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Ferulic acid pretreatment enhances dehydration-stress tolerance of cucumber seedlings

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Abstract

To examine whether ferulic acid (FA) could protect plants from dehydration stress and to investigate a mechanism for the protection, cucumber seedlings were pretreated with 0.5 mM FA for 2 d and then were exposed to dehydration induced by 10 % polyethylene glycol 6000. After pretreatment with FA, the activities of antioxidant enzymes (catalase, superoxide dismutase, and quaiacol peroxidase) in leaves were higher than under dehydration treatment alone which was in accordance with the increased transcript levels of respective genes. Moreover, the combination of FA pretreatment and dehydration reduced the content of superoxide radical, hydrogen peroxide, and malondialdehyde, and increased the relative water content and content of FA, proline, and soluble sugars in comparison with dehydration alone. We propose that pretreatment with FA protects cucumbers against dehydration stress by decrease of lipid peroxidation due to activation of antioxidant enzymes and by increase of proline and soluble sugar content in leaves.

Additional key words: catalase, *Cucumis sativus*, guaiacol peroxidase, malondialdehyde, polyethylene glycol, superoxide dismutase.

Introduction

Drought stress decreases water potential, inhibits plant growth (*e.g.* Van den Berg and Zeng 2006), disrupts ion homeostasis, and results in overproduction of reactive oxygen species (ROS), such as superoxide radical ($O_2^{\cdot-}$) and hydrogen peroxide (Thapa *et al.* 2011). The accumulation of ROS can cause lipid peroxidation (Foyer *et al.* 1994) which can damage ultrastructure, disrupt normal metabolism, and even lead to cell death. To inhibit the toxicity of ROS, plants have evolved an array of enzymatic antioxidants including superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), guaiacol peroxidase (GPX), glutathione peroxidase (GSH-Px), glutathione reductase (GR), dehydroascorbate reductase (DHAR), and monodehydroascorbate reductase (MDHAR) (Asada 1992).

Ferulic acid (FA) is a ubiquitous phenolic compound that arises from the metabolism of phenylalanine and tyrosine in plants and it accumulates in soil and influences plant growth (Turner and Rice 1975). However, in human beings, it has been shown that the hydroxyl and phenoxy groups of FA donate electrons to scavenge free radicals (Srinivasan *et al.* 2007). Based on these results, we hypothesize that also in plants, FA at a certain concentration will affect antioxidant enzyme activities. Up to date, it is unknown whether FA pretreatment enhances drought tolerance of plants.

Cucumber is sensitive to drought stress and polyethylene glycol (PEG) 6000 can be used to induce dehydration (Liu *et al.* 2009a). Therefore, in this study, cucumber seedlings were pretreated with FA and then were exposed to dehydration stress induced by 10 % (m/v) PEG 6000. Our aims were to investigate whether FA can protect cucumbers from dehydration stress and if the protection is associated with regulation of antioxidant enzyme activities and accumulation of proline and soluble sugars.

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Abbreviations: APX - ascorbate peroxidase; AsA - ascorbate; CAT - catalase; DHAR - dehydroascorbate reductase; EDTA - ethylenediaminetetraacetic acid; FA - ferulic acid; GPX - guaiacol peroxidase; GR - glutathione reductase; GSH-Px - glutathione peroxidase; HEPES - N-(2-hydroxyethyl) piperazine-N'-2-ethanesulfonic acid; MDA - malondialdehyde; MDHAR - monodehydroascorbate reductase; NBT - nitroblue tetrazolium; $O_2^{\cdot-}$ - superoxide radical; PEG - polyethylene glycol; PVP - polyvinyl pyrrolidone; ROS - reactive oxygen species; RWC - relative water content; SOD - superoxide dismutase.

