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Research article

Comparative photosynthetic and metabolic analyses reveal mechanism of improved cold stress tolerance in bermudagrass by exogenous melatonin

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ABSTRACT

Melatonin (N-acetyl-5-methoxytryptamine) has been reported to participate in plant development and abiotic stress responses. The main objective of this study was to investigate the role of melatonin in the cold-sensitive (S) and the cold-tolerant (T) bermudagrass genotypes' response to cold stress. The genotypes were treated with 100 μM melatonin and exposed to 4 °C temperature for 3 days. In both genotypes, cold stress increased the endogenous melatonin levels, and more prominently in T than S. Physiological responses indicated that exogenous melatonin triggered antioxidant activities in both genotypes, while it alleviated cell damage in the T genotype response to cold stress. Melatonin treatment under cold stress increased fluorescence curve levels for both genotypes, and higher in T than S genotypes. In both genotypes, the alterations in photosynthetic fluorescence parameters after melatonin treatment highlighted the participation of melatonin in improving photosystem response to cold stress, particularly for the cold-tolerant genotype. The metabolic analyses revealed the alterations of 44 cold-responsive metabolites in the two genotypes, mainly including carbohydrates, organic acids and amino acids. After exogenous melatonin treatment under cold condition, there was high accumulation of metabolites in the cold-tolerant regimes than their cold-sensitive counterparts. Collectively, the present study revealed differential modulations of melatonin between the cold-sensitive and the cold-tolerant genotypes in response to cold stress. This was mainly by impacting antioxidant system, photosystem II, as well as metabolic homeostasis.

Abbreviations: TBARS, TBA-reactive substances; SOD, superoxide dismutase; POD, peroxidase; OJIP curve, F_0, minimal reliable recorded fluorescence, at 20 μs with the pulse-amplitude modulation (PAM) fluorometer; F_J, fluorescence intensity at the J-step (2 ms) of OJIP; F_I, Fluorescence intensity at the I-step (30 ms) of OJIP; F_P, maximal recorded fluorescence intensity, at the peak P of OJIP; φP_0, maximum quantum yield of primary photochemistry; PI_{ABS}, PI (potential) for energy conservation from exciton to the reduction of intersystem electron; φE_0, Quantum yield of the electron transport flux from QA to QB; γRC, Probability that a PSII Chl molecule functions as RC; ΨE_0, Efficiency/probability with which a PSII trapped electron is transferred from QA to QB; φR_0, Quantum yield for reduction of end electron acceptors at the PSI acceptor side; ABS/RC, Absorbed photon flux per RC; TP_0/RC, Trapped excitation flux per RC; DI_0/RC, dissipated photon flux per RC; ET_0/RC, Electron transport flux per RC; SC, cold-sensitive genotype treated with deionized water; SM, cold-sensitive genotype treated with melatonin; TC, cold-tolerant genotype treated with deionized water; TM, cold-tolerant genotype treated with melatonin.

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1. Introduction

Low temperature is a critical environmental factor that limits agricultural productivity, survival and geographical distribution of plants. Multiple physiological, biochemical, molecular and metabolic changes take place during plants' response to cold stress. These include alterations of membrane fluidity, enzyme activity, metabolism homeostasis and genes transcription (Thomashow, 1999; Zhu et al., 2004; Chinnusamy et al., 2010; Knight and Knight, 2012).

Photosynthesis is sensitive to low temperature, which directly influences photosystem performance and photosynthetic apparatus activities (Smillie and Hetherington, 1984). Under cold stress, the efficiency of photosynthetic electron transport in plant is vividly decreased, which leads to an excessive energy generation, and even triggers photoinhibition (Krause, 1994). Kee et al. (1986) suggested that the cold-induced inhibition involves the whole chain electron transport and PS II catalyzed photochemistry. Moreover, reactive oxygen species (ROS) is formed when plants are subjected to photoinhibitory conditions, which results in serious injury of PSII components. The extent of impairment relies on the balance between damage and repair of PSII components (Chen et al., 2013). However, there is limited information concerning the effects of melatonin on photosystem in plant under low temperature.

Metabolites are the end products of cellular regulatory processes, and their levels can be estimated as the ultimate response of biological systems to genetic or environmental changes (Fiehn, 2002). Many studies have revealed that small soluble metabolites, such as sucrose, fructan, and proline, participate in plants response to abiotic stress (Stitt and Hurry, 2002). Sugars frequently accumulate in plants exposure to cold stress, especially if the temperature is insufficient to kill tissues (Levitt, 1980). In addition, soluble sugars, ROS and tetrapyrrole intermediate Mg-protoporphyrin (Mg-ProtoIX) are three major types of metabolic signals, which might be pivotal for cold signaling (Zhu et al., 2007).

