

Effects of exogenous melatonin on plant growth, root hormones and photosynthetic characteristics of trifoliolate 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 the salt stress of plants. However, it is not clear whether exogenous melatonin mitigates the negative effect on citrus plants subjected to salt stress. This study was to explore the response of exogenous melatonin (0, 50, 100, 150 $\mu\text{mol/L}$) on plant growth, root hormone levels and photosynthetic system of trifoliolate orange (*Poncirus trifoliata*) seedlings exposed to 0, 150 mmol/L NaCl for 4 weeks. The 150 mmol/L NaCl treatment significantly increased root zeatin riboside and gibberellin levels, while dramatically inhibited plant height, root auxin level, leaf photosynthesis and fluorescence indexes of seedlings. However, melatonin seedlings represented greater growth performance and dry matter quality under non-salt and salt stress. 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 effectively alleviated and the damage of NaCl stress was also reduced when seedlings were treated with melatonin. It suggests that exogenous melatonin had positive effects on enhancing salt tolerance of trifoliolate orange seedlings, which is involved in greater plant growth, higher auxin level, and stronger photosynthetic physiology, and 100 $\mu\text{mol/L}$ melatonin concentration has the best result.

Introduction

Salt stress, one of the important abiotic stresses in plants kingdom, considerably seriously restricts the normal growth of crops (Stassinou et al. 2021). Cl^- and CO_3^{2-} ions are the main factors of soil salinization, which seriously restricts agricultural development in arid areas (Wang et al. 2019). Saline-alkali soil is not conducive to the growth and development of crops, so that crop tissue is damaged, resulting in physiological drought of crops (Zhou et al. 2021). The salt in the soil will make the seeds of crops mouldy and not germinate in the soil (Zhou et al. 2021). Too much salt will cause the root of crops to rot until dehydration and finally death (Sowmyalakshmi et al. 2015). Salt stress significantly inhibited the growth and dry matter accumulation of plants, resulting in slow plant growth, decreased leaf area and photosynthetic parameters (He et al. 2005). There are 2 reasons about salt stress inhibit the photosynthesis of plants. On the one hand, the toxic effect of inorganic salt ions inhibited the activity of photosynthetic pigments; on the other hand, excessive inorganic salt ions affected the osmotic potential of plant cells and further affected the photosynthetic rate (Lu et al. 2003). Salt stress will cause the decrease of net photosynthetic rate and organic compound synthesis of plants, and eventually affect the normal growth of plants (Lu et al. 2003). Preliminary studies have shown that the increased stomatal resistance resulted 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 hormones (Mahmud et al. 2016). The mechanism of hormones in regulating the salt responses can be divided into two groups, namely, positive-related hormones (e.g., auxin- IAA, gibberellins- GAs, brassinosteroids- BRs and cytokinins- CKs) and stressed hormones (e.g., salicylic acid- SA, jasmonic acid- JA and abscisic acid- ABA) (Kosová et al. 2012).

Melatonin (MT) was first discovered in 1995 as an indoles compound, which 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 of plants and then improve the tolerance of plants under abiotic and biotic stresses which similar to auxin (Tan et al. 2012; Janas and Posmyk 2013; Sun et al. 2020). As a new member of phytohormones, melatonin alleviates the damage of plant stress by regulating the gene transcription level of antioxidant enzymes (Tan et al. 2000; Chen et al. 2018). It is 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 the appropriate concentration of melatonin can enhance the stress resistance of plants and improve its germination rate, regulate the flowering period, delay the aging of leaves, promote the formation of roots and lateral roots (Pill 1995; Tiryaki and Keles 2012; Park and Back 2012; Byeon and Back 2014; Liang et al. 2018). In cucumber, melatonin can improve germination rate and root resistance ability 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 increasing the accumulation of chlorophyll content (Zhang et al. 2003; Wang et al. 2016). In tomato, melatonin can significantly increase the chlorophyll in seedlings under salt stress (Sun et al. 2020). Application of exogenous melatonin on grape can significantly alleviate the damage of water-deficient stress, which the mechanism may be ascribed to the enhanced activity of antioxidant enzymes, increased levels of nonenzymatic antioxidants, and increased amount of osmoprotectants (Meng et al. 2015). Melatonin also can significantly increase the content of potassium ion and decrease the content of sodium ion, and then maintain the stability of ion in maize seedlings under salt stress (Jiang et al. 2016). Therefore, previous studies have shown that melatonin can enhance the stress resistance in 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). Also, citrus plants are one of salt- sensitive horticultural crops (Wu and Zou 2009; Wu et al. 2010b; Wu and Zou 2013; Zhang et al. 2017). In this background, the purpose of the present study was conducted to evaluate melatonin effects on plant growth, root hormone levels, and photosynthetic physiology of trifoliate orange (*Poncirus trifoliata* L. Raf., a citrus rootstock) under salt stress. The physiological mechanism of alleviating citrus salt stress by exogenous melatonin was discussed fully.

