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Application of Melatonin-Enhanced Tolerance to High-Temperature Stress in Cherry Radish (*Raphanus sativus* L. var. *radculus pers*)

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Abstract

The growth and development of cold-season plants are susceptible to high temperature. Melatonin is a plant growth regulator with potential to improve plant tolerance to biological and abiotic stresses. In our study, cherry radish (*Raphanus sativus* L. var. *radculus pers*) was cultured at a high temperature (35 °C/30 °C day/night) and different concentrations (0, 11.6, 17.4, 29.0, 34.8 and 67.0 mg L^{-1}) of melatonin were applied to these high-temperature stressed plants to its effects on biomass, quality, antioxidant enzyme activity, chlorophyll and endogenous hormone contents. The plants were grown under normal temperature (25 °C/20 °C) as control and high-temperature condition as HT-stress treatment. The results revealed that under high temperature with 29.0 mg L^{-1} melatonin treatment, cherry radish biomass was significantly increased by 12.9%, and the soluble protein and soluble solid were increased by 18.7 and 9.2%, respectively. The activity of antioxidant enzyme, ascorbate peroxidase and peroxidase were increased by 43.7 and 45.5%, respectively. The chlorophyll a and carotenoid contents were increased by 7.4 and 20.0% compared with the control at 27 days. The auxin and abscisic acid contents were significantly increased by 28.5 and 6.7% compared with HT at 9 days. Thus, application of optimal rate of melatonin had a positive effect on cherry radish growth under high-temperature stress.

Keywords Melatonin · High-temperature stress · Yield · Soluble protein · Antioxidant enzyme activity · Endogenous hormone

Introduction

High-temperature (HT) stress has been one of the major limiting factors for plant growing and is leading to severe decline in global crop production (Wilson et al. 2014). The obvious symptoms of high-temperature injury include rolled leaves, folded leaves, dehydration and so on (Sharma et al. 2016). Moreover, toxic metabolites and reactive oxygen species (ROS) are produced in damaged cells due to metabolic abnormalities (Wahid et al. 2007). In extreme environments, high-temperature stress accelerates crop senescence, deceases plant productivity (Porter 2005) and sometimes results in death (Locato et al. 2010). Hence, the development of measures to induce high-temperature stress tolerance is crucial in plants and attracts considerable concern (Baninasab and Ghobadi 2011). Approaches taken to develop thermostability plants include gene project (Grover et al. 2013), breeding (Jha et al. 2015), and application of growth regulators (Zou et al. 2016). However, genetic engineering is costly. In addition, breeding is time consuming and labor intensive. Generally, growth regulators, as exogenous non-nutritive chemical substances, can be conducted to the action site in the plant. Moreover, growth regulators could promote or inhibit some aspects of plant life processes at low concentrations, with low cost and no need for more manpower.

Melatonin (*N*-acetyl-5-methoxytryptamine) acts as a type of plant growth regulator and has been widely discovered in

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the plant kingdom (Fan et al. 2015; Hardeland 2016). Studies by Ahammed et al. (2018a) showed that under high-temperature stress conditions, melatonin was essential for the maintenance of photosynthetic ability of tomato. Some research indicated that the application of melatonin increased the antioxidant enzymes activity, for instance, superoxide dismutase (SOD) (Zhang et al. 2017), furthermore, it increased enzyme activity associated with the AsA-GSH period, for instance, ascorbate peroxidase (APX). In conclusion, melatonin affects photosynthesis (Arnao and Hernández-Ruiz 2015), root system structure (Pelagio-Flores et al. 2012; Zhang et al. 2014), organ development (Arnao and Hernández-Ruiz 2014), senescence (Byeon et al. 2012; Wang et al. 2013; Shi et al. 2015c), defense (Weeda et al. 2014), and stress reaction (Byeon and Back 2014; Kostopoulou et al. 2015; Zhang et al. 2015) in plants. Over the past few years, a growing number of studies have been carried out on the physiological functions of melatonin in plants. One of its most remarkable features is to act as the first line of defense against oxidative stress both inside and outside (Tan et al. 2012). Plants with relatively high-melatonin level have been discovered to be more resistant to stress (Zhang et al. 2015).

Cherry radish (*Raphanus sativus* L. var. *radculus pers*) was studied for its short growth period, abundant nutrition and good economic benefits (Liu et al. 2017). The optimum growth temperature is 5–25 °C, Supraoptimal temperatures damaged the antioxidant enzymes activities and chlorophyll content in cherry radishes (Chen et al. 2014). However, few studies have paid attention to the molecular mechanisms of melatonin application on cherry radish under heat-induced stress, although other crops, such as citrus (Kostopoulou et al. 2015) and rice (Byeon and Back 2014), have been investigated. It is hypothesized that cherry radish might be an indicator crop for reactions to high-temperature stress. However, whether the addition of exogenous melatonin could improve the heat resistance of cherry radish by changing its physiological characteristics under high-temperature stress has not been solved. In the current research, the effects of adding melatonin on antioxidant enzyme activity, chlorophyll and endogenous hormone levels were investigated by intermittent high-temperature stress on cherry radish. We found that in cherry radish, the addition of exogenous melatonin increased its antioxidant system enzyme activity, endogenous hormone content, which in turn increased its biomass and improved quality.

