DOI: 10.1007/s11099-017-0748-6 PHOTOSYNTHETICA 56 (3): 884-892, 2018

Effect of melatonin priming on photosynthetic capacity of tomato leaves under low-temperature stress

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Abstract

Melatonin has different functions in plant growth and development, especially in the protection of plants suffering from various forms of abiotic stress. We explored the effect of melatonin priming on photosynthetic activity of tomato (*Lycopersicon esculentum* L.) leaves. Our results showed that 100 μM is the optimal concentration used for alleviation of the damage to photosynthetic apparatus. Melatonin priming both in the form of leaf spray and direct root application was found to reduce the damage to photosynthetic apparatus, and increase the electron transfer rate and quantum yield of PSI and PSII photochemistry, to protect the thylakoid membrane from damage caused by low-temperature stress. Our study provides fundamental information for further research on the molecular mechanism of melatonin function in regulating photosynthesis.

Additional key words: abiotic stress; chemical priming; chlorophyll fluorescence; photoinhibition.

Introduction

Plants as sessile organisms are continuously exposed to a various environmental stresses during their growth and development, which causes a considerable yield loss. Abiotic stress, including strong light, drought, cold, and heavy metal poisoning, can result in an over-reduction of the electron transport chain (ETC) which in turn leads to photooxidation and reduces photosynthetic efficiency (Gururani *et al*. 2015, Johnson *et al*. 2015). Plants have developed a variety of adaptation mechanisms, such as movement of leaves or chloroplasts in order to reduce light absorption, screening of optical radiation, reactive oxygen species (ROS)-scavenging system, heat dissipation, state transitions, cyclic electron flow around PSI (PSI-CEF), and photorespiration (Roach and Krieger-Liszkay 2014, Derks *et al.* 2015, Baránková *et al*. 2016, Fan *et al.* 2016). In the northern of China, it is still hardly to avoid the occurrence of low-temperature stress even during cultivating in Chinese solar greenhouse. Low-temperature stress can induce the generation of ROS and the accumulation of malondialdehyde (MDA) and proline in plants, damage the ultrastructure of phloem and photosynthetic apparatus, reduce the photosynthetic efficiency (Zhang *et al*. 2014a,b; Liu *et al*. 2015c, Hao *et al*. 2016, Yang *et al*. 2016).

Received 28 December 2016, *accepted* 10 May 2017, *published as online-first* 27 June 2017.
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Abbreviations: C_a – ambient CO_2 concentration; C_i – intercellular CO_2 concentration; Chl – chlorophyll; CK – control; CK0 – plants were grown at normal temperature (day and night temperature of 25/15°C) for 3 d after pretreatment by spraying water on leaves and applying 50 mL of water on roots; CK1 – plants were grown at low temperature (day and night temperature of 15/6°C) for 3 d after pretreatment by spraying water on leaves and applying 50 mL of water on roots; E – transpiration rate; ETR_I – electron transfer rate of PSI; ETR_{II} – electron transfer rate of PSII; F_v/F_m – the maximum photochemical quantum yield of PSII; F_v'/F_m' – the efficiency of excitation energy capture by open PSII reaction center; g_s – stomatal conductance; L100 – plants were grown at low temperature (day and night temperature of 15/6°C) for 3 d after pretreatment by leaf spraying 100 μM melatonin on leaves and applying 50 mL of water on roots; L_s – stomatal limitation; MDA – malondialdehyde; NPQ – nonphotochemical quenching; P_m – PSI content; P_N – net photosynthetic rate; PSI-CEF – cyclic electron flow around PSI; q_P – photochemical quenching coefficient; R100 – plants were grown at low temperature (day and night temperature of 15/6°C) for 3 d after pretreatment by spraying water on leaves and applying 100 mL of melatonin on roots; RLC – rapid light curve; RNS – reactive nitrogen species; ROS – reactive oxygen species; WUE – water-use efficiency (= P_N/E); Y_I – quantum yield of PSI photochemistry; Y_{II} – actual quantum yield of PSII photochemistry for light-adapted state; Y_{NA} – quantum yield of nonphotochemical energy dissipation due to acceptor side limitation; Y_{ND} – quantum yield of nonphotochemical energy dissipation due to donor side limitation; Y_{NPQ} – quantum yield of regulatory energy dissipation; Y_{NO} – quantum yield of nonregulatory energy dissipation.
Acknowledgements: This work was supported by the National Key Research Program of China (2016YFD0201004); National Natural Science Foundation of China (Grant No. 31301813) and China Agriculture Research System (Grant No. CARS-25).