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Materials and methods

Plants and treatments: Cucumber (*Cucumis sativus* L. cv. Yuexiu No. 3) seedlings were cultivated at 25 °C and watered with Hoagland nutrient solution (Li *et al.* 2011). At two-leaf stage, seedlings (8 plants per group) were watered with Hoagland nutrient solution containing different concentrations (0, 0.3, 0.4, 0.5, 0.6, 0.7, and 0.8 mM) of FA for 2 d and then were watered with Hoagland nutrient solution containing 10 % PEG 6000 for further 2 d. Three biological replicates were used. Based on the results of these preliminary experiments, 32 cucumber seedlings were divided into four groups where two groups were watered with Hoagland nutrient solution containing FA at optimum concentration (0.5 mM) once a day for 2 d and other two groups were watered for 2 d with Hoagland nutrient solution only. After that, the sand for planting cucumber seedlings was rinsed with water 6 times and with Hoagland nutrient solution 6 times. Subsequently, the FA-pretreated seedlings and FA-untreated seedlings were watered with either the Hoagland nutrient solution or the Hoagland nutrient solution containing 10 % PEG 6000. After 2 d of PEG-induced dehydration stress, the second leaves from all four groups were harvested for the subsequent experiments. Three biological replicates were used.

Relative water content (RWC) in leaves was determined according to Barrs and Weatherley (1962) using leaf discs (1 cm in diameter) and floating on distilled water in a Petri dish in the darkness for 5 h. Dry mass was determined after drying at 75 °C for 48 h.

FA content: Leaf dry tissue was ground into powder. Then, 0.5 g of leaf powder was soaked with 20 cm^3 of methanol-double distilled water (30:70, v/v) for 10 min and then broken by ultrasound for 30 min. The solution was filtrated through 0.45 μm membrane and the content of FA was measured with the *Agilent 1200* rapid resolution liquid chromatography (*Agilent Technologies*, Waldbronn, Germany). The mobile phase was 0.4 % (v/v) acetic acid + acetonitrile (96:4 for 8 min, 50:50 for 10 min, 20:80 for 7 min, and 90:10 for 10 min). Chromatography was carried out at 290 nm, 25 °C, and a flow-rate of 1.0 $cm^3\ min^{-1}$. The marker substance of FA was accurately weighed and dissolved in methanol-double distilled water (30:70) to give serial concentrations within the ranges 0.0005 - 0.02 mg cm^{-3}. The standard curve was analyzed using the peak area and the FA content in a leaf sample was calculated.

Formation rate of $O_2^{\cdot-}$ was measured according to Elstner and Heupel (1976) with some modifications. After grinding with liquid nitrogen, the second leaves (0.2 g) were homogenized in 3 cm^3 of 65 mM phosphate buffer (pH 7.8) and centrifuged at 4 °C and 5 000 *g* for 10 min. Then, the supernatant (0.75 cm^3) was mixed with 0.075 cm^3 of 10 mM hydroxylamine chlorhydrate and 0.675 cm^3 of 65 mM phosphate buffer (pH 7.8). When 0.375 cm^3 of 17 mM sulfanilamide and 0.375 cm^3 of 7 mM α-naphthylamine were added, the mixture was kept at 25 °C for 20 min. After adding 2.25 cm^3 of ether and centrifugation at 4 °C and 1 500 *g* for 15 min, the absorbance was measured at 530 nm and the formation rate of $O_2^{\cdot-}$ was calculated from a standard curve of $NaNO_2$ reagent.

H_2O_2 content was determined according to the method of Bernt and Bergmeyer (1974) with modifications. Leaves (1 g) were ground with liquid nitrogen, homogenized in 3 cm^3 of 100 mM phosphate buffer (pH 6.8), and then centrifuged at 4 °C and 18 000 *g* for 20 min. The supernatant (0.5 cm^3) was mixed with 2.5 cm^3 of 83 mM sodium phosphate (pH 7.0), 0.005 % (m/v) *o*-dianisidine, and 1 mM peroxidase. After incubation at 30 °C for 10 min, 0.5 cm^3 of 1 M perchloric acid was added to stop the reaction. The absorbance was determined at 436 nm.

Membrane lipid peroxidation was determined by measuring malondialdehyde (MDA) content (Dhindsa *et al.* 1981). Leaf samples (0.2 g) were ground with liquid nitrogen and homogenized in 4 cm^3 of 10 % (m/v) trichloroacetic acid. After centrifugation at 4 °C and 10 000 *g* for 15 min, 1 cm^3 of supernatant was mixed with 1 cm^3 of 0.6 % (m/v) thiobarbituric acid and then heated at 95 °C for 30 min. The absorbance of reaction solution was determined at 450, 532, and 600 nm, and the MDA content was estimated by using the formula: $MDA\ [\mu M] = 6.45\ (A_{532} - A_{600}) - 0.56\ A_{450}$.

