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Exogenous sucrose influences antioxidant enzyme activities and reduces lipid peroxidation in water-stressed cucumber leaves

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Abstract

To investigate whether exogenous sucrose can protect cucumber from water stress, cucumber (*Cucumis sativus* L.) seedlings were pretreated with 90 mM sucrose or 90 mM mannitol for 1 d and then were dehydrated with 10 % (m/v) polyethylene glycol (PEG) 6000 for further 1 d. Dehydration inhibited plant growth and decreased osmotic potential and relative water content (RWC) in leaves. The pretreatment with 90 mM sucrose further reduced the osmotic potential but increased the RWC and alleviated the growth inhibition. Compared with the PEG treatment alone, the combination of sucrose + PEG increased the activities of superoxide dismutase, guaiacol peroxidase, glutathione reductase, dehydroascorbate reductase, monodehydroascorbate reductase, ascorbate peroxidase, and glutathione peroxidase, and elevated the content of endogenous sucrose, glucose, and fructose together with the activities of soluble acid invertase and neutral invertase. This was in accordance with the enhanced transcription of genes encoding copper/zinc superoxide dismutase, guaiacol peroxidase, and glutathione reductase. Furthermore, the sucrose pretreatment decreased the content of malondialdehyde and hydrogen peroxide and increased the content of ascorbate, reduced glutathione, and proline under the dehydration. Taken together, the pretreatment with 90 mM sucrose, but much less with mannitol, induced antioxidants, proline, and soluble sugars and thus reduced dehydration-caused damage to the cucumber seedlings.

Additional key words: ascorbate-glutathione cycle, *Cucumis sativus*, fructose, gene expression, glucose, H_2O_2, invertase, malondialdehyde, polyethylene glycol, proline.

Introduction

Drought is a severe environmental stress that induces reactive oxygen species (ROS) including superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) (Wu *et al.* 2013). The accumulation of ROS damages lipids and proteins within cells, resulting in plant growth inhibition (Li *et al.* 2013). To alleviate the toxicity induced by ROS, plants have evolved an antioxidant system including superoxide dismutase (SOD) (Mutlu *et al.* 2013), guaiacol peroxidase (GPX), glutathione peroxidase (GSH-Px), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR) (Li *et al.* 2013), and the reduced glutathione (GSH) and ascorbate (AsA) (Huang *et al.* 2013).

Sucrose represents the major transport form of photosynthates (Ward *et al.* 1998) and plays different roles in plants. At a low concentration, sucrose serves as substrate or signal for stress-induced alterations, whereas it functions directly as protective agent at higher concentrations (Uemura and Steponkus 2003, Rolland *et al.* 2006). The application of sucrose has a positive effect on AsA accumulation of harvested broccoli florets (Nishikawa *et al.* 2005). In the root suspension culture of *Morinda citrifolia*, the supplement of sucrose at a certain concentration regulates the increase of antioxidant enzyme activities and thereby decreases oxidative

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Abbreviations: APX - ascorbate peroxidase, AsA - ascorbate; DHAR - dehydroascorbate reductase; GPX - guaiacol peroxidase; GR - glutathione reductase; GSH - glutathione; GSH-Px - glutathione peroxidase; MDHAR - monodehydroascorbate reductase; NI - neutral invertase; PEG - polyethylene glycol; ROS - reactive oxygen species; RWC - relative water content; SAI - soluble acid invertase; SOD - superoxide dismutase.
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damage (Baque *et al*. 2012). In *Arabidopsis thaliana*, a treatment with sucrose enhances anoxia tolerance and abolishes ROS stress during extended darkness (Loreti *et al*. 2005, Rosenwasser *et al*. 2011). Moreover, sucrose confers a high tolerance of *Arabidopsis* seedlings to the herbicide atrazine, which is associated with transcriptional reprograming genes involved in ROS defence (Ramel *et al*. 2007) and with the activation of antioxidant enzymes (Ramel *et al*. 2009). These observations argue a possible interaction between sucrose and ROS signalling in plants in response to stress conditions. Therefore, we hypothesize that an exogenous sucrose treatment might increase plant tolerance to drought stress through affecting antioxidant enzyme activities and mitigating damage by ROS.