Selection of some exogenous chemical priming can regulate plant metabolism and improve plant resistance. For example, exogenous $CaCl_2$ treatment can enhance photosynthesis of tomato seedlings under low night temperature stress (Liu *et al.* 2015c, Savvides *et al.* 2016). In recent years, the use of melatonin in improving plant resistance has received increasing attention. Experiments have shown that melatonin is found in many plant cells, the content of endogenous melatonin is dependent on different growth conditions (Beilby *et al*. 2015). Melatonin can act directly as an effective antioxidant, decreasing concentrations of ROS and reactive nitrogen species (RNS), can enhance plant resistance by reducing environmental stress and biological stress damage (Liu *et al.* 2015b, Reiter *et al.* 2015, Wang *et al.* 2013). Melatonin can also act as a membrane stabilizer, playing an important role in membrane fluidity. As a biostimulator, melatonin modifies the expression of redox-network genes involved in the antistress response, and fortifies plants by optimizing photosynthesis-related parameters and repressing senescence genes (Arnao and Hernández-Ruiz 2014, 2015, Nawaz *et al*. 2015). Overexpression of the melatonin biosynthetic genes elevates melatonin contents in transgenic plants and enhanced tolerance to abiotic stresses.

Previous studies have found that the addition of 10 μM melatonin increased the *Chara australis*'s actual quantum yield of PSII photochemistry by 34% (Lazár *et al.* 2013). Similarly, 100 μM exogenous melatonin treatment improved the photosynthesis of pennisetum, especially of the cold-resistant genotypes (Hu *et al.* 2016). Wang *et al*. (2013) found that when 100 μM melatonin was added to soils under drought conditions and the resulting oxidative stress was eased and leaf senescence was delayed. They also found that melatonin helped to maintain better function of PSII under drought. Depending on the pathway, the subcellular site of melatonin synthesis is either the cytoplasm or chloroplasts, which may differentially affect the mode of melatonin action in plants. Chloroplast is a main generation site of ROS, thus, melatonin is likely to be involved in the regulation of photosynthesis due to its ability to remove free radicals (Beilby *et al*. 2015, Back *et al*. 2016). However, there are very few reports about the influence of melatonin on photosynthesis when the plants grow under adverse environmental conditions, especially low-temperature stress. Tomato is an important vegetable cultivated in the solar greenhouses in China, where low-temperature stress is common in the winter. The understanding of the regulatory role of melatonin on photosynthesis of tomato under low-temperature stress has a certain guiding significance to vegetable production. In the present study, chlorophyll (Chl) fluorescence measurement technology together with gasometric and electrochromic shift measurement were used to explore the effects of melatonin priming on photosynthetic capacity of tomato leaves under low-temperature stress.

Materials and methods

Plants and growth conditions: The experiments were conducted at Shenyang Agricultural University vegetable test site in northeastern China (longitude: 123.56°E; latitude: 41.82°N) during June–October 2015. Tomato seeds of a popular variety 'Liao Yuan Duo Li' (*Lycopersicon esculentum* L.) were germinated and transferred to plastic pots (13 cm × 13 cm) at the two-leaf stage under a normal cultivation management in greenhouse with average day/night temperatures of 25/15°C under natural light [approximately 600 μmol(photon) m^{-2} s^{-1}] at a relative humidity of 60%. We started testing when the tomato plants reached the six-leaf stage. The plants grew in nutrition plastic pots (13 cm × 13 cm) and were watered according to normal cultivation management.

Melatonin priming and low-temperature stress: First, we carried out screening tests in order to choose a proper concentration. Melatonin priming was performed by applying different concentrations of melatonin in the form of leaf spray and root application before low-temperature stress. Leaf spray with water and root application with 50 ml of water was regarded as control (CK). The leaf spraying was stopped when water started dropping from the leaves (about 15 mL per plant). This process was repeated in the morning and afternoon for three consecutive days. The low-temperature treatment was conducted during the subsequent three days in *Phytotron* with day/night temperature of 15/6°C, respectively, relative humidity of 60% and light intensity of 600 μmol(photon) m^{-2} s^{-1}. We chose the optimal melatonin concentration by comparing the maximum photochemical quantum yield of PSII (F_v/F_m) and a parameter representing the quantity of efficient PSI complex (P_m). We finally determined 100 μM as the optimal concentration for leaf spray and root application.

