan et al. 2000; Chen et al. 2018). It has been found that both the synthesis of melatonin in plants and the exogenous application of melatonin can effectively improve the adaptability of plants to various stresses (Tan et al. 2000; Chen et al. 2018). Earlier studies found that appropriate concentrations of melatonin can enhance the stress resistance of plants and can improve germination rates, regulate the flowering period, delay the aging of leaves, and promote the formation of roots and lateral roots (Tiryaki and Keles 2012; Park and Back 2012; Byeon and Back 2014; Liang et al. 2018). Application of exogenous melatonin to grape can significantly alleviate the damage caused by water-deficient stress, due to enhanced activity of antioxidant enzymes, increased levels of nonenzymatic antioxidants, and increased amounts of osmoprotectants (Meng et al. 2015). Melatonin also can significantly increase the content of potassium ions and decrease the content of sodium ions, and maintain the stability of ions in maize seedlings under salt stress (Jiang et al. 2016). In cucumber, melatonin can improve the germination rate and root growth under drought stress while at the seedling stage, melatonin also inhibited the adverse effects of drought stress by increasing the photosynthetic rate of leaves and the accumulation of chlorophyll (Zhang et al. 2003; Wang et al. 2016). In addition, MT protects plants against salt stress, such as increasing the chlorophyll contents of tomato seedlings, improving photosynthesis and redox homeostasis in watermelon, and promoting the growth and antioxidant capacity of naked oat seedlings (Li et al. 2017; Gao et al. 2019; Sun et al. 2020). Therefore, previous studies have shown that melatonin can enhance the stress resistance of plants.

Citrus, as an important economic crop, is widely cultivated around the world (Wu et al. 2010a; Zhang et al. 2016; He et al. 2019) and are salt-sensitive horticultural crops (Wu and Zou 2009, 2013; Wu et al. 2010b; Zhang et al. 2017). Due to this, the purpose of the present study was to evaluate the effects of melatonin on plant growth, root hormone levels, and photosynthetic physiology of trifoliate orange Citrus (Poncirus) trifoliata L., a citrus rootstock under salt stress.

Materials and methods

Experimental design.

The experiment was arranged in a $2^2$ factorial completely randomized blocked design: soil with or without NaCl (150 mmol/L) and 4 concentrations of melatonin (MT, N-acetyl-5-methoxytryptamine. Sigma, USA; MT: 0, 50, 100, 150 µmol/L). Each treatment was replicated 6 times, and each replicate had 3 seedlings, for a total of 144 seedlings.

Plant culture.

Five-leaf-old trifoliate orange seedlings were transplanted into 2.0 L pots containing autoclaved (0.11 MPa, 121 ℃, 1.5 h) substrates (soil : sand = 3 : 1, v/v). Two weeks after transporting, the salt and melatonin treatments were applied. The NaCl and MT solutions were watered weekly into each pot. The salt and melatonin treatments were maintained for four weeks until the plants were harvested. The seedlings were grown in a glasshouse of Yangtze University campus between March to May, 2021.

Plant assessment.

At harvest, plant heights and stem diameters were determined. The seedlings were divided into roots and shoots and the dry weights of these parts determined.

Chlorophyll contents, including chlorophyll a (Chla), chlorophyll b (Chlb) and total chlorophyll (Chla + Chlb), were determined using their absorbance at wavelengths of 665, 649 and 470 nm using a spectrophotometer (UH5300, Hitachi of Japan) based on the protocol of Pazurkiewicz-Kocot et al. (2011).

Leaf photosynthetic characteristics, including net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO$_2$ concentration (Ci) and transpiration rate (Tr), were determined using a portable photosynthetic system analyzer (Li-6400, Li-Cor of USA) based on the protocol of Fan et al. (2021).

Leaf chlorophyll fluorescence kinetic parameters, including PSII reaction center actual photochemical efficiency ($\phi$PSII), PSII effective light quantum yield (Fv'/Fm'), photochemical quenching coefficient (qP) and non-photochemical quenching coefficient (NPQ), were determined using a luminoscope (Handy-PEA, Lufthansa of England) based on the protocol of Baker et al. (2008).