Extraction and assays of antioxidant enzymes: After grinding with liquid nitrogen, leaves (0.2 g) were suspended in 2 cm^3 of ice-cold N-(2-hydroxyethyl) piperazine-N'-2-ethanesulfonic acid (HEPES) buffer (25 mM, pH 7.8) which contained 2 % (m/v) polyvinyl pyrrolidone (PVP) and 0.2 mM ethylenediaminetetra-acetic acid (EDTA)-Na_2. The homogenate was centrifuged at 4 °C and 12 000 *g* for 20 min and the supernatant was collected and used for determining the activities of SOD (EC 1.15.1.1), CAT (EC 1.11.1.6), GPX (EC 1.11.1.7), and GR (EC 1.6.4.2) (Ramiro *et al.* 2006). GSH-Px (EC 1.11.1.9) was extracted by suspending 0.3 g of liquid nitrogen-ground leaves in 0.3 cm^3 of 25 mM HEPES buffer containing 0.2 mM EDTA and 2 % PVP (pH 7.8). To extract APX (EC 1.11.1.11), 0.3 g of liquid nitrogen-ground leaves was homogenized in 25 mM HEPES buffer (pH 7.8) containing 0.2 mM EDTA, 2 % PVP and 2 mM ascorbate (AsA). After grinding with liquid nitrogen, 0.2 g of leaves was suspended in 2 cm^3 of $KNaHPO_4$ buffer containing 1 mM EDTA (Flohe and Gunzler 1984) to extract DHAR (EC 1.8.5.1) and MDHAR (EC 1.6.5.4).

To measure the SOD activity at 560 nm, a reaction solution comprised 130 mM methionine, 50 mM phosphate buffer (pH 7.8), 20 μM riboflavin, 75 μM nitroblue tetrazolium (NBT), and 0.015 cm^3 of enzyme extract (Dhindsa *et al.* 1981). One unit of SOD activity was defined as the amount of enzyme that gave half-maximal inhibition of NBT reduction. A reaction mixture, which contained 0.022 cm^3 of enzyme extract, 100 mM phosphate buffer (pH 7.8), and 10 mM H_2O_2, was used to assay the CAT activity at 240 nm *via* the decomposition of H_2O_2 (coefficient of absorbance, $\varepsilon = 40\ M^{-1}\ cm^{-1}$) (Pereira *et al.* 2002). The activity of GPX was determined at 470 nm according to guaiacol oxidation ($\varepsilon = 6.39\ mM^{-1}\ cm^{-1}$) and the reaction solution consisted of 0.05 % (m/v) guaiacol, 15 mM H_2O_2, 100 mM phosphate buffer (pH 7.0), and 0.03 cm^3 of enzyme extract (Ramiro *et al.* 2006). The activity of GSH-Px was determined at 412 nm using H_2O_2 as a substrate according to Xue *et al.* (2001). To assess the activity of APX at 290 nm, H_2O_2-dependent oxidation of ascorbate, AsA ($\varepsilon = 2.8\ mM^{-1}\ cm^{-1}$) was used (Zhu *et al.* 2004). The activity of MDHAR was determined at 340 nm by monitoring the oxidation of NADPH ($\varepsilon = 6.2\ mM^{-1}\ cm^{-1}$) (Hoque *et al.* 2007). The activity of DHAR was measured at 265 nm based on the formation of AsA ($\varepsilon = 14\ mM^{-1}\ cm^{-1}$) (Doulis *et al.* 1997). The activity of GR was assayed by the absorbance change at 340 nm due to oxidation of NADPH ($\varepsilon = 6.2\ mM^{-1}\ cm^{-1}$) (Foyer and Halliwell 1976).

To calculate antioxidant enzyme activities, protein in the enzyme extracts was measured by the method of Bradford (1976) using a standard calibration curve constructed from bovine serum albumin and was expressed in mg cm^{-3} (Skrebsky *et al.* 2008).