Cucumber is sensitive to drought since it has a shallow root system and large leaves. In this study, sucrose-pretreated cucumber seedlings were subjected to a water stress induced by polyethylene glycol (PEG) 6000 (Li *et al*. 2013). We aimed to examine whether the sucrose application could protect cucumber leaves from dehydration stress and if so, whether the protective effect involved the regulation of antioxidant enzymes. In addition, our aim was to investigate the role of soluble acid invertase (SAI), neutral invertase (NI) and proline in dehydration stress mitigated by exogenous sucrose.

Materials and methods

Plants and treatments: Cucumber (*Cucumis sativus* L. cv. Jinchun No. 4) seedlings were cultivated in sand saturated with a Hoagland nutrient solution at a temperature of 25 °C, a relative humidity of 75 %, an irradiance of 300 μmol m^{-2} s^{-1}, and a 12-h photoperiod (Li *et al*. 2011). When the second leaves were fully expanded, the seedlings (eight plants per group) were watered with the Hoagland nutrient solution containing different concentrations (0, 70, 90, and 110 mM) of sucrose for 1 d and with nutrient solution containing 10 % (m/v) PEG 6000 for further 1 d. Three biological replicates were used. Based on the results of these preliminary experiments, 48 cucumber seedlings were divided into 6 groups where 2 groups were watered with the Hoagland nutrient solution only, and 2 groups were watered with the Hoagland nutrient solution containing the optimum concentration (90 mM) of sucrose, and last 2 groups were watered with the Hoagland nutrient solution containing 90 mM mannitol. After one day, the sand was rinsed six times with water and six times with the Hoagland nutrient solution. Then the seedlings were watered with either the Hoagland nutrient solution or the Hoagland nutrient solution containing 10 % PEG 6000. After one day, the second leaves were harvested from all the plant groups for the subsequent experiments. Three biological replicates were used. The measurement of plant growth was performed according to Sun *et al*. (2012).

Determinations of relative water content (RWC) and osmotic potential: Leaf discs (1 cm in diameter) were randomly taken from the middle portion of the second leaves. After the determination of fresh mass (FM), they were floated on distilled water in a Petri dish in darkness for 5 h and the water saturated mass (SM) was determined. Then the discs were dried at 75 °C for 48 h to determine the dry mass (DM). RWC [%] = (FM - DM)/(SM - DM) × 100 (Lee *et al*. 2013).

Cucumber leaves were frozen in liquid nitrogen, thawed, and their osmotic potential was determined as described by Bajji *et al*. (2001) using an osmometer (*Vapro5520*, *Wescor*, Logan, USA).

Malondialdehyde (MDA) and H_2O_2 content: MDA was measured following procedures that were described by Dhindsa *et al*. (1981) and modified by Xu *et al*. (2008). Absorbances was read at 450, 532, and 600 nm using a spectrophotometer (*TU-1810*, *Beijing Purkinje General Instrument Co.*, Beijing, China).

H_2O_2 content was determined according to Bernt and Bergmeyer (1974) with some modifications. After grinding with liquid nitrogen, leaf samples (0.3 g) were homogenized in 0.9 cm^3 of an ice-cold sodium phosphate buffer (100 mM, pH 6.8) and centrifuged at 4 °C and 18 000 *g* for 20 min. Then, the supernatant (0.15 cm^3) was mixed with 0.75 cm^3 of a reagent consisting of 83 mM sodium phosphate (pH 7.0), 0.005 % (m/v) *o*-dianisidine, and 1 mM peroxidase. The mixture was incubated at 30 °C for 10 min. The reactions were subsequently stopped by adding 0.5 cm^3 of 1 M perchloric acid. After centrifugation at 5 000 *g* for 5 min, the absorbance of supernatant was measured at 436 nm, and the content of H_2O_2 in leaves was calculated from a standard curve.

Rate of $O_2^{\cdot-}$ formation was analyzed according to Elstner and Heupel (1976) with some modifications. Liquid nitrogen-ground leaves (0.2 g) were homogenized with 1 cm^3 of a 65 mM phosphate buffer (pH 7.8) and then centrifuged at 4 °C and 5 000 *g* for 10 min. Subsequently, the supernatant (0.5 cm^3) was mixed with a reagent containing 0.05 cm^3 of 10 mM hydroxylamine chlorhydrate, 0.45 cm^3 of the 65 mM phosphate buffer (pH 7.8), 0.25 cm^3 of 17 mM sulfanilamide, and 0.25 cm^3 of 7 mM α-naphthylamine. The mixture was kept at 25 °C for 20 min and then 1.5 cm^3 of diethyl ether was added. After centrifugation at 4 °C and 1 500 *g* for 15 min, the absorbance was measured at 530 nm. The formation rate of $O_2^{\cdot-}$ in leaves was calculated from a standard curve of a $NaNO_2$ reagent.

