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Melatonin reduces oxidative stress and promotes drought tolerance in young *Coffea arabica* L. plants

Cleide Nascimento Campos[a,*], Roniel Geraldo Ávila[b], Kamila Rezende Dázio de Souza[b], Lillian Magalhães Azevedo[b], Jose Donizeti Alves[b]

[a] Departamento de Biologia Vegetal, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, SP, 13083 – 862, Brazil
[b] Departamento de Biologia, Setor de Fisiologia Vegetal, Universidade Federal de Lavras, Lavras, MG, CP 3037, Brazil

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ABSTRACT

Water deficit severely compromises the homeostatic balance of plants and suggests a redox imbalance in cells. Melatonin is described as a promoter of stress tolerance in plants that induces the expression of stress-related genes, reduces lipid peroxidation and increases the antioxidant system. To analyze the influence of exogenous melatonin on the promotion of tolerance to water deficit, we applied concentrations of 300 μM and 500 μM to *Coffea arabica* L. seedlings. Here, we show that the intensity of the short-term responses to melatonin may vary according to the concentration of melatonin. We also report that lower concentrations of melatonin (300 μM) promote an increase in the root system and the protection of the photosynthetic apparatus, allowing greater gas exchange, greater carboxylation efficiency and higher chlorophyll contents. Moreover, melatonin improved the activity of the enzymatic and nonenzymatic antioxidant systems and reduced lipid peroxidation. In addition, we demonstrated that higher concentrations (500 μM) caused negative effects on stress tolerance, thus demonstrating a toxic level. Overall, these results demonstrated that melatonin mediates the signaling for water deficit responses by acting as an inductor of tolerance, most likely enhanced by increased carboxylation efficiency and antioxidant systems. This study provides evidence that exogenous melatonin protects coffee against water deficit.

1. Introduction

Coffee has great importance for the world and stands out as the second most traded commodity worldwide. Coffee belongs to the genus Coffea and has two large species of world importance, *Coffea arabica* and *Coffea canefora*, known as arabica coffee and conilon, respectively (CONAB, 2015). However, the coffee plantations of Brazil are comprised primarily of cultivars of *Coffea arabica*. This species offers products of good quality and great acceptance by the consumer market (Nogueira, 2005). Arabica coffee beans produce finer and more refined beverages, as well as having an intense aroma and the most varied types of tastes, body and acidity (Semmelroch and Grosch, 1996). Genetic and breeding researchers have developed several coffee cultivars and lines from the Arabica variety, including the cultivar Catuaí (Nogueira, 2005). The Catuaí coffee cultivar shows great adaptability, as well as high productivity, in most of the coffee regions in Brazil and around the world. This cultivar exhibits short height, allowing a higher density of planting and facilitating crop and phytosanitary treatments. In addition, the Catuaí cultivar offers important characteristics, such as producing abundantly during the first years of harvest, being less affected by deficiencies of calcium, magnesium and zinc, and being more resistant to coffee rust (Blank et al., 1991). Such characteristics arouse great interest in this coffee cultivar among coffee producers around the world.

For maintaining this productivity, growers tend to expand its cultivation to increasing areas, resulting in exposure to edaphoclimatic adversities. Water scarcity is one of the most severe adversities. Water scarcity is a limiting environmental factor and severely compromises the homeostatic balance of the plants. In general, responses to water deficit begin with the increased endogenous production of abscisic acid (ABA), which induces the increased expression of several water stress response genes and activates a signaling cascade that leads to reduced stomatal conductance (*gs*) and consequently, internal carbon dioxide concentration (*Ci*) (Osakabe et al., 2014). In addition, there is a reduction in the carboxylation activity of the ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) enzyme due to the low levels of its substrate, CO_2 (Xu et al., 2013).

To function with this reduction in the carboxylation activity of

* Corresponding author at: Núcleo de pesquisa em Fisiologia e Bioquímica, Instituto de Botânica, Caixa Postal: 68041, CEP: 04045-972, São Paulo, SP, Brazil.
E-mail address: cleide125@gmail.com (C.N. Campos).

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Rubisco, NAPH accumulates, and $NADP^+$ is unavailable to receive the electrons from the end of the photochemical stage, resulting in electron accumulation (Porcar-Castell et al., 2014). These electrons are then donated to molecular oxygen generating superoxide radicals, which together with other reactive species, can damage the photosynthetic apparatus, as well as the peroxidation of lipid membranes and chlorophylls (Nishiyama et al., 2011). Due to this low photosynthetic assimilation, there is a reduction in the synthesis of sucrose and deprivation of the supply of soluble sugars to maintain metabolism (Hammond and White, 2008). Additionally, this disaccharide can be hydrolyzed to form hexoses and allocated in reserve organs for later uses (Xu et al., 2015). This allocation of assimilates from the aerial part to the roots can increase root growth by increasing water uptake by roots.

To control these imbalances caused by low photosynthetic assimilation and other physiological processes that lead to the formation of ROS, plants have a cellular antioxidant defense system consisting of enzymes, such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT), and nonenzymatic components, such as ascorbate and glutathione, which together act to neutralize the reactive oxygen species (ROS), protecting the cells from possible oxidative damage (Sharma et al., 2012). In addition to these antioxidant defense mechanisms, the plant's tolerance to water deficit involves the accumulation of organic solutes, such as proline, that function to maintain its osmotic adjustment. In this sense, proline increases the resistance to dehydration, helping to reduce the water potential, since it allows the movement of water to the interior of the cells, minimizing the damage caused by excess ions (Burritt, 2012). Proline forms a layer of hydration around proteins preventing them from being damaged by the stress conditions (Burritt, 2012). In this manner, the drought increases the accumulation of osmolytes for water retention or to avoid excessive loss of water.

