African Journal of Biotechnology Vol. 11(22), pp. 6064-6074, 15 March, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1354
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Effects of exogenous spermidine on photosynthesis, xanthophyll cycle and endogenous polyamines in cucumber seedlings exposed to salinity

Sheng Shu#, Ling-Yun Yuan#, Shi-Rong Guo*, Jin Sun and Chao-Jie Liu

Key Laboratory of Southern Vegetable Crop Genetic Improvement, College of Horticulture, Nanjing Agricultural University, Ministry of Agriculture, Nanjing 210095, China.

Accepted 22 July, 2011

The effects of exogenous spermidine (Spd, 1 mmol·L^{-1}) on photosynthetic characteristics, xanthophylls cycle components and endogenous polyamines levels were investigated in cucumber seedlings subjected to salt stress (75 mmol·L^{-1} NaCl). Chlorophyll contents and net photosynthetic rate (P_N) of cucumber seedlings showed a significant decrease under salinity but an increase with exogenous Spd application. Salt stress caused a remarkable decline in the maximum quantum efficiency (F_v/F_m) and the actual efficiency of photosystem II (ΦPSⅡ), where an increase was observed in the constitutive loss processes (ΦNO). Application of exogenous Spd significantly decreased ΦNO and enhanced regulated non-photochemical energy loss (ΦNPQ) in the salt-stressed plants. Spd treatment caused an increase in the size of xanthophyll cycle pool (VAZ) and further enhanced de-epoxidation of the xanthophyll cycle (DEPS) under salt stress. These results suggest that exogenous Spd alleviated salt-mediated decline in photosynthetic efficiency through the enhanced involvement of the energy dissipation that is dependent on the xanthophyll cycle. In addition, foliar spray Spd significantly increased the free, bound and conjugated polyamines in the leaves of the salt stressed plants. Spd also increased the free putrescine (Put)/(Spd+Spm) ratio and decreased bound and conjugated Put/(Spd+Spm) under salinity. Thus, we conclude that Spd can alleviate salt-induced damage on cucumber seedlings by regulating the levels of endogenous polyamines, which was associated with an improvement in the photochemical efficiency of PSII of the salt stressed plants.

Key words: Cucumber, endogenous polyamines, photosynthetic characteristics, salt stress, spermidine.

INTRODUCTION

Soil salinity is one of the main environmental factors that affect crop production at least in 20% of irrigated land worldwide (Sudhir and Murthy, 2004). Salt stress severely depresses a wide range of physiological metabolism and biochemical reactions, such as photosynthesis, enzymes activity and protein synthesis (Munns and Tester, 2008). However, salt stress which resulted in the suppression of photosynthetic capacity might be ascribed to stomatal closure (Meloni et al., 2003), chlorophyll content loss (Sudhir and Murthy, 2004), inhibition of Ribulose 1,5bisphosphate carboxylase oxygenase (Rubisco) activity (Brugnoli and Björkman, 1992; Ziska et al., 1990), and degradation of membrane proteins in photosynthetic apparatus (Khan and Ungar, 1997).

High salinity induced a decrease in photosynthetic efficiency is often associated with inhibition of photosystem II (PSII) (Lu and Vonshak, 2002; Kalaji et al., 2010; Xia et al., 2004). PSII has been considered the main site of salt stress-mediated damage to electron transport processes (Baker, 1991; Mehta et al., 2010), yet the damage site of PSII seems to vary with the severity and duration of stress (Lakshmi et al., 1996; Misra et al., 1997) and plant species (Megdiche et al., 2008).

Misra et al. (2001) reported that salinity affects PSII

*Corresponding author. E-mail: srguo@njau.edu.cn. Tel: +86 25 8439 5267. Fax: +86 25 8439 5267.

#Both authors contributed equally to this work.

photochemical efficiency, primary charge separation and pigment-protein complexes of thylakoid membranes. Allakhverdiev et al. (2000) via analyzing Chl fluorescence suggested that the photochemical reaction center complex was damaged by NaCl-treated in *Cyanobacterium* cells. However, the decrease in photosynthesis induced by salt stress enhanced the amount of excess excitation energy in chloroplasts, which, if not safely dissipated, may result in damage to photosystem II (PSII) due to an over-reduction of reaction center (Demmig-Adams and Adams III, 1992).

Plants have evolved many mechanisms against excess excitation energy which damages photosynthetic apparatus. Xanthophyll cycle is one of the most effective mechanisms of photoprotection to avoid photodamage by dissipating excess excitation energy (Adams III et al., 2001). Increased thermal dissipation can protect photosystem II against damage via decreasing excess redox of photosystem II and electron transfer chain (Demming-Adams and Adams III, 1996). In this process, the formation of pH gradient across the thylakoid membrane activates the de-epoxidation of violaxanthin (V) to zeaxanthin (Z) and anteraxanthin (A), facilitating the thermal dissipation of excess excitation energy (Eskling et al., 1997). Xanthophyll cycle dependent energy dissipation down regulates the photochemical efficiency of PSII, thus protecting the reaction centers from photooxidation (Aranjuelo et al., 2008).