Enzyme activities and the content of non-enzymatic antioxidants: For determination of enzyme activities, leaf samples were homogenized with an ice-cold HEPES buffer. SOD activity was assessed according to Hwang *et al*. (1999), and 1 unit of activity was defined as the amount of SOD enzyme that gave half-maximal inhibition of nitro blue tetrazolium reduction. GPX was measured according to Ramiro *et al*. (2006), GSH-Px according to Xue *et al*. (2001), APX according to Zhu *et al*. (2004), DHAR according to Doulis *et al*. (1997), MDHAR according to Hoque *et al*. (2007), and GR according to Foyer and Halliwell (1976). SAI and NI activities were assayed according to Batta and Singh (1986) and Huang *et al*. (2013), respectively. The content of protein in the homogenates was measured according to Bradford (1976).

The GSH content in leaves was determined according to Guri (1983), and the content of oxidized glutathione (GSSG) was measured using a GSH and GSSG assay kit (Beyotime Institute of Biotechnology, Jiangsu, China). The content of AsA and total ascorbate were determined at 525 nm (Kampfenkel *et al*. 1995). The content of oxidized ascorbate was estimated from the difference between the total ascorbate and the AsA content.

Proline and sugar content: Liquid nitrogen-ground leaves (0.2 g) were homogenized in 5 cm^3 of 3 % (m/v) sulfosalicylic acid, and the proline content was measured at 520 nm according to Bates *et al*. (1973).

Soluble sugars were quantified by the anthrone-sulfuric acid assay (Yemm and Willis 1954), and their content was calculated using glucose as standard. The content of sucrose, glucose, and fructose were determined according to procedures that were described by Ozaki *et al*. (2009) and modified by Huang *et al*. (2013).

Transcriptions of *Cu/Zn-SOD*, *GPX* and *GR* genes: Real-time PCR was performed according to Li *et al*. (2013) with modifications. Forward primers of *Cu/Zn-SOD*, *GPX*, *GR*, and α-*tubulin* (a housekeeping control) genes were: 5'-GACTGGGCCACATTTCAACC-3', 5'-ACCGCCTCATCAACTTCAAC-3', 5'-TGATGAGG CTTTGAGTTTAGAGGAG-3', and 5'-CACTACACC GTTGGAAAGGAAA-3', whereas their reverse primers were: 5'-GCCTTGCCATCTTCACCAA-3', 5'-ATCTAG TGGGTGTGACTGGGTC-3', 5'-AACTTTGGCACC CATACCATTC-3', and 5'-CAAAAGGAGGGAGCC GAGA-3', respectively. The annealing temperatures were 59 °C for *Cu/Zn-SOD* and *GPX*, and 61 °C for *GR*. The expressions of *Cu/Zn-SOD*, *GPX*, and *GR* were normalized against the corresponding α-*tubulin* expression.

Statistical analysis: Data were expressed as means ± SE. Differences were tested with one-way *ANOVA* and the least significant difference (LSD) test. $P < 0.05$ was considered to be significant.

Results

In comparison to the 0, 70, and 110 mM sucrose applications, the pretreatment with 90 mM sucrose led to the highest fresh mass of the second leaf and the fresh and dry masses of shoots and roots in the PEG-treated seedlings (Table 1). Under the PEG-induced dehydration, the 90 mM sucrose pretreatment resulted in the lowest content of MDA and H_2O_2, as well as $O_2^{\cdot-}$ production, and the highest activities of SOD and GPX in the second leaves. Therefore, 90 mM was chosen as the suitable concentration of sucrose in the following experiments.

The fresh masses of the second leaves and shoots and the dry masses of roots and shoots markedly increased

Table 1. Changes in plant growth, content of MDA and H_2O_2, $O_2^{\cdot-}$ production, and activities of SOD and GPX in the second leaf when cucumber seedlings were pretreated with different concentrations of sucrose for 1 d and then exposed to 10 % (m/v) PEG 6000 for further 1 d. Means ± SE, $n = 3$. Different letters indicate statistically significant differences between treatments at $P < 0.05$.

