

*Full Length Research Paper*

# Effects of proline on photosynthesis, root reactive oxygen species (ROS) metabolism in two melon cultivars (*Cucumis melo* L.) under NaCl stress

Zhiming Yan, Shirong Guo\*, Sheng Shu, Jin Sun and Takafumi Tezuka

College of Horticulture, Nanjing Agricultural University, Nanjing 210095, China.

Accepted 4 November, 2011

**Effects of 0.2 mM proline applied to saline nutrient solution on biomass, chlorophyll content, photosynthetic parameters, reactive oxygen species and antioxidant enzymes activities of two melon cultivars (cv. Yuhuang and cv. Xuemei) were examined. Results indicate that exogenous proline increased the fresh and dry weights of both melon cultivars under NaCl stress, raised their chlorophyll content, net photosynthetic rate (*Pn*), actual efficiency of photosystem II ( $\Phi$ PSII), enhanced the activity of SOD, POD, CAT, APX, DHAR and GR in their roots, lowered the superoxide anion radical level and reduced the hydrogen peroxide ( $H_2O_2$ ) content and malondialdehyde (MDA) content. Exogenous proline also alleviate salinity-induced damage of membrane in both melon cultivars. In conclusion, proline treatment enhanced the salinity tolerance of both melon plants and alleviated their salinity-induced damage. However, all the above effects of proline were markedly more significant in cv. Xuemei than in cv. Yuhuang, suggesting that proline had different effects on different cultivars of melon plants.**

**Key words:** Proline, photosynthesis, reactive oxygen species, antioxidant enzymes, salt tolerance, NaCl stress.

## INTRODUCTION

Salinity stress is one of the most common abiotic factors that inhibit crop growth and productivity by reducing the photosynthetic capacity of plants (Parida and Das, 2005; Ashraf and Foolad, 2007; Subrahmanyam, 2008). The reduced photosynthesis is not only caused by stomatal closure that reduces stomatal conductance (*gs*), transpiration rate (*Tr*), intercellular  $CO_2$  concentration (*Ci*), and net photosynthesis rate (*Pn*) but also by non-stomatal factors that decrease photosystem II (PSII) efficiency (Zhang et al., 2009). The inhibition of photosynthetic capacity may vary with species (Dubey, 1994).

High salinity increases the levels of reactive oxygen

species (ROS) in plants, such as superoxide radicals, hydrogen peroxide, singlet oxygen and hydroxyl radicals (Scandalios, 1993; Kangasjärvi et al., 1994; Larson, 1995). ROS damage normal metabolism via oxidation of membrane lipids, proteins and plant nucleic acids (Smirnoff, 1993; Gómez et al., 1999; Hernández et al., 2001). Plants develop various defensive mechanisms to cope with salinity-induced damage by accumulating such compatible solutes as proline, glycinebetaine and sugars, and by up-regulating antioxidant enzymes and  $Na^+/H^+$  antiporters (Parida and Das, 2005).

Recently, molecular biology techniques, such as transgenic approaches, have been employed to enhance salinity tolerance in plants. Transgenic tobacco plants over-producing proline exhibit significantly reduced levels of reactive oxygen species (ROS) and improve tolerance to NaCl (Hong et al., 2000). *Arabidopsis* receiving an antisense proline dehydrogenase cDNA displays enhanced accumulation of proline and constitutive tolerance to salinity (up to 600 mM NaCl) (Nanjo et al., 2003). However, attempts to improve tolerance to salinity through conventional plant breeding or transgenic methods could

\*Corresponding author. E-mail: [srguo@njau.edu.cn](mailto:srguo@njau.edu.cn). Tel/Fax: +86-25-8439 5267.

**Abbreviations:** ROS, Reactive oxygen species; *Pn*, net photosynthetic rate;  $H_2O_2$ , hydrogen peroxide; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase; DHAR, dehydroascorbate reductase; GR, glutathione reductase.

require excessive time and hard work; but, application of exogenous proline (Hua and Guo, 2002; Kaya et al., 2007; Hoque et al., 2007; Ozden et al., 2009), silicon (Zhu et al., 2004), spermidine (Roy et al., 2005; Duan et al., 2008), or ascorbic acid (Athar et al., 2007) could also improve tolerance to salinity in plants and was speedier and more convenient.

The accumulation of proline is essential for plants under osmotic stress. Furthermore, salt stress up-regulates the key enzyme,  $\Delta$ 1-pyrroline-5-carboxylate synthase (P5CS) that are required for proline biosynthesis in *Arabidopsis* (Hare et al., 1999), which implies that a proper concentration of proline is involved in the osmotic potential of some plants under stress. According to Roy et al. (1993), 30 mM proline is the most effective concentration for improving seed germination and seedling growth in rice plants subjected to salt stress; higher concentrations of exogenous proline (40 or 50 mM) suppress seedling growth and reduce the  $K^+/Na^+$  ratio. Okuma et al. (2000) have reported that growth of tobacco cells suspension-cultured under salt stress is promoted by exogenous proline (10 mM), which seems due to proline-mediated protection of enzymes and membranes. Hoque et al. (2007b) have found that exogenous proline mitigates the detrimental effects of salt stress on tobacco plants by enhancing the activities of antioxidant enzymes. However, Krishnamurthy and Bhagwat (1993) have shown that proline has no beneficial effect on the salinity-induced accumulation of either  $Na^+$  or  $Cl^-$  in rice leaves. The function of proline in tolerance to salinity in plants is still unclear and requires further study.

Therefore, the present study investigated the effect of proline on ROS metabolism and photosynthesis in melon seedlings subjected to salt stress and determined if the mechanism of increased salt stress tolerance in melon plants in the presence of exogenous proline would involve the regulation of ROS metabolism and photosynthesis.

