Scientia Horticulturae 121 (2009) 138–143

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Exogenous paraquat changes antioxidant enzyme activities and lipid peroxidation in drought-stressed cucumber leaves

Zhong-Jing Liu [a], Xiao-Long Zhang [a], Ji-Gang Bai [a,*], Bang-Xia Suo [a], Pei-Lei Xu [a], Lin Wang [b]

[a] State Key Laboratory of Crop Biology, College of Life Sciences, Shandong Agricultural University, Tai'an 271018, Shandong, PR China
[b] Beijing Entry-Exit Inspection and Quarantine Bureau, Beijing 100029, PR China

ARTICLE INFO

Article history:
Received 1 July 2008
Received in revised form 21 January 2009
Accepted 23 January 2009

Keywords:
Antioxidant
Cucumber
Drought
Malonaldehyde
Paraquat

ABSTRACT

In order to examine whether paraquat modifies the functioning of antioxidants and oxidative stress levels in drought-stressed plants, a cucumber cultivar (*Cucumis sativus* cv. Yuexiu no. 3) was grown hydroponically for 2 days. Drought stress, which was induced by polyethylene glycol (PEG), increased the contents of malonaldehyde (MDA), superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) in cucumber leaves, while pretreatment of paraquat decreased them. Under drought stress induced by PEG, we observed the decreased contents of MDA, H_2O_2 and O_2^- in paraquat-pretreated plants in comparison to unpretreated stressed plants. Drought stress and paraquat both increased the activities of antioxidants such as superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), guaiacol peroxidase (GPX, EC 1.11.1.7), ascorbate peroxidase (APX, EC 1.11.1.11), dehydroascorbate reductase (DHAR, EC 1.8.5.1), monodehydroascorbate reducatase (MDHAR, EC 1.6.5.4), glutathione reductase (GR, EC 1.6.4.2), reduced glutathione (GSH) and reduced ascorbate (AsA). But the combined effect of paraquat application and drought stress resulted in the highest activities of antioxidants. So paraquat is able to moderate the activities of scavenging system enzymes and to influence oxidative stress intensity under drought stress induced by PEG.

1. Introduction

Drought occurs in many parts of the world every year. The environmental stress induces the excessive generation of reactive oxygen species (ROS) such as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) (Mittler, 2002) in plants. ROS can cause lipid peroxidation and lead to the death of cells (Imlay, 2003). To alleviate the damage from ROS, plants evolve enzymatic antioxidants such as superoxide dismutase (SOD), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), catalase (CAT), dehydrateascorbate reducatase (DHAR), monodehydroascorbate reductase (MDAR) and glutathione reductase (GR) and non-enzymatic antioxidants including both of the reduced ascorbic acid (AsA) and glutathione (GSH) (Xu et al., 2008).

ROS not only induce oxidative damage but also work as a second messenger in signal transduction pathways at low concentration (Chamnongpol et al., 1998). Pretreatment of H_2O_2 increases the antioxidant activities of plants, and hence meliorates the damage from environmental stresses such as chilling (Yu et al., 2002) and salt (De Azevedo Neto et al., 2005). Paraquat (PQ) is a pneumotoxic herbicide and will block photosynthesis in light to give the free radical form. Oxygen can rapidly reconvert this free radical thus resulting in the production of O_2^- (Ananieva et al., 2004; Takizawa et al., 2007). Concentrations of PQ above 2 mM cause significant damage to photosystem activity (Chagas et al., 2008). However, in the presence of PQ, antioxidant enzymes are usually induced in leaves due to the generation of O_2^- (Casano et al., 1999; Ekmekci and Terzioglu, 2005). We hypothesized that pretreatment with a low concentration of PQ would act like H_2O_2 and enhance the ability of plants to resist draught by changing the antioxidant activities. However, It has not been reported that PQ supply could alleviate the stress of draught.

Polyethylene glycol (PEG) 6000 can mimic water stress (Turkan et al., 2005). Cucumber (*Cucumis sativus*) has a shallow root system and large leaves. It is sensitive to drought stress and must be irrigated well everyday. Several days of poor irrigation will block their growth and development. In this paper, cucumber seedlings were pretreated with 10 μM PQ and then were watered with Hoagland solution containing 10% PEG 6000 to investigate the effects of PQ on antioxidant activities and lipid peroxidation in drought-stressed leaves.

* Corresponding author. Tel.: +86 538 8242656x8447; fax: +86 538 8226399.
E-mail address: baijg@sdau.edu.cn (J.-G. Bai).

Abbreviations: APX, ascorbate peroxidase; AsA, reduced ascorbate; CAT, catalase; DHAR, dehydrateascorbate reductase; GPX, guaiacol peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; MDA, malonaldehyde; MDHAR, monodehydroascorbate reducatase; O_2^-, superoxide radical; PEG, polyethylene glycol; PQ, paraquat; ROS, reactive oxygen species; SOD, superoxide dismutase.

0304-4238/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.scienta.2009.01.032

2. Materials and methods

2.1. Plant materials and treatments

Seeds of *Cucumis sativus* cv. Yuexiu no. 3 were incubated on moist pledgets at 25 °C for 2 days and then were planted into 10 cm of plastic pots containing sand. Seedlings of the cucumber were grown at 25 °C and 12 h light (600 μmol m^{-2} s^{-1})/12 h dark and were watered twice every day with the Hoagland 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, cucumber seedlings were selected and divided into four groups for treatments.