Parameters	0	70	90	110 mM sucrose
Leaf f.m. [mg plant^{-1}]	1120.27 ± 41.10b	1202.90 ± 80.90ab	1254.20 ± 20.00a	1197.50 ± 81.80ab
Root f.m. [mg plant^{-1}]	1254.70 ± 71.70b	1589.20 ± 8.35a	1715.50 ± 102.00a	1422.80 ± 57.50b
Root d.m. [mg plant^{-1}]	64.50 ± 4.99b	75.20 ± 2.41ab	84.77 ± 3.80a	76.50 ± 3.01ab
Shoot f.m. [mg plant^{-1}]	3245.90 ± 68.40b	3441.90 ± 58.70b	3925.00 ± 79.40a	3416.73 ± 89.73b
Shoot d.m. [mg plant^{-1}]	209.00 ± 8.02b	244.80 ± 6.43b	276.80 ± 4.05a	228.90 ± 4.82b
Wilting leaves [%]	81.94 ± 3.67a	40.28 ± 5.01c	19.44 ± 2.78d	63.89 ± 1.39b
MDA [nmol g^{-1}(f.m.)]	7.80 ± 0.19a	5.45 ± 0.06c	4.95 ± 0.05d	5.71 ± 0.01b
$O_2^{\cdot-}$ [nmol g^{-1}(f.m.) min^{-1}]	4.25 ± 0.11a	3.45 ± 0.04b	3.26 ± 0.02c	4.35 ± 0.04a
H_2O_2 [nmol g^{-1}(f.m.)]	154.83 ± 1.43a	117.56 ± 1.43b	106.09 ± 2.48c	123.29 ± 2.48b
SOD [U mg^{-1}(protein)]	12.04 ± 0.42c	14.12 ± 0.18b	16.99 ± 0.46a	13.82 ± 0.83bc
GPX [nmol mg^{-1}(protein) min^{-1}]	272.40 ± 5.24c	366.10 ± 2.47b	414.60 ± 6.23a	205.20 ± 2.60d

Table 2. Changes in plant growth, RWC, osmotic potential, content of MDA and H_2O_2, specific activities of antioxidant enzymes, transcription of *Cu/Zn-SOD*, *GPX* and *GR* genes, content of AsA, GSH, proline, soluble sugars, endogenous sucrose, fructose, and glucose, and activities of SAI and NI in the second leaves after 90 mM sucrose or 90 mM mannitol pretreatments and PEG-induced dehydration. Means ± SE, *n* = 3. Different letters indicate statistically significant differences between treatments at $P < 0.05$.