## MATERIALS AND METHODS

### Plant materials and culture conditions

Melon (*Cucumis melo* L. cv. Yuhuang and cv. Xuemei) seeds were sterilized with 1% (w/v) sodium hypochlorite for 10 min and were rinsed thoroughly with sterile distilled water. The rinsed seeds were placed on two layers of filter paper moistened with distilled water in Petri dishes at  $28 \pm 1^\circ C$  so as to germinate. The germinated seeds were planted in plastic boxes containing perlite in a greenhouse at 25 to  $30^\circ C$  (light period) and 15 to  $18^\circ C$  (dark period). After emergence of the 2<sup>nd</sup> leaf, seedlings of uniform size were transplanted into plastic vessels containing 25 L of full-strength Hoagland's nutrient solution (pH 6.3 to 6.5, EC 2.0 to 2.2 dS  $m^{-1}$ ). The nutrient solution was vigorously aerated using an air pump at intervals of 20 min to keep dissolved oxygen (DO) at 7.8 to 8.2 mg  $L^{-1}$ . The relative humidity in the greenhouse was between 60 and 70%. The light intensity was controlled between 800 to 1000  $\mu mol\ m^{-2}\ s^{-1}$ . After pre-cultured for four days, the seedlings were then cultured in four different nutrient solutions designated as (a) control: full-strength Hoagland's solution; (b) NaCl: full-strength

Hoagland's solution + 100 mM NaCl; (c) proline: full-strength Hoagland's solution + 0.2 mM proline (This concentration was determined by preliminary experiment.); and (d) NaCl + proline: full-strength Hoagland's solution + 100 mM NaCl + 0.2 mM proline. Troughs were arranged by a completely randomized block design with three replicates, providing a total of 12 containers with a total of 36 plants per treatment. Solutions were renewed every 2 days. Root samples of melon cultivars used to measure ROS content and activities of antioxidant enzymes were harvested in triplicate at 3 days and 5 days after starting treatment with the solutions as described above. At 5 days after treatment started, photosynthesis and chlorophyll fluorescence of plants were measured in triplicate, and 15 plants per treatment were harvested to measure their growth.

### Measurement of fresh and dry weights

After the melon plants were washed in distilled water, their fresh weight was measured. The weighed plants were then dried at  $70^\circ C$  for 72 h and their dry weight was measured.

### Assay of antioxidant enzyme activity

To measure superoxide dismutase (SOD) in the roots of melon plants, preparations were made as described by Giannopolitis and Ries (1977). The reaction mixture (3 ml) contained 50  $\mu M$  nitro blue tetrazolium (NBT), 1.3  $\mu M$  riboflavin, 13 mM methionine, 75  $\mu M$  disodium-EDTA, 50 mM Na-phosphate (pH 7.8) and 30  $\mu l$  of the enzyme fraction of the preparation. The test tubes containing the reaction mixture (3 ml) were irradiated with fluorescent lamps at 50  $\mu mol\ m^{-2}\ s^{-1}$  for 15 min. Absorbance at 560 nm was measured with a spectrophotometer (JH723, Shanghai, China). SOD activity was estimated based on the amount of protein in the enzyme extraction that caused a 50% inhibition of the photochemical reduction of NBT.

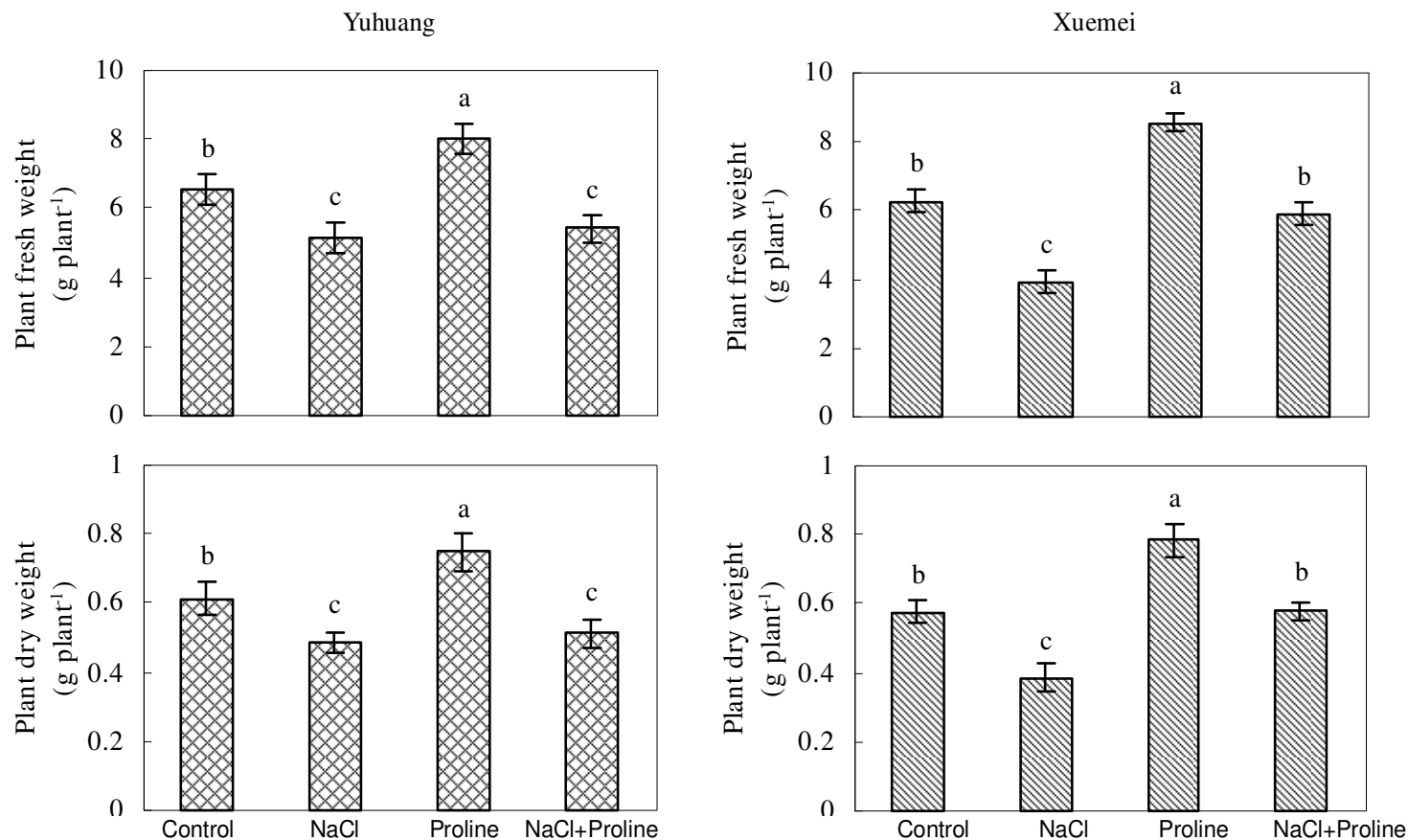
Sampling and assaying of catalase (CAT) and peroxidase (POD) in the roots of melon plants were performed as described by Chance and Maehly (1955). To assay CAT activity, the reaction mixture (3 ml) was made of 50 mM potassium phosphate (pH 7.0), 5.9 mM  $H_2O_2$  and 0.1 ml of the crude extract. The reaction was carried out with observation of the absorbance changed at 240nm. To examine POD, the reaction mixture (3 ml) was prepared with 50 mM sodium phosphate (pH 5.0), 20 mM guaiacol, 40 mM  $H_2O_2$  and 0.1 ml of crude extract. The reaction was carried out with observation of the change in absorbance at 470 nm.