In our preliminary experiments, cucumber seedlings were separately pretreated with four concentrations of PQ (1, 5, 10 and 15 μM) in moderate light (100 μmol m^{-2} s^{-1}) for 0.5, 1, 2 and 4 h and then were put into darkness for 24 h to resume before being exposed to drought stress induced by PEG. The plants, which were treated with 10 μM PQ for 1 h, resisted drought stress well. Therefore, two groups of cucumber seedlings were watered with 15 ml of 10 μM PQ (Syngenta), and others were treated with 15 ml of H_2O. After kept in light (100 μmol m^{-2} s^{-1}) for 1 h, all plants were successively eluted 10 times with Hoagland solution and then were put into darkness for 24 h to resume. Two groups of seedlings, which were pretreated with PQ, were separately watered with 15 ml of Hoagland solution and 15 ml of Hoagland solution + 10% PEG and were, respectively, named as PQ pretreatment and PQ + PEG treatment. The other groups of cucumbers were watered with either of the two types of nutrient solutions as indicated above and were, respectively, named as control and PEG treatment. Every plant was subjected to 25 °C and 12 h light (600 μmol m^{-2} s^{-1})/12 h dark and was watered twice per day. The second leaves were collected at 0 h before pretreatment of PQ and at 1, 25, 49 and 73 h after pretreatment of PQ and were used for the subsequent experiments with three replicates. Each sample included 8 seedlings.

2.2. Determination of malonaldehyde content

Malonaldehyde (MDA) was extracted with 10% trichloroacetic acid and was determined at 450, 532 and 600 nm with 0.6% thiobarbituric acid as described by Dhindsa et al. (1981).

2.3. Determination of the formation rate of O_2^-

The formation rate of O_2^- was determined at 530 nm according to the method of Elstner and Heupel (1976) and was calculated from a standard curve of $NaNO_2$.

2.4. Determination of H_2O_2 content

When 2 g of fresh leaves were homogenized in 2 ml of refrigerated acetone and were centrifuged at 3000 × *g* for 10 min, the supernatant was used for the H_2O_2 content assay as described by Mukherjee and Choudhuri (1983).

2.5. Activities determination of antioxidant enzyme

After homogenized with liquid nitrogen, 0.3 g of leaves were suspended in 3 ml of ice-cold HEPES buffer (25 mM, pH 7.8) which contained 0.2 mM EDTA and 2% PVP. The homogenate was centrifuged at 4 °C and 12,000 × *g* for 20 min, and the resulting supernatant was used for determination of SOD, GPX, CAT, DHAR, MDHAR and GR (Ramiro et al., 2006). The HEPES buffer (25 mM, pH 7.8) containing 0.2 mM EDTA, 2% PVP and 2 mM AsA was used for APX extraction.

Activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) according to the method of Hwang et al. (1999). One unit of the enzyme activity was defined as the amount of enzyme required to result in a 50% inhibition of the reduction rate of NBT under assay conditions.

Activity of GPX was measured at 470 nm according to the method of Ramiro et al. (2006). The CAT activity was surveyed as the decline in absorbance at 240 nm (Pereira et al., 2002). Activity of APX was determined at 290 nm as described by Chen and Asada (1989). The DHAR activity was determined by monitoring the reduction of dehydroascorbate at 265 nm according to Doulis et al. (1997). Activity of MDHAR was measured at 340 nm as described by Hossain et al. (1984). The GR activity was measured at 340 nm according to the method of Lee and Lee (2000). Absorbance change of 0.1 in 1 min was defined as one unit of CAT, GPX, APX, GR, DHAR and MDHAR.

2.6. Content assay of non-enzymatic antioxidants

The ground leaves (0.6 g) were suspended in 6 ml of 1% (W/V) oxalic acid and were centrifuged at 4 °C and 8000 × *g* for 10 min. The supernatant was used for the content determination of AsA and total ascorbate (T-AsA). The content of AsA was measured according to the methods of Klein and Perry (1982) and Raghu et al. (2007), while the content of T-AsA was determined as described by Mukherjee and Choudhuri (1983). The absorbance was measured at 500 nm, and the contents of the two ascorbates were calculated from a standard curve of ascorbic acid. Oxidized ascorbate was estimated from the difference of T-AsA and AsA.

Cucumber leaves (0.03 g) were homogenized in 0.3 ml of 5% trichloroacetic acid, and then were centrifuged for 10 min at 4 °C and 10,000 × *g*. The supernatants were used to determine the content of GSH (Guri, 1983). The content of oxidized glutathione (GSSG) was measured using the GSH and GSSG assay kit (Beyotime Institute of Biotechnology, China).

2.7. Statistics

Data were expressed as means ± standard errors. Differences were analyzed with one-way ANOVA and Least Significant Difference (LSD) (Williams et al., 2006). *P*-values of <0.05 were considered to be significant.

