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Caffeic acid pretreatment enhances dehydration tolerance in cucumber seedlings by increasing antioxidant enzyme activity and proline and soluble sugar contents

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ABSTRACT

To determine the mitigative effects of exogenous caffeic acid (CA) on dehydration stress and the physiological mechanisms underlying these effects in plants, cucumber seedlings were pretreated with CA and exposed to dehydration conditions induced by 10% polyethylene glycol (PEG) 6000. Among the concentrations of CA examined, treatment with 100 μm CA caused the greatest reduction in the levels of superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and malonaldehyde under dehydration stress. Following 2 days of pretreatment with 100 μm CA, the activities of superoxide dismutase (EC 1.15.1.1), catalase (EC 1.11.1.6), glutathione peroxidase (EC 1.11.1.9), ascorbate peroxidase (EC 1.11.1.11), glutathione reductase (EC 1.6.4.2), dehydroascorbate reductase (EC 1.8.5.1) and monodehydroascorbate reductase (EC 1.6.5.4) increased. When the CA-pretreated seedlings were subjected to dehydration, the activities of these antioxidant enzymes further increased and were higher than those observed under dehydration treatment alone, which was in accordance with the increased transcript levels of *copper/zinc superoxide dismutase*, *manganese superoxide dismutase* and *guaiacol peroxidase* genes and coincided with the increased contents of reduced glutathione and ascorbate. Meanwhile, CA + PEG treatment mitigated growth inhibition, reduced the osmotic potential and enhanced the increases in proline and soluble sugar levels observed in response to PEG treatment, and this treatment also increased the contents of endogenous CA and reduced the levels of $O_2^{\bullet-}$, H_2O_2 and malonaldehyde in the leaves. Therefore, pretreatment with 100 μm CA increases the deposition of endogenous CA, antioxidant enzyme activities and proline and soluble sugar contents in cucumber leaves, which may protect the seedlings from dehydration stress. This work lays the foundation for studies investigating the application of CA to seedlings as drought-resistance or water-retaining agents.

1. Introduction

Drought stress is a serious threat to plant growth, and it causes the accumulation of reactive oxygen species (ROS) including superoxide radical ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) (Proietti et al., 2013). The overproduction of ROS damages membranes, DNA and proteins and even leads to cell death (Naliwajski and Skłodowska, 2014). To mitigate the adverse effects of ROS, plants have evolved enzymatic and non-enzymatic antioxidants (Sen and Alikamanoglu, 2013), including superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), ascorbate peroxidase (APX) (Doupis et al., 2013), glutathione peroxidase (GSH-Px), glutathione reductase (GR), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR) (Li et al., 2013), reduced glutathione (GSH) and ascorbate (AsA) (Namdjoyan and Kermanian, 2013). Accordingly, the expression of the *SOD*, *GPX* and *CAT* genes is induced in drought-tolerant chrysanthemum cultivars under water stress (Sun et al., 2013). The transcription of *SOD* and *CAT* is activated in cucumber under dehydration following glucose pretreatment (Huang et al., 2013). Nitric oxide treatment enhances chilling tolerance in banana fruit by increasing the activities of antioxidant enzymes and inducing the expression of antioxidant-related genes (Wu et al., 2014).

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Exogenous caffeic acid (CA, 3,4-dihydroxycinnamic acid) disrupts plant–water relations and inhibits the growth of leafy spurge (Barkosky et al., 2000). However, at certain concentrations and exposure durations, CA is a potent antioxidant (Laranjinha et al., 1995; Chen and Ho, 1997; Gulcin, 2006), and it increases oxidation resistance in many different systems (Meyer et al., 1998; Fukumoto and Mazza, 2000). Exogenous CA reduces the defensive freezing behavior of mice and inhibits the emotional abnormality produced by conditioned fear stress (Takeda et al., 2002). Treatment with exogenous CA protects *Salmonella typhimurium* from bleomycin-induced oxidative stress (Stagos et al., 2004). In plants, the application of CA increases soybean yields under reduced light intensity (Krishna and Surinder, 2003). Since an antioxidant enzyme gene regulates the activities of other antioxidant enzymes in plants (Shin et al., 2013) and the antioxidant system is interactional, we hypothesized that pretreatment with the potent antioxidant CA would affect antioxidant enzyme activities in plants; when CA-pretreated plants are exposed to drought conditions, the antioxidant enzyme activities will be further altered, thereby protecting plants from drought stress. To the best of our knowledge, there is no report that exogenous CA alleviates the damage in plants caused by drought stress.

Cucumber is the fourth most important vegetable crop worldwide (Lv et al., 2012). Cucumber is mainly consumed as raw fruit, but it also has various medicinal values (Shah et al., 2013). Cucumber has a shallow root system and large leaves and is sensitive to water stress. It is therefore important to mitigate drought stress in cucumber using various compounds, as described by Ouda et al. (2007). Moreover, as plants suffer from cellular dehydration under drought stress, polyethylene glycol (PEG) 6000 can be used to induce dehydration stress under laboratory conditions in a uniform, repeatable manner; this treatment alters ROS production in plants (Huang et al., 2013; Li et al., 2013).