Preparation for and assay of ascorbate peroxidase (APX) in the melon roots were performed as described by Nakano and Asada (1981). The reaction mixture (3 ml) was made of 50 mM potassium phosphate (pH 7.0), 0.1 mM disodium-EDTA, 0.1 mM  $H_2O_2$ , 0.5 mM reduced ascorbate (AsA) and the enzyme fraction of the preparation. The activity was determined by following the change in  $A_{290}$ .

Preparation for and assay of dehydroascorbate reductase (DHAR) in the roots were done as described by Nakano and Asada (1981). The reaction mixture contained 50 mM potassium phosphate (pH 7.0), 2.5 mM reduced glutathione (GSH), 0.1 mM dehydroascorbic acid (DHA) and the enzyme fraction of the preparation. The activity was determined by following the change in  $A_{265}$ .

Preparation for and assay of glutathione reductase (GR) in the roots were carried out according to the method by Foster and Hess (1980). The reaction mixture consisted of 50 mM Tris-HCl (pH 7.5), 3 mM  $MgCl_2$ , 0.15 mM NADPH, 0.5 mM GSSG (oxidized glutathione) and the enzyme fraction of the preparation. The activity was determined by following the change in  $A_{340}$ .

Protein levels in the enzyme fraction were quantified using the Bradford assay (1976), with bovine serum albumin as the standard.



**Figure 1.** Effects of 0.2 mM exogenous proline application on fresh weight and dry weight of melon plants exposed to 100 mM NaCl for 5 d. Vertical bars represent the mean  $\pm$  SE of three independent experiments ( $n=3$ ). Different letters above bars indicate significant differences at  $P < 0.05$ .

#### Measurement of ROS production and membrane damage

The superoxide production rate, hydrogen peroxide content, malondialdehyde (MDA) content and electrolyte leakage of the melon roots were measured according to the methods by Elstner and Heupel (1976), Patterson et al. (1984), Heath and Packer (1968) and Gong et al. (1998), respectively.

#### Measurement of photosynthesis and chlorophyll fluorescence

Net photosynthetic rate ( $P_n$ ) was measured using a portable photosynthesis system (LI-6400, LI-COR, Lincoln, NE, USA). The  $P_n$  of melon cultivars was measured under the following conditions: temperature, 25°C; relative humidity (RH) in the chamber, 70%; external  $\text{CO}_2$  concentration,  $380 \pm 10 \mu\text{mol mol}^{-1}$ ; and light intensity,  $800 \mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetic photon flux density. The maximum efficiency of photosystem II (PSII) photochemistry ( $F_v/F_m$ ) was measured with a portable fluorometer (PAM 2100, Walz, Germany) after 30 min of dark adaptation. The actual efficiency of photosystem II ( $\Phi\text{PSII}$ ) was calculated as described by Lu et al. (2003).

#### Determination of chlorophyll content

A fresh leaf sample (1 g) of melon cultivars was ground in 90% acetone to obtain the chlorophyll fraction which was then centrifuged at 1,000 g for 15 min at 4°C. The absorbance of the chlorophyll

solution was measured with a spectrophotometer at  $A_{663}$  and  $A_{645}$ . Chlorophyll concentrations were estimated on a leaf fresh-weight basis, according to a modified version of the method by Strain and Svec (1966).

#### Statistical analysis

All the experiments were performed in triplicate. Significant differences were determined by Duncan's multiple range test using SAS software (SAS Institute Inc., Cary, N.C., USA). Differences were considered significant at  $p < 0.05$ .

## RESULTS

### Fresh and dry weights

Figure 1 shows that 5 days of 100 mM NaCl treatment significantly decreased the fresh and dry weights of both melon cultivars and that the decrease was greater in cv. Xuemei than in cv. Yuhuang, implying that cv. Yuhuang was more tolerant to salt than cv. Xuemei. However, application of exogenous 0.2 mM proline to salinized nutrient solution alleviated the decrease in fresh and dry

weights which was more significant in cv. Xuemei than in cv. Yuhuang. Under non-saline conditions, exogenous proline increased the fresh and dry weights of both melon cultivars.

### Chlorophyll content, photosynthesis and chlorophyll fluorescence

Table 1 shows that after 5 days of treatment with salt stress (NaCl), net photosynthesis rate ( $P_n$ ) and actual efficiency of photosystem II ( $\Phi_{PSII}$ ) of the leaves of both cultivars were significantly decreased as compared to the control, and the decrease was greater in cv. Xuemei than in cv. Yuhuang. Salinity caused a significant increase in maximum quantum efficiency of PSII ( $F_v/F_m$ ) and chlorophyll content in cv. Xuemei leaves but not in cv. Yuhuang leaves. Exogenous proline significantly alleviated the decrease of  $P_n$ ,  $F_v/F_m$ ,  $\Phi_{PSII}$  and chlorophyll content in both melon cultivars under saline conditions, and the effect was more remarkable in cv. Xuemei than in cv. Yuhuang. However, under non-saline conditions, exogenous proline exerted no obvious effect on those parameters in either melon cultivar.

### ROS production and membrane damage

The superoxide anion radical level and  $H_2O_2$  content in the roots of both melon cultivars were significantly increased after 3 days of salt stress, and they reached the highest values at 5 days. But the two values were greater in the plants of cv. Xuemei than in those of cv. Yuhuang (Figure 2 A, B, C and D). Compared with NaCl alone, exogenous proline reduced the superoxide anion radical level and the  $H_2O_2$  content in the roots of both cultivars at 5 days. Proline treatment under saline conditions had no effect on the anion radical level and the  $H_2O_2$  content in cv. Yuhuang plants at 3 days (Figure 2 A and C). The malondialdehyde (MDA) content and the electrolyte leakage in the roots were increased by NaCl treatment at 3 and 5 days. But the two values were greater in cv. Xuemei than in cv. Yuhuang (Figure 2 E and F). The MDA content and electrolyte leakage levels were significantly reduced by exogenous proline in the roots of both melon cultivars under salt stress, but the effect was more remarkable in cv. Xuemei than in cv. Yuhuang under salt stress. Under non-saline conditions, exogenous proline displayed no significant differences in both cultivars at 3 and 5 days of treatment.