Polyamines (PAs) are small aliphatic polycation growth regulators implicate in a wide range of fundamental processes in plants, such as cell division, growth and development, senescence and plants in response to abiotic stress (Bais and Ravishankar, 2002; Zapata et al., 2008). The most common PAs in plants are di-amine (putrescine, Put), tri-amine (spermidine, Spd), and tetra-amine (spermine, Spm). PAs also occur as free, soluble bound and soluble conjugated forms. Kotzabasis et al. (1993) using high performance liquid chromatography (HPLC) showed that mesophyll cells, intact chloroplasts, thylakoid membranes, the light-harvesting complexes and PSII complexes are enrich in the three main polyamines. The effects of polyamines on photosynthesis efficiency of plants in response to environmental stresses have become a research focus. In green alga, it was shown that the bound Put content of the thylakoid membrane was increased in environments with high CO_2 concentrations, which caused an increase in reaction center density and led to an increased photosynthetic rate (Logothetis et al., 2004). An increase in conjugated Put content can stabilize the thylakoid membrane, thus enhancing resistance of tobacco plants to ozone pollution (Navakoudis et al., 2003) and UV-B radiation (Lütz et al., 2005). Low temperature stress reduced the content of Put as well as the Put/Spm ratio in thylakoids and the light-harvesting complexes LHCII in *Phaseolus vulgaris* L., leading to a decrease in photosynthetic electron transport rate and inactivation of the PSII reaction center (Sfakianaki et al., 2006).

Recently, some published studies showed that increased endogenous levels of PAs induced by spraying suitable concentration of exogenous PAs are able to improve photosynthetic efficiency in several environmental stresses. It is well established that application of exogenous polyamines can protect plants against oxidative damage and lipid peroxidation (Tang and Newton, 2005), increase active reaction center population (Dernetriou et al., 2007), energize the cell through stimulation of ATP synthesis (Ioannidis et al., 2007), and improve the photosynthetic capacity by increasing the level of the photochemical efficiency of PSII under salinity (Zhang et al., 2009). Although the involvement of polyamines in plant responses to abiotic stresses have been studied by a number of works, the mechanism of exogenous Spd alleviated salt-induced inhibition of photochemical efficiency in higher plants remain until today largely unknown. Therefore, in this study, cucumber was selected as the test material due to its high sensitive to salinity. The objective of this study was to determine the effects of exogenous Spd on photosynthesis and fluorescence, xanthophyll cycle components and endogenous PAs levels of cucumber leaves, so that we can better understand the possible mechanism of Spd in alleviating the damage induced by salt stress.

MATERIALS AND METHODS

Plant material and growth conditions

The seeds of cucumber (*Cucumis sativus* L., cv. Jingyou No. 4) were germinated for 24 h in a thermostat at 29°C in Petri plates lined with two layers of filter paper moistened with sterile distilled water. The germinated seeds were sown in washed quartz sand. The seedlings were grown in a greenhouse in Nanjing Agricultural University, which was kept at 25 to 30°C with a maximum PPFD of about 1,200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and relative humidity of 75 to 80%. When the seedlings expanded two true leaves, they were transplanted to plastic containers (the length, width and depth were 55, 40 and 10 cm, respectively) containing 20 L of full-strength Hoagland solution (pH 6.5±0.1, EC 2.0 to 2.2 $dS \cdot m^{-1}$), with 12 plants per container. Nutrient solution was aerated using an air pump at an interval of 30 min to keep dissolved oxygen at 8.0±0.2 $mg \cdot L^{-1}$.

Salt and Spd treatments

After two days of pre-culture under control conditions, NaCl and Spd treatments were commenced by adding NaCl to the nutrient solution and by foliar spray of 1 mM Spd, respectively. NaCl concentrations were increased by 25 mM increments every day until a final concentration of 75 mM was reached. The experimental plots included four treatments: (a) C; 0 mM NaCl+0 mM Spd, (b) CS; 0 mM NaCl+1 mM Spd, (c) S; 75 mM NaCl+0 mM Spd, and (d) SS; 75 mM NaCl+1 mM Spd. Containers were arranged in a completely randomized block design with three replicates per treatment, with a total of 36 plants per treatment. Solutions were renewed every two days. After seven days of the final concentration of salt treatment, the third fully expended leaf numbered basipetally starting at the uppermost fully expended leaves was used to measure photosynthesis, chlorophyll fluorescence and chemical analyses. Samples used for the chemical analyses were kept frozen at -80°C until analysis.