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 $\mu\text{mol/L}$). So there were 8 treatments: 0 mmol/L NaCl+0 $\mu\text{mol/L}$ MT, 0 mmol/L NaCl+50 $\mu\text{mol/L}$ MT, 0 mmol/L NaCl+100 $\mu\text{mol/L}$ MT, 0 mmol/L NaCl+150 $\mu\text{mol/L}$ MT, 150 mmol/L NaCl+0 $\mu\text{mol/L}$ MT, 150 mmol/L NaCl+50 $\mu\text{mol/L}$ MT, 150 mmol/L NaCl+100 $\mu\text{mol/L}$ MT, 150

mmol/L NaCl+150 μ mol/L MT. Each treatment was replicated 6 times, and each replicate had 3 seedlings, for a total of 144 seedlings.

Plant culture

The 5-leaf-old trifoliolate orange seedlings were transplanted into 2.0 L pots containing autoclaved (0.11 MPa, 121 °C, 1.5 h) substrates (soil : sand = 3 : 1, v/v). After two weeks of transplanted, salt and melatonin treatments were applied. The designed NaCl and MT solutions were weekly watered into each pot. Such salt and melatonin treatments were maintained for four weeks until the plants were harvested. These seedlings were grown in a glasshouse of Yangtze University campus between March to May, 2021.

Variable determinations

At harvesting, plant height and stem diameter were determined by a ruler. The whole seedlings were divided into the roots and the shoots for gain the data of total dry weight, root dry shoot and shoot dry weight.

chlorophyll content, including chlorophyll a (Chla), chlorophyll b (Chlb) and total chlorophyll content (Chla+Chlb), were determined for absorbance at wavelength of 665, 649 and 470 nm by spectrophotometer (UH5300, Hitachi of Japan).

Leaf photosynthetic characteristics, including net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO₂ concentration (Ci) and transpiration rate (Tr), were determined by portable photosynthetic system analyzer (Li-6400, Li-Cor of USA).

Leaf chlorophyll fluorescence kinetic parameters, including PSII reaction center actual photochemical efficiency (ϕ PSII), PSII effective light quantum yield (Fv'/Fm'), photochemical quenching coefficient (qP) and non-photochemical quenching coefficient (NPQ), were determined by 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 by the protocol of He et al. (2019) and were determined by liquid chromatography-mass spectrometry (LC-MS) based on the protocol of Kojima and Sakakibar (2012).

Statistical analysis

The data were statistically analyzed according to the variance (ANOVA) (SAS software 8.1v), and the significant difference between the treatments was compared with the Duncan's multiple range tests at $P < 0.05$.

Results

Plant growth performance

As shown in Table 1, salt stress had suppressed trifoliolate orange seedlings' growth observably. 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). 4 concentrations of MT treated seedlings showed better growth performance, especially the 100 $\mu\text{mol/L}$ MT. Treated with 100 $\mu\text{mol/L}$ MT conferred significantly increased plant height, stem diameter, total plant dry weight, root and shoot dry weight by 5.5%, 2.5%, 4.5%, 5.0% and 2.5% under non-salt stress and by 21.75%, 27.8%, 11.9%, 11.9% and 14.7% under salt stress (Table 1). Therefore, the damage of salt stress on plant growth was reduced when seedlings were treated with melatonin.

Table 1
Effects of exogenous melatonin (MT) on plant growth performance of trifoliolate orange seedlings under non-salt (0 mmol/L) and salt stress (150 mmol/L) conditions.