Then we conducted resistance experiments using the optimal concentration. For control treatment (CK0), plants

Treatment	Leaf spray	Root application
CK	H_2O	50 mL H_2O
L5	5 μM melatonin	50 mL H_2O
L50	50 μM melatonin	50 mL H_2O
L100	100 μM melatonin	50 mL H_2O
L150	150 μM melatonin	50 mL H_2O
L250	250 μM melatonin	50 mL H_2O
R5	H_2O	5 mL melatonin
R50	H_2O	50 mL melatonin
R100	H_2O	100 mL melatonin
R150	H_2O	150 mL melatonin
R250	H_2O	250 mL melatonin

were grown at normal temperature (day and night temperature of 25/15°C) for 3 d after pretreatment by spraying water on leaves and applying 50 mL of water on roots. For low-temperature treatment (CK1), plants were grown at low temperature (day and night temperature of 15/6°C) for 3 d after pretreatment by spraying water on leaves and applying 50 mL of water on roots. For melatonin treatment (R100), plants were grown at low temperature (day and night temperature of 15/6°C) for 3 d after pretreatment by spraying water on leaves and applying 100 mL of melatonin on roots. For melatonin treatment (L100), plants were grown at low temperature (day and night temperature of 15/6°C) for 3 d after pretreatment by leaf spraying 100 µM melatonin on leaves and applying 50 mL of water on roots (Fig. 1). The pretreatment process was repeated in the morning and afternoon for three consecutive days as described for concentration screening test

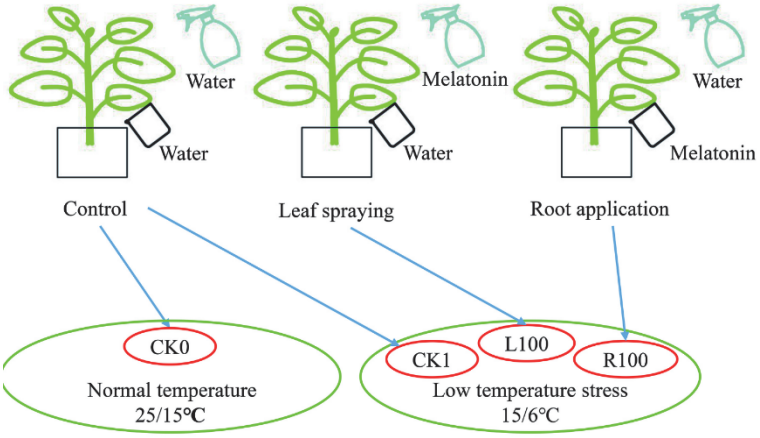

Fig. 1. Schematic diagram of melatonin priming and cold-stress treatments in the experiment. CK0 – plants grown at normal temperature (day/night temperatures of 25/15°C) for 3 d after pretreatment by spraying water on leaves and applying 50 mL of water on roots. CK1 – plants grown at low temperature (day/night temperatures of 15/6°C) for 3 d after pretreatment by spraying water on leaves and applying 50 mL of water on roots. L100 – plants grown at low temperature (day/night temperatures of 15/6°C) for 3 d after pretreatment by leaf spraying 100 µM melatonin on leaves and applying 50 mL of water on roots. R100 – plants grown at low temperature (day/night temperatures of 15/6°C) for 3 d after pretreatment by spraying water on leaves and applying 50 mL of 100 µM melatonin on roots.

Gas-exchange parameters and quantum yield of PSI and PSII: Gas-exchange parameters and activities of PSII and PSI were measured by *GFS-3000* (*Heinz Walz*, Germany) and *DUAL-PAM-100* measuring systems (*Heinz Walz*, Germany), the measurement and the parameter calculation was performed in *GFS-Win* and *Dual PAM v1.19* software, respectively. The operation was performed using software standard protocols with appropriate modification (Zhang *et al*. 2014a). The fourth leaf of each plant was used for this measurement. Each leaf was subjected to dark adaptation for 30 min prior to the measurement. The leaf area monitored by the measuring head was 1.3 cm^2 with atmospheric CO_2 concentration of about 400 ± 20 µmol mol^{-1}, the intensity of saturation pulse light (red light) was 10,000 µmol(photon) m^{-2} s^{-1}, intensity of actinic light (red light) was 630 µmol(photon) m^{-2} s^{-1}. All measurements were carried out at room temperature (25 ± 2°C). Gas-exchange parameters include the net photosynthetic rate (P_N), stomatal conductance (g_s), atmospheric CO_2 concentration (C_a), transpiration rate (E), intercellular CO_2 concentration (C_i), leaf stomatal limitation ($L_s = 1 - C_i/C_a$) and the maximum water-use efficiency ($WUE = P_N/E$). PSII fluorescence parameters mainly include the maximum photochemical quantum yield of PSII (F_v/F_m), actual quantum yield of PSII photochemistry (Y_{II}) for light-adapted state, the quantum yield of nonregulatory energy dissipation (Y_{NO}), and the quantum yield of regulatory energy dissipation (Y_{NPQ}) (Lazár 2015). PSI fluorescence parameters mainly include the leaf PSI content (P_m), quantum yield of PSI photochemistry (Y_I), quantum yield of nonphotochemical energy dissipation due to donor side limitation (Y_{ND}), and quantum yield of nonphotochemical energy dissipation due to acceptor side limitation (Y_{NA}) (Schreiber and Klughammer 2008b).