Root endogenous hormones, including indole acetic acid (IAA), zeatin riboside (ZR), gibberellin (GAs) and
brassinolide (BRs), were extracted using the protocol of He et al. (2019) and were determined by liquid chromatography-mass spectrometry (LC-MS) based on the protocol of Kojima and Sakakibara (2012).

**Statistical analysis**

Statistical analysis was carried out using two-way ANOVA followed by post hoc test analysis computer software Data Processing System (Version DPSv7.5). Post hoc mean comparison was performed using the LSD tests. A value of P<0.05 was considered significant.

**Results**

For all data sets analyzed the effect of salt-treatment was significant (P<0.05 in all cases).

Plant growth performance.

As shown in Table 1, salt stress suppressed seedling growth. Plant height, stem diameter, total dry weight, shoot and root dry weight were significantly decreased in the salt treatments compared to the non-salt treatments, irrespective of MT status (Table 1). MT treatment had no effect on the growth of the non-salt stressed plants. Under salt stress, plant height was increased by 19.7%, 21.7%, 18.7% with 50, 100 and 150 μmol/L MT treatments, and total dry weight, root and shoot dry weight were significantly increased compared to the appropriate control by 11.9%, 11.9%, 14.7% by the 100 μmol/L MT treatment. 50 μmol/L MT also ameliorated reductions in root dry weight (Table 1). For these data sets, the interactions between the salt and MT treatments were not significant.

Leaf photosynthetic pigment content.

Salt stress significantly decreased leaf photosynthesis pigment indices. Compared to the non-salt treatments, NaCl significantly decreased the levels of chlorophyll a (Chla), chlorophyll b (Chlb) and total chlorophyll content

| Treatments | Plant height (cm) | Stem diameter (mm) | Total dry weight (g) | Root dry weight (g) | Shoot dry weight (g) |
|------------|------------------|--------------------|---------------------|--------------------|---------------------|
| 0 mmol/L NaCl 0 μmol/L MT | 25.13±0.67abc | 2.81±0.07a | 2.01±0.05ab | 0.80±0.01a | 1.22±0.04a |
| 50 μmol/L MT | 25.25±0.80ab | 2.82±0.09a | 2.08±0.06a | 0.83±0.01a | 1.24±0.05a |
| 100 μmol/L MT | 25.61±0.73a | 2.88±0.09a | 2.10±0.09a | 0.84±0.02a | 1.25±0.05a |
| 150 μmol/L MT | 26.12±0.87ab | 2.81±0.08a | 2.07±0.07a | 0.83±0.01a | 1.23±0.05a |
| 150 mmol/L NaCl 0 μmol/L MT | 20.01±0.66d | 2.52±0.06b | 1.68±0.04d | 0.67±0.01c | 1.02±0.05c |
| 50 μmol/L MT | 23.95±0.63c | 2.55±0.06b | 1.79±0.04cd | 0.72±0.03b | 1.06±0.05bc |
| 100 μmol/L MT | 24.35±0.66bc | 2.59±0.06b | 1.88±0.04bc | 0.75±0.02b | 1.17±0.04ab |
| 150 μmol/L MT | 23.76±0.56c | 2.56±0.07b | 1.77±0.03cd | 0.71±0.01bc | 1.08±0.03bc |
| ANOVA NaCl*** | MT*** | Stem diameter*** | Total dry weight*** | Root dry weight*** | Shoot dry weight*** |
| NaCl×MT ns | ns | ns | ns |

The data presented are means (n=6) and standard errors. The different letters followed within the same column indicate the significant differences at P≤0.05. ** means P≤0.05, *** means P≤0.01, ns means not significant. The same as below.