CA functions as an antioxidant and plays a role in the scavenging of ROS. Therefore, in the current study, we pretreated cucumber seedlings with 100 μM CA and exposed the seedlings to dehydration stress induced by 10% PEG 6000. One of our aims was to determine whether CA could protect plants from dehydration stress and whether the protective effect is associated with the regulation of antioxidant enzymes. Proline and soluble sugars are important osmotic regulators that can mitigate stress (Xi et al., 2013). It was also our aim to investigate the effect of exogenous CA on dehydration tolerance based on the contents of proline and soluble sugars. Our work not only contributes to elucidating the physiological mechanism of dehydration stress mitigated by CA in plants, but it also lays a theoretical and practical foundation for studies investigating the application of CA to seedlings as drought-resistance or water-retaining agents.

2. Materials and methods

2.1. Plant materials and treatments

Seedlings of cucumber (*Cucumis sativus* cv. Jinchun no. 4) were cultivated in sand with Hoagland nutrient solution according to Liu et al. (2009) until the two-leaf stage. Then, 32 cucumber seedlings were divided into four groups (eight plants per group) and watered separately for 2 d with different concentrations (0, 75, 100 and 125 μM) of CA. All seedlings were then exposed to 1 day of dehydration stress treatment induced by 10% PEG 6000. Based on the results of this preliminary experiment, 56 cucumber seedlings were selected and divided into seven groups, where one group was used to determine physiological parameters at 0 d and three groups were watered with Hoagland nutrient solution containing the optimal concentration (100 μM) of CA, while the remaining three groups were watered with Hoagland nutrient solution lacking CA. The six groups of cucumber seedlings were grown in growth chambers (12 h photoperiod, 300 μmol photons m^{-2} s^{-1} and day/night temperature of 25/18 °C) for 2 d. Then, one group of CA-pretreated seedlings and one group of CA-untreated seedlings were subjected to physiological parameter analysis on day 2 of treatment. The sand used to plant the remaining seedlings was rinsed six times with water and six times with Hoagland nutrient solution. Subsequently, two groups of CA-pretreated seedlings were watered for 1 d with Hoagland nutrient solution and Hoagland nutrient solution containing 10% PEG 6000, respectively; the groups were designated "CA pretreatment" and "CA + PEG treatment", respectively. Two groups of cucumbers that were not pretreated with CA were treated as above; the groups were designated the "control" and "PEG treatment" groups, respectively. Three biological replicates were performed per treatment.

2.2. Determination of relative water content (RWC)

The RWC was determined according to Barrs and Weatherley (1962) using leaf discs (1 cm in diameter).

2.3. Analysis of osmotic potential

Cucumber leaves were frozen in liquid nitrogen, and their osmotic potential was determined according to Bajji et al. (2001).

2.4. Examination of endogenous CA contents

The endogenous CA contents were determined according to Wang et al. (2004) with the modifications of Li et al. (2013).

2.5. Malondialdehyde content assay

Malondialdehyde (MDA) was extracted from the samples with 10% trichloroacetic acid, and its content was measured with the method described by Dhindsa et al. (1981) as modified by Xu et al. (2008).

2.6. Examination of the rate of $O_2^{\bullet -}$ formation

The rate of $O_2^{\bullet -}$ formation was measured based on the method of Elstner and Heupel (1976) and was calculated from a standard curve of $NaNO_2$ reagent.

2.7. Measurement of H_2O_2 content

The H_2O_2 content was determined according to Bernt and Bergmeyer (1974) using a standard curve of H_2O_2 reagent.

2.8. Analysis of antioxidant enzyme activities

The leaf samples (0.1 g) were ground in liquid nitrogen and suspended in 1 ml of ice-cold HEPES buffer (25 mM, pH 7.8) containing 0.2 mM EDTA and 2% PVP to determine the activities of SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), GPX (EC 1.11.1.7), DHAR (EC 1.8.5.1) and MDHAR (EC 1.6.5.4) (Ramiro et al., 2006). The enzymes GR (EC 1.6.4.2) and GSH-Px (EC 1.11.1.9) were extracted by suspending 0.12 g of liquid nitrogen-ground leaves into 0.12 ml of 25 mM HEPES buffer (pH 7.8, containing 2% PVP and 0.2 mM EDTA). HEPES buffer (25 mM, pH 7.8) containing 0.2 mM EDTA, 2% PVP and 2 mM ascorbate was used for APX (EC 1.11.1.11) extraction. Enzyme activities were determined according to the following protocols: SOD, Hwang et al. (1999); CAT, Pereira et al. (2002); GPX, Ramiro et al. (2006); GSH-Px, Xue et al. (2001); APX, Zhu et al. (2004); MDHAR, Hoque et al. (2007); DHAR, Doulis et al. (1997) and GR, Foyer and