### Antioxidant enzymes activities

Compared to the control, the activities of antioxidant enzymes such as SOD, CAT, APX, DHAR and GR in the roots of both melon cultivars were decreased by salinity

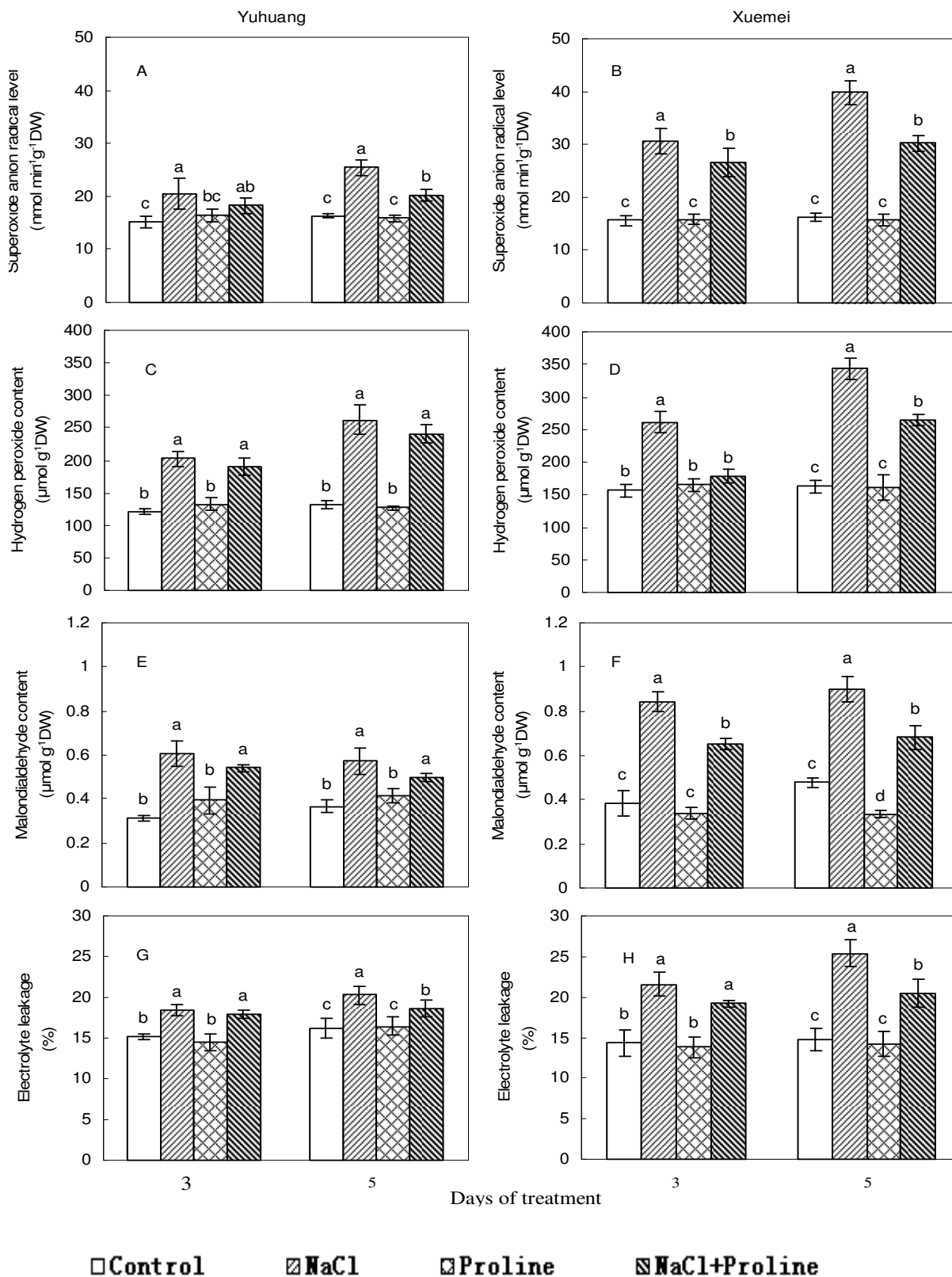
stress. But the decrease was more obvious in the roots of cv. Xuemei than in those of cv. Yuhuang (Figures 3 and 4). Furthermore, this phenomenon in cv. Xuemei melon was more robust at 5 days than at 3 days of NaCl treatment. In contrast, the activity of POD in both melon cultivars was increased by NaCl treatment (Figure 3 C and D). Exogenous proline without NaCl increased the activities of SOD (Figure 3 B/5 days), POD (Figure 3 C and D), CAT (Figure 3 F) and APX (Figure 4 A/5 days, B /3 days). The activities of the other enzymes were not affected or were slightly suppressed (Figure 3 A, B/3 days, E; Figure 4 A/3 days, B/5 days, C, D, E, F). The effects of exogenous proline with NaCl on antioxidant enzymes were in two patterns: [1] activities of SOD (Figure 3 B), POD (Figure 3 D), CAT (Figure 3 F), APX (Figure 4 A/5 days B) and DHAR (Figure 4 D) were significantly enhanced; [2] activities of SOD (Figure 3 A), POD (Figure 3 C), CAT (Figure 3 E), APX (Figure 4 A/3 days), DHAR (Figure 4 C), and GR (Figure 4 E) were not significantly increased. In general, exogenous proline under salt stress enhanced the activity of antioxidant enzymes, but this effect was better in cv. Xuemei than in cv. Yuhuang.