| Treatments | Chla (mg/g) | Chlb (mg/g) | Chla+Chlb (mg/g) | Chla/Chlb |
|------------|------------|------------|-----------------|-----------|
| 0 mmol/L NaCl 0 μmol/L MT | 1.72±0.06ab | 0.72±0.02b | 2.41±0.05bc | 2.28±0.05ab |
| 50 μmol/L MT | 1.79±0.04ab | 0.75±0.03ab | 2.51±0.04ab | 2.38±0.07a |
| 100 μmol/L MT | 1.81±0.06a | 0.79±0.04a | 2.61±0.07a | 2.27±0.06ab |
| 150 μmol/L MT | 1.73±0.06ab | 0.76±0.01ab | 2.47±0.05b | 2.29±0.07ab |
| 150 mmol/L NaCl 0 μmol/L MT | 1.05±0.03d | 0.48±0.01c | 1.48±0.03d | 2.26±0.05ab |
| 50 μmol/L MT | 1.22±0.03c | 0.56±0.02d | 1.88±0.03c | 2.27±0.07ab |
| 100 μmol/L MT | 1.67±0.04b | 0.65±0.01c | 2.30±0.02d | 2.40±0.09a |
| 150 μmol/L MT | 1.23±0.02c | 0.57±0.02d | 1.83±0.05d | 2.19±0.05b |
| ANOVA NaCl*** | MT*** | Chla+Chlb*** | Chla/Chlb ns |
| NaCl×MT ns | ns | ns | ns |

Note: Chla- chlorophyll a, Chlb- chlorophyll b, Chla+Chlb- total chlorophyll content, Chla/Chlb- chlorophyll a / chlorophyll b;
(Chla + Chlb), irrespective of MT status (Table 2). Under non-salt stress, the 100 µmol/L MT treatment significantly increased leaf Chlb, Chla + Chlb by 9.7%, and 8.3%. Under salt stress, compared with control group, MT-treated seedlings contained higher concentrations of Chla and Chlb with the 100 µmol/L treatment showing the least reduction. As for Chla + Chlb, only the 50 µmol/L MT treatment performed significantly differently with a 27.0% increase compared to the control. However, there were no significant difference of Chla/Chlb in all treatments, irrespective of salt stress and MT (Table 2). Regarding the interactions between the main effects, only those relating to Chla and Chla + Chlb were significant.

Leaf photosynthetic parameters.

As shown in Table 3, salt stress significantly decreased the leaf photosynthetic parameters of the seedlings. Compared to the non-salt treatment, 150 mmol/L NaCl dramatically decreased net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO₂ concentration (Ci) and transpiration rate (Tr) by 26.3%, 49.2%, 40.0% and 29.6% (Table 3). MT treatment had no significant effects on Pn, Gs, Ci and Tr under non-salt stress conditions. However, 100 µmol/L MT notably decreased the reductions in Pn, Gs, Ci and Tr by 22.9%, 72.8%, 57.3% and 24.4% and under salt stress, compared with 0 µmol/L MT. The plants in the 50 and 100 µmol/L MT treatments also had significantly higher Gs than the control by 68.5 and 72.8%, respectively (Table 3). For these data sets, the interactions between the main effects were significant.

Leaf fluorescence parameters.

Compared to the non-salt treatments, 150 mmol/L NaCl significantly decreased PSII reaction center photochemical efficiency (ϕPSII), PSII effective light quantum yield (Fv’/Fm’) and photochemical quenching coefficient (qP) by 27.4%, 28.1% and 25.3% (Table 4); MT treatment had no significant effects on on these parameters under non-salt stress conditions. However, under salt stress, MT notably increased ϕPSII, Fv’/Fm’ and qP by 22.2%, 31.1%, 24.4%, 12.9%, 25.8%, 14.5%, and 16.2%, 25.0%, 16.2%, respectively, compared to the appropriate control. The greatest increase in Fv’/Fm’ and qP occurred with MT given at 100 µmol/L (Table 4). However, the level of

### Table 3

| Treatments | Pn (µmol/m².s) | Gs (µmol/m².s) | Ci (µmol/mol) | Tr (mmol/m².s) |
|------------|----------------|----------------|---------------|----------------|
| 0 mmol/L NaCl | 8.15 ± 0.16a | 1.81 ± 0.04a | 200.01 ± 4.31ab | 2.80 ± 0.07a |
| 50 µmol/L MT | 8.24 ± 0.14a | 1.82 ± 0.05a | 210.08 ± 8.37a | 2.83 ± 0.08a |
| 100 µmol/L MT | 8.50 ± 0.14a | 1.82 ± 0.04a | 212.10 ± 7.42a | 2.84 ± 0.09a |
| 150 µmol/L MT | 8.19 ± 0.13a | 1.81 ± 0.05a | 209.07 ± 7.91a | 2.83 ± 0.09a |
| 150 mmol/L NaCl | 6.01 ± 0.14d | 0.92 ± 0.03c | 120.68 ± 4.34d | 1.97 ± 0.04d |
| 50 µmol/L MT | 6.96 ± 0.16bc | 1.55 ± 0.05b | 169.79 ± 6.12c | 2.22 ± 0.06c |
| 100 µmol/L MT | 7.39 ± 0.22b | 1.59 ± 0.04b | 189.88 ± 6.88b | 2.45 ± 0.07b |
| 150 µmol/L MT | 6.77 ± 0.20c | 1.01 ± 0.02c | 168.77 ± 4.63c | 2.21 ± 0.08c |

