Scientia Horticulturae 162 (2013) 20–30

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Exogenous glucose regulates activities of antioxidant enzyme, soluble acid invertase and neutral invertase and alleviates dehydration stress of cucumber seedlings

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ARTICLE INFO

Article history:
Received 30 January 2013
Received in revised form 26 July 2013
Accepted 27 July 2013

Keywords:
Antioxidant
Glucose
Neutral invertase
Polyethylene glycol 6000
Soluble acid invertase

ABSTRACT

To investigate proposed alleviative effects of exogenous glucose on dehydration stress and the physiological mechanisms behind, seedlings of *Cucumis sativus* L. cv. Jinchun no. 4 were pretreated with glucose for 3 days and then were exposed to dehydration conditions induced by 10% polyethylene glycol (PEG) 6000 for 2 days. Based on the results of our initial experiments, 20 mM glucose was chosen since it mitigated growth inhibition caused by dehydration and as well resulted in the lower levels of malondialdehyde, superoxide radical ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) under dehydration stress than other concentrations of glucose. After three days of exposure to 20 mM glucose, the levels of reduced glutathione, ascorbate, proline, soluble sugars, glucose and fructose in leaves were increased, and the activities of superoxide dismutase (EC 1.15.1.1), catalase (EC 1.11.1.6), glutathione reductase (EC 1.6.4.2), soluble acid invertase (SAI, EC 3.2.1.26) and neutral invertase (NI, EC 3.2.1.27) were enhanced. When the glucose-pretreated seedlings were exposed to dehydration stress for 2 days, the levels of reduced glutathione, ascorbate, proline, soluble sugars, glucose and fructose and the activities of superoxide dismutase, catalase, glutathione reductase, SAI and NI were changed further and were higher than PEG treatment alone, which was in accordance with the increased transcript levels of *copper/zinc superoxide dismutase*, *manganese superoxide dismutase* and *catalase* genes. Meanwhile, we observed the mitigated growth inhibition in glucose + PEG treatment in comparison to PEG treatment. We showed that pretreatment with 20 mM glucose induced antioxidants, SAI, NI, proline and soluble sugars in leaves, and it thus can protect cucumber seedlings from dehydration stress. These indicate that exogenous glucose may have the application possibility for a future practical trial of stress reduction.

1. Introduction

Drought occurs in many parts of the world every year. It inhibits plant growth (van den Berg and Zeng, 2006) and generates reactive oxygen species (ROS) including superoxide radical ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) in plants (Li and Staden, 1998). The excessive accumulation of ROS induces lipid peroxidation (Blokhina et al., 2003), adversely affects normal metabolism of plants and even leads to cell death. In order to eliminate the damage of ROS, plants evolve an array of enzymatic antioxidants including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione reductase (GR) and non-enzymatic antioxidants such as the reduced glutathione (GSH) and ascorbate (AsA) (Asada, 1992). Among which, SOD dismutates $O_2^{\bullet -}$ into H_2O_2 (Vyas and Kumar, 2005), while CAT and GSH-Px and GSH can regulate H_2O_2 levels (Alscher et al., 1997; Noctor and Foyer, 1998). Meantime, AsA directly scavenges ROS (Thomas et al., 1992), and GR is a key enzyme in GSH regeneration cycle (Luster and Donaldson, 1987). In recent years, growing attention has been focused on the mechanism of action of sugars. It has been shown that sucrose treatment abolishes ROS stress in *Arabidopsis* leaves during extended darkness (Rosenwasser et al., 2011). Meanwhile, glucose pretreatment decreases the extent of lipid peroxidation and preserves the quality of watermelon seedlings for low-temperature storage (Jiang et al., 2012). Therefore, we hypothesize that pretreatment with exogenous sugars decreases the levels of ROS through regulating antioxidants, and it thereby enhances the tolerance of plants to drought stress. Hu et al. (2009) have studied the photosynthesis-related characteristics affected by exogenous glucose in wheat

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http://dx.doi.org/10.1016/j.scienta.2013.07.031

seedlings under water stress. However, there is no report that whether antioxidants are involved in glucose-induced alleviation of drought stress.

During drought stress, plants suffer from dehydration of their cells and tissues. And polyethylene glycol (PEG) 6000 is used to induce dehydration conditions to ensure the uniform and repeatable water potential in roots (Liu et al., 2009). On the other hand, sugars trigger many stress responsive genes in *Arabidopsis* and play a role in environmental responses (Price et al., 2004). In early spring of North China, glucose or other kinds of sugars are applied to pollinating solutions to protect flowers and young fruits of fruit trees from damage of low temperature. These indicate the practical significance of sugars in stress reduction. Moreover, cucumber is sensitive to dehydration stress. When breeding materials of cucumber are cultured, seedlings are often withered in a short period of time due to water deficit (Liu et al., 2009, 2010). If cucumber seedlings are exposed to drought for a long time, the whole leaves will be dried and could not be used to determine physiological parameters. Hence, in this study, seedlings of cucumber were addressed. To ensure the repeatability of experimental data, they were kept in growth chambers. Then, the plantlets were pretreated with glucose for 3 days and exposed to dehydration stress induced by 10% PEG 6000 for a short period of time (2 days). Our aims were to investigate whether exogenous glucose can increase cucumber's dehydration stress tolerance and to what extent the induced protective mechanisms are based on antioxidant enzyme activities. Due to the fact that soluble acid invertase (SAI) and neutral invertase (NI) are important enzymes in carbohydrate metabolism in plants (Batta and Singh, 1986) and proline plays an important role in counteracting to hyperosmotic stresses (Delauney and Verma, 1993), it was also our aim to examine the effects of exogenous glucose on dehydration stress tolerance based on levels of proline and carbohydrate metabolism-related SAI, NI and endogenous soluble sugars. Sugars have several additional roles including maintaining osmotic potential and signaling energy status, while mannitol is one of the important osmotic regulators (Dhanda et al., 2004). In this study, exogenous mannitol was used as an osmotic control to analyze the effects of glucose pretreatment under dehydration stress. Our work may elucidate a physiological mechanism of dehydration stress alleviated by glucose and lay the theoretical and practical foundation for the application of glucose in stress reduction of seedlings.