Transcript levels of *Cu/Zn-SOD*, *Mn-SOD*, *CAT*, and *GPX* genes: Total RNA from cucumber leaves was isolated with the *TRIzol* reagent (*Invitrogen*, Carlsbad, USA) and then reverse-transcribed to cDNA using the *Quantscript RT* kit (*Cwbio*, Beijing, China). PCR primers of *Cu/Zn-SOD*, *Mn-SOD*, *CAT*, *GPX,* and *actin* genes were designed (Table 1). Real-time PCR was performed using 10 mm^3 of 2 × ultra *SYBR* mixture with *Rox* (*Cwbio*) to each well containing 2 mm^3 of template cDNA and 0.8 mm^3 of 5 pM of each forward and reverse primers in a 20-mm^3 reaction system. The PCR parameters included an initial denaturation step at 95 °C for 10 min, followed by amplification at 95 °C for 15 s, and 40 cycles at 61 °C for 1 min. The gene expression levels were normalized against the corresponding *actin* levels. The $2^{-\Delta\Delta Ct}$ comparative CT method was used to calculate the different expression of *Cu/Zn-SOD*, *Mn-SOD*, *CAT*, and *GPX*. Three independent experiments were done. PCR amplification was conducted with the *CFX96*™ real-time system (*Bio-Rad*, Hercules, USA) and the results were analyzed using the *Bio-Rad CFX* manager software.

Determination of proline and soluble sugar content: After grinding in liquid nitrogen, leaf samples (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).

The soluble sugars were quantified by the anthrone-sulfuric acid assay and content was calculated using glucose as a standard (Yemm and Willis 1954).

Statistical analysis: The data, which were assayed in triplicate, were expressed as means ± SD. Differences were analyzed by two-way *ANOVA* and the least significant difference (LSD) test. *P*-values < 0.05 were considered to be significant. The analyses were performed using *SPSS* software (*v. 13.0* for *Windows*, *SPSS Inc.*, Chicago, USA).

Table 1. Forward (F) and reverse (R) primers used in this study.

Sequence	Acc. number	Primer sequences (5'-3')		Product size [bp]
Cu/Zn-SOD	EF121763	F: GACTGGGCCACATTTCAACC	R: GCCTTGCCATCTTCACCAA	108
Mn-SOD	EF203086	F: CAATGGCGGAGGTCACATTA	R: AGAGCAAGCCACACCCATC	195
CAT	EF468517	F: AATGGCCGGAGGATGTGA	R: CCAACGACATAGAGAAAGCCAAC	111
GPX	M91373	F: ACCGCCTCATCAACTTCAAC	R: ATCTAGTGGGTGTGACTGGGTC	132
Actin	AB010922	F: GGTCGTGACCTTACTGATGC	R: CAATAGAGGAACTGCTCTTTGC	166

Results

Application of 0.5 mM FA led to the highest ($P < 0.05$) fresh and dry masses when compared to other concentrations (0, 0.3, 0.4, 0.5, 0.6, 0.7, and 0.8 mM) (Table 2). Under PEG-induced dehydration, pretreatment with 0.5 mM FA resulted in the lower ($P < 0.001$) MDA content than at all other concentrations of FA, and in the lower content of $O_2^{\cdot-}$ and H_2O_2 ($P < 0.01$) than at 0, 0.4, 0.7, and 0.8 mM FA. This study also examined whether exogenous FA influenced antioxidant enzyme activities under dehydration conditions. In comparison to control,

Table 2. Changes in plant growth [g plant^{-1}], $O_2^{\cdot-}$ production rate [nmol g^{-1}(f.m.) min^{-1}], content of MDA [nmol g^{-1}(f.m.)] and H_2O_2 [nmol g^{-1}(f.m.)], and activities of SOD [U mg^{-1}(protein)] and CAT [nmol(H_2O_2) mg^{-1}(protein) min^{-1}] in the second leaves of cucumber after FA pretreatment for 2 d and 10 % PEG-induced dehydration stress for 2 d. Means ± SE, n = 3. Different letters indicate statistically significant differences between treatments at $P < 0.05$.