**ANOVA**

| NaCl | MT | NaCl×MT |
|------|----|---------|
| *** | *** | *** |

**Note:** Pn- net photosynthetic rate, Gs- stomatal conductance, Ci- intercellular CO₂ concentration and Tr- transpiration rate.

### Table 4

| Treatments | ϕPSII | Fv’/Fm’ | qP | NPQ |
|------------|-------|---------|----|-----|
| 0 mmol/L NaCl | 0.62 ± 0.03ab | 0.85 ± 0.01ab | 0.91 ± 0.02ab | 0.60 ± 0.02d |
| 50 µmol/L MT | 0.63 ± 0.02ab | 0.86 ± 0.02ab | 0.92 ± 0.01a | 0.59 ± 0.02d |
| 100 µmol/L MT | 0.66 ± 0.02a | 0.88 ± 0.03a | 0.93 ± 0.03a | 0.58 ± 0.01d |
| 150 µmol/L MT | 0.63 ± 0.02ab | 0.81 ± 0.02bc | 0.91 ± 0.01a | 0.60 ± 0.02d |
| 150 mmol/L NaCl | 0.45 ± 0.01d | 0.62 ± 0.01c | 0.68 ± 0.02d | 1.57 ± 0.05a |
| 50 µmol/L MT | 0.55 ± 0.01c | 0.70 ± 0.01d | 0.79 ± 0.02c | 1.22 ± 0.04b |
| 100 µmol/L MT | 0.59 ± 0.01bc | 0.78 ± 0.02c | 0.85 ± 0.03b | 1.05 ± 0.03c |
| 150 µmol/L MT | 0.56 ± 0.01c | 0.71 ± 0.01d | 0.79 ± 0.01c | 1.21 ± 0.04b |

**ANOVA**

| NaCl | MT | NaCl×MT |
|------|----|---------|
| *** | *** | *** |

**Note:** ϕPSII- PSII reaction center actual photochemical efficiency, Fv’/Fm’- PSII effective light quantum yield, qP- photochemical quenching coefficient and NPQ- non-photochemical quenching coefficient.
non-photochemical quenching coefficient (NPQ) was significantly increased by 161.7% on salt stress, compared with non-salt stress (Table 4), and MT had no effect of NPQ under non-salt-stress conditions, but reduced NPQ to varying degrees particularly at 100 µmol/L, under salt-stress. As a consequence, the damage caused by salt stress on leaf fluorescence parameters was partially restored when seedlings were treated with melatonin. For all data sets there were significant interactions between the main effects.

Root endogenous hormones.

The roots were harvested to measure the levels of indole-acetic acid (IAA), zeatin riboside (ZR), gibberellin (GAs) and brassinolide (BRs). Compared with the non-salt treated seedlings, the salt stressed seedlings had significantly lower levels of IAA (Table 5). By contrast, salt stress observably increased the levels of ZR, GAs and BRs by 29.4%, 44.4%, and 2.3% (Table 5). In non-stressed plants, statistical analysis showed no effects of MT on GAs, BR and ZR. For IAA, under 100 and 150 µmol/L MT-treatment IAA was increased. For the salt-stressed plants, no MT treatment had any effect on ZR, and the 100 µmol/L MT treatment caused small increases in GA and BR (Table 5). However, the decrease IAA caused by salt stress was partly ameliorated by treatment with MT, particularly at 100 µmol/L. For the IAA or GAs data sets, the interactions between the main effects were significant.