2. Materials and methods

2.1. Plant materials and treatment conditions

Seeds of cucumber (*Cucumis sativus* L. cv. Jinchun no. 4) were incubated on moist gauze at 25 °C. After 2 days, each seedling was planted into a 10-cm plastic pot filled with sands. All plants were grown in a solar greenhouse at 25/18 °C (day/night) and were watered twice per day with 100 ml of Hoagland nutrient solution, which contained 5 mM KNO_3, 5 mM $Ca(NO_3)_2$, 1 mM $NH_4H_2PO_4$, 2 mM $MgSO_4$, 10 μM $MnSO_4$, 50 μM H_3BO_3, 0.7 μM $ZnSO_4$, 0.2 μM $CuSO_4$, 0.01 μM $(NH_4)_6Mo_7O_{24}$ and 70 μM Fe-EDTA-Na_2. At the two-leaf stage, uniform cucumber seedlings were selected for treatments in the preliminary and formal experiments.

The preliminary experiments were performed to select the optimum concentration of glucose to reduce PEG-induced dehydration stress. Four groups of cucumber seedlings (eight plants per group) were separately watered with the Hoagland nutrient solution containing different concentrations (0, 10, 20, 30 mM) of glucose for 3 days and then were watered with the Hoagland nutrient solution containing 10% PEG 6000 for 2 days. Three different sets of plants grown at different times were used for the preliminary experiments.

Based on the results of the preliminary experiments, 20 mM glucose was used for the formal experiments. 80 cucumber seedlings were divided into ten groups (eight plantlets each group) to study the effects of glucose pretreatment on dehydration-stressed cucumbers in the formal experiments. The second leaves from one group of seedlings were harvested at 0 day to investigate the physiological parameters of cucumbers before treatments. Meantime, the remaining nine groups of cucumber seedlings were kept in growth chambers at 25/18 °C (day/night) with a photoperiod of 12 h light (300 μmol m^{-2} s^{-1})/12 h dark and were watered once per day. Among which, three groups of seedlings were watered with the Hoagland nutrient solution only, and three groups were watered with the Hoagland nutrient solution containing 20 mM glucose, while another three groups were watered with the Hoagland nutrient solution containing 20 mM mannitol. After 3 days, the second leaves from one group of glucose-pretreated and one group of mannitol-pretreated seedlings and one group of plantlets untreated with glucose and mannitol were harvested to investigate the physiological parameters of cucumbers at 3 days after the start of experiments. Then, the sand for planting the remaining six groups of cucumber seedlings was rinsed with water for 6 times and with the Hoagland nutrient solution for 6 times. Subsequently, two groups of seedlings, which were untreated with glucose and mannitol, were respectively watered with the Hoagland nutrient solution and the Hoagland nutrient solution containing 10% PEG 6000, and they were separately named as "control" and "PEG treatment". Two groups of glucose-pretreated seedlings were watered with the solutions as described above and were designated "glucose pretreatment" and "glucose + PEG treatment", respectively. Two groups of mannitol-pretreated cucumbers were also separately watered with the solutions as described above, and they were named as "mannitol pretreatment" and "mannitol + PEG treatment", respectively. After 2 days of PEG-induced dehydration stress, the second leaves began to be withered in the PEG treatment group and were separately harvested from 6 treatment groups to investigate the physiological parameters of cucumbers at 5 days after the start of experiments. Three different sets of plants grown at different times were used for the formal experiments.

2.2. Measurement of malondialdehyde (MDA) content in cucumber leaves

The MDA content in the second leaves was determined at 450, 532 and 600 nm following the procedures that were described by Dhindsa et al. (1981) and modified by Xu et al. (2008).

2.3. Determination of H_2O_2 level in cucumber leaves

According to the procedures that were described by Bernt and Bergmeyer (1974) and modified by Zhang et al. (2012), leaf samples (0.3 g) were ground with liquid nitrogen, homogenized in 3 ml of ice-cold 100 mM sodium phosphate buffer (pH 6.8), and then were centrifuged at 4 °C and 18,000 × *g* for 20 min. The extracting solution (0.15 ml) was mixed with 0.008 ml of peroxidase and 0.75 ml of reaction reagent, which contained 83 mM sodium phosphate (pH 7.0) and 0.005% o-dianisidine. The reaction mixture was then incubated at 30 °C for 10 min, and the reaction was stopped through adding 0.15 ml of 1 M perchloric acid. After centrifugation at 5000 × *g* for 5 min, the absorbance was measured at 436 nm.

2.4. Determination of the formation rate of $O_2^{\bullet -}$

The formation rate of $O_2^{\bullet -}$ in the second leaves was determined according to Elstner and Heupel (1976) with some modifications. Leaf samples (0.1 g) were ground with liquid nitrogen, extracted