Materials and Methods

Melatonin was purchased from Shanghai Aladdin Bio-Chem Technology Co (Shanghai, China) and cherry radish seeds were obtained from the BEJO China (Shanghai, China). Two seeds were sown at 0.3 cm depth in each nursery box (10 × 8 × 7 cm) filled with container substrates. The nursery boxes were placed in an artificial climate incubator (50–60% relative humidity, 25 °C/20 °C day/night temperature) with a light intensity of 15,000 lx and photoperiod of 16/8 h (day/night). Seedlings were subjected treatments with six rates (0, 11.6, 17.4, 29.0, 34.8, 67.0 mg L^{-1}) of melatonin and two temperature regimes (25 °C/20 °C or 35 °C/30 °C for day/night) during 27-day growing period. The growth period of cherry radish is normally about 27 days (Liu et al. 2017). The details of seven treatments were presented in Table 1 and each treatment was replicated three times. Five solutions of melatonin were prepared based on treatments by diluting the stock solution (6.96 g L^{-1}). The diluted melatonin solution (10 mL) with 40-mL Hoagland solution (Hoagland and Arnon 1950) per pot was applied as substrate irrigation at 7, 16, and 25 days. The Hoagland solution was used as fertilizers for plant to grow and for enhancing distribution of melatonin in the root zone. Tissue samples were collected after each high-temperature stress treatment at 9, 18 and 27 days (Table 1).

Antioxidant Enzyme and Malondialdehyde (MDA) assay

Under the chilled condition of liquid nitrogen cooling, leaf samples were pestle uniformly with a mortar in 0.05 mol L^{-1} phosphate buffer (pH 7.8). The homogenate was screening by filter paper and centrifuged at 4 °C for 20 min at 10,000×*g*. All spectrophotometric analyses were conducted on a SHIMADZU UV-2450 spectrophotometer (Kyoto, Japan). In the light of Stewart and Bewley's method (1980), Determination

Table 1 Experimental treatments

Treatment ID	HT (control for HT-stress)	MT-1	MT-2	MT-3	MT-4	MT-5	CK
Melatonin (mg L^{-1})	0	11.6	17.4	29.0	34.8	67.0	0
1–7 days	25 °C/20 °C (day/night)						25 °C/20 °C (day/night)
7–9 days	35 °C/30 °C (day/night) high-temperature stress for 48 h						
9–16 days	25 °C/20 °C (day/night)						
16–18 days	35 °C/30 °C (day/night) high-temperature stress for 48 h						
18–25 days	25 °C/20 °C (day/night)						
25–27 days	35 °C/30 °C (day/night) high-temperature stress for 48 h						

of SOD activity by measuring its capacity to inhibit photochemical reduction of nitroblue tetrazolium. Catalase (CAT) activity was determined according to the method of Patra et al. (1978), and the absorbance at 240 nm decreased due to the decrease in the extinction of H_2O_2. Peroxidase (POD) activity was determined by the increase in absorbance at 470 nm caused by guaiacol oxidation. The MDA content in fresh leaves was determined by the method of thiobarbituric acid reaction (Heath and Packer 1968).

Assay of Chlorophyll Content of Leaves

The chlorophyll level was measured in the light of Knudson and others' method (Knudson et al. 1977). Fresh leaf tissue (0.5 g) was extracted in the dark with 2 mL of 95% (v/v) ethanol for 24 h, and the concentration of chlorophyll a, b and carotenoid in the extract was determined by a spectrophotometer (SHIMADZU UV-2450, Kyoto, Japan), and the absorbance readings were taken at 665, 649 and 470 nm, respectively.

Quality, Rubisco, ascorbate peroxidase, glutathione reductase (GR) activity, auxin (IAA), abscisic acid (ABA) and proline (Pro) were estimated using the ELISA kit from Shanghai HengYuan Biological Technology Co. Ltd. (Shanghai, China) as formerly narrative (Shi et al. 2015a, b).

Observation of the Ultrastructure of the Chloroplast

The chloroplast ultrastructure was observed as characterized by Xu et al. (2008). Leaves were cut with scissors into pieces of about 1 mm^2. The leaf pieces were impregnated in the transmission electron microscope in a 0.1 M phosphate buffer (pH 7.4) for 2 h and then soaked in the identical buffer with 1% osmic acid for 5 h. After dehydration in acetone and embedded in 812 resin, ultrathin sections were cut and stained continuously for 15 min with uranyl acetate in pure ethanol and leas citrate, and observed under a HITACHI transmission electron microscope (Carl Zeiss, Göttingen, Germany) at 80 kV acceleration voltage.

Statistical Analyses

Microsoft Excel 2010 was used for processing data and SigmaPlot 12.5 was used for drawing of figures. Data were analyzed using analyses of variance (ANOVAs) with mean separations performed with Duncan tests ($P \leq 0.05$), all conducted in SAS Version 9.2, (SAS, 2012).