Melatonin (N-acetyl-5-methoxytryptamine) is a ubiquitous and highly conserved molecule occurring even in evolutionarily distant organisms. Melatonin was first described in *Gonyaulax polyedra* in the year 1991 (Poeggeler et al., 1991). Subsequently, progressive research regarding its presence and importance in plants has been rigorously conducted (Posmyk et al., 2009). Melatonin was first discovered in higher plants by Dubbels et al. (1995) and Hattori et al. (1995). Melatonin participates in multiple developmental processes including governing growth of roots and shoots (Zhang et al., 2014), circadian rhythms regulation, photosynthesis promotion, chlorophyll preservation (Arnao and Hernández-Ruiz, 2009; Tan et al., 2012), leaf senescence delay (Byeon et al., 2012), and alleviation of oxidative damage induced by reactive oxygen species (ROS) burst (Tan et al., 2012). Furthermore, has been reported to improve plants tolerance against multiple abiotic stresses, such as cold, drought as well as salt (Bajwa et al., 2014; Shi et al., 2015a; Wang et al., 2014).

Bermudagrass (*Cynodon dactylon* (L).Pers.), is a major turf species for park, sport fields as well as lawns (Shi et al., 2015a, 2015b; Fan et al., 2014). As a widely cultivated warm-season turfgrass, low temperature is considered to be a major environmental factor limiting its usage (Fan et al., 2014). However, there is insufficient information regarding bermudagrass in response to cold stress. Thus, cold tolerance improvement in this botanical species is urgent for turf-grass engineering.

This study was designed to investigate the possible role of melatonin in bermudagrass response to cold stress, particularly focusing on modulations of antioxidant enzymes activities, photosynthesis and metabolisms. Two bermudagrass genotypes that were filtrated by our team, the cold-sensitive (S) genotype 'WBGg-17' and the cold-tolerant (T) genotype 'WBD128', combined with exogenous melatonin treatment, were exposed to cold stress. The effects of exogenous melatonin on cell damage and underlying antioxidant responses were determined. Photosynthetic and comparative metabolic analyses were also performed. This study provided some insights into the possible mechanisms behind the differences in cold tolerance ability modulated by exogenous melatonin in bermudagrass.

2. Materials and methods

2.1. Plant materials and growth conditions

Two wild genotypes of bermudagrass [*Cynodon dactylon* (L).Pers.] were used in this study. They are the cold sensitive 'WBGg-17' and the cold tolerant 'WBD128', which were collected from Xiaojiang city, Zhejiang province, China (N 27°34.258, E 120°27.383) and Baise city, Guangxi province, China (N24°51.397, E 106°33.288), respectively. Uniform stolons of the two cultivars were planted in plastic pots (7.5 cm in diameter and 9.0 cm deep) that were filled with matrix (brown coal soil). Several drainage holes were drilled at the bottom of the pot to allow excess water to drain out and to enhance soil aeration. For bermudagrass establishment, the pots were kept in the greenhouse for 2 months with a growth condition of 12-h-light (260 μmol m^{-2} s^{-1}, 30 °C)/12-h-dark (25 °C) and 40% relative humidity. During the establishing period, bermudagrass was watered daily and fertilized every two weeks with full-strength Hoagland nutrient solution.

2.2. Treatments

After establishment, the plants were transferred into two growth chambers (LSC-339CF, Xingxing Group Co.,Ltd, Zhejiang, China) with different growth conditions. The condition of control chamber was 12-h-light (260 μmol m^{-2} s^{-1}, 30 °C)/12-h-dark (25 °C) and 70% relative humidity, while cold-treated chamber were similar with control, except for 4 °C (day/night) temperature. In the study, 100 μM melatonin was applied, while double distilled water was used as the control. The plants were subjected to four treatment regimes: (i) the cold-sensitive genotype treated with deionized water (SC), (ii) the cold-tolerant genotype treated with deionized water (TC), (iii) the cold-sensitive genotype treated with melatonin (SM) and (iv) the cold-tolerant genotype treated with melatonin (TM). The plants in each growth chamber were watered and sprayed daily with 100 mL of deionized water or melatonin for 3 days. After treatment, bermudagrass leaves were collected for physiological and metabolomic assays, and chlorophyll (Chl) α fluorescence transient was recorded at 3 d of cold treatment.

2.3. Chlorophyll (Chl) α fluorescence transient

The data of chlorophyll (Chl) α fluorescence transient was measured by pulse-amplitude modulation (PAM) fluorometer (PAM 2500, Heinz Walz GmbH). The third fully expanded bermudagrass leaves were collected at 3 d of cold treatment. After 30 min pre-adaption in the dark, red light of 3000 μmol photons m^{-2} s^{-1} triggered the OJIP transients, which was aimed to ensure closure of all reaction centers of PSII to estimate a real maximal fluorescence intensity (Chen et al., 2014). Subsequently, the strong light pulses triggered the Chl α fluorescence emission that was subsequently measured and digitized between 10 us and 320 ms. The data of OJIP transients was processed by using the JIP-test as reported by Chen et al. (2014).