Parameters	0	0.3	0.4	0.5	0.6	0.7	0.8 mM FA
Leaf f.m.	0.53±0.06b	0.62±0.05b	0.66±0.05b	0.72±0.03a	0.65±0.04b	0.63±0.02b	0.35±0.01c
Leaf d.m.	0.07±0.01b	0.07±0.00b	0.08±0.01b	0.10±0.01a	0.09±0.00b	0.08±0.00b	0.05±0.00c
Root f.m.	0.44±0.05b	0.42±0.00b	0.56±0.06b	0.62±0.04a	0.56±0.04b	0.49±0.06b	0.40±0.03b
Root d.m.	0.04±0.00b	0.04±0.00b	0.05±0.00b	0.06±0.01a	0.05±0.01b	0.05±0.01b	0.04±0.00b
Shoot f.m.	1.61±0.05b	1.65±0.07b	1.79±0.09b	2.31±0.10a	1.80±0.06b	1.74±0.06b	1.46±0.05b
Shoot d.m.	0.15±0.01b	0.16±0.01b	0.16±0.01b	0.21±0.01a	0.16±0.01b	0.15±0.01b	0.14±0.00b
$O_2^{\cdot-}$	2.65±0.09a	2.42±0.06a	2.19±0.09bc	1.89±0.03d	2.02±0.06cd	2.16±0.13ab	2.26±0.13a
MDA	7.80±0.19a	6.18±0.26b	6.08±0.10b	4.95±0.05e	5.45±0.06d	5.71±0.01c	7.21±0.18a
H_2O_2	80.29±2.48a	67.39±4.30a	53.06±2.87ab	35.86±5.17c	43.03±3.79bc	60.23±1.43a	60.23±1.43a
SOD	39.78±0.59e	41.10±0.42e	62.32±0.75c	81.27±0.32a	74.24±0.36b	57.75±0.45b	41.28±0.43e
CAT	143.76±1.68d	145.45±4.54cd	163.17±1.84b	181.12±5.25a	158.11±1.42bc	142.23±3.03d	122.59±9.77d

Table 3. Changes in plant growth, RWC, content of FA, MDA, and H_2O_2, $O_2^{\cdot-}$ production rate, activities of antioxidant enzymes, relative expression levels of *Cu/Zn-SOD*, *Mn-SOD*, *CAT*, and *GPX*, and content of proline and soluble sugars in the second leaves of cucumber after FA pretreatment and PEG-induced dehydration stress. Means ± SE, n = 3. Different letters indicate statistically significant differences between treatments at $P < 0.05$.