3. Results and discussion

3.1. Content of MDA

MDA is an indicator of lipid peroxidation (Ohkawa et al., 1979). It is demonstrated that PEG results in the increasing of MDA contents (Chang and Kao, 1997), and this is related to the damage of lipid membrane. Zhang et al. (2007) have found that uniconazole decreases the MDA content in drought-stressed soybean leaves. In this paper, PQ application increased the MDA content in cucumber leaves at 1 h, but did not enhance the content significantly at 25 h (Fig. 1A). At 73 h, the MDA content was the lowest in PQ pretreatment and was the highest in PEG treatment. In comparison to PEG treatment, treatment of PQ + PEG made the MDA content to decrease more. According to the result of ANOVA, PQ significantly ($P < 0.01$) decreases the MDA content, and PEG increases ($P < 0.01$) it. This indicates that 10% PEG results in the serious oxidative stress. PQ influences the lipid membrane after pretreatment, but improves the lipid peroxidation when cucumber is transferred into drought stress induced by PEG. The pretreatment of a mild oxidative stress is responsible for reducing the deleterious effects of drought on plants.

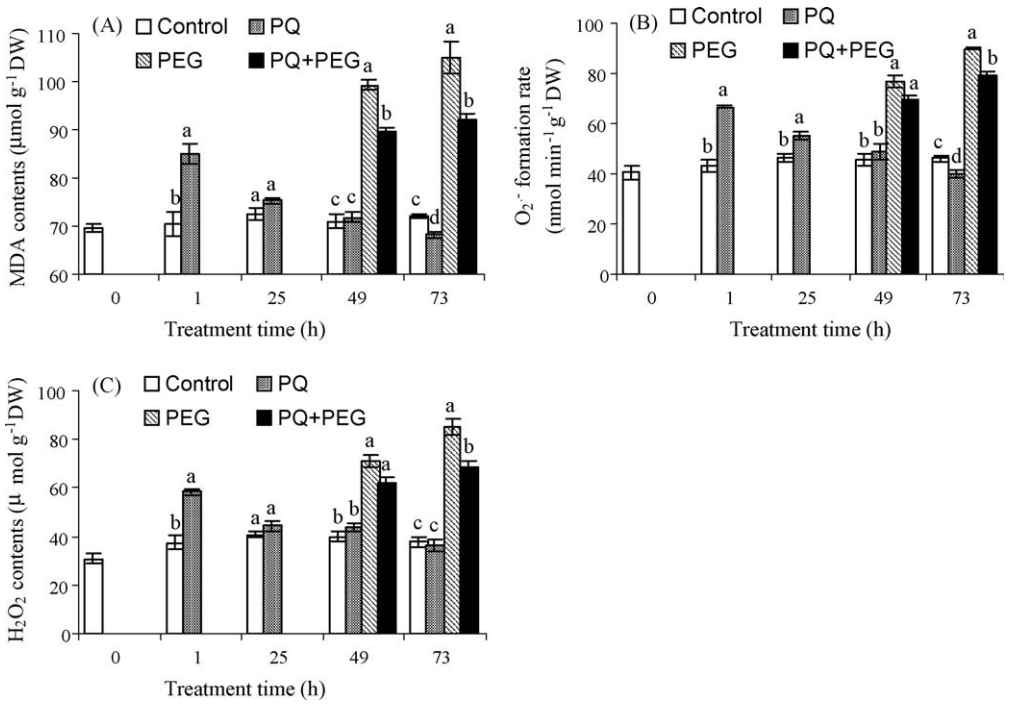

Fig. 1. Effects of PQ on MDA content (A), O_2^- formation rate (B) and H_2O_2 content (C) in cucumber leaves. Control, watering with Hoagland solution; PEG, watering with Hoagland solution + 10% PEG; PQ, pretreating with 10 μM PQ and watering with Hoagland solution; PQ + PEG, pretreating with 10 μM PQ and watering with Hoagland solution + 10% PEG. Bars represent standard errors. Values with the same letter are not significantly different at $P < 0.05$. *F*-values: PQ pretreatment and PEG treatment are 21.26** and 251.72** for MDA content, and 50.89** and 1191.51** for O_2^- formation rate, and 11.81** and 228.32** for H_2O_2 content, respectively. Significance of ANOVA: **$P < 0.01$.

3.2. Formation rate of O_2^- and content of H_2O_2

PQ generates O_2^- in light (Ananieva et al., 2004), and PQ-induced oxidative damages are also related to excess H_2O_2 (Chagas et al., 2008). So the formation rate of O_2^- (Fig. 1B) and the content of H_2O_2 (Fig. 1C) were increased at 1 h. At 25 h, PQ has been rinsed, therefore, the formation rate of O_2^- in PQ pretreatment achieved a 0.17-fold decrease, while the content of H_2O_2 was not significantly higher than control. Environmental stresses such as drought induce the overproduction of ROS, which can damage lipid membrane and increase the content of MDA (Smirnoff, 1993). At 73 h, PEG treatment resulted in the highest contents of O_2^- and H_2O_2 in cucumber leaves, while PQ pretreatment made the O_2^- to be the lowest and allowed H_2O_2 content to have no difference with control. Compared with PEG treatment, treatment of PQ + PEG significantly decreased the contents of the two kinds of ROS. So PEG increases ($P < 0.01$) the formation rate of O_2^- and the content of H_2O_2, but PQ significantly ($P < 0.01$) decreases the ones. This is consistent with contents of MDA. The data suggest that pretreatment of PQ decreases the lipid peroxidation by reducing the contents of ROS under drought stress induced by PEG.