Photochemical efficiency of PSII and electron transport rate: Rapid light curves (RLCs) were measured after the determination of slow Chl fluorescence induction kinetics. The light intensity gradient of RCL were 29, 37, 55, 113, 191, 113, 349, 520, 778; 1,197; 1,474 µmol(photon) m^{-2} s^{-1}. The duration of each light intensity was 30 s and the saturation pulse was 10,000 µmol(photon) m^{-2} s^{-1} for 300 ms. The following parameters recorded by the RLC were used in the analysis: photochemical quenching coefficient [$q_P = (F_m' - F)/(F_m' - F_0')$], the efficiency of excitation energy capture by open PSII reaction center [$F_v'/F_m' = (F_m' - F_0')/F_m'$], electron transfer rate of PSI

(ETR_I), electron transfer rate of PSII (ETR_{II}).

Integrity of thylakoid membrane and ATP-synthase activity: The dual-beam 550–515 nm difference signal (the electrochromic shift) was monitored simultaneously by using the *P515/535* module of the *Dual-PAM-100* and the automated routines provided by the *DUAL-PAM* software with minor modifications (Schreiber and Klughammer 2008b, Zhang *et al.* 2014a). After 1 h of dark adaptation, P_{515} changes induced by saturating single turnover flashes were recorded to evaluate the integrity of the thylakoid membrane. After 6 min of preillumination at 630 µmol (photon) m^{-2} s^{-1} and 4 min of dark adaptation, P_{515} changes induced by saturating single turnover flashes were recorded to evaluate ATP-synthase activity. Preillumination activates the reversible ATP-synthase in the thylakoid membrane, thus increasing the H^+ conductivity of the membrane. The decay of the P_{515} signal reflects the relaxation of the flash-induced membrane electric potential difference (created by charge separation in the two photosystems and electrogenic electron transport of the Q-cycle at the cytochrome *b/f* complex) by H^+ efflux through the ATP-synthase of H^+ counter ions (K^+, Mg^{2+}, Cl^-) through the membrane. A functionally intact photosynthetic apparatus is characterized by a slow decay after dark-adaptation (high membrane integrity) and a fast decay after illumination (high ATP-synthase activity) (Schreiber and Klughammer 2008a, Suzuki *et al*. 2011, Lu *et al*. 2017, Lyu and Lazár 2017).

Statistical analysis: Experiment was repeated twice and the quantitative assessment was conducted on randomly selected samples from four independent biological replicates. One way analysis of variance (*ANOVA*) was performed in *SPSS version 22* (*SPSS*, Chicago, USA). Comparisons between the mean values were accomplished by the least significant difference test at the level of $p<0.05$ (*) and $p<0.01$ (**). All graphs were made using *SigmaPlot version 12.0* (*Systat*) using means and standard deviation of each data point.

Results

Concentration screening: In order to assess the effects of leaf spraying and root application with different concentrations of melatonin on photochemical reaction of tomato leaves under low-temperature stress, we first carried out concentration screening test. As shown in Fig. 2, F_v/F_m of tomato under low-temperature stress was the lowest for water pretreatment (CK). In contrast, melatonin priming with different concentrations of melatonin through leaf spraying and root application improved the photochemical efficiency of PSII. With the increase in the concentration, both treatments first showed an increasing and then a decreasing trend. The F_v/F_m ratio of L100, L150, R100, and R150 were significantly higher than that of CK; P_m also showed a similar trend as F_v/F_m, CK treatment had the lowest P_m value (1.73) (Fig. 2). PSII was expected to be sensitive to some environmental stresses; in this study, we additionally found that PSI of tomato leaves was also very sensitive to low-temperature stress, which suggested that melatonin priming can reduce damage to PSI and PSII of tomato leaves under low-temperature stress. We used the two indicators to screen the optimum concentration of melatonin priming. The F_v/F_m and P_m values of L100 and R100 were the largest among the different concentrations used for the priming treatments. Therefore, 100 µM represented the optimal concentration of melatonin for leaf spraying and root application.