Discussion

Salt stress is one of the most problematic abiotic stresses affecting plants in agriculture worldwide. In saline soils, plants try to neutralize the effects of salt stress by physiological changes, leading to decreases in both oxidative and osmotic stresses (Stassinos et al. 2021). Melatonin, an indoleamine widely found in animals and plants, is considered as a candidate phytohormone that affects responses to a variety of biotic and abiotic stresses, such as salt stress (Wei et al. 2015; Li et al. 2019). In the present study, exogenous melatonin treatment alleviated the inhibition of trifoliate orange seedling growth under 150 mmol/L NaCl stress to a certain extent, which is in line with the earlier result reported by Zhang et al. (2014) that melatonin treatment could improve the germination of *Pennisetum alopecuroides* (Linn.) seeds and alleviate the negative effect of salt stress on their subsequent growth. In addition, exogenous melatonin treatment can effectively promote dry matter accumulation, leaf elongation rate and alleviate the inhibition of plant height under salt stress, which imples that melatonin can improve plant resistance to salt stress through osmotic regulation (Li et al. 2019). Also, melatonin promotes soybean growth, seed production, and stress (salt and drought) tolerance by regulating cell division, photosynthesis, carbohydrate metabolism, fatty acid biosynthesis, and ascorbate metabolism (Wei et al. 2015).

When plants are under stress, the photosynthetic rate and the level of chlorophyll in leaves decreases (Harizanova and Koleva-Valkova 2019). The main tissue in which photosynthesis occurs is the leaf, and the amount of chlorophyll directly affects the ability of plants to carry out photosynthesis (Demming and Adams 1996). Salt stress not only affects the synthesis of chlorophyll but also accelerates the decomposition of chlorophyll, resulting in a decrease of chlorophyll (Schreiber et al. 1998). Our results showed that NaCl stress led to the degradation of chlorophyll in leaves, and exogenous melatonin alleviated the damage of NaCl stress on chlorophyll a and chlorophyll b in trifoliate orange leaves, as reported by Kostopoulos et al. (2014) in citrus. Salt stress can also decrease the net photosynthetic rate of plants, reduce the synthesis of organic matter, and ultimately affect the normal growth of plants (Centritto et al. 2003). In cotton, salt stress significantly inhibited seedling growth (Zhang et al. 2014) in *Pennisetum alopecuroides* (Linn.) seeds

| Treatments | IAA (ng/g FW) | ZR (ng/g FW) | GAs (ng/g FW) | BRs (ng/g FW) |
|------------|--------------|--------------|---------------|---------------|
| 0 mmol/L NaCl 0 µmol/L MT | 60.13 ± 0.48b | 5.81 ± 0.05b | 6.01 ± 0.05c | 6.81 ± 0.01c |
| 50 µmol/L MT | 61.25 ± 0.54b | 5.82 ± 0.06b | 6.08 ± 0.03c | 6.83 ± 0.02c |
| 100 µmol/L MT | 66.51 ± 0.56a | 5.80 ± 0.06b | 6.10 ± 0.07c | 6.88 ± 0.03c |
| 150 µmol/L MT | 66.12 ± 0.72a | 5.81 ± 0.05b | 6.07 ± 0.05c | 6.83 ± 0.04c |
| 150 mmol/L NaCl 0 µmol/L MT | 50.01 ± 0.38c | 7.52 ± 0.04a | 8.68 ± 0.04b | 6.97 ± 0.01b |
| 50 µmol/L MT | 52.95 ± 0.39d | 7.53 ± 0.05a | 8.59 ± 0.04b | 7.02 ± 0.02b |
| 100 µmol/L MT | 55.35 ± 0.60c | 7.52 ± 0.05a | 8.89 ± 0.03a | 7.11 ± 0.01a |
| 150 µmol/L MT | 52.76 ± 0.56d | 7.51 ± 0.05a | 8.57 ± 0.02b | 7.01 ± 0.02b |

ANOVA

| NaCl | *** | *** | *** | *** |
| MT | *** | ns | *** | *** |
| NaCl×MT | *** | ns | *** | ns |

Note: IAA- indole acetic acid, ZR- zeatin riboside, GAs- gibberellin and BRs- brassinolide.
growth and biomass accumulation, and decreased leaf area and Pn, Gs, Ci and Tr, which is in line with our results (He et al. 2005a, b). In this study, salt stress decreased leaf Pn, Gs, Ci and Tr while melatonin increased them partially, which is agreement with previous work in cucumber (Harizanova and Koleva-Valkova 2019).