Parameters	Control	Sucrose	PEG	Sucrose+PEG	Mannitol+PEG
Leaf f.m. [g plant^{-1}]	1.22 ± 0.02b	1.30 ± 0.01a	0.84 ± 0.03d	1.11 ± 0.03c	0.82 ± 0.07d
Root f.m. [g plant^{-1}]	1.41 ± 0.01a	1.53 ± 0.05a	1.37 ± 0.01b	1.49 ± 0.03a	1.21 ± 0.02c
Root d.m. [mg plant^{-1}]	63.00 ± 1.05bc	73.30 ± 1.50a	60.60 ± 0.68c	64.30 ± 0.38b	54.30 ± 0.80d
Shoot f.m. [g plant^{-1}]	2.99 ± 0.01b	3.26 ± 0.06a	2.10 ± 0.03d	2.52 ± 0.05c	2.14 ± 0.10d
Shoot d.m. [mg plant^{-1}]	213.90 ± 5.66b	234.40 ± 2.57a	187.00 ± 0.33c	210.50 ± 4.22b	187.40 ± 0.86c
RWC [%]	75.57 ± 2.41b	85.71 ± 1.22a	62.51 ± 2.49c	80.97 ± 1.17b	56.93 ± 3.62c
Osmotic potential [MPa]	-0.85 ± 0.01a	-0.96 ± 0.01c	-0.93 ± 0.01b	-1.05 ± 0.00d	-0.91 ± 0.01b
MDA [nmol g^{-1}(f.m.)]	9.37 ± 0.17d	8.38 ± 0.09e	13.41 ± 0.37a	10.60 ± 0.28c	11.73 ± 0.22b
H_2O_2 [nmol g^{-1}(f.m.)]	114.69 ± 2.48c	86.03 ± 1.43d	154.83 ± 1.43a	106.09 ± 2.48c	133.33 ± 1.43b
SOD [U mg^{-1}(protein)]	7.30 ± 0.39d	12.74 ± 0.12c	14.31 ± 0.26b	17.16 ± 0.46a	15.02 ± 0.29b
GPX [nmol mg^{-1}(protein) min^{-1}]	190.80 ± 2.51e	332.50 ± 0.00c	272.40 ± 5.24d	414.60 ± 6.23a	380.40 ± 4.48b
GSH-Px [μmol mg^{-1}(protein) min^{-1}]	9.03 ± 0.04c	9.41 ± 0.11b	7.92 ± 0.11d	12.63 ± 0.11a	7.75 ± 0.09d
APX [nmol mg^{-1}(protein) min^{-1}]	84.80 ± 2.45d	115.40 ± 2.89b	101.00 ± 3.07c	139.70 ± 2.54a	103.30 ± 2.98c
MDHAR [nmol mg^{-1}(protein) min^{-1}]	59.40 ± 1.35b	61.10 ± 1.59b	49.90 ± 0.85d	78.70 ± 0.20a	54.55 ± 0.61c
DHAR [nmol mg^{-1}(protein) min^{-1}]	67.70 ± 0.49d	73.10 ± 0.33c	60.70 ± 0.34e	81.20 ± 0.57a	76.43 ± 0.50b
GR [nmol mg^{-1}(protein) min^{-1}]	3.01 ± 0.01d	3.88 ± 0.03b	3.84 ± 0.08b	5.85 ± 0.12a	3.56 ± 0.06c
Cu/Zn-SOD	0.80 ± 0.02c	1.14 ± 0.02b	1.17 ± 0.01b	1.50 ± 0.10a	1.23 ± 0.02b
GPX	0.75 ± 0.05d	1.55 ± 0.02b	1.00 ± 0.00c	1.86 ± 0.01a	1.76 ± 0.03a
GR	0.74 ± 0.01d	0.83 ± 0.00c	1.00 ± 0.00b	1.18 ± 0.01a	1.03 ± 0.02b
AsA [μg g^{-1}(f.m.)]	94.30 ± 0.38b	97.14 ± 0.44a	66.96 ± 0.22e	82.93 ± 0.79c	73.74 ± 0.58d
GSH [μg g^{-1}(f.m.)]	788.88 ± 3.27c	870.53 ± 5.66b	864.00 ± 6.53b	971.77 ± 3.27a	805.21 ± 8.64c
AsA/oxidized ascorbate	4.39 ± 0.25bc	5.46 ± 0.14a	4.03 ± 0.12c	4.63 ± 0.09b	4.39 ± 0.15bc
GSH/GSSG	4.19 ± 0.05d	6.35 ± 0.06a	4.26 ± 0.07d	5.50 ± 0.03b	4.81 ± 0.03c
Proline [μg g^{-1}(f.m.)]	10.48 ± 0.11d	11.46 ± 0.32c	12.73 ± 1.06b	16.86 ± 0.31a	8.09 ± 0.53e
Soluble sugars [mg g^{-1}(f.m.)]	5.25 ± 0.09e	6.91 ± 0.10d	7.17 ± 0.09c	8.46 ± 0.07b	8.88 ± 0.08a
Endogenous sucrose [mg g^{-1}(f.m.)]	0.48 ± 0.01c	1.65 ± 0.08a	1.01 ± 0.01b	1.75 ± 0.04a	0.99 ± 0.02b
Fructose [μg g^{-1}(f.m.)]	251.10 ± 0.42d	300.90 ± 7.22cd	369.90 ± 7.56c	660.60 ± 36.3a	493.20 ± 4.49b
Glucose [μg g^{-1}(f.m.)]	229.10 ± 1.34c	266.30 ± 8.28b	168.70 ± 5.01d	297.60 ± 4.24a	285.40 ± 1.89ab
SAI [nmol g^{-1}(protein) min^{-1}]	41.17 ± 0.33c	47.00 ± 0.33b	33.50 ± 0.33d	53.50 ± 0.50a	40.50 ± 0.33c
NI [nmol g^{-1}(protein) min^{-1}]	146.00 ± 0.33d	175.67 ± 0.33c	180.00 ± 0.50b	265.00 ± 0.67a	176.33 ± 0.33c

