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Research Paper

Melatonin pretreatment improves vanadium stress tolerance of watermelon seedlings by reducing vanadium concentration in the leaves and regulating melatonin biosynthesis and antioxidant-related gene expression

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ABSTRACT

Vanadium (V) is an important heavy metal with ubiquitous presence in the Earth's crust, but limited information is available as to its effect on plants and management strategies. Melatonin is a widely studied biomolecule; it acts as an antioxidant and a signaling molecule that enhances the abiotic stress tolerance of plants. Melatonin improves copper, zinc, and cadmium tolerance in plants. In this study, we investigated the response of watermelon seedlings to V stress and the potential role of melatonin in enhancing V stress tolerance of watermelon seedlings. The results showed that seedlings pretreated with melatonin (0.1 μM) exposed to V (50 mg/L) had a higher relative chlorophyll content (SPAD index), photosynthetic assimilation, and plant growth compared with non-melatonin pretreated seedlings. Melatonin pretreatment lowered leaf and stem V concentrations by reducing V transport from root to shoot. Melatonin pretreatment enhanced superoxide dismutase (SOD) and catalase (CAT) activities, and reduced the hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) content of watermelon seedlings, by regulating melatonin biosynthesis and gene expression for superoxide dismutase, peroxidase, ascorbate peroxidase, glutathione peroxidase, and glutathione S-transferase. So far as we know, these results are the first evidence that melatonin improves plant growth of watermelon seedlings under vanadium stress conditions. Considering these observations, melatonin can be utilized to reduce the availability of V to plants, and improve plant growth and V stress tolerance.

1. Introduction

Vanadium (V) is the fifth most abundant trace element among the transitional metals found in the Earth's crust (Amorim et al., 2007). The V concentration reported in the Earth's crust varies from 10 to 220 mg/kg, whereas the soils that are in human use contain higher amounts (Perron, 2001; Moskalyk and Alfantazi, 2003; Teng et al., 2006; Sachin et al., 2011). V adversely affects plant growth and development through altering enzymatic activities, gene expression, production of reactive oxygen species (ROS) (Imtiaz et al., 2015a; Reiter et al., 2015), and cellular metabolism (Vaccarino et al., 1983; Imtiaz et al., 2016). Higher levels of V influence the formation of lateral roots, and leaves show chlorosis due to the destruction of chlorophyll (Alan et al., 2005; Imtiaz et al., 2015a,b, 2017). V application at 25 mg/L to chickpea plants substantially increased the H_2O_2 and malondialdehyde (MDA) content, leading to reduced root length, shoot length, root growth, and fresh weight (Imtiaz et al., 2016). V and other heavy metal stresses reduce the protein content of maize plants, leading to disturbance in normal metabolic functioning of plant tissues (Tanyolaç et al., 2007). V is a chemical analogue of phosphorous (P); thus, V affects the plant absorption capacity of P, an important and essential plant nutrient. V also affects the activities of enzymes of which P is a major component (Imtiaz et al., 2017).

Watermelon is an important fruit grown on a commercial scale across the world. The world's watermelon production exceeds 110 million tons, with 67% of watermelons being produced in China (FAO, 2014). There is limited information regarding the effect of V on the growth of watermelon seedlings. Similarly, there is also lack of data regarding the potential of melatonin to enhance the V stress tolerance of watermelon seedlings. Melatonin (N-acetyl-5-methoxytryptamine) is

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a low-molecular-weight biomolecule utilized as a biostimulant (Arnao and Hernandez-Ruiz, 2014). It is ubiquitous in plants and animals and exhibits pleiotropic biological activities (Hardeland et al., 2011; Arnao and Hernandez-Ruiz, 2014; Reiter et al., 2014; Nawaz et al., 2016a). Prior to 1995, melatonin was thought to be an animal hormone when it was discovered in Japanese morning glory (*Pharbitis nil*) and numerous edible plants (Van-Tassel et al., 1995, 2001; Hattori et al., 1995; Dubbels et al., 1995). Subsequently, the presence of melatonin has been confirmed in a large number of plant species belonging to the families *Rosaceae*, *Vitaceae*, *Poaceae*, *Apiaceae*, and *Brassicaceae* (Reiter et al., 2015); some plants possess melatonin in large amounts (Nawaz et al., 2016a). The role of melatonin in germplasm storage (Zhao et al., 2011; Uchendu et al., 2013), seed germination and seedling growth (Zhang et al., 2013; Aguilera et al., 2015; Arnao and Hernandez-Ruiz, 2014, 2015), root growth and development (Hernandez-Ruiz et al., 2005; Park and Back, 2012), fruit ripening, salt stress (Li et al., 2012; Zhang et al., 2014; Wei et al., 2015), low-temperature stress (Shi et al., 2015a), high-temperature stress (Byeon and Back, 2014; Hernandez et al., 2015), drought stress (Wang et al., 2013), high-light-intensity stress, ultraviolet radiation stress (Afreen et al., 2006; Zhang et al., 2012), herbicide and other chemical stresses (Park et al., 2013), and plant senescence (Wang et al., 2013; Arnao and Hernandez-Ruiz, 2014, 2015; Shi et al., 2015b) has been uncovered.