Parameters	Control	FA	PEG	FA + PEG
Leaf f.m. [mg plant^{-1}]	790.67±5.17a	780.63±10.3a	658.93±22.3b	778.80±15.8a
Leaf d.m. [mg plant^{-1}]	84.60±2.22a	83.67±2.22a	70.00±3.60b	82.10±1.74a
Root f.m. [mg plant^{-1}]	1030.27±65.2a	1004.37±66.3a	718.33±31.7b	908.53±48.1a
Root d.m. [mg plant^{-1}]	44.90±2.19a	43.83±2.49a	33.30±1.90b	43.40±1.39a
Shoot f.m. [mg plant^{-1}]	3143.53±166.3a	2954.20±248.5a	2112.60±168.4b	2922.80±228.7a
Shoot d.m. [mg plant^{-1}]	207.63±12.3a	192.13±14.2a	141.33±8.35b	173.93±4.16a
RWC [%]	59.00±0.00a	60.00±0.00a	42.00±3.00b	61.00±2.00a
FA [μg g^{-1}(d.m.)]	26.88±0.04b	27.55±0.00a	25.64±0.02d	26.45±0.02c
MDA [nmol g^{-1}(f.m.)]	11.58±0.10b	9.58±0.21c	12.13±0.10a	10.95±0.25b
H_2O_2 [nmol g^{-1}(f.m.)]	64.53±2.87c	68.83±1.43c	106.09±2.48a	87.46±3.79b
$O_2^{\cdot-}$ [nmol g^{-1}(f.m.) min^{-1}]	2.72±0.06b	2.39±0.07c	3.01±0.06a	2.65±0.03b
SOD [U mg^{-1}(protein)]	37.39±0.64c	42.81±1.33b	31.87±0.68d	57.33±0.58a
CAT [nmol mg^{-1}(protein) min^{-1}]	119.31±1.64c	115.61±0.90c	158.30±6.40b	180.02±3.34a
GPX [nmol mg^{-1}(protein) min^{-1}]	1801.56±17.0b	1752.88±9.73b	1625.84±19.5c	2450.53±15.5a
GSH-Px [nmol mg^{-1}(protein) min^{-1}]	2599.97±79.0c	2895.01±119.7c	3931.80±126.8b	5083.76±231.1a
APX [nmol mg^{-1}(protein) min^{-1}]	287.31±0.00d	300.75±3.17c	505.96±0.00b	549.87±0.00a
MDHAR [nmol mg^{-1}(protein) min^{-1}]	108.25±4.03b	107.35±8.00b	9.48±2.74c	172.79±3.33a
DHAR [nmol mg^{-1}(protein) min^{-1}]	119.39±1.28c	123.48±1.10c	197.06±1.54b	340.70±1.08a
GR [nmol mg^{-1}(protein) min^{-1}]	5.80±0.15c	6.58±0.03b	6.57±0.11b	15.29±0.21a
Cu/Zn-SOD	0.89±0.02c	0.69±0.02d	1.13±0.02b	1.24±0.03a
Mn-SOD	0.94±0.05c	1.15±0.01b	0.70±0.05d	1.20±0.08a
CAT	1.00±0.01c	0.87±0.07c	1.12±0.03b	1.32±0.05a
GPX	1.03±0.05a	0.58±0.03bc	0.51±0.03c	0.64±0.03b
Proline [μg g^{-1}(f.m.)]	11.28±0.32d	13.73±0.78c	21.88±0.65b	28.87±0.65a
Soluble sugar [mg g^{-1}(f.m.)]	1.27±0.01d	1.45±0.03c	2.68±0.04b	3.14±0.03a

0.4 - 0.7 mM FA increased ($P < 0.001$) SOD activities in dehydration-stressed leaves, and 0.4 - 0.6 mM FA resulted in the enhanced ($P < 0.01$) CAT activities. Among all concentrations of FA, 0.5 mM FA led to the highest ($P < 0.01$) activities of SOD and CAT under PEG-induced dehydration stress. Therefore, 0.5 mM was chosen as the suitable concentration of FA for following experiments.

The fresh and dry masses of the second leaves, roots, and shoots, and the leaf RWC all showed non-significant

($P > 0.05$) difference between the FA pretreatment and control groups (Table 3). Compared to the control, PEG treatment significantly ($P < 0.05$) decreased all fresh and dry masses and RWC in the second leaves. In the FA + PEG treatment group, the seven parameters were higher ($P < 0.05$) than those in the PEG treatment group. So exogenous FA significantly ($P < 0.05$) alleviated the growth inhibition induced by PEG.

Compared to the control group, the FA content in leaves of the FA pretreatment groups was enhanced ($P < 0.05$). In leaves of the PEG treatment group, the FA content was lower ($P < 0.05$) than in the control, FA pretreatment or FA + PEG treatment groups.

The formation rate of $O_2^{\cdot-}$ and the content of MDA in leaves of the FA pretreatment group were lower ($P < 0.05$) than those in the control group, and the content of H_2O_2 was not different ($P > 0.05$). Among all groups, the formation rate of $O_2^{\cdot-}$ and the content of H_2O_2 and MDA were the highest ($P < 0.05$) in the PEG treatment group and they were significantly ($P < 0.05$) decreased in the FA + PEG treatment group (Table 3).