### DISCUSSION

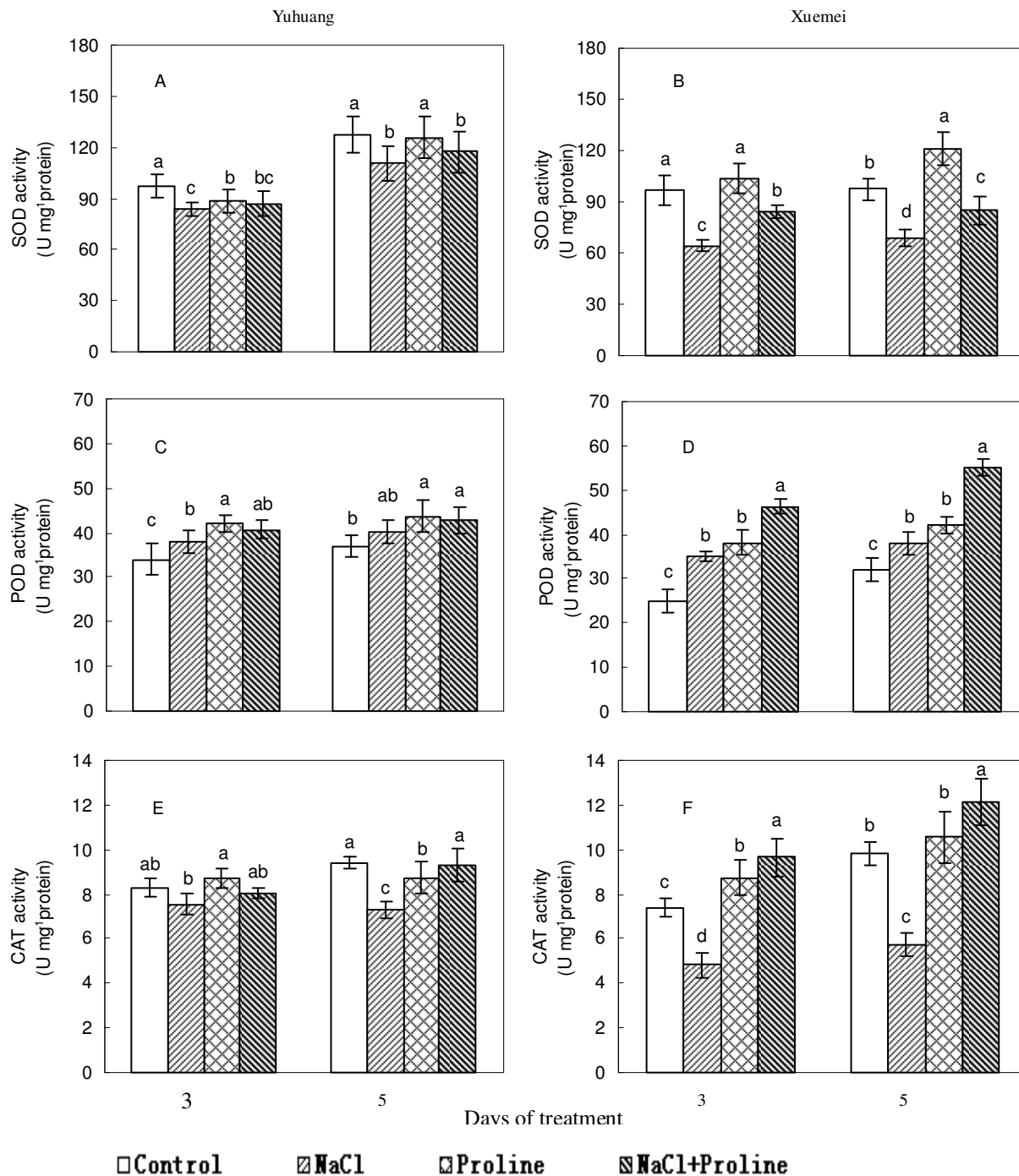
Some previous studies focused on one cultivar of a plant in investigating the alleviating effect of exogenous chemicals under salt stress (Kaya et al., 2007; Tuna et al., 2007, etc). Others studies investigated the effect on two cultivars of a plant and found that the exogenous chemical had different effects on different cultivars because these cultivars of the same plant possessed different tolerance to salt (Athar et al., 2007; Duan et al., 2008, etc). Inspired, we chose two cultivars of melon plant in the present research on the effect of exogenous proline on the physiology of melon under salt stress, believing that such results would be more significant. Our results confirmed this inference, as will be discussed in what follows.

Previous studies demonstrated that exogenous proline played an important role in the stress tolerance of plants (Roy et al., 1993; Okuma et al., 2004; Kumar and Yadav, 2009), which was shown in the present study too. Kaya et al (2007) reported that proline applied to the leaves of cv. "Tempo F1" increased the dry weight of shoot and root of the melon subjected to salt stress. We also found the positive effect of proline on melons under salt stress. However, this effect was more remarkable in cv. Xuemei than in cv. Yuhuang. As shown in Figure 1, exogenous proline significantly increased the fresh and dry weights of cv. Xuemei but did not in cv. Yuhuang (Figure 1). This result of the study differed from those of Kaya et al. (2007) in that proline had different effects on different cultivars of melon. Similar results were reported by other researchers (Athar et al., 2007; Duan et al., 2008).

In the present study, under non-salinity condition,



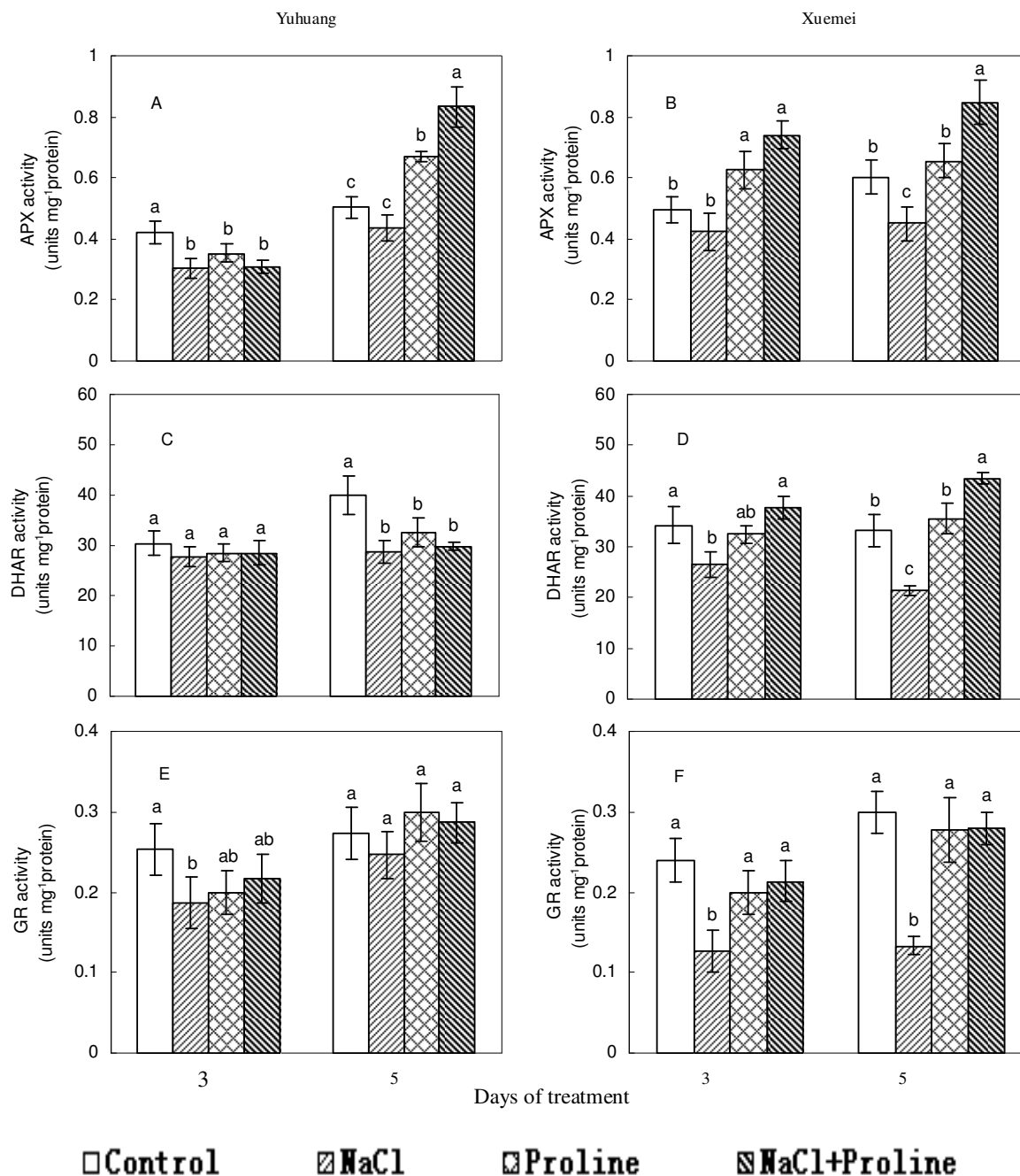
**Figure 2.** Effects of 0.2 mM exogenous proline application on superoxide production rate, hydrogen peroxide content, malondialdehyde content and electrolyte leakage in the roots of melon seedlings exposed to 100 mM NaCl for 3 and 5 d. Vertical bars represent the mean ± SE of three independent experiments (n=3). Different letters above bars indicate significant differences at P < 0.05.