Thus, we conducted this study to assess the effect of melatonin pretreatment to reduce the adverse effects of V on plant growth and development of watermelon seedlings, and to investigate the mechanism involved. So far as we know, we are the first to report the effect of V on watermelon seedlings and the potential of melatonin to improve plant growth under V stress conditions.

2. Materials and methods

2.1. Plant material and treatments

This study was conducted at Huazhong Agricultural University, China (latitude 30° 27′ N, longitude 114° 20′ E, and altitude 22 m above sea level). In this study, a watermelon cultivar "Zaojia 8424" (*Citrullus lanatus*) was used and seeds were sown in 50-cell plug trays filled with a mixture (1:1:1) of peat, perlite, and vermiculite (v/v). After the emergence of two leaves, plants were shifted to hydroponic conditions containing half-strength Hoagland solution. After five days, half of the seedlings were shifted to full-strength Hoagland solution, while the other half was shifted to full-strength Hoagland solution containing 0.1 μM melatonin. After three days, half of the melatonin pretreated and non-melatonin pretreated seedlings were transferred to full-strength nutrient solution while the other half were transferred to nutrient solution containing 50 mg/L V. The concentration of V (50 mg/L) and melatonin (0.1 μM) was selected based on our preliminary studies, because the response of V to different plant species varies considerably. Similarly, the response of melatonin varies for plant species, method of application [foliar application or root application (hydroponic conditions, substrate conditions, and soil conditions)], and the time of exposure to melatonin. During preliminary studies we observed that at higher concentrations (>0.1 μM) melatonin reduces the plant growth while at lower concentration (0.1 μM) it improves root growth and root morphology; thus we pretreated watermelon seedlings with 0.1 μM melatonin. To select the vanadium (V) concentration, we conducted a preliminary study and applied V ranging from 0 mg/L to 100 mg/L to the watermelon seedlings grown under hydroponic conditions. We selected 50 mg/L for further study because, below this level, symptoms of V stress (leaf injury) and reduction in plant growth were not obvious during five to seven days after V application. Thus, on the basis of symptoms of leaf injury (leaf burning/chlorophyll destruction) and growth data (dry weight reduction), we selected a concentration of 50 mg/L V (Fig. S1). In this study, we utilized ammonium metavanadate (NH_4VO_3) as a source of V. Plants were allowed to grow under V stress conditions for seven days, and plant samples were collected for the measurement of different parameters. The solution was changed every four days to avoid the deficiency of a specific ion. The nutrient solution was aerated every two hours throughout the day and night with an air pump. The experiment was conducted in a greenhouse, and the temperature of the greenhouse varied from 16 °C to 30 °C, while the relative humidity varied from 55% to 95% during the experimental stage.

2.2. Plant growth

To measure plant growth, six uniform plants from each replicate were harvested and their fresh weight was measured using an electric balance (MSE24P-1-CE-DA, Cubis®, Sortorious, Goettingen, Germany). Then seedlings were placed in paper bags, labeled, and put in the oven at 105 °C for 15 min and then at 70 °C for 72 h. Afterwards, the dry weight (DW) was measured.

2.3. SPAD index and leaf photosynthesis parameters

The relative chlorophyll content of leaves (third leaf from the top) was measured using SPAD-502 Chlorophyll Meter (Minolta Camera Co., Ltd., Japan). Photosynthetic assimilation, stomatal conductance, intercellular CO_2 concentration, and transpiration rate of watermelon leaves were measured using a portable photosynthesis system (Li-6400XT, LI-COR, Lincoln, Nebraska, USA). The measuring chamber was controlled to maintain leaf temperature, CO_2 concentration, and photosynthetic photon-flux density at 25 °C, 360 μM/mol, and 800 μM/m^2/s, respectively.

2.4. Root morphology

Roots of three uniform plants from each replicate were harvested, washed with deionized water, and the root morphology was studied. The root scanning was performed using Imagery Scan Screen (Epson Expression 11000XL, Regent Instruments, Canada), and root image analysis was conducted via WinRHIZO 2003a software (Regent Instruments, Canada).

2.5. Ion analysis

To measure the V concentration of leaves, stem, and root, six plants from each replicate were harvested and washed with deionized water. The dried plant samples were ground and then digested with HNO_3 and $HClO_4$ (v/v, 4:1). The V concentration was measured using an atomic absorption spectrophotometer (200 AA series, Agilent Technologies, Santa Clara, USA). P concentration was measured using the phosphomolybdate blue method (Murphy and Riley, 1962), while K concentration was measured through inductively coupled plasma optical emission spectrometry (IRIS Advantage, Thermo Jarrell Ash/Baird, Massachusetts, USA).

2.6. Hydrogen peroxide (H_2O_2), malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT) measurement

The root and leaf samples for the measurement of H_2O_2 and MDA content and SOD and CAT activities were taken after three days and seven days of V application. The root and leaves of three plants from each replicate were harvested and immediately frozen in liquid nitrogen. These samples were temporarily stored at −80 °C and were then utilized for further analysis.

To measure H_2O_2 and MDA, 0.1 g of frozen samples were taken, ground to a powder in liquid nitrogen, and extracted with a buffer (900 μL), following the instructions described in H_2O_2 and MDA kits (A064, A003-3) purchased from Nanjing Jiancheng Bioengineering Institute, Nanjing, China. H_2O_2 and MDA contents were recorded at a