Melatonin (*N*-acetyl-5-methoxytryptamine) is described as a stress tolerance promoter in plants (Arnao and Hernández-Ruiz, 2014; Jiang et al., 2016; Shi et al., 2015), since its synthesis is induced in plants exposed to biotic and abiotic stresses. Melatonin acts to limit oxidative stress by inducing the expression of stress related genes, increasing the enzymatic antioxidant system and reducing lipid peroxidation and hydrogen peroxide (H_2O_2) levels (Reiter et al., 2015). Melatonin positively regulates the mRNA expression of several biotic and abiotic stress response genes, such as the C-repeat binding factors (CBFs)/drought response element binding factors (DREB1 s) in *Arabidopsis thaliana* (Shi et al., 2015), increases the activity of antioxidant enzymes (Wang et al., 2013; Jiang et al., 2016) and reduces the levels of malondialdehyde (MDA - Jiang et al., 2016) and H_2O_2 (Wang et al., 2013). In addition, melatonin induces increased levels of ascorbate, since this molecule is a secondary antioxidant that signals retrograde to the nucleus under low photosynthetic status. Moreover, plants treated with melatonin showed higher growth rates than those not treated (Wang et al., 2016), probably enhanced by the increased sugar metabolism and photosynthetic rates (Zhao et al., 2015).

Although the benefits of melatonin in mitigating the damage caused by abiotic stresses are known, there is no data in the literature regarding the induction of the tolerance to water deficit in coffee. In light of the responses triggered by melatonin in plants exposed to low water status, concentrations of 300 μM and 500 μM melatonin were applied to coffee seedlings to analyze the influence of exogenous melatonin on the promotion of the tolerance to water deficit. Therefore, we show that the intensity of the short-term responses to melatonin may vary according to the concentration of melatonin. Overall, these results demonstrated that melatonin mediates the signaling for drought stress responses by acting as an inductor of tolerance, most likely enhanced by increased carboxylation efficiency, antioxidant system, and root systems. This study provides evidence of water deficit protection in coffee by exogenous melatonin.

2. Material and methods

2.1. Experimental design

Seedlings of *Coffea arabica* L. cv. Catuaí 144 were transferred to polypropylene pots containing 3 kg of standard substrate for coffee seedlings, consisting of sifted subsoil, sand and tanned corral manure and sieved in ratios of 2: 1: 1 plus fertilizer containing nitrogen, phosphorus and potassium in the proportions 4: 14: 8.

After transplantation, the plants were acclimated for four weeks and then were subjected to the following treatments: Control (CT), in which the plants were irrigated, and the substrate moisture was maintained close to the field capacity; Water deficit (WD) in which the plants were kept at 40% of the maximum moisture retention capacity; Melatonin at 300 μM + water deficit (M300) where melatonin was applied to the soil, and the plants were maintained at 40% moisture retention capacity, and Melatonin at 500 μM + water deficit (M500) in which melatonin was applied to the soil, and the plants were maintained at 40% of the maximum moisture retention capacity.

The determination of the CT condition, in which the soil was maintained close to the maximum moisture retention capacity, was performed using the direct method. A soil sample was oven dried, weighed and placed in a container with water, and the surface of the container was covered by a plastic bag to avoid evaporation until it reached saturation by capillarity. The maximum water retention capacity was determined by the difference between the dry and the saturated weight. The melatonin stock solution was prepared by dissolving melatonin in ethanol (50 mg of melatonin in 1 mL of ethanol). After the stock solution was prepared, it was diluted to obtain the appropriate concentrations that were used for the application, and the volume was brought to 100 mL with distilled water. In each pot, 100 mL of melatonin solution was applied directly to the soil in a single application. Before the treatments, all the pots were weighed to maintain the humidity at 100%. Following this procedure, melatonin was applied at concentrations of 300 and 500 μM, and the weight of the pots was monitored daily to maintain them at 40% of field capacity. In the water deficit treatment plants not treated with melatonin, 100 mL of distilled water were applied, so that they had the same soil moisture as those treated with melatonin.

The evaluation and data collection were performed in two periods. The first evaluation was performed twenty days after the treatments when the first symptoms appeared, and all the pots were irrigated until the soil reached the field capacity. The second evaluation was performed 24 h after rehydration.

The experiment was conducted in a completely randomized design (CRD) and carried out in a 4 × 2 factorial scheme with four treatments (CT, WD, M300 and M500), two collection times (20 and 21 days of treatment) and three replicates. Each experimental unit consisted of two pots containing one plant each with one plant used for destructive analysis and the other for nondestructive analysis, totaling 48 plants. The variables were analyzed for normality using the Shapiro-Wilk test ($p \geq 0.05$). Those variables were subjected to an analysis of variance using the ASSISTAT 7.7 beta (Silva and Azevedo, 2002) statistical program whose means among the treatments were compared by the Scott-Knott test ($p \leq 0.05$).

2.2. Water potential

The water potential of the plants was measured predawn using a pressure chamber (PMS Instruments Company, USA) as described by Scholander et al. (1964). One leaf per plant that was part of the second pair of fully expanded leaves was used.

2.3. Vegetative growth

The plants were divided into shoots (stalk and leaves) and roots, and