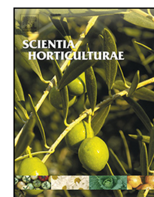
Scientia Horticulturae 219 (2017) 264–271

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti



Exogenous melatonin ameliorates cold-induced damage in tomato plants

Fei Ding, Bin Liu, Shuoxin Zhang*

College of Forestry, Northwest A&F University, 3 Taicheng Rd., Yangling, Shaanxi 712100, China

ARTICLE INFO

Article history:
Received 8 January 2017
Received in revised form 19 March 2017
Accepted 21 March 2017
Available online 24 March 2017

Keywords:
Antioxidant potential
Cold tolerance
Melatonin
Metabolites
Photosynthetic capacity
Solanum lycopersicum

ABSTRACT

Low temperature is an adverse environmental factor posing damage to tomato plants and causing huge loss of yields. Thus, seeking an effective way of ameliorating cold damage is important for sustainable tomato production. Melatonin is a crucial molecule involved in plant abiotic stress responses. In this study, we investigated the role of exogenous melatonin in amelioration of cold damage in tomato plants. Lower malondialdehyde (MDA) content and electrolyte leakage, greater activities of antioxidant enzymes, and higher levels of non-enzymatic antioxidants were observed in melatonin-pretreated plants than in non-melatonin-pretreated plants under cold stress. Gene expression analyses showed that exogenous melatonin substantially promoted expression of cold-responsive genes, including *SlICE*, *SlCBF* and *SlP5CS*, under cold condition. Notably, *SlSBP*, a gene encoding a Calvin cycle enzyme sedoheptulose-1,7-bisphosphatase (SBPase), was considerably induced in melatonin-treated plants under cold stress, consistent with the observed increase in photosynthetic carbon assimilation. Analyses of metabolites revealed that levels of polyamines, sucrose, and proline were significantly enhanced following cold treatment in melatonin-pretreated plants. Collectively, our data provide evidence for the ameliorative effects of melatonin on cold-induced damage to tomato plants. Our work also provides a case study that exogenous application of melatonin may be potentially employed as a strategy to improve cold tolerance in tomato production.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

Low temperature stress is one of the greatest challenges in growth and development of warm-climate plants. Low temperatures cause a wide range of disruptions at the physiological, molecular and metabolic levels in plants (Chinnusamy et al., 2010; Shi et al., 2015). One of the destructive effects of cold is that it induces damages to cell membranes, leading to decreased membrane fluidity and disrupted ion homeostasis in plants (Steponkus et al., 1998; Beck et al., 2004). Cold stress adversely impacts photosynthesis by disrupting electron transport chain in chloroplasts and mitochondria, which inevitably gives rise to excessive reactive oxygen species (ROS) (Allen and Ort, 2001; Fan et al., 2015). Excessive accumulation of ROS causes damage to DNA, proteins, and lipids (Apel and Hirt, 2004; Cheng and Song, 2006). Moreover, cold-induced accumulation of ROS affects carbon fixation by inactivating key enzymes, such as SBPase, involved in the Calvin cycle (Ding et al., 2017b). Cold stress also alters metabolic status in plants. In cold-stressed plants, the levels and activities of enzymes involved in key pathways of metabolism are generally changed and as a consequence, plant metabolome is considerably altered (Cook et al., 2004; Zhu et al., 2007).

To cope with adverse environmental conditions, such as drought and low temperature, plants have evolved multiple strategies. In response to elevated production of ROS under cold condition, plants employ an efficient detoxifying networks, including increased activities of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), and non-enzymatic antioxidants, including glutathione and ascorbic acid (Shi et al., 2015). Moreover, metabolites, such as carbohydrates and amino acids, are generally accumulated to protect plants against stress-induced osmotic injury. These metabolites are compatible solutes important for responses to abiotic stresses (Stitt and Hurry, 2002; Krasensky and Jonak, 2012). At the molecular level, plants respond to cold stress by inducing the expression of genes encoding transcription factors, including C-repeat-binding factor 1 (CBF1)/Drought Response Element Binding factor 1b (DREB 1b), CBF2/DREB 1c, and CBF3/DREB 1a. These CBFs are capable of binding to the promoter regions of cold responsive genes, thus regulating their expression (Stockinger et al., 1997; Gilmour et al., 1998). Other

* Corresponding author.
E-mail address: sxzhang@nwsuaf.edu.cn (S. Zhang).

http://dx.doi.org/10.1016/j.scienta.2017.03.029
0304-4238/© 2017 Elsevier B.V. All rights reserved.

transcription factors, such as C_2H_2 zinc-finger transcription factors ZAT10 and ZAT12, are involved in the regulation of CBFs during cold responses in plants (Zhou et al., 2011).

Melatonin (*N*-acetyl-5-methoxytryptamine) is a well-known hormone mediating many biological processes in animals. Since its identification in plants by two research groups in 1995, melatonin has drawn widespread attention among plant biologists (Dubbels et al., 1995; Hattori et al., 1995). Melatonin has been demonstrated to be involved in growth, development and stress responses in plants. As a free radical scavenger, melatonin is able to directly scavenge ROS in cellular compartments, thereby mitigating oxidative stress in plants (Reiter et al., 2015). Moreover, melatonin protects plants against a variety of environmental stresses, such as cold, heat, salinity and drought (Bajwa et al., 2014; Shi et al., 2015; Ding et al., 2017a). Recent studies have reported that melatonin-mediated cold tolerance is associated with the regulation of cold-responsive genes including *ZAT6*, *ZAT10*, *ZAT12*, *CBFs*, and *CAMTA* (Bajwa et al., 2014; Shi and Chan, 2014).