Photosynthetic gas-exchange parameters directly reflect the photosynthetic carbon assimilation ability. Low-temperature stress significantly reduced the P_N of tomato leaves (Table 1). The P_N values of L100 and R100 treatments were 7.80 µmol m^{-2} s^{-1} and 7.09 µmol m^{-2} s^{-1}, respectively, and were significantly higher than that of CK1, which was 5.01 µmol m^{-2} s^{-1}. Low-temperature stress significantly reduced the *E*, the values for CK1, L100, and R100 treatments were all lower than that of CK0. Besides, *E* of L100 and R100 were significantly different when compared with CK1. Low-temperature stress also significantly influenced the C_i, g_s, L_s, and WUE. The C_i values of L100 and R100 were slightly lower than that of CK0, while g_s of L100 and R100 was significantly higher than that of CK1. WUE of CK0 was the highest

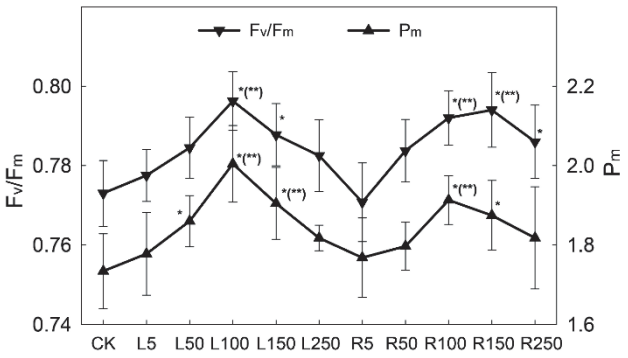

Fig. 2. Influence of different concentration of melatonin on F_v/F_m and P_m of tomato leaves under low-temperature stress. Melatonin priming was performed before low-temperature stress, CK – leaf spray with water and root application with 50 ml of water. L5, L50, L100, L150, L250 – root application with 50 mL of water and leaf spray with 5, 50, 100, 150, 250 µM melatonin, respectively; R5, R50, R100, R150, R250 – leaf spray with water and root application with 50 mL of 5, 50, 100, 150, 250 µM melatonin by using a beaker, respectively. F_v/F_m – the maximum photochemical quantum yield of PSII, P_m – the quantity of efficient PSI complex. Values are means of four replicates ± SD. Statistically significant differences of every sample to the CK are marked by asterisks (*$p<0.05$, **$p<0.01$).

(4.51 μmol mmol^{-1}), while CK1 had the lowest value. Similarly, WUE of L100 and R100 were significantly higher than that of CK1. These results suggest that L100 and R100 treatments can indirectly reduce the influence of low-temperature stress on photosynthetic gas exchange. Moreover, L100 seemed to be more effective than R100.

Quantum yield of PSII photochemistry: The efficiency of excitation energy capture by open PSII reaction center (F_v'/F_m') and q_P were determined because their product equals Y_{II}, which is widely applied to estimate the photochemical reaction efficiency of PSII of plants under severe environment stress conditions (Lazár 2015). F_v'/F_m' and q_P decreased with the increase in light intensity, F_v'/F_m' and q_P of CK1 were significantly lower than that of CK0, almost at all light intensities (Fig. 3*A,B*). L100 and R100 treatment slightly increased both F_v'/F_m' and q_P, suggesting that melatonin priming increased both the maximal photochemical efficiency of PSII and as well as the amount of open PSII centers under low-temperature stress. It was also evident that L100 was more effective than R100 probably because more of the melatonin reached its target if applied *via* the leaf.

Light energy distribution and electron transport rate: The Y_I, Y_{II}, and Y_{NO} of tomato under low temperature were significantly reduced compared with CK0 (Fig. 4*A,B*). On the other hand, Y_{NA} and Y_{NPQ} significantly increased under the low-temperature stress compared with CK0. It suggested that low-temperature stress reduced absorbed light energy distribution to photochemical reaction of PSI and PSII, and strengthened the usage of absorbed light energy distribution for the nonphotochemical reactions. While, the Y_{ND} and Y_{NO} showed no difference between the treatments, the L100 and R100 treatments increased Y_I and Y_{II} and reduced Y_{NA} and Y_{NPQ} compared with CK1. It suggests that melatonin priming, therefore, had a certain influence that was beneficial to absorbed light energy distribution to photochemical reaction under the low temperature conditions. ETR_I and ETR_{II} of both PSII and PSI were significantly lower compared with CK0, especially under moderate and high light conditions. L100 and R100 treatment significantly increased ETR_{II} and ETR_I of tomato leaves under low-temperature stress, which indicated that melatonin priming both by spraying on the leaves and root application increased the electron transfer rate of PSI and PSII under low-temperature stress. In addition, ETR_{II} was saturated at strong light intensities, whereas ETR_I was not (Fig. 5*A,B*).