Compared to the control group, the activities of SOD, APX, and GR were significantly ($P < 0.05$) increased in leaves of the FA pretreatment group, whereas the activities of CAT, GPX, GSH-Px, DHAR, and MDHAR did not show significant ($P > 0.05$) difference between the FA pretreatment and control groups. In leaves of the PEG treatment group, the activities of CAT, GSH-Px, APX, DHAR, and GR were higher ($P < 0.01$) than those in the control group, whereas the activities of SOD, GPX, and MDHAR were lower ($P < 0.05$). In comparison to the PEG treatment group, the activities of SOD, CAT, GPX, GSH-Px, APX, MDHAR, DHAR, and GR were higher ($P < 0.05$) in leaves of the FA + PEG treatment group.

To complete the effects of FA on antioxidant enzyme activities under dehydration stress, the transcript levels of *Cu/Zn-SOD*, *Mn-SOD*, *CAT*, and *GPX* genes in leaves were determined by real-time PCR. Compared to the control group, FA pretreatment decreased ($P < 0.01$) the mRNA levels of *Cu/Zn-SOD* and *GPX* and increased ($P < 0.05$) the expression of *Mn-SOD*. The transcription of *CAT* did not change. In comparison with the control, PEG pretreatment increased ($P < 0.05$) the expression of *Cu/Zn-SOD* and *CAT* and decreased ($P < 0.05$) the expression of *Mn-SOD* and *GPX*. In the FA + PEG treatment group, the transcript levels of *Cu/Zn-SOD*, *Mn-SOD*, *CAT*, and *GPX* were all higher ($P < 0.05$) than those in the PEG treatment group.

Compared to the control group, the content of proline and soluble sugars in leaves of the FA pretreatment group increased significantly ($P < 0.05$). Both the proline and soluble sugar content was the highest ($P < 0.01$) in the FA + PEG treatment group and was the second highest ($P < 0.01$) in the PEG treatment group.

Discussion

PEG-induced dehydration stress lowers water potential in plants and adversely affects plant growth (Van den Berg and Zeng 2006). In this study, the same results were obtained in the PEG treatment group. FA can inhibit plant growth (Turner and Rice 1975), however, 0.5 mM FA did not suppress cucumber growth in the current experiment. Pretreatment with 0.5 mM FA alleviated the growth inhibition caused by PEG. These results suggest that exogenous FA at a certain concentration can be used to increase dehydration tolerance of cucumber seedlings which is supported by the report that cinnamic acid pretreatment mitigates growth inhibition of drought-stressed cucumber seedlings (Sun *et al.* 2012).

Application of exogenous silicon increases silicon content in chilling-stressed cucumber leaves whereas chilling treatment alone decreases endogenous silicon content in seedlings (Liu *et al.* 2009b). In this study, similarly, PEG-induced dehydration stress reduced FA content in cucumber leaves and the combination of exogenous FA and dehydration stress mitigated the reduction of FA content. The higher content of FA in the FA-pretreated dehydration-stressed cucumber leaves was consistent with the mitigation of growth inhibition.

Drought stress induces ROS including $O_2^{\cdot-}$ and H_2O_2. We got the same result that the content of $O_2^{\cdot-}$ and H_2O_2 in leaves were elevated under PEG-induced dehydration stress. Application of 5-aminolevulinic acid reduces ROS content in cucumber leaves under drought stress (Li *et al.* 2011). Similarly, pretreatment with FA suppressed the ROS production under PEG-induced dehydration stress in this paper. When 0.5 mM FA was combined with PEG treatment, the decreased content of ROS corresponded to the increased FA content in leaves.

PEG-induced dehydration stress resulted in a dramatic increase in the MDA content (Table 3) and FA pretreatment alleviated the increase in MDA content. So the change of MDA and ROS levels are identical in this paper. This is similar with the report that pretreatment with paraquat results in fewer ROS and thereby reduces damage to membrane lipids under drought (Liu *et al.* 2009a).

Bian and Jiang (2009) have found that SOD activity is reduced in drought-stressed roots. Similarly, PEG decreased the SOD activity in cucumber leaves in this study. Cinnamic acid can increase SOD activities in plants (Ye *et al.* 2006) and the similar results showed that FA could induce SOD activities in leaves whether cucumber seedlings were subjected to PEG-induced dehydration stress or not (Table 2, 3). In FA-pretreated PEG-stressed leaves in this study, the higher SOD

activity coincided with the decreases of $O_2^{\cdot-}$ and MDA content and was consistent with the alleviation of growth inhibition. It could be suggested that FA pretreatment can reduce lipid peroxidation by modulating the amount of $O_2^{\cdot-}$ *via* SOD under stress and thereby increases the ability of cucumber to resist PEG-induced dehydration stress. The increase of SOD activity thus induces higher tolerance to oxidative stress. Our results with SOD can be supported by the report that exogenous 5-aminolevulinic acid increases SOD activity and thereby decreases $O_2^{\cdot-}$ content and improves plant growth under salt stress (Naeem *et al.* 2011).