**Figure 3.** Effects of exogenous 0.2 mM proline application on the activities of SOD, POD and CAT in the roots of melon seedlings exposed to 100 mM NaCl for 3 and 5 d. Vertical bars represent the mean  $\pm$  SE of three independent experiments ( $n=3$ ). Different letters above bars indicate significant differences at  $P < 0.05$ .

proline enhanced the growth of both melon cultivars (Figure 1), which might be that proline acted as a nutrient and contributed to cell division and cell enlargement in the plant (Kumar and Sharman, 1989; Okuma et al., 2004). Under salt stress, proline significantly increased the

growth of cv. Xuemei but not of cv. Yuhuang. It could be inferred that proline not only functioned as a nutrient but also possessed some defensive mechanisms for damaged plants under salt stress. These mechanisms were: Promoting photosynthesis, maintaining enzyme activity



**Figure 4.** Effects of exogenous 0.2 mM proline application on the activities of APX, DHAR and GR in the roots of melon seedlings exposed to 100 mM NaCl for 3 and 5 d. Vertical bars represent the mean  $\pm$  SE of three independent experiments (n=3). Different letters above bars indicate significant differences at  $P < 0.05$ .

and scavenging ROS.

NaCl stress was reported to seriously affect plant photosynthesis (Demetriou et al., 2007). It reduced  $P_n$  in cucumber cultivars (Duan et al., 2008) and decreased the chlorophyll content in melon leaves (Kaya et al., 2007). Salt stress in the present study decreased  $P_n$ ,  $\square$ PSII and chlorophyll content in the leaves of cv. Xuemei and cv.

Yuhuang. We also observed that salt stress caused decrease in Fv/Fm of Xuemei melon, but not in Fv/Fm of Yuhuang melon; and these results also showed parallel trend with Chl a and Chl b (Table 1). Similar results were obtained by Lutts et al. (1996). The decrease of  $P_n$  in Yuhuang melon due to salt stress might be caused by the stomatal factor, but that in Xuemei melon might be caused

**Table 1.** Effects of exogenous 0.2 mM proline application on the net photosynthetic rate ( $P_n$ ), maximum quantum efficiency of PSII ( $F_v/F_m$ ), actual efficiency of photosystem II ( $\Phi_{PSII}$ ), and chlorophyll content of melon seedlings exposed to 100 mM NaCl for 5 days.

Cultivar	Treatment	$P_n$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$F_v/F_m$	$\Phi_{PSII}$	Chla [ $\text{mg g}^{-1}$ (FM)]	Chlb [ $\text{mg g}^{-1}$ (FM)]	Total Chl [ $\text{mg g}^{-1}$ (FM)]
Yuhuang	Control	$26.4 \pm 1.3^a$	$0.82 \pm 0.01^a$	$0.59 \pm 0.01^a$	$0.84 \pm 0.02^a$	$0.28 \pm 0.01^a$	$1.12 \pm 0.08^a$
	NaCl	$20.2 \pm 1.6^c$	$0.82 \pm 0.02^a$	$0.55 \pm 0.01^b$	$0.79 \pm 0.02^a$	$0.25 \pm 0.03^b$	$1.04 \pm 0.04^a$
	Proline	$26.9 \pm 0.9^a$	$0.84 \pm 0.01^a$	$0.60 \pm 0.03^a$	$0.86 \pm 0.04^a$	$0.28 \pm 0.02^a$	$1.14 \pm 0.08^a$
	NaCl + Proline	$24.1 \pm 1.2^b$	$0.82 \pm 0.01^a$	$0.60 \pm 0.02^a$	$0.84 \pm 0.03^a$	$0.29 \pm 0.01^a$	$1.13 \pm 0.05^a$
	Control	$25.4 \pm 1.5^a$	$0.82 \pm 0.01^a$	$0.60 \pm 0.01^{ab}$	$0.82 \pm 0.02^a$	$0.26 \pm 0.02^a$	$1.08 \pm 0.03^a$
Xue mei	NaCl	$15.8 \pm 0.4^c$	$0.77 \pm 0.01^b$	$0.51 \pm 0.02^c$	$0.63 \pm 0.01^c$	$0.21 \pm 0.02^c$	$0.84 \pm 0.06^b$
	Proline	$25.7 \pm 1.6^a$	$0.83 \pm 0.02^a$	$0.61 \pm 0.04^a$	$0.81 \pm 0.03^a$	$0.27 \pm 0.01^a$	$1.08 \pm 0.04^a$
	NaCl + Proline	$23.3 \pm 1.3^b$	$0.82 \pm 0.03^a$	$0.57 \pm 0.02^b$	$0.79 \pm 0.03^b$	$0.24 \pm 0.01^b$	$1.04 \pm 0.05^a$

Each value is the mean  $\pm$  SE of three independent experiments ( $n = 3$ ). Different letters indicate significant differences between treatments ( $P < 0.05$ ). Comparisons are only valid between treatments of the same cultivar.

by the non-stomatal factor. Exogenous proline treatment alleviated the decrease in  $P_n$ ,  $\Phi_{PSII}$  and chlorophyll contents of the two melon cultivars under salt stress (Table 1), suggesting that proline improved photosynthetic efficiency in leaves of both melons cultivars possibly through alleviating salinity induced detrimental effects of photosynthetic pigments. Table 1 also indicated that the effect of proline was greater in cv. Xuemei than in cv. Yuhuang.