In recent years, great progress has been made in elucidating the role of melatonin in responses to abiotic stresses in a number of species, however, little information is available on the effects of melatonin on cold tolerance in tomato plants, which is an important horticultural crop but is highly sensitive to low temperatures because of its tropical origin. Cold stress negatively impacts almost all developmental stages in tomato plants and severely limits tomato yields (Park et al., 2004; Zushi et al., 2012). Thus, it is of practical significance to explore melatonin-mediated cold tolerance in tomato production. The objectives of the present work were to determine the potential role of melatonin in amelioration of cold damage to tomato plants and to investigate the possible mechanisms of melatonin-mediated responses to cold stress at the physiological, molecular and metabolic levels in tomato plants.

2. Materials and methods

2.1. Plant materials, growth conditions and treatment

Tomato (*Solanum lycopersicum* L. cv. MicroTom) seeds were sterilized and germinated at 25 °C in the dark on filter paper in petri dishes. Germinated seeds were then planted individually in 12 cm × 12 cm plastic pots filled with peat and vermiculite (3/1 v/v). Plants were grown under following conditions: 380 μmol mol^{-1} of CO_2, photon flux density of 400 μmol m^{-2} s^{-1}, day/night temperature of 25/20 °C, relative humidity of 60% and a photoperiod of 14 h.

At the four-leaf stage, tomato plants were sprayed one time a day either with 100 μmol melatonin (Sigma-Aldrich, St. Louis, USA) solution or with distilled water for 3 days. Then, plants were subjected to cold stress (4 °C) for 48 h and control plants were grown under standard conditions. Leaf samples were harvested at 0, 24 and 48 h for analyses of MDA, H_2O_2, $O_2^{\bullet-}$ and antioxidant enzymes. Leaf samples were harvested at 0, 12, 24, 36 and 48 h for analysis of electrolyte leakage. Leaf samples were harvested at 48 h for analyses of GSH, AsA, *SlSBP* expression, SBPase activity and metabolites. Leaf samples were harvested at 0, 1, 3, 6, 12 and 24 h for expression analyses of *SlICE1*, *SlCBF1* and *SlP5CS*.

2.2. Determination of carbon assimilation rates and maximum quantum efficiency of photosystem II

Photosynthetic rates were measured on young fully expanded leaves with a portable photosynthesis system (LI-COR Biosciences, USA). Tomato plants were dark adapted for 30 min and the minimal fluorescence from a dark-adapted leaf (Fo) was measured with a portable fluorometer (PAM-2000, Walz, Germany) and following a saturating pulse, the maximal fluorescence from a dark-adapted leaf (Fm) was obtained, which allowed to calculate the maximum quantum efficiency (Fv/Fm).

2.3. Determination of malonaldehyde (MDA) content

MDA content was determined following a previous study (Fan et al., 2015). MDA was extracted using thiobarbituric acid (TBA), and was quantified by determining the absorbance of the supernatant at 450, 532, and 600 nm.

2.4. Electrolyte leakage assay

Leaf samples were collected at 0, 12, 24, 36 and 48 h following cold treatment for the determination of electrolyte leakage. Electrolyte leakage was measured according to Ishitani et al. (1998).

2.5. Determination of ROS accumulation

Leaf samples from tomato plants were collected at 24 and 48 h for the determination of H_2O_2 and $O_2^{\bullet-}$ accumulation. The content of H_2O_2 was measured by monitoring the A410 of titanium-peroxide complex as described by Patterson et al. (1984). $O_2^{\bullet-}$ was detected with nitroblue tetrazolium (NBT) method following a previous study (Jabs et al., 1996).

2.6. Determination of antioxidant enzyme activities

Enzyme was extracted by homogenizing 0.2 g of fresh leaf sample in 50 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% polyvinylpyrrolidone (w/v). The extraction was centrifuged and the supernatant was collected for the assay of SOD, CAT, POD and APX following previous studies (Beauchamp and Fridovic, 1971; Nakano and Asada, 1981; Cakmak and Marschner, 1992)

2.7. Determination of GSH and AsA

The content of AsA was determined according to Logan et al. (1998). The crude extract of AsA was obtained by homogenizing 0.1 g fresh leaves in 6% (v/v) cold $HClO_4$. The supernatant after centrifuging the crude extract was collected for further analysis. AsA was assayed by determining the absorbance difference of the supernatant at 265 nm in 200 mM sodium acetate buffer (pH 5.6) before and after 15-min incubation with 1.5 units of AsA oxidase.

GSH was measured by an enzymatic cycling assay method as described in previous studies (Griffith, 1980). The crude extract was obtained by homogenizing 0.1 g fresh leaves in 5% sulfosalicylic acid and then centrifuged at 14,000*g* for 10 min at 4 °C. For measurement of GSH, the supernatant was mixed with buffers. The GR was added to the supernatant and the increase in absorbance at 412 nm was used for calculating the content of GSH.

2.8. Determination of transcript abundance by quantitative real-time PCR

Total RNA was extracted from tomato leaves and was used for cDNA synthesis by PrimeScript® reverse transcriptase according to standard protocols. Quantitative real-time PCR was performed using SYBR® Premix Ex TaqTM (TaKaRa) according to manufacturer's instructions. Each real-time PCR reaction was performed in 25 μL final volume on iQ5 Multicolor Real-Time PCR Detection System (BIO-RAD, USA) under the following program: 1 cycle of 30 s at 95 °C, followed by 40 cycles of 5 s at 95 °C, 30 s at 60 °C. The primers for *SlSBP* were CGTGACATCTCCAACAGCTAA GG (Forward) and CATCGCTGCTGTAACCTCCAG (Reverse). The primers for *SlICE1*

Download English Version:

https://daneshyari.com/en/article/5769732

Download Persian Version:

https://daneshyari.com/article/5769732

Daneshyari.com