It has been shown that the activities of CAT (Manivannan *et al.* 2008) and APX (Wang *et al.* 2009) were increased under drought stress, and the GSH-Px activity was elevated under salt stress (Ben-Hayyim *et al.* 1993) whereas the GPX activity was decreased in response to drought stress (Goicoechea *et al.* 2005). In this study, we observed the similar results that PEG-induced dehydration stress increased the activities of CAT, GSH-Px, and APX and decreased the GPX activity. When the FA-pretreated plants were subjected to PEG-induced dehydration stress, the activities of CAT, GPX, GSH-Px, and APX were higher than those in the PEG treatment alone. And this result is similar to the report that paraquat pretreatment enhances the activities of CAT, GPX, GSH-Px, and APX under salt stress (Lin *et al.* 2011). In the FA + PEG treatment in this study, the higher activities of CAT, GPX, GSH-Px, and APX coincided with the decreased H_2O_2 content.

Based on the metal co-factor, SODs in plants are mainly classified into Cu/Zn-SOD, Mn-SOD, and Fe-SOD but Lee and Lee (2000) reported that Fe-SOD has not been observed in cucumber. Therefore, not only the expression of *CAT* and *GPX* genes but also the transcript levels of *Cu/Zn-SOD* and *Mn-SOD* were assessed to demonstrate the effects of exogenous FA on antioxidant enzyme activities. In FA + PEG treatment in comparison to PEG treatment, the increased expression of *Cu/Zn-SOD*, *Mn-SOD*, *CAT,* and *GPX* genes were consistent with the higher activities of SOD, CAT, and GPX confirming that FA affects gene expression of antioxidant enzymes under PEG-induced dehydration stress.

The activity of MDHAR is decreased under salt conditions (Chaparzadeh *et al.* 2004) and the activities of DHAR and GR are increased in *Agropyron cristatum* leaves under water stress (Shan *et al.* 2012). Similarly, in this study, the activities of MDHAR were reduced and the activities of DHAR and GR were enhanced in the PEG treatment group. Compared to PEG treatment alone, the FA + PEG treatment increased the activities of these three enzymes (Table 3). This is similar to the report that exogenous proline increases MDHAR, DHAR, and GR activities under salt stress (Hoque *et al.* 2007). The alterations of MDHAR, GR, and DHAR activities in the FA + PEG treatment in this study were consistent with the variation of APX activity suggesting that exogenous FA can activate MDHAR, DHAR, and GR to regenerate the substrate for APX under PEG-induced dehydration stress.

Under abiotic stresses, compatible osmolytes including proline and soluble sugars are accumulated in many plants (Hoque *et al.* 2007). Similarly, proline and soluble sugar content increased in cucumber seedlings treated with PEG. The FA pretreatment and PEG increased the content of proline and soluble sugars to a greater extent than PEG treatment alone suggesting that proline and soluble sugars are involved in FA-induced changes in dehydration-stressed seedlings. This can be supported by the report that exogenous uniconazole enhances the levels of proline and soluble sugars and induces the tolerance of cucumber seedlings to drought stress (Zhang *et al.* 2007).

In conclusion, pretreatment with 0.5 mM FA alleviates growth inhibition in cucumbers caused by PEG-induced dehydration stress. The pretreatment enhances FA content in leaves, increases antioxidant enzyme activities under PEG-induced dehydration stress, and thereby decreases lipid peroxidation and elevates proline and soluble sugar content in dehydration-stressed leaves. Thus, pretreatment with 0.5 mM FA enhances dehydration tolerance of cucumber seedlings through antioxidant enzymes, proline, and soluble sugars.

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