Treatments		Plant height (cm)	Stem diameter (mm)	Total dry weight (g)	Root dry weight (g)	Shoot dry weight (g)
0 mmol/L NaCl	0 $\mu\text{mol/L}$ MT	25.13 \pm 1.26b	2.81 \pm 0.15a	2.01 \pm 0.12a	0.80 \pm 0.02a	1.22 \pm 0.08a
	50 $\mu\text{mol/L}$ MT	26.25 \pm 1.59ab	2.82 \pm 0.16a	2.08 \pm 0.11a	0.83 \pm 0.02a	1.24 \pm 0.09a
	100 $\mu\text{mol/L}$ MT	26.51 \pm 1.34a	2.88 \pm 0.17a	2.10 \pm 0.19a	0.84 \pm 0.03a	1.25 \pm 0.11a
	150 $\mu\text{mol/L}$ MT	26.12 \pm 1.98ab	2.81 \pm 0.12a	2.07 \pm 0.13a	0.83 \pm 0.02a	1.23 \pm 0.12a
150 mmol/L NaCl	0 $\mu\text{mol/L}$ MT	20.01 \pm 1.01d	2.52 \pm 0.10b	1.68 \pm 0.09c	0.67 \pm 0.01c	1.02 \pm 0.07c
	50 $\mu\text{mol/L}$ MT	23.95 \pm 1.36c	2.55 \pm 0.12b	1.79 \pm 0.08bc	0.72 \pm 0.02bc	1.06 \pm 0.08bc
	100 $\mu\text{mol/L}$ MT	24.35 \pm 1.79bc	2.59 \pm 0.15b	1.88 \pm 0.07b	0.75 \pm 0.03b	1.17 \pm 0.11b
	150 $\mu\text{mol/L}$ MT	23.76 \pm 1.47c	2.56 \pm 0.14b	1.77 \pm 0.06bc	0.71 \pm 0.01bc	1.08 \pm 0.06bc

Leaf photosynthetic pigment content

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

and total chlorophyll content (Chla+Chlb), irrespective of MT status (Table 2). The MT treatments, especially the 100 $\mu\text{mol/L}$ MT, significantly increased leaf Chla, Chlb, Chla+Chlb by 5.3%, 8.9%, and 8.3% under non-salt stress and by 59.0%, 35.4%, and 55.4% under salt stress (Table 2). However, there had no significant difference of Chla/Chlb in all treatments, irrespective of salt stress and MT (Table 2). Hence, the damage of salt stress on leaf photosynthesis pigment was reduced when seedlings were treated with melatonin.

Table 2
Effects of exogenous melatonin (MT) on photosynthetic pigment content in leaves of trifoliolate orange seedlings under non-salt (0 mmol/L) and salt stress (150 mmol/L) conditions.

Treatments		Chla (mg/g)	Chlb (mg/g)	Chla+Chlb (mg/g)	Chla/Chlb
0 mmol/L NaCl	0 $\mu\text{mol/L}$ MT	1.72±0.16ab	0.72±0.05ab	2.41±0.12b	2.28±0.12a
	50 $\mu\text{mol/L}$ MT	1.79±0.11a	0.75±0.06a	2.51±0.11ab	2.38±0.20a
	100 $\mu\text{mol/L}$ MT	1.81±0.14a	0.79±0.07a	2.61±0.19a	2.27±0.13a
	150 $\mu\text{mol/L}$ MT	1.73±0.12ab	0.76±0.02a	2.47±0.13ab	2.29±0.17a
150 mmol/L NaCl	0 $\mu\text{mol/L}$ MT	1.05±0.05d	0.48±0.03c	1.48±0.09d	2.26±0.14a
	50 $\mu\text{mol/L}$ MT	1.22±0.06c	0.56±0.04bc	1.88±0.08c	2.27±0.16a
	100 $\mu\text{mol/L}$ MT	1.67±0.09b	0.65±0.03b	2.30±0.07b	2.40±0.21a
	150 $\mu\text{mol/L}$ MT	1.23±0.07c	0.57±0.05bc	1.83±0.11c	2.19±0.11a

Note: Chla- chlorophyll a, Chlb- chlorophyll b, Chla+Chlb- total chlorophyll content, Chla/Chlb- chlorophyll a / chlorophyll b; The different letters followed within the same column indicate the significant differences at $P < 0.05$. The same as below.