Results

Effects of Melatonin on Cherry Radish Biomass and Quality

As a cool season crop, cherry radish is relatively susceptible to high temperatures. As shown by the CK treatment, high-temperature stress significantly inhibited cherry radish plant growth. However, at 27 days, compared with HT, the inhibition was alleviated by 17.4 and 29.0 mg L^{-1} MT, especially in the 29.0 mg L^{-1} MT treatment. Compared with that in the HT treatment, the biomass (fresh weight of whole radish) in the MT-2 and MT-3 treatments was increased by 7.6 and 12.9% (Fig. 1). The soluble protein, soluble solid and vitamin C (Vc) content in the MT-2 treatment were significantly increased compared to HT (Table 2). The soluble protein and soluble solid in the MT-3 treatment were increased by 18.7 and 9.2%, respectively, compared to HT. Both of these indexes in the MT-3 treatment were significantly increased compared with CK. Furthermore, the NO_3^- content in the MT-2 and MT-3 treatments were significantly decreased compared with CK. These results indicated that MT improves plant growth and radish quality. It appears that there is an optimal range of MT concentrations for mitigating high-temperature stress.

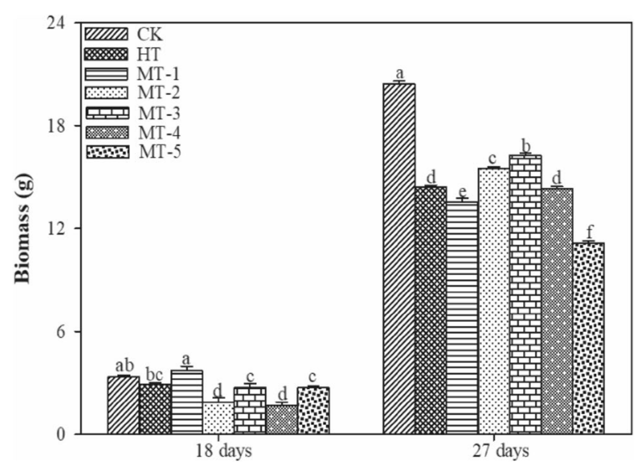

Fig. 1 Biomass of cherry radish with different treatments. CK (25 °C/20 °C day/night temperature treatment without melatonin); HT (35 °C/30 °C day/night temperature treatment without melatonin); MT-1(35 °C/30 °C day/night temperature treatment with 11.6 mg L^{-1} melatonin); MT-2 (35 °C/30 °C day/night temperature treatment with 17.4 mg L^{-1} melatonin); MT-3 (35 °C/30 °C day/night temperature treatment with 29.0 mg L^{-1} melatonin); MT-4 (35 °C/30 °C day/night temperature treatment with 34.8 mg L^{-1} melatonin); MT-5 (35 °C/30 °C day/night temperature treatment with 67.0 mg L^{-1} melatonin). Bar heights represent means and error bars represent ± SE. Within each graph, means followed with the same letter were significantly different based on a one-way ANOVA followed by Duncan's multiple-range tests ($P \leq 0.05$)

Table 2 Quality of cherry radish root with different treatments of cherry radishes

Treatment	Soluble protein (mg g^{-1} FW)	Soluble solid (%)	Vc content (mg g^{-1})	NO_3^- content (mg kg^{-1})
CK	32.55 ± 0.30e	4.09 ± 0.02bc	1.88 ± 0.02e	145.8 ± 3.72b
HT	43.06 ± 0.12c	3.93 ± 0.04 cd	2.13 ± 0.01b	133.2 ± 5.17bc
MT-1	30.14 ± 0.42f	4.45 ± 0.19a	1.95 ± 0.02d	99.0 ± 1.59d
MT-2	48.38 ± 0.43b	4.25 ± 0.06ab	2.37 ± 0.01a	106.8 ± 4.12d
MT-3	51.12 ± 0.46a	4.29 ± 0.06ab	2.10 ± 0.01b	128.4 ± 3.43c
MT-4	36.38 ± 0.11d	3.76 ± 0.05de	1.90 ± 0.02e	148.2 ± 7.09b
MT-5	51.36 ± 0.53a	3.52 ± 0.12e	2.05 ± 0.01c	183.6 ± 5.59a

CK (25 °C/20 °C day/night temperature treatment without melatonin); HT (35 °C/30 °C day/night temperature treatment without melatonin); MT-1(35 °C/30 °C day/night temperature treatment with 11.6 mg L^{-1} melatonin); MT-2 (35 °C/30 °C day/night temperature treatment with 17.4 mg L^{-1} melatonin); MT-3 (35 °C/30 °C day/night temperature treatment with 29.0 mg L^{-1} melatonin); MT-4 (35 °C/30 °C day/night temperature treatment with 34.8 mg L^{-1} melatonin); MT-5 (35 °C/30 °C day/night temperature treatment with 67.0 mg L^{-1} melatonin). Means within each column followed by the same letters were not significantly different based on one-way ANOVAs followed by Duncan's multiple-range tests ($P \leq 0.05$)

Effects of Melatonin on the Antioxidant Enzyme Activities of Cherry Radish

Because of the oxidative stress of cherry radish caused by high-temperature treatment, then we discussed the behavior of the antioxidant enzymes. It demonstrated that high-temperature stress decreased the activity of SOD, POD, and CAT at 27 days significantly (Table 3), while exogenous application of suitable MT concentration to the high-temperature growth medium markedly enhanced the enzymes activity. SOD, POD, and CAT activities in leaves of cherry radishes growing at the high temperature were increased by 26.0, 45.5 and 76.5%, respectively, compared with HT, at 27 days.