3.3. Activities of antioxidants

O_2^- can be dismutated into H_2O_2 by SOD (Bowler et al., 1992) in the chloroplast, mitochondrion, cytoplasm and peroxisome. The increasing of SOD activity induces the higher tolerance to oxidative stress (Bowler et al., 1991). Under stress of salt (Liang et al., 2003) and drought (Gong et al., 2005), the addition of silicon increases SOD activity. Our present results showed that the SOD activity of cucumber leaves was enhanced in PQ pretreatment during treatment time (Fig. 2A). After the pretreated plants were resumed for 24 h, the enzyme activity increased by 2%, indicating that pretreatment of PQ induces the expression of SOD slightly. At 73 h, the pretreated plants have been transferred into PEG conditions for 2 days, and the SOD activity in treatment of PQ + PEG was the highest, indicating that the enzyme is further induced. The SOD activity in leaves of PEG treatment was higher than control, but was lower than the one in treatment of PQ + PEG. This is negatively correlated with the change of O_2^- formation rate. So pretreatment of PQ increases the ability of cucumber leaves to dismutate O_2^- via SOD under drought stress induced by PEG.

H_2O_2, which is resulted from the action of SOD, can rapidly diffuse across membranes and is toxic (Foyer et al., 1997). To scavenge this kind of ROS, plants evolve an antioxidant system, including the ascorbate–glutathione cycle, CAT and GPX. The ascorbate–glutathione cycle is found in the chloroplasts and cytosol (Foyer et al., 1994). In this cycle, APX plays an important role in removing H_2O_2 when catalyzing the oxidization of AsA (Asada, 1999). After treated with silicon, there is an increase of APX activity in salt-stressed cucumbers (Zhu et al., 2004). During the treatment time of this study, the APX activity of cucumber leaves was gradually increased in PQ pretreatment (Fig. 2B). At 25 h, the enzyme activity of PQ pretreatment increased by 17%. After the pretreated cucumber seedlings were exposed to PEG stress for 2 days, the APX activities in leaves of PQ pretreatment and PEG treatment were both higher than control. In comparison to PEG treatment, treatment of PQ + PEG made the APX activity to increase more. This result is negatively correlated with the contents of H_2O_2, indicating that pretreatment of PQ can decrease the lipid peroxidation induced by H_2O_2 via APX under drought stress. The ascorbate–glutathione cycle needs regeneration of AsA that relies on GSH-dependent DHAR and GR (a key enzyme in GSH regeneration cycle) or NADH-dependent MDHAR (Luster and Donaldson, 1987). PEG increases the activity of APX and GR (Turkan et al., 2005). In tobacco plants treated by PQ, the activities of DHAR and GR are activated (Miyagawa et al., 2000). Pretreatment of salicylic acid increases the activities of GR, DHAR and MDHAR in heat-stressed mustard seedlings (Dat et al., 1998). In the current experiment, PQ pretreatment gradually increased the