Table 1. Effect of melatonin priming on photosynthetic gas exchange parameters of tomato leaves under low temperature stress. Values are means of four replicates ± SD. *Different letters* in the same column indicate significant difference between treatments at $p \leq 0.05$ by using LSD test. P_N – net photosynthetic rate, E – transpiration rate, C_i – intercellular CO_2 concentration, g_s – stomatal conductance, L_s – leaf stomatal limitation, WUE – water-use efficiency.

Treatment	P_N [μmol m^{-2} s^{-1}]	E [mmol m^{-2} s^{-1}]	C_i [μmol mol^{-1}]	g_s [mmol m^{-2} s^{-1}]	L_s	WUE [μmol mmol^{-1}]
CK0	9.49 ± 0.19^a	2.11 ± 0.08^a	348.17 ± 14.05^a	200.98 ± 10.46^a	0.18 ± 0.01^c	4.51 ± 0.25^a
CK1	5.01 ± 0.16^c	1.56 ± 0.05^b	259.42 ± 10.04^b	110.23 ± 7.60^c	0.41 ± 0.01^a	3.22 ± 0.07^c
L100	7.80 ± 0.18^b	1.94 ± 0.04^b	339.80 ± 8.49^a	179.07 ± 5.30^b	0.24 ± 0.02^b	4.02 ± 0.09^a
R100	7.09 ± 0.15^b	1.87 ± 0.05^b	321.09 ± 9.80^a	167.25 ± 7.94^b	0.25 ± 0.02^b	3.80 ± 0.13^b

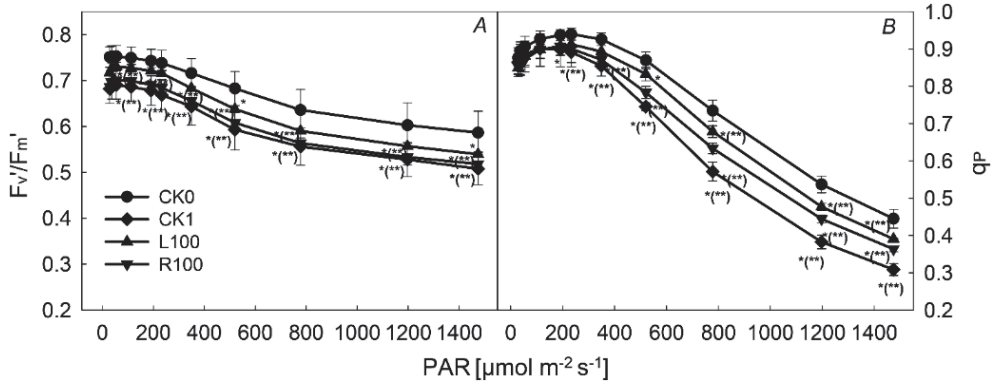

Fig. 3. Effect of melatonin priming on rapid light curves of F_v'/F_m' (*A*) and q_P (*B*) of tomato leaves under low-temperature stress. F_v'/F_m' – efficiency of excitation energy capture by open PSII reaction center, q_P – photochemical quenching coefficient of PSII. Values are means of four replicates ± SD. Statistically significant differences of every sample to the CK0 are marked by asterisks (*p<0.05, **p<0.01).

Activity of ATP-synthase and thylakoid membrane integrity: A saturating single turnover flash induced an extremely rapid P_{515} signal increase, which reflected the primary charge separation at PSI and PSII reaction centers. In intact, dark-adapted samples, this rapid rise was followed by a much slower rise phase ($t_{1/2}$ approx. 20 ms), the rate of the ensuing signal decay was highly dependent on the physiological state of the sample, in particular with respect to membrane integrity and preillumination.