High-temperature treatment inhibited activities of APX and GR in cherry radish leaves at 9 days, whereas a suitable concentration of MT application alleviated this inhibition (Table 3). For instance, MT-3 treatment improved APX and GR activity by 6.6% and 90.6%, respectively, compared to HT at 9 days. The application of MT-3 decreased the accumulation of MDA in leaves of the high-temperature-stressed plants during the growth period (Table 3).

Effects of Melatonin on the Chlorophyll Content of Cherry Radish

Suitable MT concentration improved chlorophyll a and carotenoid significantly (Fig. 2a) contents in plant leaves, compared with HT. Chlorophyll a and carotenoid content were increased by 7.4% and 20.0% in MT-3 treatment, compared to HT. Compared with HT, MT-2 treatment significantly increased chlorophyll b (Fig. 2a) content by 12.2%. Nevertheless, the difference in Rubisco activity was not significant

Effects of Melatonin on Endogenous Hormone and Pro Content of Cherry Radish

The contents of IAA and ABA in leaves rapidly increased after high-temperature stress in the MT-2 and MT-3 treatments at 9 days (Fig. 3a, b), and then decreased later at 18 and 27 days. MT-3 treatment significantly increased IAA and ABA contents by 28.5% and 6.7%, respectively, compared to HT at 9 days. Compared with HT, the melatonin content in leaves of cherry radish increased at the beginning period and decreased in the later period of growth, and the Pro content showed the opposite trend.

Discussion

Under high-temperature stress at 35 °C, plants develop various strategies to cushion the damage due to high temperature, including strategies at the biochemical, physiological and transcriptome levels. Cherry radish is a cool-season crop and is relatively susceptible to high-temperature stress. In the study, the results showed that the heat treatment had a certain inhibitory effect on the growth and physiological parameters of cherry radish, and the suitable MT concentration pretreatment had a certain effect on the inhibition of high-temperature stress. Similar results were observed that the application of exogenous melatonin improved the high-temperature tolerance of arabidopsis (Antoniou et al. 2017) and drought tolerance of alfalfa (Zhang et al. 2013), respectively. Additionally, under oxidative stress, the exogenous application of melatonin was shown to increase the bermudagrass roots' fresh weight and plant height and then promote growth (Shi et al. 2015c). The results indicated that MT might be effective in improving the heat tolerance in crops.

Table 3 Antioxidant enzyme activities and MDA content of cherry radishes

Treatment	SOD activity (U g^{-1} FW)			POD activity (U g^{-1} min^{-1} FW)		
	9 days	18 days	27 days	9 days	18 days	27 days
CK	821.83 ± 20.15a	801.68 ± 4.61a	643.57 ± 14.96c	13.80 ± 0.46b	7.20 ± 0.60d	6.50 ± 0.26d
HT	593.79 ± 14.28c	743.34 ± 22.15b	509.96 ± 15.73d	3.59 ± 0.10e	6.60d ± 0.92d	5.78 ± 0.17e
MT-1	573.14 ± 9.18bc	561.28 ± 23.27c	453.33 ± 6.14e	6.67 ± 0.64d	7.93 ± 0.13 cd	11.96 ± 0.13a
MT-2	–	527.35 ± 5.65c	685.25 ± 6.75b	–	9.00 ± 0.35c	7.82 ± 0.37c
MT-3	829.34 ± 22.38a	527.65 ± 15.98c	642.46 ± 7.28c	15.46 ± 0.90b	13.20 ± 0.35a	8.41 ± 0.22c
MT-4	649.01 ± 14.70b	539.11 ± 3.02c	726.48 ± 12.51a	9.49 ± 0.14c	11.51 ± 0.16b	8.02 ± 0.08c
MT-5	686.58 ± 18.13b	525.93 ± 28.41c	716.72 ± 35.64ab	21.68 ± 0.99a	9.22 ± 0.41b	9.48 ± 0.19b
Treatment	CAT activity (U g^{-1} min^{-1} FW)			MDA content (μmol g^{-1} FW)		
	9 days	18 days	27 days	9 days	18 days	27 days
CK	23.90 ± 0.52b	18.83 ± 1.21a	11.32 ± 0.12a	3.54 ± 0.16a	3.98 ± 0.19e	9.28 ± 0.16a
HT	21.27 ± 0.48b	14.39 ± 2.00b	4.69 ± 0.19e	2.67 ± 0.12b	7.90 ± 0.29a	6.13 ± 0.05e
MT-1	16.73 ± 0.93c	11.43 ± 0.53bc	4.71 ± 0.11e	1.34 ± 0.15d	5.30 ± 0.24 cd	6.66 ± 0.15d
MT-2	–	14.22 ± 0.38b	7.50 ± 0.12c	–	4.65 ± 0.16d	7.33 ± 0.06c
MT-3	20.80 ± 0.64b	11.96 ± 0.80bc	8.28 ± 0.09b	1.67 ± 0.09 cd	4.70 ± 0.16d	5.48 ± 0.06f
MT-4	21.80 ± 1.80b	10.32 ± 0.12c	7.09 ± 0.11d	0.06 ± 0.01e	6.56 ± 0.13b	7.99 ± 0.10b
MT-5	29.36 ± 1.33a	9.17 ± 0.23c	8.31 ± 0.09b	1.97 ± 0.15c	5.56 ± 0.27c	5.63 ± 0.19f
Treatment	APX activity (mIU L^{-1})			GR activity (IU L^{-1})		
	9 days	18 days	27 days	9 days	18 days	27 days
CK	98.24 ± 0.58b	95.07 ± 0.48c	55.45 ± 1.28f	374.83 ± 5.43e	357.15 ± 3.16e	279.19 ± 5.84f
HT	86.06 ± 1.38d	117.57 ± 0.93a	79.24 ± 0.83e	309.32 ± 5.79 g	514.69 ± 5.12a	601.22 ± 5.15a
MT-1	90.57 ± 1.13c	80.90 ± 1.05d	97.80 ± 0.43c	559.40 ± 3.25b	521.44 ± 3.16a	532.06 ± 1.87b
MT-2	99.04 ± 1.40b	65.94 ± 1.08e	94.17 ± 0.64d	350.91 ± 1.38f	461.13 ± 1.80c	408.14 ± 3.81d
MT-3	91.76 ± 1.26c	65.68 ± 0.74e	113.90 ± 1.23b	589.55 ± 5.99a	412.78 ± 3.25d	402.38 ± 1.87d
MT-4	117.44 ± 0.83a	56.54 ± 1.03f	81.35 ± 0.73e	406.19 ± 2.29d	334.27 ± 3.64f	427.23 ± 1.43c
MT-5	54.29 ± 1.39e	101.16 ± 0.40b	127.08 ± 1.45a	530.80 ± 1.87b	483.49 ± 1.38b	353.75 ± 3.74e