due to the sucrose treatment in comparison to the untreated control, but the fresh mass of roots was not affected (Table 2). Compared to the control, the fresh masses of the second leaves, shoots, and roots and the dry mass of shoots decreased after the PEG treatment, and the dry mass of roots was not altered. In comparison to the PEG treatment, the sucrose + PEG treatment significantly enhanced the fresh masses of the second leaves, shoots, and roots and dry masses of shoots and roots, whereas the mannitol + PEG treatment significantly decreased the fresh and dry masses of roots and did not markedly change the fresh masses of the second leaves and the fresh and dry masses of shoots. So the PEG-induced dehydration significantly inhibited plant growth, and the pretreatment with sucrose, but not with mannitol, alleviated the growth inhibition under the dehydration.

Compared to the control, RWC in the second leaves dramatically increased by the sucrose treatment, whereas it remarkably decreased by the PEG treatment. In comparison to the PEG treatment, RWC obviously enhanced in the sucrose + PEG treatment and had no change in the mannitol + PEG treatment. The osmotic potential in the second leaves significantly decreased by the sucrose and PEG treatments when compared with the control. In comparison to the PEG treatment, the sucrose + PEG treatment obviously reduced the osmotic potential, whereas the mannitol + PEG treatment did not alter it (Table 2).

As compared to the untreated control, the content of MDA and H_2O_2 were significantly reduced in the second leaves by the sucrose treatment and markedly increased by the PEG treatment. In comparison to the PEG treatment, the content of MDA and H_2O_2 decreased more by the sucrose + PEG treatment than by the mannitol + PEG treatment.

The activities of SOD, GPX, GSH-Px, APX, DHAR, and GR in the second leaves significantly increased by the sucrose treatment in comparison to the control, and

the activity of MDHAR did not change. When compared the PEG treatment with the control, the activities of SOD, GPX, APX, and GR significantly enhanced, and the activities of GSH-Px, MDHAR, and DHAR decreased. The activities of SOD, GPX, GSH-Px, APX, MDHAR, DHAR, and GR remarkably increased under the sucrose + PEG treatment in comparison to the PEG treatment. Compared the mannitol + PEG treatment with the PEG treatment, the activities of SOD, GSH-Px and APX did not change, and the activities of GPX, MDHAR, and DHAR significantly increased, whereas the activity of GR was reduced (Table 2).

The expression of *Cu/Zn-SOD*, *GPX,* and *GR* genes significantly increased at the sucrose or PEG treatments in comparison to the control. Compared with the PEG treatment, the *Cu/Zn-SOD*, *GPX*, and *GR* expressions remarkably increased by the sucrose + PEG treatment. In the mannitol + PEG treatment in comparison to the PEG treatment, the *GPX* expression was induced, whereas the transcriptions *Cu/Zn-SOD* and *GR* were not changed.

Compared to the control, the content of AsA and GSH and the ratios of AsA/oxidized ascorbate and GSH/GSSG in the second leaves significantly increased under the sucrose treatment. The content of AsA and the ratio of AsA/oxidized ascorbate were remarkably reduced by the PEG treatment in comparison to the control, and the GSH content significantly increased, whereas the ratio of GSH/GSSG had no changes. Compared to the PEG treatment, the sucrose + PEG treatment markedly enhanced the content of AsA and GSH and the ratios of AsA/oxidized ascorbate and GSH/GSSG in the leaves. In the mannitol + PEG treatment, the content of AsA and the ratio of GSH/GSSG increased, and the GSH content decreased when compared to the PEG treatment.

The content of proline, soluble sugars, endogenous sucrose, and glucose significantly increased in the second leaves when compared the sucrose treatment with the untreated control, and the content of fructose had no obvious change. As compared with the control, the PEG treatment significantly reduced the glucose content and increased the content of proline, soluble sugars, fructose, and endogenous sucrose. In comparison to the PEG treatment, the content of proline, soluble sugars, endogenous sucrose, glucose, and fructose were significantly elevated by the sucrose + PEG treatment. Compared the mannitol + PEG treatment with the PEG treatment, the former remarkably increased the content of soluble sugars, glucose, and fructose, and decreased the proline content; the content of endogenous sucrose did not change.