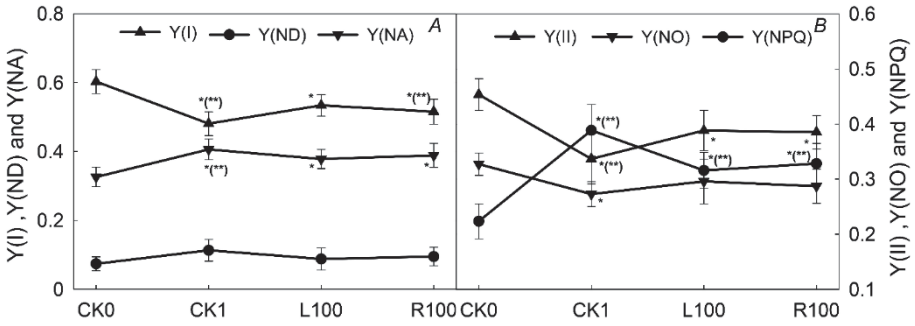

Fig. 4. Effect of melatonin priming on light energy distribution of PSI (*A*) and PSII (*B*) of tomato leaves under low-temperature stress. Y_{II} – the actual quantum yield of PSII photochemistry for light adapted state, Y_{NPQ} – the quantum yields of nonregulatory energy dissipation, Y_{NO} – the quantum yield of regulatory energy dissipation, Y_I – the quantum yield of PSI photochemistry, Y_{ND} – quantum yield of nonphotochemical energy dissipation due to donor side limitation of PSI, Y_{NA} – quantum yield of nonphotochemical energy dissipation due to acceptor side limitation of PSI. Y_{NO} and Y_{NPQ} are related to PSII and Y_{ND} and Y_{NA} are related to PSI. Values are means of four replicates ± SD. Statistically significant differences of every sample to the CK0 are marked by asterisks (*p<0.05, **p<0.01).

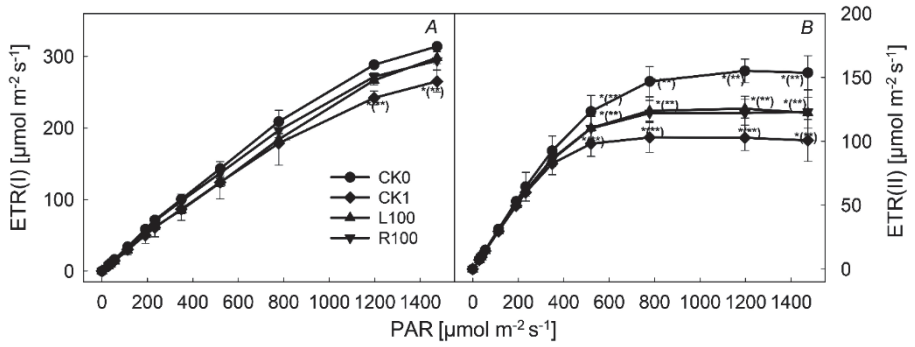

Fig. 5. Effect of melatonin priming on ETR_I (*A*) and ETR_{II} (*B*) of tomato leaves under low-temperature stress. ETR_I – electron transfer rate of PSI, ETR_{II} – electron transfer rate of PSII. Values are means of four replicates ± SD. Statistically significant differences of every sample to the CK0 are marked by asterisks (*p<0.05, **p<0.01).

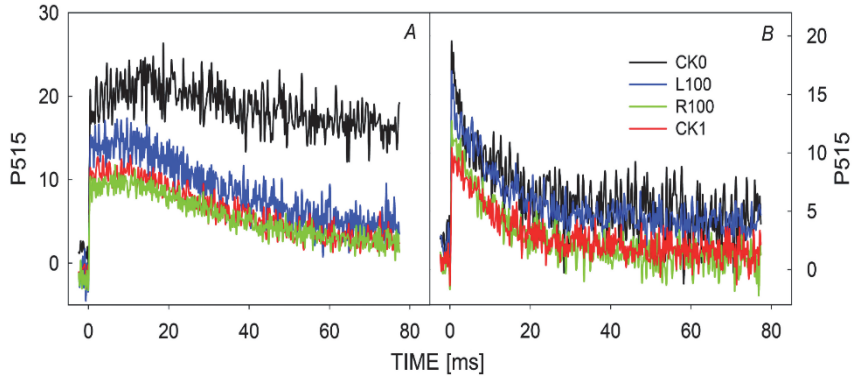

Fig. 6. Effect of melatonin priming on the fast P_{515} signal of tomato leaves under low-temperature stress, (*A*) for thylakoid membrane, changes in the P_{515} signal of tomato leaves after 1 h of dark adaptation. (*B*) ATPase activity, changes in the P_{515} signal of tomato leaves after 6 min of preillumination at 630 µmol(photon) m^{-2} s^{-1} followed by 4 min of dark adaptation.

Our observations of faster decay after dark adaptation (Fig. 6*A*) and a slower decay after illumination (Fig. 6*B*) with low-temperature treatment (CK1) both showed damage to the thylakoid membrane and ATP-synthase after low-temperature stress. The P_{515} induction curves of L100 and R100 treatments were higher than that of CK1 after dark adaptation (Fig. 6*A*), indicating that melatonin priming slightly reduced the damage to thylakoid membrane under low-temperature stress. The P_{515} signal of L100 and R100 were similar with CK1 after illumination, which showed that the influence of melatonin on ATP-synthase was not obvious (Fig. 6*B*).