Leaf photosynthetic parameters

As shown in Table 3, salt stress significantly decreased leaf photosynthetic parameters of seedlings. Compared to the non-salt treatment, 150 mmol/L NaCl dramatically decreased the levels of 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). Although, MT treatment had not significant effects on Pn, Gs, Ci and Tr under non-salt stress. However, 100 $\mu\text{mol/L}$ MT notably increased Pn, Gs, Ci and Tr by 22.9%, 72.8%, 57.3% and 24.4% under salt stress (Table 3). So, the decrease of salt stress on leaf photosynthesis was partially promoted when seedlings were treated with melatonin.

Table 3

Effects of exogenous melatonin (MT) on photosynthetic parameters of trifoliolate orange seedlings leaves under non-salt (0 mmol/L) and salt stress (150 mmol/L) conditions.

Treatments		Pn ($\mu\text{mol}/\text{m}^2.\text{s}$)	Gs ($\mu\text{mol}/\text{m}^2.\text{s}$)	Ci ($\mu\text{mol}/\text{mol}$)	Tr ($\text{mmol}/\text{m}^2.\text{s}$)
0 mmol/L NaCl	0 $\mu\text{mol}/\text{L}$ MT	8.15 \pm 0.76ab	1.81 \pm 0.12a	200.01 \pm 10.19ab	2.80 \pm 0.15a
	50 $\mu\text{mol}/\text{L}$ MT	8.24 \pm 0.59a	1.82 \pm 0.13a	210.08 \pm 20.15a	2.83 \pm 0.19a
	100 $\mu\text{mol}/\text{L}$ MT	8.50 \pm 0.34a	1.82 \pm 0.10a	212.10 \pm 18.10a	2.84 \pm 0.22a
	150 $\mu\text{mol}/\text{L}$ MT	8.19 \pm 0.46ab	1.81 \pm 0.16a	209.07 \pm 19.17a	2.83 \pm 0.26a
150 mmol/L NaCl	0 $\mu\text{mol}/\text{L}$ MT	6.01 \pm 0.41c	0.92 \pm 0.07c	120.68 \pm 11.99c	1.97 \pm 0.11c
	50 $\mu\text{mol}/\text{L}$ MT	6.96 \pm 0.39bc	1.55 \pm 0.12b	169.79 \pm 15.58bc	2.22 \pm 0.12bc
	100 $\mu\text{mol}/\text{L}$ MT	7.39 \pm 0.63b	1.59 \pm 0.11b	189.88 \pm 16.07b	2.45 \pm 0.21b
	150 $\mu\text{mol}/\text{L}$ MT	6.77 \pm 0.41bc	1.00 \pm 0.04c	168.77 \pm 14.09bc	2.21 \pm 0.19bc

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

Leaf fluorescence parameters

Salt stress changes leaf fluorescence parameters of seedlings. Compared to the non-salt treatments, 150 mmol/L NaCl significantly decreased the levels of PSII reaction center actual 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). Although, MT treatment had not significant effects on ϕPSII , Fv'/Fm' and qP under non-salt stress. However, under salt stress, 3 concentrations of MT notably increased ϕPSII , Fv'/Fm' and qP by 22.2%, 31.1%, 24.4%, and 12.9%, 25.8%, 14.5%, and 16.2%, 25.0%, 16.2%, respectively (Table 4). However, the level of non-photochemical quenching coefficient (NPQ) was significantly increased by 161.7% on salt stress, compared with non-salt stress (Table 4). And, MT reduced NPQ to varying degrees, irrespective of salt status. In consequence, the damage of salt stress on leaf fluorescence parameters was partially restored when seedlings were treated with melatonin.

Table 4

Effects of exogenous melatonin (MT) on leaf fluorescence parameters of trifoliate orange seedlings under non-salt (0 mmol/L) and salt stress (150 mmol/L) conditions.

Treatments		ϕ PSII	Fv'/Fm'	qP	NPQ
0 mmol/L NaCl	0 μ mol/L MT	0.62 \pm 0.06a	0.85 \pm 0.05a	0.91 \pm 0.02a	0.60 \pm 0.04d
	50 μ mol/L MT	0.63 \pm 0.05a	0.86 \pm 0.06a	0.92 \pm 0.03a	0.59 \pm 0.05d
	100 μ mol/L MT	0.66 \pm 0.04a	0.88 \pm 0.07a	0.93 \pm 0.09a	0.58 \pm 0.03d
	150 μ mol/L MT	0.63 \pm 0.05a	0.81 \pm 0.05a	0.91 \pm 0.03a	0.60 \pm 0.05d
150 mmol/L NaCl	0 μ mol/L MT	0.45 \pm 0.03c	0.62 \pm 0.02c	0.68 \pm 0.05c	1.57 \pm 0.13a
	50 μ mol/L MT	0.55 \pm 0.04b	0.70 \pm 0.04b	0.79 \pm 0.06b	1.22 \pm 0.12b
	100 μ mol/L MT	0.59 \pm 0.03ab	0.78 \pm 0.05ab	0.85 \pm 0.07ab	1.05 \pm 0.09c
	150 μ mol/L MT	0.56 \pm 0.01b	0.71 \pm 0.02b	0.79 \pm 0.05b	1.21 \pm 0.11b