Chlorophyll fluorescence is an effective probe of photosynthesis, through which almost all changes of photosynthesis can be detected (Mimuro et al. 1999). Chlorophyll fluorescence can be used to determine PSII reaction center photochemical efficiency (φPSII), PSII effective light quantum yield ($F_{v'}/F_{m'}$), photochemical quenching coefficient (qP) and non-photochemical quenching coefficient (NPQ) (Farzad et al. 2007). This study showed that salt stress markedly increased NPQ and significantly decreased φPSII, $F_{v'}/F_{m'}$ and qP. Furthermore, to a degree, melatonin alleviated these effects. As the photochemical efficiency of the PSII reaction center, the decrease of φPSII implies that salt stress inhibits photosynthesis (Han et al. 2010). The decrease of $F_{v'}/F_{m'}$ is due to the fact that photosynthetic electron transfer not being carried out in time (Shibata et al. 2012). The decrease of qP indicates that PSII could not transfer photosynthetic electrons efficiently after being damaged, and the effective reaction light has been decreased (Havaux et al. 1991). NPQ is a non-photochemical quenching index reflecting chlorophyll absorption and transformation, and is an effective heat dissipation element used to resist light damage (Tietz et al. 2017). In this experiment, NaCl stress leded to an increase in NPQ, resulting in a decrease in PSII and photosynthetic rate. Melatonin increased φPSII, $F_{v'}/F_{m'}$ and qP and decrease the NPQ in trifoliate orange seedlings under salt stress, implying that melatonin can effectively improve PSII photochemical efficiency and photosynthetic rate of leaves, which is consistent with previous studies on maize and celery (Ye et al. 2016). Thus, melatonin can improve the photosynthetic capacity of plants under NaCl stress.

IAA plays an important role in regulating plant growth under adverse stresses (Iqbal and Ashraf 2007; Zhang et al. 2013, 2018, 2019). As a cytokinin, ZR has been reported to have the ability to enhance plant tolerance to salt and temperature stress (Javid et al. 2011). GAs is an essential for many plants in response to abiotic stress and also take part in plant growth and development (Colebrook et al. 2014). BRs, a kind of steroid hormone, are necessary for plant growth and development, and can tolerate environmental stresses by inducing antioxidant activities (Bajguz and Piotrowska-Niczyporuk 2014). IAA significantly enhanced the tolerance of salt stress in maize (Kaya et al. 2013). Our study showed that there was a significant decrease in root IAA levels under salt stress. However, melatonin notably increased root IAA concentrations. This result is similar to the findings of Liu et al. (2016). Furthermore, IAA is closely related to the growth and development of plant roots (Liu et al. 2018). Therefore, the melatonin effect on IAA is effectively associated with melatonin-induced growth improvement, root modification and salt tolerance. Salt stress slightly increased root ZR, GAs and BRs levels, but melatonin had no or little effect on these hormones. Therefore, melatonin appears to increase plant salt tolerance mainly through interactions with auxin.

Conclusions

Salt stress significantly increased root zeatin riboside, gibberellin and brassinolide levels, while reduces plant growth, root auxin levels, leaf photosynthesis and fluorescence mediate of seedlings. In salt stress, melatonin only increased plant height. However, melatonin seedlings represented similar growth performance and dry matter quality under non-salt stress. At the same time, melatonin (100 μmol/L) appears to interact with the root hormones (IAA, GAs and BRs) except ZR. Furthermore, the effects of salt stress on the net photosynthetic rate, stomatal conductance, maximum photochemical efficiency, PSII effective photon yield, photochemical quenching and other indicators of seedlings leave were practically alleviated when the seedlings were treated with 100 μmol/L melatonin.

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Declarations

Ethical Statements In consideration of the publication, we hereby warrant and undertake: 1. This article is an original work and no portion of the study has been published or is under consideration for publication elsewhere. 2. None of the authors has any potential conflict of interest related to this manuscript. 3. All authors have contributed to the work, and they have agreed to submit the manuscript.

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