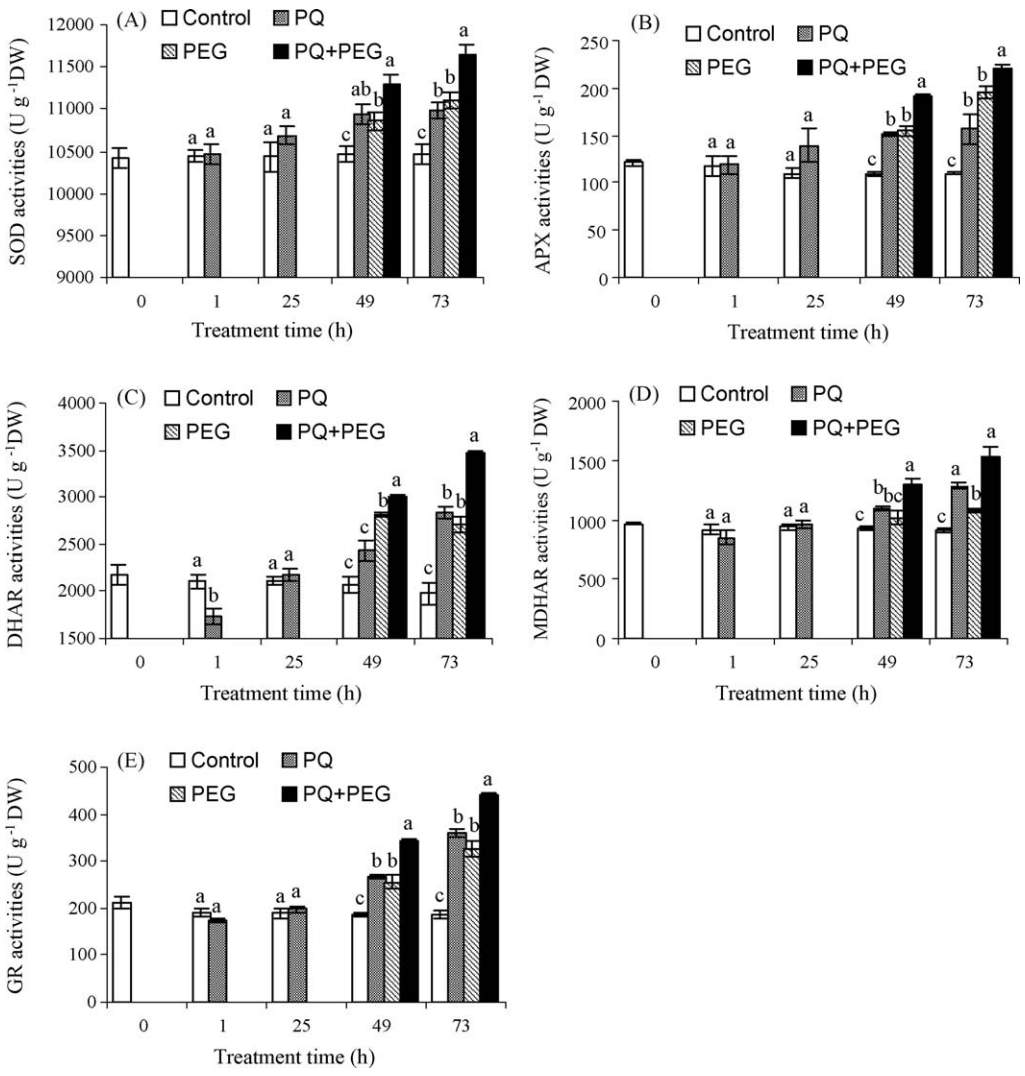

Fig. 2. Effects of PQ on activities of SOD (A), APX (B), DHAR (C), MDHAR (D) and GR (E). Control, watering with Hoagland solution; PEG, watering with Hoagland solution + 10% PEG; PQ, pretreating with 10 μM PQ and watering with Hoagland solution; PQ + PEG, pretreating with 10 μM PQ and watering with Hoagland solution + 10% PEG. Bars represent standard errors. Values with the same letter are not significantly different at $P < 0.05$. *F*-values: PQ pretreatment and PEG treatment are 24.79** and 37.08** for SOD activity, and 17.02** and 73.62** for APX activity, and 99.52** and 70.51** for DHAR activity, and 76.73** and 19.51** for MDHAR activity, and 172.87** and 104.53** for GR activity, respectively. Significance of ANOVA: **$P < 0.01$.

activities of DHAR (Fig. 2C), MDHAR (Fig. 2D) and GR (Fig. 2E) in cucumber leaves and also enhanced the contents of AsA (Fig. 4A) and GSH (Fig. 4B) and the ratios of AsA/oxidized ascorbate (Fig. 4C) and GSH/GSSG (Fig. 4D) during treatment time. At 73 h, the activities of DHAR, MDHAR and GR in PQ pretreatment and treatments of PEG and PQ + PEG were separately higher than control. Compared with control, PQ pretreatment and treatments of PEG or PQ + PEG also made the contents of AsA and GSH and the ratios of AsA/oxidized ascorbate and GSH/GSSG to increase more. Treatment of PQ + PEG resulted in the strongest activities of DHAR,

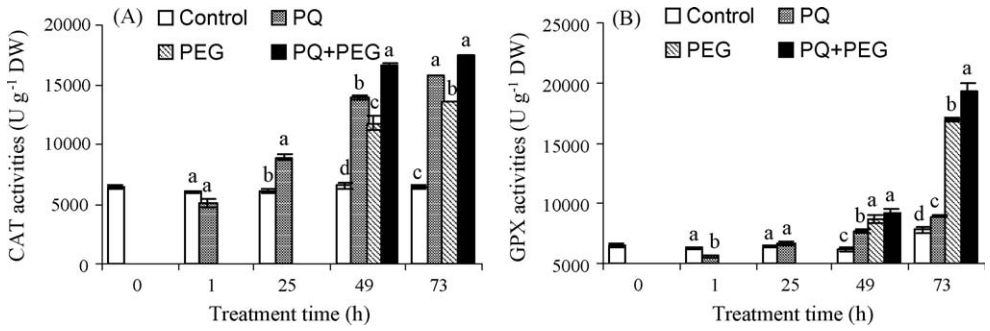

Fig. 3. Effects of PQ on activities of CAT (A) and GPX (B) in cucumber leaves. Control, watering with Hoagland solution; PEG, watering with Hoagland solution + 10% PEG; PQ, pretreating with 10 μM PQ and watering with Hoagland solution; PQ + PEG, pretreating with 10 μM PQ and watering with Hoagland solution + 10% PEG. Bars represent standard errors. Values with the same letter are not significantly different at $P < 0.05$. *F*-values: PQ pretreatment and PEG treatment are 277.10** and 121.90** for CAT activity, and 21.67** and 682.29** for GPX activity, respectively. Significance of ANOVA: **$P < 0.01$.