Note: ϕ PSII- PSII reaction center actual photochemical efficiency, Fv'/Fm' - PSII effective light quantum yield, qP- photochemical quenching coefficient and NPQ- non-photochemical quenching coefficient.

Root endogenous hormones

The trifoliate orange seedlings roots have been harvested for measuring the levels of phytohormones, such as indoleacetic acid (IAA), zeatin riboside (ZR), gibberellin (GAs) and Brassinolide (BRs). Compared with non-salt seedlings, salt stress seedlings had significantly lower level of IAA (Table 5). By contrast, salt stress observably increased the levels of ZR and GAs by 29.4 % and 44.4% (Table 5). However, there has no difference of BRs concentration between non-salt and salt treatments. With regards to MT, its treatments increased root IAA level under non-salt and salt stress conditions in varying degrees, of which 100 μ mol/L MT has the best result (Table 5). However, MT had not significant effects on root ZR, GAs and BRs of trifoliate orange seedlings, irrespective of non-salt and salt stress (Table 5).

Table 5

Effects of exogenous melatonin (MT) on concentrations of root endogenous hormones of trifoliate orange seedlings under non-salt (0 mmol/L) and salt stress (150 mmol/L) conditions.

Treatments		IAA (ng/g FW)	ZR (ng/g FW)	GAs (ng/g FW)	BRs (ng/g FW)
0 mmol/L NaCl	0 μ mol/L MT	60.13 \pm 1.26ab	5.81 \pm 0.15b	6.01 \pm 0.12b	6.80 \pm 0.02a
	50 μ mol/L MT	61.25 \pm 1.59ab	5.82 \pm 0.16b	6.08 \pm 0.11b	6.83 \pm 0.02a
	100 μ mol/L MT	66.51 \pm 1.34a	5.80 \pm 0.17b	6.10 \pm 0.19b	6.88 \pm 0.03a
	150 μ mol/L MT	66.12 \pm 1.98a	5.81 \pm 0.12b	6.07 \pm 0.13b	6.83 \pm 0.02a
150 mmol/L NaCl	0 μ mol/L MT	50.01 \pm 1.01c	7.52 \pm 0.10a	8.68 \pm 0.09a	6.97 \pm 0.01a
	50 μ mol/L MT	52.95 \pm 1.36bc	7.53 \pm 0.12a	8.59 \pm 0.08a	7.02 \pm 0.02a
	100 μ mol/L MT	55.35 \pm 1.79b	7.52 \pm 0.15a	8.88 \pm 0.07a	7.11 \pm 0.03a
	150 μ mol/L MT	52.76 \pm 1.47bc	7.51 \pm 0.14a	8.57 \pm 0.06a	7.01 \pm 0.01a

Note: IAA- indole acetic acid, ZR- zeatin riboside, GAs- gibberellin and BRs- brassinolide.

Discussion

Salt stress is one of the most problematic abiotic stress affecting plants in agriculture worldwide. In saline soils, plants try to neutralize the effects of salt stress by physiological changes, leading to the decreasing of both oxidative and osmotic stresses (Stassinis 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 as reported by Zhang et al. (2014) that melatonin treatment could improve the germination ability of *Pennisetum alopecuroides* (Linn.) seeds and alleviate the negative effect of salt stress on its 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 imply that melatonin improved plant resistance to salt stress through osmotic regulation (Li et al. 2019). Also, melatonin promotes soybean growth, seed production, and stresses (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 of leaves were decreases (Harizanova and Koleva-Valkova, 2019). The main medium of plant photosynthesis is leaf,

and the amount of chlorophyll content directly affects the ability of plant to carry out photosynthesis (Demming and Adams, 1996). Salt stress not only affect the synthesis of chlorophyll but also accelerate the decomposition of chlorophyll, resulting in the decrease of chlorophyll content (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 similarly reported by Kostopoulou et al. (2014) in citrus. Salt stress could 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 the seedlings growth and biomass accumulation, and decreased leaf area and the indexes of Pn, Gs, Ci and Tr, which is in line with our results (He et al. 2005). 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). The possible mechanism is that melatonin can improve the content of chlorophyll, promote the activity of PSII and effectively regulate the photosynthesis of plant leaves under salt stress, resulting in improve the salt tolerance of plants.