On the 9th day of the table, MT-2 treatment was unable to determine the relevant indicators due to insufficient sampling weight at seedling stage CK (25 °C/20 °C day/night temperature treatment without melatonin); HT(35 °C/30 °C day/night temperature treatment without melatonin); MT-1 (35 °C/30 °C day/night temperature treatment with 11.6 mg L^{-1} melatonin); MT-2 (35 °C/30 °C day/night temperature treatment with 17.4 mg L^{-1} melatonin); MT-3 (35 °C/30 °C day/night temperature treatment with 29.0 mg L^{-1} melatonin); MT-4 (35 °C/30 °C day/night temperature treatment with 34.8 mg L^{-1} melatonin); MT-5 (35 °C/30 °C day/night temperature treatment with 67.0 mg L^{-1} melatonin). Means within each column followed by the same letters were not significantly different based on one-way ANOVAs followed by Duncan's multiple-range tests ($P \leq 0.05$)

Melatonin Modulates Activity of Enzymatic Antioxidants Under High-Temperature Stress

Generally, high-temperature stress stimulates the formation of ROS, which leads to electrolyte leakage and lipid peroxidation, and the antioxidant enzyme activities were increased following high-temperature treatment. Among them, the superoxide radical was disproportionated by SOD to H_2O_2 and was further scavenged by CAT and peroxides (such as POD) by conversion into H_2O. Exogenous MT application was confirmed to alleviate oxidative damage caused by abiotic stresses to maintain ROS homeostasis (Shi et al. 2015a; Xu et al. 2016; Yadu et al. 2018). Additionally, some researchers have revealed that exogenous melatonin increased the level of endogenous melatonin in *CAFFEIC ACIDO-METHYLTRANSFERASE 1*-silenced tomato and mitigated the high-temperature-induced oxidative stress (Ahammed et al. 2019). It also symbolized the vital role of exogenous melatonin in crop antioxidant stress. In the study, in the 29.0 mg L^{-1} MT treatment, the SOD, POD and CAT activities were increased, compared to HT at 27 days (Table 3), but the level of oxidants was reduced in cherry radish after exogenous application of 29.0 mg L^{-1} MT which improved heat-stress tolerance in cherry radish by enhancing biomass (Fig. 1). The results were consistent with the discovers from other researchers who confirmed that MT pretreatments mitigated oxidative stress in crops (Xu et al. 2016;