Previous studies showed that plants under salt stress accumulated extra ROS that damaged chloroplasts, proteins, and lipids in plants (Adám et al., 1989; Scandalios, 1993; Kaya et al., 2007), and led to apoptosis-like cell death and/or necrosis (Fath et al., 2001; Van and Dat, 2006; Banu et al., 2009). The present study also showed that salt stress markedly raised the superoxide anion radical level,  $\text{H}_2\text{O}_2$  and MDA contents in the roots of both melon cultivars (Figure 2 A, B, C, D, E, F). Exogenous proline treatment under salt stress markedly decreased the superoxide anion radical level,  $\text{H}_2\text{O}_2$  content and MDA content in the roots of the Xuemei melon (Figure 2 B, D, F). Under conditions without NaCl, proline treatment exhibited no change in superoxide anion radical level or  $\text{H}_2\text{O}_2$  content in both the melon cultivars. However, the MDA content in the roots of the Xuemei melon was decreased at 5 days after proline treatment (Figure 2 F). These phenomena suggested that proline might act as a scavenger of ROS (Ashraf and Foolad, 2007) or induce the activation of antioxidant enzymes (Hoque et al., 2007a, b, c) to allow scavenging of ROS.

Antioxidant enzymes activities under salt stress were not consistent in previous researches, some showing increase in enzymes (Meneguzzo et al., 1999; Meloni et al., 2003; Duan et al., 2008) while others indicating decrease (Meneguzzo et al., 1999; Hoque et al., 2007c). In the present research, SOD, CAT, APX, DHAR, GR, except POD decreased under salt stress (Figures 3 and

4). Proline treatment with salt increased the activities of all these enzymes. Inferably, proline maintained enzyme activities in melon under stress. These enzymes scavenged extra ROS which was induced by salinity. This phenomenon indicated that besides its direct function of scavenging ROS (in the above paragraph), proline also performed this function by maintaining the activities of enzymes. Similar results were obtained in previous studies (Hoque et al., 2007a, c). However, proline had a greater effect in maintaining enzyme activities in cv. Xuemei than in cv. Yuhuang due to their different salinity tolerance.

It was also found that proline decreased electrolyte leakage in both the melon cultivars, indicating that proline alleviated the membrane damage in these two cultivars and enhanced their tolerance to NaCl stress.

## Conclusion

The present study demonstrates that proline was involved in the salinity tolerance of melon plants. It enhanced salinity tolerance through promoting photosynthesis, maintaining enzyme activities, scavenging ROS and protecting membrane. However, these effects varied on different cultivars.

## ACKNOWLEDGEMENTS

This work was supported by the National Basic Research Program of China (973 Program; No. 2009CB119000). We thank Prof. Fei Rong Gu of College of Foreign Studies, NJAU for language editing of the manuscript.

## REFERENCES

- Adám A, Farkas T, Somlyai G, Hevesi M, Király Z (1989). Consequence of  $\text{O}_2^-$  generation during a bacterially induced