Chlorophyll fluorescence is an effective probe of photosynthesis, through which almost all changes of photosynthesis can be detected (Mimuro et al. 1999). Chlorophyll fluorescence contains PSII reaction center actual photochemical efficiency (ϕPSII), PSII effective light quantum yield (Fv'/Fm'), photochemical quenching coefficient (qP) and non-photochemical quenching coefficient (NPQ) (Farzad et al. 2007). This study showed that salt stress markedly increased NPQ while significantly decreased ϕPSII , Fv'/Fm' and qP. Furthermore, melatonin alleviated the increase and decrease degree of them. As actual photochemical efficiency of the PSII reaction center, the decrease of ϕPSII indicated that the photosynthetic electron decreased under carbon fixation, resulting in the decrease of net photosynthetic rate, which explained the mechanism of salt stress inhibiting photosynthesis (Han et al. 2010). The decrease of Fv'/Fm' is due to the fact that photosynthetic electron transfer is not carried out in time after light capture, but directly consumes light energy through chlorophyll transformation, which leads to the decrease of PSII (Shibata et al. 2012). The index of qP reflects the degree to which the photoreaction center is effectively applied in the photochemical reaction. The decrease of qP indicated that PSII could not transfer photosynthetic electrons smoothly after being damaged, and the effective reaction light decreased (Havaux et al. 1991). NPQ is a non-photochemical quenching index, reflecting the process of 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 led to an increase in NPQ, resulting in a decrease in PSII and photosynthetic rate. Melatonin increased the index of ϕPSII , Fv'/Fm' 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 an cytokinin (CTK), ZR has been reported to have the ability to enhance plant salt tolerance and temperature stress (Javid et al. 2011). GAs are 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 hormones, are necessary for plant growth and development, and can tolerate environmental stresses by inducing antioxidant activities (Bajguz and Piotrowska-Niczyporuk, 2014). IAA could 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 of melatonin and non-melatonin trifoliate orange seedlings under the salt stress versus the non-salt stress. At the same time, melatonin treatment notably increased root IAA concentration. The 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. However, the salt stress significantly increased root ZR and GAs levels, while melatonin has no effect with ZR and GAs in this study. Perhaps there has no interaction between melatonin and ZR or GAs. In this study, melatonin did not significantly alter root BRs concentration in non-salt and salt trifoliate orange seedlings. Perhaps in salt stress, the regulation of melatonin to other phytohormones is sufficient for plants to resist salt stress. So, melatonin increased plant salt tolerance mainly through interaction with auxin.

Conclusion

The salt stress significantly increased root zeatin riboside and gibberellin levels, while dramatically inhibited plant height, root auxin level, leaf photosynthesis and fluorescence indexes of seedlings. However, melatonin seedlings represented greater growth performance and dry matter quality under non-salt and salt stress. 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 leaves were effectively alleviated and the damage of salt stress was also reduced when seedlings were treated with melatonin. Under salt stress, melatonin can promote the growth of trifoliate orange seedlings, significantly increase the content of photosynthetic pigments in seedlings leaves, effectively improve PSII photochemical efficiency and photosynthetic rate of seedlings, and then enhance the salt tolerance of seedlings. Melatonin enhances plant salt tolerance in 2 ways: one is via direct pathways, such as the direct clearance of reactive oxygen species; the other is via an indirect pathway by enhancing photosynthetic efficiency, antioxidant enzyme activity and metabolite content, and by regulating transcription factors associated with salt stress. In addition, many results consider that melatonin probably achieved its promotional roles in plants through enhancement of genes involved in cell division, photosynthesis, phytohormone, carbohydrate metabolism, fatty acid biosynthesis, and ascorbate metabolism. Our results demonstrate that melatonin has significant potential for improvement of plant growth and salt tolerance. Further study should uncover more about the molecular mechanisms of melatonin's function in plant kingdom.

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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