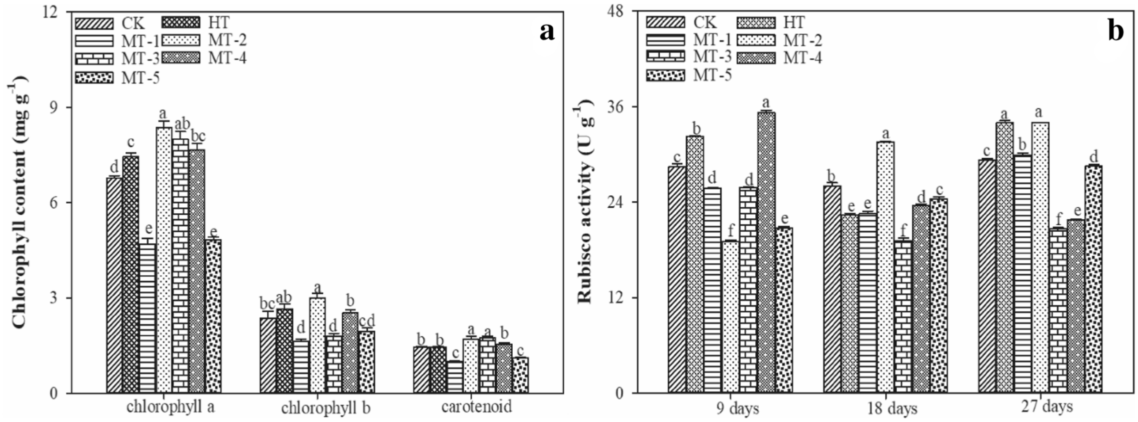

Fig. 2 Chlorophyll a, b, and carotenoid contents (**a**) at 27 days and Rubisco activity (**b**) of cherry radish with different treatments. CK (25 °C/20 °C day/night temperature treatment without melatonin); HT(35 °C/30 °C day/night temperature treatment without melatonin); MT-1(35 °C/30 °C day/night temperature treatment with 11.6 mg L^{-1} melatonin); MT-2 (35 °C/30 °C day/night temperature treatment with 17.4 mg L^{-1} melatonin); MT-3 (35 °C/30 °C day/night temperature treatment with 29.0 mg L^{-1} melatonin); MT-4 (35 °C/30 °C day/night temperature treatment with 34.8 mg L^{-1} melatonin); MT-5 (35 °C/30 °C day/night temperature treatment with 67.0 mg L^{-1} melatonin). Bar heights represent means and error bars represent ± SE. Within each graph, means followed with the same letter were significantly different based on a one-way ANOVA followed by Duncan's multiple-range tests ($P \leq 0.05$)

Antoniou et al. 2017). Thence, the reduction of oxidants and the increase of antioxidant enzyme in MT pretreated seedlings were associated with an increase in crop heat tolerance.

As crops grow, some stress resistance develops and there is no need to produce more GR. Therefore, the GR content of cherry radish was higher in early phase d and reduced in the middle and later phase. Adding suitable MT in the early growth stage promoted the generation of GR and improved the resistance of seedlings to high-temperature stress. The APX could promote the generation of ascorbic acid in addition to improving the stress resistance of crops (Khan et al. 2011). The content of APX in MT-3 treatment was higher than that in the HT in the early growth stage of cherry radish but only by a small amount. The reason for the significant increase of APX activity in the later growth stage was the synthesis of ascorbic acid. With the increase of cherry radish fruit, the quality was improved. MDA was a key index to measure the extent of membrane lipid peroxidation, and its reduction also confirmed that the addition of an appropriate concentration of MT can promote the antioxidant system removal of reactive oxygen species to resist high-temperature stress. Flavonoids such as epigallocatechin-3-gallate also showed great effect on improving crop stress resistance, which increased the antioxidant enzyme activity of tomato under salt stress and reduced oxidative stress (Ahammed et al. 2018b). Compared with epigallocatechin-3-gallate, melatonin exhibited the same resistance, both of which increased SOD, POD, APX enzyme activity and relieved stress damage.

Melatonin Alleviated High-Temperature Stress by Enhancing Chlorophyll Content

Ahammed et al. (2018a) indicated that the lack of endogenous melatonin resulted in a decrease in chlorophyll content in tomato, while exogenous addition significantly increased the chlorophyll fluorescence parameters. In the study, adding appropriate concentration of melatonin treatment improved the chlorophyll content of cherry radish, even when the self-defense system of cherry radish was perfect in the later growth stage. It also indicated that adding melatonin at appropriate concentration could alleviate the damage to chloroplasts and thylakoids caused by high-temperature stress to a certain extent in Fig. 4. Compared to CK, HT treatment increased contents of chlorophyll a (Fig. 2). Previous studies indicated similar results for lemon (Martin et al. 1995) and tomato (Camejo and Torres 2001) possibly due to the light-harvesting complexes to stress condition.

Effects of Exogenous Melatonin on Endogenous Hormone Under High-Temperature Stress

Plant hormones play a pivotal role in resisting biological and abiotic stress and regulating crop developmental processes (Robert-Seilaniantz et al. 2007). In some plants, stress tolerance has been related to morphological adaptations, which is obviously regulated by specific hormones (Arbona and Go'mez-Cadenas 2008; Etehadnia et al. 2008). The vital role of IAA in plant growth and development has already been indicated (Di et al. 2016; Zhao 2010). In the research, the IAA content trend in the melatonin-treated plants was