In comparison to the untreated control, the activities of SAI and NI in the second leaves significantly enhanced by the sucrose pretreatment. Compared the PEG treatment with the control, the activity of SAI decreased and the activity of NI increased by the PEG treatment. As compared with the PEG treatment alone, the sucrose + PEG treatment significantly enhanced the activities of SAI and NI, and the mannitol + PEG treatment increased the activity of SAI but decreased the activity of NI.

Discussion

Although the preliminary and main experiments in this study were performed at different time and used different seedlings, the same result was obtained: the pretreatment with 90 mM sucrose alleviated growth inhibition under PEG-induced dehydration. This is in agreement with a report that glucose pretreatment protects cucumber seedlings from dehydration stress (Huang *et al.* 2013). The mitigating effect of sucrose on dehydration indicates that sucrose might be applied as ingredient of water-retaining agents which effectively enhance water use efficiency and crop yield (Wen *et al.* 2012). Compared to exogenous sucrose, 90 mM mannitol showed different influence on RWC and plant growth under PEG (Table 2) suggesting that sucrose probably did not act only as osmotic regulator when it alleviated the dehydration stress.

The accumulation of MDA indicates the injury of lipid membranes (Hameed *et al.* 2013). In this study, the MDA content in leaves increased under the PEG-induced dehydration but less in the sucrose-pretreated seedlings. This further suggests that exogenous sucrose alleviated the dehydration stress in cucumbers. Since MDA is caused by ROS (Perveen *et al.* 2013), the change of MDA content in the current experiment is consistent with our result that sucrose pretreatment also mitigated the increase in H_2O_2 under PEG. In agreement with this finding, a pretreatment with caffeic acid leads to a less ROS accumulation and thereby reduces damage to membrane lipids under dehydration (Wan *et al.* 2014).

Salt increases the activities of SOD, APX, and GR (Lin *et al.* 2011), whereas PEG elevates the GPX activity (Li *et al.* 2011) and decreases the activities of GSH-Px, MDHAR, and DHAR (Sun *et al.* 2012) in cucumber. In the current experiment, the PEG treatment resulted in the increased SOD, APX, GR, GPX, GSH-Px, and DHAR activities. However, the increases in H_2O_2 and MDA under the PEG treatment indicate that the production of ROS probably exceeded the capacity of antioxidant enzymes to eliminate it. In this study, the pretreatment with sucrose elevated the activities of SOD, GPX, GSH-Px, APX, DHAR, and GR under the PEG-induced dehydration. This response was accompanied by the increased transcription of *Cu/Zn-SOD*, *GPX* and *GR* genes and coincided with the decreased content of H_2O_2 and MDA (Table 2). This suggests that sucrose pretreatment can increase dehydration resistance of plants

by regulating lipid peroxidation *via* increased activities of antioxidant enzymes.

Under the PEG treatment, the content of AsA decreased and the content of GSH increased. The same results of AsA (Lin *et al*. 2011) and GSH (Huang *et al*. 2013) have been observed under salt stress and dehydration, respectively. Under the PEG-induced dehydration, exogenous sucrose not only enhanced the content of AsA and GSH, but also elevated the ratios of AsA/oxidized ascorbate and GSH/GSSG (Table 2).

The content of proline and soluble sugars in cucumber increased under the PEG treatment. Khalid *et al*. (2010) observed the similar results in *Pelargonium odoratissimum*. In the current experiment, the application of sucrose increased the proline and soluble sugars content under the PEG-induced stress. Similarly, pretreatment with ferulic acid enhances the levels of proline and soluble sugars under dehydration stress (Li *et al*. 2013). The higher content of soluble sugars in the sucrose-pretreated PEG-stressed leaves was confirmed by the elevated content of endogenous sucrose, glucose, and fructose, and by the enhanced activities of SAI and NI (Table 2) indicating that proline and soluble sugars were involved when exogenous sucrose protected the dehydration-stressed seedlings.

In conclusion, the pretreatment with 90 mM sucrose increased RWC and alleviated growth inhibition under the PEG-induced dehydration. Furthermore, it elevated the activities of SOD, GPX, DHAR, MDHAR, APX, GSH-Px, GR, SAI, and NI, and enhanced the content of AsA, GSH, proline, soluble sugars, endogenous sucrose, glucose, and fructose, but decreased the content of MDA and H_2O_2 in the dehydration-stressed leaves. We conclude that the sucrose pretreatment regulated antioxidants, proline, and soluble sugars, thus mitigating the dehydration stress of cucumber.

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