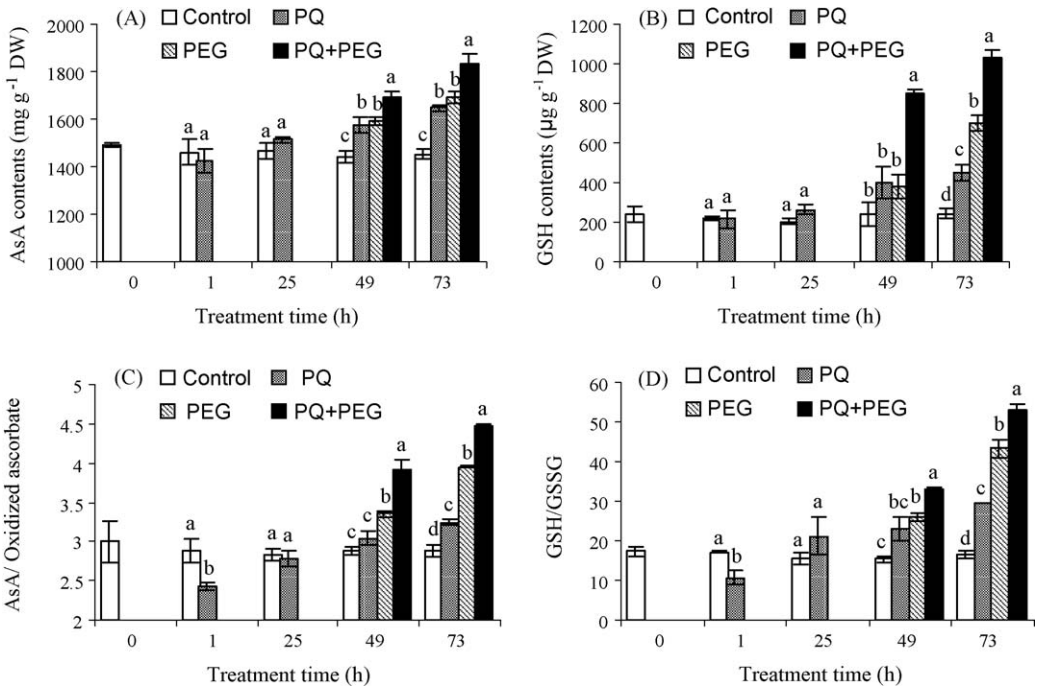

Fig. 4. Effects of PQ on contents of AsA (A), GSH (B), and ratios of AsA/oxidized ascorbate (C) and GSH/GSSG (D) in cucumber leaves. Control, watering with Hoagland solution; PEG, watering with Hoagland solution + 10% PEG; PQ, pretreating with 10 μM PQ and watering with Hoagland solution; PQ + PEG, pretreating with 10 μM PQ and watering with Hoagland solution + 10% PEG. Bars represent standard errors. Values with the same letter are not significantly different at $P < 0.05$. *F*-values: PQ pretreatment and PEG treatment are 44.02** and 70.72** for AsA content, and 53.12** and 201.64** for GSH content, and 109.23** and 721.02** for AsA/Oxidized ascorbate, and 31.68** and 142.21** for GSH/GSSG, respectively. Significance of ANOVA: $^{**}P < 0.01$.

MDHAR and GR, and the highest contents of AsA and GSH, and the biggest ratios of AsA/oxidized ascorbate and GSH/GSSG. This suggests that AsA and GSH are regenerated well in the ascorbate–glutathione cycle and APX can remove H_2O_2 by catalyzing the oxidization of AsA, when cucumber seedlings are pretreated with PQ and then are transferred into drought stress.

CAT is a main enzyme to eliminate H_2O_2 in the mitochondrion and microbody (Shigeoka et al., 2002). The enhanced CAT activity has been found in treatments of acid rain (Wyrwicka and Skłodowska, 2006). Pretreatment of salicylic acid increases the enzyme activity in lead-stressed rice seedlings (Chen et al., 2007). GPX may be against the accumulation and toxicity of H_2O_2 in the apoplast (Shigeoka et al., 2002). The enzyme activity is increased in PEG treatment (Turkan et al., 2005) and is enhanced significantly by foliar spraying with Spd or Spm under $CdCl_2$ treatment (Zhao and Yang, 2008). In this study, the activities of CAT (Fig. 3A) and GPX (Fig. 3B) were gradually increased in cucumber leaves of PQ pretreatment during treatment time. At 25 h, the activities of CAT and GPX were increased by 73 and 19%, respectively. After the pretreated cucumber seedlings were exposed to PEG stress for 2 days, the activities of the two enzymes in treatment of PQ + PEG were the highest, and they were higher in PQ pretreatment and treatment of PEG or PQ + PEG than that in control. Thereby, the expression of CAT and GPX can be induced slightly by pretreatment of PQ and is further increased when the pretreated cucumber is under PEG treatment. Lipid peroxidation induced by H_2O_2 can be decreased by pretreatment of PQ via CAT and GPX under drought stress induced by PEG.

AsA and GSH not only act as substrates in the ascorbate–glutathione cycle, but also perform non-enzymatically. Their higher contents have been owed to alleviate the injury by ROS (Kumar et al., 2003; Schonhof et al., 2007). The recent result (Fig. 4) shows that PQ and PEG significantly ($P < 0.05$) increase the contents of GSH and AsA in cucumber leaves. Treatment of PQ + PEG made the contents of the two antioxidants increased more than treatment of PEG, indicating that PQ can increase the ability of cucumber leaves to elimination ROS via the higher contents of GSH and AsA under drought stress induced by PEG treatment.

In conclusion, drought stress induced by PEG would increase the contents of O_2^- and H_2O_2, and thereby enhanced the content of MDA in cucumber leaves. When plants were pretreated with PQ and exposed to drought stress induced by PEG, the activities of antioxidant enzymes such as SOD, APX, DHAR, MDHAR, GR, CAT and GPX and the contents of AsA and GSH were increased gradually during treatment time. The combined effect of PQ application and drought stress resulted in the highest activities of antioxidants and the lowest content of MDA. Therefore, the bipyridylium herbicide PQ could lead to the higher activities of antioxidants in light condition (Casano et al., 1999; Ekmekci and Terzioglu, 2005) via generating O_2^- (Ananieva et al., 2004). When PQ pretreatment seedlings are transferred to drought stress, the antioxidants are further modified so as to play a role in decreasing the lipid peroxidation of membrane (Yu et al., 2002; De Azevedo Neto et al., 2005). PQ pretreatment could protect cells from being damaged by ROS in drought-stressed cucumber leaves.