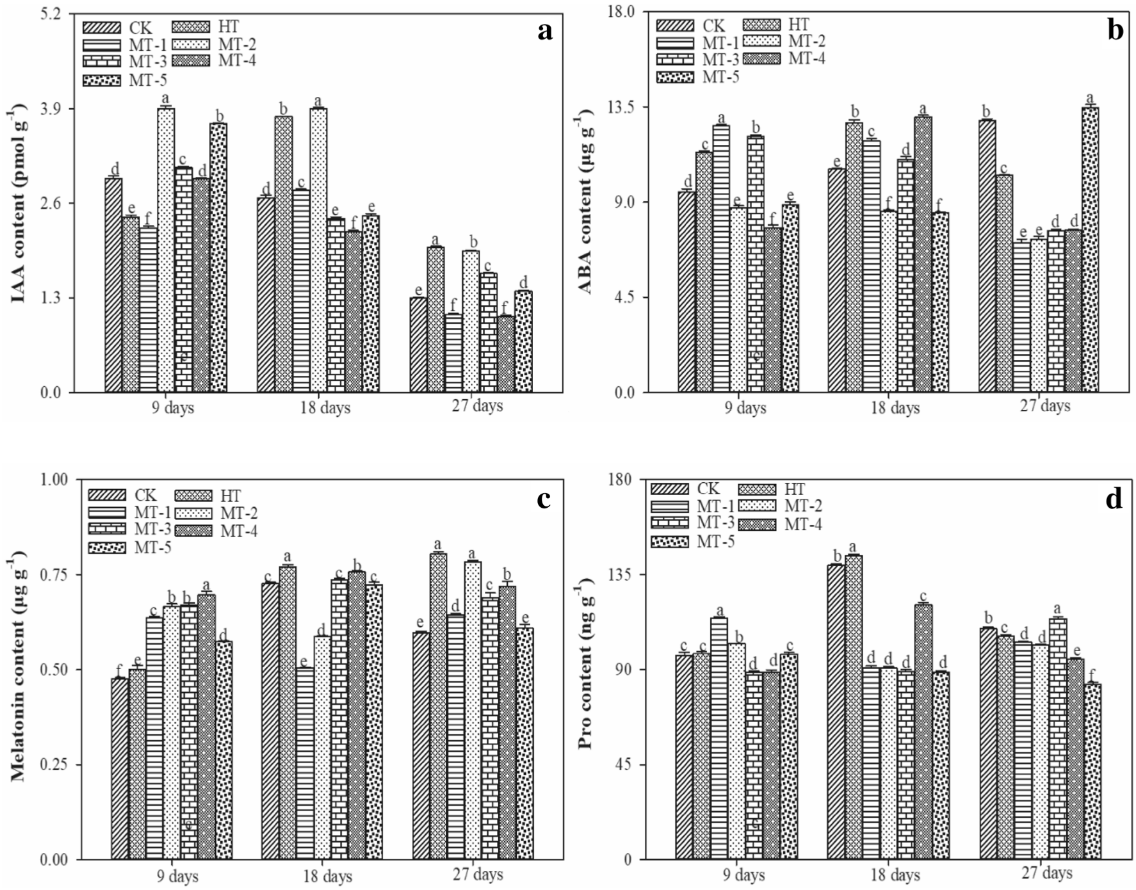

Fig. 3 IAA (**a**), ABA (**b**), melatonin (**c**) and Pro contents (**d**) of cherry radish with different treatments. CK (25 °C/20 °C day/night temperature treatment without melatonin); HT (35 °C/30 °C day/night temperature treatment without melatonin); MT-1(35 °C/30 °C day/night temperature treatment with 11.6 mg L^{-1} melatonin); MT-2 (35 °C/30 °C day/night temperature treatment with 17.4 mg L^{-1} melatonin); MT-3 (35 °C/30 °C day/night temperature treatment with 29.0 mg L^{-1} melatonin); MT-4 (35 °C/30 °C day/night temperature treatment with 34.8 mg L^{-1} melatonin); MT-5 (35 °C/30 °C day/night temperature treatment with 67.0 mg L^{-1} melatonin). Bar heights represent means and error bars represent ± SE. Within each graph, means followed with the same letter were significantly different based on a one-way ANOVA followed by Duncan's multiple-range tests ($P \leq 0.05$)

consistent during the whole growing season. Compared with HT, plants with melatonin had higher IAA content in early stage than in later, but the IAA content of HT was the highest in the middle and late stages of growth. It might be that there is greater demand for IAA at the seedling growth stage, and the addition of more melatonin promotes plants to produce more IAA to promote growth and resist stress. The decrease in IAA content in the late stage of growth was, on the one hand, related to weaker demand for IAA, on the other hand, the content of melatonin in the plant's later period has decreased, and the promotion of IAA was not significant. Many reports have shown that ABA is involved in regulating plant growing and ambient stresses (Bai et al. 2013). With plant growth, the higher the melatonin content, the higher the ABA content, but the increase in ABA content in the later period of plant growth would inhibit the growth of plants, so adding a suitable concentration of melatonin could relieve stress with no inhibitory effect on plant growth.

As a known penetrant, Pro maintains the osmotic adjustment of plants and algae under abiotic stress conditions. Pro was accumulated more higher under various stresses, it was supposed to act as a compatible solute and free radical scavenger to remove excess ROS, reconstitute cell redox balance and stabilize subcellular structure (Lv et al. 2011). Compared with HT, MT-3 treatment for 27 days significantly increased the Pro content and resisted harm due to stress, increasing crop yield.