Discussion

This study used the F_v/F_m and P_m to screen the priming concentration of melatonin and identified 100 μM as the optimal concentration for spraying on the leaves and root application in order to improve photosynthesis of tomato under low-temperature stress. Low temperature was found to reduce the operational efficiency of leaf photosynthesis. Melatonin priming both in the form of leaf spray and direct root application was found to reduce the damage to photosynthetic apparatus, make more of the absorbed light used for photochemistry and make photosynthesis more tolerant to the low temperature. We also found that leaf spray treatment was better than the root application in alleviating damage to photosynthesis due to low-temperature stress.

Photoinhibition of PSI is more dangerous than PSII, because the photoinhibition of PSI is difficult to recover and it can also cause secondary damage (Nishiyama *et al.* 2011). Low temperature can induce accumulation of ROS and reduce the light intensity at which photoinhibition may occur, damage photosynthetic apparatus, and indirectly reduce photosynthetic efficiency. Plant species have evolved several physiological and molecular protective mechanisms to reduce damage from cold stress. When cold stress was prolonged, there was an increase in the expression of cold-responsive genes coding for transporters, responses to stimuli and stress, regulation of defense response, as well as genes related to signal transduction of all phytohormones (Zhu 2016, Zhang *et al*. 2017). The first protective reaction after photoinhibition of plants is the heat dissipation (q_E) of the excess energy when photoinhibition occurs (Ahn *et al.* 2008), while cyclic electron transfer mediated by proton gradient regulation 5 (PGR5) and PGR5-like1 (PGRL1) proteins and NAD(P)H dehydrogenase (NDH) complexes is another important mechanism to alleviate photoinhibition and lower the PSII light damage (Suorsa 2015, Takahashi *et al.* 2009).

Li *et al*. (2016) found that exogenous melatonin application enhances the drought priming-induced cold tolerance by modulating subcellular antioxidant systems and abscisic acid concentrations in barley. Lazár *et al.* (2013) suggest that melatonin protection against ROS covers not only Chl, but also photosynthetic proteins in general. Chloroplasts are the center of photosynthesis and the main production site of oxygen free radicals, and melatonin likely plays an important role in photosynthesis because it is mainly synthesised in chloroplasts in plants. Previous study has found that 100 μM melatonin treatment improves the Chl fluorescence of Bermuda grass under low-temperature stress and improves the sugar and acid content (Fan *et al.* 2015). Liu *et al*. (2015a) found that pretreatment of 100 μM melatonin significantly alleviated the effect of drought stress on PSII activity of tomato seedling and maintained high photosynthetic capacity. Most recent researchers suggest that exogenous melatonin increased the chilling tolerance of chloroplast in cucumber seedlings by accelerating the ascorbate-glutathione cycle and by regulating photosynthetic electron flux (Zhao *et al*. 2016). Similarly, exogenous melatonin also improves the photosynthetic activities of tomato seedlings under salt stress, where the mechanism could be in beneficial roles of melatonin on redox regulation of photosynthetic electron transport and synthesis of D1 protein (Zhou *et al*. 2016). Our results are, therefore, consistent with the findings of Liu *et al*. (2015a). Both abiotic and biotic stress produce a significant increase in endogenous melatonin contents, and endogenous circadian rhythms in melatonin concentrations has been demonstrated in some species (Arnao and Hernández-Ruiz 2015), but the involvement of endogenous melatonin in regulating photosynthesis is still unknown.

Several hormones, osmotic regulators, and antioxidants have been reported as chemical priming reagents. These substances, such as salicylic acid, brassinolide, calcium ion, sodium nitroprusside, polyamine, melatonin, and abscisic acid, generally need a small dosage for their quick and remarkable effect (Ziogas *et al.* 2015, Borges *et al.* 2014). Our study showed that 100 μM melatonin priming on leaves or roots both alleviated the damage of low-temperature stress on tomato seedling and significantly enhanced leaf photosynthetic performance. We also found that spraying on the leaves was better than the root application. This study is a preliminary exploration of regulatory role of melatonin priming on photosynthetic capacity, however, more studies are needed to explore the regulatory mechanism of melatonin on the photosynthetic electron transport chain. Using melatonin to increase crop resistance and alleviate photoinhibition is an important agricultural management measure, and the role of melatonin priming mechanism still needs extensive research in the future.

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