References

Ananieva, E.A., Christov, K.N., Popova, L.P., 2004. Exogenous treatment with Salicylic acid leads to increased antioxidant capacity in leaves of barley plants exposed to paraquat. J. Plant Physiol. 161, 319–328.

Asada, K., 1999. The water–water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Annu. Rev. Plant Physiol. Plant Mol. Biol. 50, 601–639.

Bowler, C., Slooten, L., Vandenbranden, S., De Rycke, R., Botterman, J., Sybesma, C., Van Montagu, M., Inzé, D., 1991. Manganese superoxide dismutase can reduce cellular damage mediated by oxygen radicals in transgenic plants. EMBO J. 10, 1723–1732.

Bowler, C., Van Montagu, M., Inzé, D., 1992. Superoxide dismutase and stress tolerance. Annu. Rev. Plant Physiol. Plant Mol. Biol. 43, 83–116.

Casano, L.M., Martín, M., Zapata, J.M., Sabater, B., 1999. Leaf age- and paraquat concentration-dependent effects on the levels of enzymes protecting against photooxidative stress. Plant Sci. 149, 13–22.

Chagas, R.M., Silveira, J.A.G., Rafael, V., Ribeiro, R.V., Vitorello, V.A., Carrer, H., 2008. Photochemical damage and comparative performance of superoxide dismutase and ascorbate peroxidase in sugarcane leaves exposed to paraquat-induced oxidative stress. Pestic. Biochem. Physiol. 90, 181–188.
Chamnongpol, S., Willekens, H., Moeder, W., Langebartels, C., Sandermann Jr., H., Van Montagu, M., Inze, D., Van Camp, W., 1998. Defense activation and enhanced pathogen tolerance induced by H_2O_2 in transgenic tobacco. Proc. Natl. Acad. Sci. U.S.A. 95, 5818–5823.
Chang, C.J., Kao, C.H., 1997. Paraquat toxicity is reduced by metal chelators in rice leaves. Physiol. Plant. 101, 471–476.
Chen, G.X., Asada, K., 1989. Ascorbate peroxidase in tea leaves: occurrence of two isozymes and the differences in their enzymatic and molecular properties. Plant Cell Physiol. 30, 987–998.
Chen, J., Zhu, C., Li, L.P., Sun, Z.Y., Pan, X.B., 2007. Effects of exogenous salicylic acid on growth and H_2O_2-metabolizing enzymes in rice seedlings under lead stress. J. Environ. Sci. 19, 44–49.
Dat, J.F., Foyer, C.H., Scott, I.M., 1998. Changes in salicylic acid and antioxidants during induced thermo tolerance in mustard seedlings. Plant Phyisol. 118, 1455–1461.
De Azevedo Neto, A.D., Prisco, J.T., Enéas-Filho, J., Medeiros, J.V.R., Gomes-Filho, E., 2005. Hydrogen peroxide pre-treatment induces salt-stress acclimation in maize plants. J. Plant Physiol. 162, 1114–1122.
Dhindsa, R.S., Plumb-Dhindsa, P., Thorpe, T.A., 1981. Leaf senescence: correlated with increase leaves of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. J. Exp. Bot. 32, 93–101.
Doulis, A.G., Debian, N., Kingston-Smith, A.H., Foyer, C.H., 1997. Differential localization of antioxidants in maize leaves. Plant Physiol. 114, 1031–1037.
Ekmekci, Y., Terzioglu, S., 2005. Effects of oxidative stress induced by paraquat on wild and cultivated wheats. Pestic. Biochem. Physiol. 83, 69–81.
Elstner, E.F., Heupel, A., 1976. Inhibition of nitrite formation from hydroxylammoniumchloride: a simple assay for superoxide dismutase. Anal. Biochem. 70, 616–620.
Foyer, C.H., Lelandais, M., Kunert, K.J., 1994. Photooxidative stress in plants. Physiol. Plant. 92, 696–717.
Foyer, C.H., Lopez-Delgado, H., Dat, J.F., Scott, I.M., 1997. Hydrogen peroxide and glutathione-associated mechanisms of acclamatory stress tolerance and signaling. Physiol. Plant. 100, 241–254.
Gong, H., Zhu, X., Chen, K., Wang, S., Zhang, C., 2005. Silicon alleviates oxidative damage of wheat plants in pots under drought. Plant Sci. 169, 313–321.
Guri, A., 1983. Variation in glutathione and ascorbic acid content among selected cultivars of *Phaseolus vulgaris* prior to and after exposure to ozone. Can. J. Plant Sci. 63, 733–737.
Hossain, M.A., Nakano, Y., Asada, K., 1984. Monodehydroascorbate reductase in spinach chloroplasts and its participation in regeneration of ascorbate for scavenging hydrogen peroxide. Plant Cell Physiol. 25, 385–395.
Hwang, S.Y., Lin, H.W., Chern, R.H., Lo, H.F., Li, L., 1999. Reduced susceptibility to water logging together with high-light stress is related to increases in superoxide dismutase and catalase activities in sweet potato. Plant Growth. Regul. 27, 167–172.
Imlay, J.A., 2003. Pathways of oxidative damage. Annu. Rev. Microbiol. 57, 395–418.