Effect of High-Temperature Stress to Biomass of Cherry Radish

Under high-temperature stress condition, plants could respond to high-temperature stress by improving the antioxidant enzyme activity, chlorophyll or endogenous hormone content and then sustain biomass production. Our results showed that the cherry radish growth was significantly

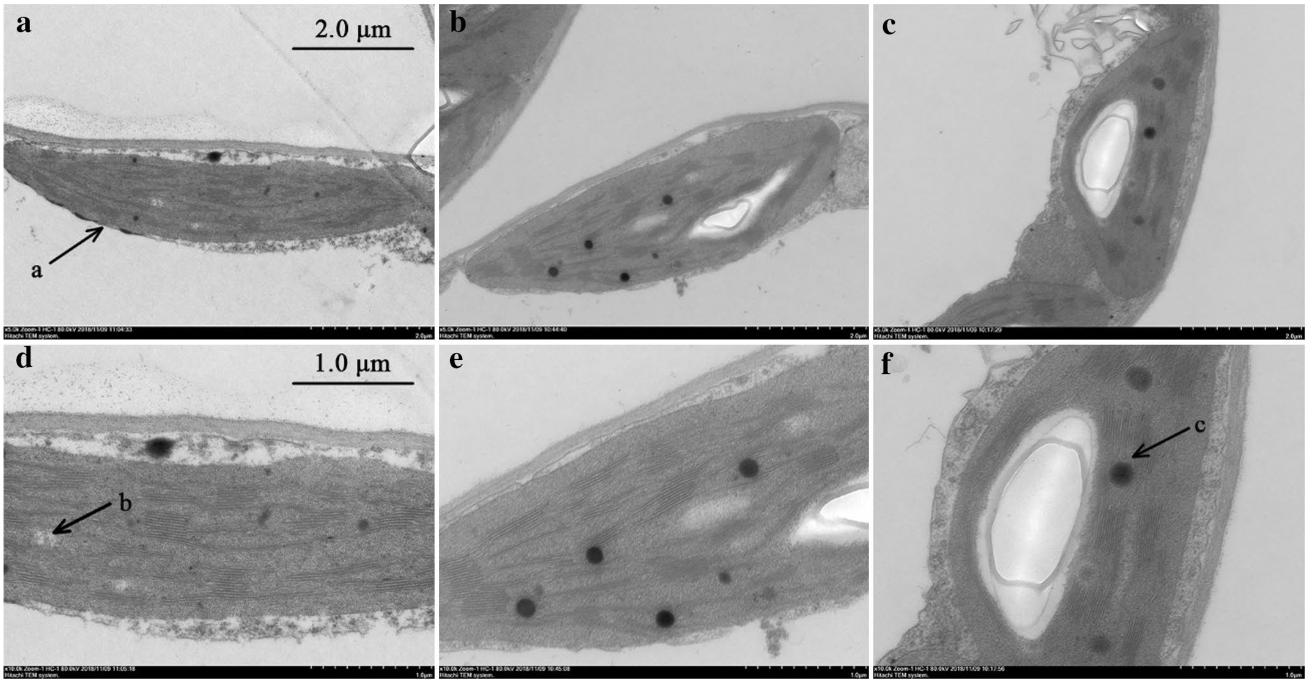

Fig. 4 Ultrastructure of chloroplasts (**a**–**c**) and thylakoid (**d**–**f**) of cherry radish with different treatments. **a**, **d** CK (25 °C/20 °C day/night temperature treatment without melatonin); **b**, **e** HT (35 °C/30 °C day/night temperature treatment without melatonin); **c**, **f** MT-3 (35 °C/30 °C day/night temperature treatment with 29.0 mg L^{-1} melatonin). Chloroplast outer membrane shown by arrow a, Granum lamellae shown by arrow b, Osmiophilic globules shown by arrow c. Scale bars for chloroplasts and thylakoids are 2 μm and 1 μm, respectively

inhibited by high-temperature stress and the tolerance of cherry radish to high-temperature stress was peaked by adding 29.0 mg L^{-1} melatonin at 27th day. High dose of melatonin did not increase biomass significantly. Shi et al. (2015c) showed that adding melatonin promoted fresh fruit weight and plant height of bermudagrass under oxidative stress. Liu et al. (2015) also showed the same trend in the alleviation salt stress of tomato by applying melatonin.

Conclusions

The results from the present study indicate that high-temperature stress at 35 °C affected cherry radish growth, and that adding melatonin could enhance the ability of cherry radish to resist stress. The optimal concentration was 29.0 mg L^{-1}. At this concentration, biomass was significantly increased by 12.9%, the soluble protein content was improved by 18.7%. The application of this compound enhanced the antioxidant enzymes activity and alleviated lipid peroxidation, among them, the POD was significantly increased by 45.5%. Moreover, the carotenoid and IAA contents were significantly increased 20.0% and 28.5%, compared with the control, which in turn improved the high-temperature stress resistance of cherry radish. These discoveries provide theoretical basis for the future application of plant growth regulators in substrate culture.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest.

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