- hypersensitive reaction in tobacco: deterioration of membrane lipids. *Physiol. Mol. Plant Pathol.* 34: 13-26.
- Ashraf M, Foolad MR (2007). Roles of glycine betain and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.* 59: 206-216.
- Athar H, Khan A, Ashraf M (2007). Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. *Environ. Exp. Bot.* 63: 224-231.
- Banu NA, Hoque MA, Watanabe SM, Matsuoka K, Nakamura Y, Shimoishi Y, Murata Y (2009). Proline and glycinebetaine induce antioxidant defense gene expression and suppress cell death in cultured tobacco cells under salt stress. *J. Plant Physiol.* 166(2): 146-156.
- Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Ann. Biochem.* 72: 248-254.
- Chance M, Maehly AC (1955). Assay of catalases and peroxidases. *Meth. Enzymol.* 2: 764-817
- Demetriou G, Neonaki C, Navakoudis E, Kotzabasis K (2007). Salt stress impact on the molecular structure and function of the photosynthetic apparatus-the protective role of polyamines. *Biochim. Biophys. Acta.* 1767(4): 272-280.
- Duan JJ, Li J, Guo SR, Kang YY (2008). Exogenous spermidine affects polyamine metabolism in salinity-stressed *Cucumis sativus* roots and enhances short-term salinity tolerance. *J. Plant Physiol.* 165: 1620-1635.
- Dubey RS (1994). Protein synthesis by plants under stressful conditions. In: M. Pessarakli (ed.), *Handbook of plant and crop stress*. Marcel Dekker Inc. New York. pp. 277-299.
- Elstner EF, Heupel A (1976). Inhibition of nitrate formation from hydroxylammonium chloride: a simple assay for superoxide dismutase. *Anal. Biochem.* 70: 616-620.
- Fath A, Bethke PC, Jones RL (2001). Enzymes that scavenge reactive oxygen species are down-regulated prior to gibberellic acid-induced programmed cell death in barley aleurone. *Plant Physiol.* 126: 156-166.
- Foster JG, Hess JL (1980). Responses of superoxide dismutase and glutathione reductase activities in cotton leaf tissue exposed to an atmosphere enriched in oxygen. *Plant Physiol.* 66: 482-487.
- Giannopolitis CN, Ries SK (1977). Superoxide dismutases occurrence in higher plants. *Plant Physiol.* 59: 309-314.
- Gómez JM, Hernández JA, Jiménez A, Del RLA, Sevilla F (1999). Differential response of antioxidative enzymes of chloroplast and mitochondria to long-term NaCl stress of pea plants. *Free Radic. Res.* 31: 11-18.
- Gong M, Li YJ, Chen SZ (1998). Abscisic acid-induced thermotolerance in maize seedlings is mediated by calcium and associated with antioxidant systems. *J. Plant Physiol.* 153: 488-496.
- Hare PD, Cress WA, Van SJ (1999). Proline synthesis and degradation: a model system for elucidating stress-related signal transduction. *J. Exp. Bot.* 50: 413-434.
- Heath RL, Packer L (1968). Photoperoxidation in isolated chloroplasts. I. Kinetic and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* 125(1): 189-198.
- Hernández JA, Ferrer MA, Jiménez A, Barceló AR, Sevilla F (2001). Antioxidant systems and O<sub>2</sub><sup>-</sup>/H<sub>2</sub>O<sub>2</sub> production in the apoplast of pea leaves. Its relation with salt-induced necrotic lesions in minor veins. *Plant Physiol.* 127: 817-831.
- Hong Z, Lakkineni K, Zhang Z, Verma DPS (2000). Removal of feedback inhibition of 1-pyrroline-5-carboxylate synthetase results in increased proline accumulation and protection of plants from osmotic stress. *Plant Physiol.* 122: 1129-1136.
- Hoque MA, Banu MNA, Nakamura Y, Shimoishi Y, Murata Y (2007a). Proline and glycinebetaine enhance antioxidant defense and methylglyoxal detoxification systems and reduce NaCl-induced damage in cultured tobacco cells. *J. Plant Physiol.* 165: 813-824.
- Hoque MA, Banu MNA, Okuma E, Amako K, Nakamura Y, Shimoishi Y (2007b). Exogenous proline and glycinebetaine increase NaCl-induced ascorbate-glutathione cycle enzyme activities, and proline improves salt tolerance more than glycinebetaine in tobacco Bright Yellow-2 suspension-cultured cells. *J. Plant Physiol.* 164: 1457-1468.
- Hoque MA, Okuma E, Banu MNA, Nakamura Y, Shimoishi Y, Murata Y (2007c). Exogenous proline mitigates the detrimental effects of salt stress more than exogenous betaine by increasing antioxidant enzyme activities. *J. Plant Physiol.* 164: 553-561.
- Hua B, Guo WY (2002). Effect of exogenous proline on SOD and POD activity of soybean callus under salt stress. *Acta Agric. Boreali-Sin.* 17(3): 37-40.
- Kangasjärvi K, Talvinen J, Utriainen M, Karjalainen R (1994). Plant defense systems induced by ozone. *Plant Cell Environ.* 17: 783-794.
- Kaya C, Tuna AL, Ashraf M, Altunlu H (2007). Improved salt tolerance of melon (*Cucumis melo* L.) by the addition of proline and potassium nitrate. *Environ. Exp. Bot.* 60(3): 397-403.
- Krishnamurthy R, Bhagwat KA (1993). Effect of foliar application of proline on the salt stressed rice seedlings. *Acta Agron. Acad. Sci. Hung.* 42: 267-272.
- Kumar V, Sharma DR (1989). Effect of exogenous proline on growth and ion content in NaCl stressed and non-stressed cells of mungbean, *Vigna radiata* var. *radiata*. *Indian J. Exp. Biol.* 27: 813-815.
- Kumar V, Yadav SK (2009). Proline and betaine provide protection to antioxidant and methylglyoxal detoxification systems during cold stress in *Camellia sinensis* (L.) O. Kuntze. *Acta Physiol. Plant.* 31: 261-269.
- Larson RA (1995). Plant defense against oxidative stress. *Arch. Insect Biochem. Physiol.* 29: 175-186.
- Lu CM, Qiu NW, Wang BS, Zhang JH (2003). Salinity treatment shows no effects on photosystem II photochemistry, but increases the resistance of photosystem II to heat stress in halophyte *Suaeda salsa*. *J. Exp. Bot.* 54(383): 851-860.
- Lutts S, Kinet JM, Bouharmont J (1996). NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Ann. Bot.* 78: 389-398.
- Meloni DA, Oliva MA, Martinez CA, Cambraia J (2003). Photosynthesis and ability of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Environ. Exp. Bot.* 49: 69-76.
- Meneguzzo S, Navari IF, Izzo R (1999). Antioxidative responses of shoots and roots of wheat to increasing NaCl concentrations. *J. plant Physiol.* 155: 274-280.
- Nakano Y, Asada K (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.* 22: 867-880.
- Nanjo T, Fujita M, Seki M, Kato T, Tabata S, Shinozaki K (2003). Toxicity of free proline revealed in an Arabidopsis T-DNA-tagged mutant deficient in proline dehydrogenase. *Plant Cell Physiol.* 44: 541-548.
- Okuma E, Murakami Y, Shimoishi Y, Tada M, Murata Y (2004). Effects of exogenous application of proline and betaine on the growth of tobacco cultured cells under saline conditions. *Soil Sci. Plant Nutr.* 50: 1301-1305.
- Okuma E, Soeda K, Tada M, Murata Y (2000). Exogenous proline mitigates the inhibition of growth of *Nicotiana tabacum* cultured cells under saline conditions. *Soil Sci. Plant Nutr.* 46: 257-263.
- Ozden M, Demirel U, Kahraman A (2009). Effects of proline on antioxidant system in leaves of grapevine (*Vitis vinifera* L.) exposed to oxidative stress by H<sub>2</sub>O<sub>2</sub>. *Sci. Hort-Amsterdam.* 119: 163-168.
- Parida AK, Das AB (2005). Salt tolerance and salinity effects on plants: A review. *Ecotoxicol Environ. Saf.* 60(3): 324-349.
- Patterson BD, Mackae EA, Ferguson IB (1984). Estimation of hydrogen peroxide in plant extracts using titanium (w). *Anal. Biochem.* 139: 487-492.
- Roy D, Basu N, Bhunia A, Banerjee SK (1993). Counteraction of exogenous L-proline with NaCl in salt-sensitive cultivar of rice. *Biol. Plant.* 35: 69-72.
- Roy P, Niyogi K, SenGupta DN, Ghosh B (2005). Spermidine treatment to rice seedlings recovers salinity stress-induced damage of plasma membrane and PM-bound H<sup>+</sup>-ATPase in salt-tolerant and salt-sensitive rice cultivars. *Plant Sci.* 168: 583-591.
- Scandalios JG (1993). Oxygen stress and superoxide dismutases. *Plant Physiol.* 101: 7-12.
- Smirnov N (1993). The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytol.* 125: 27-58.
- Strain HH, Svec WA (1966). Extraction, separation, estimation and isolation of chlorophylls. In: Vernon, L.P., Seely, G.R. (Eds.). *The Chlorophylls*. Academic Press. NY. pp. 21-66.

- Subrahmanyam D (2008). Effects of chromium toxicity on leaf photosynthetic characteristics and oxidative changes in wheat (*Triticum aestivum* L.). *Photosynthetica*, 46: 339–345.
- Tuna AL, Kaya C, Ashraf M, Altunlu H, Yokas I, Yagmur B (2007). The effects of calcium sulphate on growth, membrane stability and nutrient uptake of tomato plants grown under salt stress. *Environ. Exp. Bot.* 59: 173-178.
- Van BF, Dat JF (2006). Reactive oxygen species in plant cell death. *Plant Physiol.* 141(2): 384-390.
- Zhu ZJ, Wei GQ, Li J, Qian QQ, Yu JQ (2004). Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber(*Cucumis sativus* L.). *Plant Sci.* 167: 527-533.