Klein, B.P., Perry, A.K., 1982. Ascorbic acid and vitamin A activity in selected vegetables from different geographical areas of the United States. J. Food Sci. 47, 941–945.
Kumar, S., Singla-Pareek, S.L., Reddy, M.K., Sopory, S.K., 2003. Glutathione: biosynthesis, homeostasis and its role in abiotic stresses. J. Plant Biol. 30, 179–187.
Lee, D.H., Lee, C.B., 2000. Chilling stress-induced changes of antioxidant enzymes in the leaves of cucumber: in gel enzyme activity assays. Plant Sci. 159, 75–85.
Liang, Y., Chen, Q., Liu, Q., Zhang, W., Ding, R., 2003. Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt-stressed barley (*Hordeum vulgare* L.). J. Plant Physiol. 160, 1157–1164.
Luster, D.G., Donaldson, R.P., 1987. Orientation of electron transport activities in the membrane of intact glyoxysomes isolated from castor bean endosperm. Plant Physiol. 85, 796–800.
Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7, 405–410.
Miyagawa, Y., Tamoi, M., Shigeoka, S., 2000. Evaluation of the defense system in chloroplasts to photooxidative stress caused by paraquat using transgenic tobacco plants expressing catalase from *Escherichia coli*. Plant Cell Physiol. 41, 311–320.
Mukherjee, S.P., Choudhuri, M.A., 1983. Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. Physiol. Plant. 58, 166–170.
Ohkawa, H., Ohishi, N., Yaqi, K., 1979. Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358.
Pereira, G.J.G., Molina, S.M.G., Lea, P.J., Azevedo, R.A., 2002. Activity of antioxidant enzymes in response to cadmium in *Crotalaria juncea*. Plant Soil 239, 123–132.
Raghu, V., Platel, K., Srinivasan, K., 2007. Comparison of ascorbic acid content of *Emblica officinalis* fruits determined by different analytical methods. J. Food Compos. Anal. 20, 529–533.
Ramiro, D.A., Guerreiro-Filho, O., Mazzafera, P., 2006. Phenol Contents, oxidase activities, and the resistance of coffee to the leaf miner *Leucoptera coffeella*. J. Chem. Ecol. 32, 1977–1988.
Schonhof, I., Kläring, H.P., Krumbein, A., Clauβben, W., Schreiner, M., 2007. Effect of temperature increase under low radiation conditions on phytochemicals and ascorbic acid in greenhouse grown broccoli. Agric. Ecosyst. Environ. 119, 103–111.
Shigeoka, S., Ishikawa, T., Tamoi, M., Miyagawa, Y., Takeda, T., Yabuta, Y., Yoshimura, K., 2002. Regulation and function of ascorbate peroxidase isoenzymes. J. Exp. Bot. 53, 1305–1319.
Smirnoff, N., 1993. The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol. 125, 27–58.
Takizawa, M., Komori, K., Tampo, Y., Yonaha, M., 2007. Paraquat-induced oxidative stress and dysfunction of cellular redox systems including antioxidative defense enzymes glutathione peroxidase and thioredoxin reductase. Toxicol. In Vitro 21, 355–363.
Turkan, I., Bor, M., Ozdemir, F., Koca, H., 2005. Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius* Gray and drought-sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. Plant Sci. 168, 223–231.
Williams, J.P., Duncan, S.E., Williams, R.C., Mallikarjunan, K., Eigel III, W.N., ?Keefe, S.F., 2006. Flavor fade in peanuts during short-term storage. J. Food Sci. 71, 265–269.
Wyrwicka, A., Skłodowska, M., 2006. Influence of repeated acid rain treatment on antioxidative enzyme activities and on lipid peroxidation in cucumber leaves. Environ. Exp. Bot. 56, 198–204.
Xu, P.L., Guo, Y.K., Bai, J.G., Shang, L., Wang, X.J., 2008. Effects of long-term chilling on ultrastructure and antioxidant activity in leaves of two cucumber cultivars under low light. Physiol. Plant. 132, 467–478.
Yu, C.W., Murphy, T.M., Sung, W.W., Lin, C.H., 2002. H_2O_2 treatment induces glutathione accumulation and chilling tolerance in mung bean. Funct. Plant. Biol. 29, 1081–1087.
Zhang, M.C., Duan, L.S., Tian, X.L., He, Z.P., Li, J.M., Wang, B.M., Li, Z.H., 2007. Uniconazole-induced tolerance of soybean to water deficit stress in relation to changes in photosynthesis, hormones and antioxidant system. J. Plant Physiol. 164, 709–717.
Zhao, H.Z., Yang, H.Q., 2008. Exogenous polyamines alleviate the lipid peroxidation induced by cadmium chloride stress in *Malus hupehensis* Rehd. Sci. Hortic. 116, 442–447.
Zhu, Z., Wei, G., Li, J., Qian, Q., Yu, J., 2004. Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber (*Cucumis sativus* L.). Plant Sci. 167, 527–533.