Photosynthesis and chlorophyll fluorescence parameters

Photosynthetic parameters were measured using a portable photosynthesis system (LI-6400, LI-COR Inc, USA) that maintained leaf temperature at 25°C, relative humidity in the leaf chamber at 70%, external CO_2 concentration at 380±10 µmol·mol^{-1} and light intensity at 1,000 µmol photons m^{-2}·s^{-1}. The relative contribution of stomatal limitation to photosynthesis, which is the proportional decrease in light-saturated (A) attributed to stomatal component (Ls), was calculated according to Farquhar and Sharkey (1982):

$Ls=1-Ci/Ca$

Where, Ca is the ambient CO_2 concentration.
Chlorophyll fluorescence was measured using a portable fluorometer (PAM 2100, Walz, Germany) as described by Lu et al. (2003). The maximum quantum yield of PSII (F_v/F_m) was determined after dark adapted for 30 min. The initial Chl fluorescence yield (F_o) was determined in low modulated measuring light (<0.1 µmol m^{-2}·s^{-1}), and a 0.8 s pulse of saturating white light (8,000 µmol m^{-2}·s^{-1}) was applied to obtain the maximum fluorescence yield (F_m). To determine the minimal fluorescence level during illumination (F_o'), a black cloth was rapidly placed around the leaf and the leaf-clip holder in the presence of far red light in order to fully oxidize the PSII centers. Upon darkening, the leaf fluorescence dropped to the F_o' level and immediately rose again within several seconds. The steady-state fluorescence level (F_s) and the maximum fluorescence level (F_m') during exposure to illumination were also measured, respectively. Fluorescence parameters were measured on light-adapted leaves using the equations of Genty et al. (1989) as follows:

Efficiency of open PSII centers $(F_v'/F_m') = (F_m'-F_o')/F_m'$

Photochemical quenching coefficient $(qP) = (F_m'-F_s)/(F_m'-F_o')$

Non-photochemical quenching coefficient $(q_N) = (F_m-F_m')/(F_m'-F_o')$

The actual PSII efficiency $\Phi PSII = (F_m'-F_s)/F_m'$

Relative electron transport rate $(rETR) = \Phi PSII \times PPFD \times 0.5 \times 0.84$

Where, PPFD is the photosynthetic photon flux density incident on the leaf, 0.5 is the factor that assumes equal distribution of energy between the two photosystems, and 0.84 is the assumed leaf absorbance. Non-regulated non-photochemical energy loss in PSII (ΦNO), equivalent to constitutive loss processes and regulated non-photochemical energy loss in PSII (ΦNPQ) were calculated by Kramer et al. (2004).

Leaf areas

The area of expanded leaves on a plant was measured using a scanner (EPSON EXPERSSION 1680) and with an image analysis software (WinRHIZO, Regent Instruments, Canada).

Chlorophyll content

Chlorophylls were extracted with a mixture of ethanol, acetone and water at the volume ratio of 4.5: 4.5: 1 and quantified according to the method described by Holden (1965).

Xanthophyll cycle components

The pigment extraction and HPLC analyses of xanthophylls cycle components, violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z) were carried out as previously described by Demming-Adams and Adams III. (2006). In short, leaf segments were plunged into liquid nitrogen and homogenized in a mortar in the presence of a small amount of Na_2CO_3. The pigments were quantitatively extracted with 100% acetone for 1 min in the dark. The extract was centrifuged for 10 min at 2,500 × *g* and at 4°C and the supernatant was filtered through AnotropTM microfilters (0.2 um; Merck, Darmstadt, Germany). Pigments were separated by the gradient reversed-phase HPLC using a spherisorb C18 column (4.6 by 250 mm, 5 µm Kromasil) with a two solvent system. Solvent A (pH=7.5): acetonitrile: methanol: Tris-HCl =72: 8:3 (v/v/v), Solvent B: methanol: hexane = 5:1 (v/v), this Solvent A was followed by a 4 min linear gradient, then into a second Solvent B , run time 30 min at a flow rate of 1.5 ml·min^{-1}, photometric detection at 440 nm and temperature controlled at 25°C. Extracts were injected (10 µl) using an injector. The de-epoxidation state of the xanthophyll cycle (DEPS) was calculated as:

$(Z+A)/(V+A+Z)$

Endogenous polyamines content

To analyze the contents of various forms of polyamines in the photosynthetic apparatus, a method described by Duan et al. (2008) was followed with slight modifications. 0.2 g fresh leaf samples were homogenized in 1.6 ml of 5% (w/v) cold perchloric acid (PCA) and incubated on ice for 1 h. After centrifugation for 20 min at 12,000 × *g* and at 4°C, the supernatant was used to determine free and conjugated polyamines and the pellet to determine bound polyamines, described as follows: for conjugated polyamines, 0.7 ml of PCA extract was mixed with 5 ml of 6 N HCl and hydrolyzed at 110°C for 18 h in flame-sealed glass ampoules to convert the conjugated polyamines to free ones. After acid hydrolysis, the hydrolysate was evaporated at 70 to 80°C, and the residue was suspended in 2 ml of 5% PCA and then centrifuged. For bound polyamines, the pellet was washed at least four times with 4 ml of 5% PCA and centrifuged for 5 min at 4,000 × *g*. Then the supernatant was discarded and the pellet was acid hydrolyzed as described above. Polyamines recovered from the hydrolyzed supernatant, the non-hydrolyzed supernatant and the hydrolyzed pellet were benzoylated according to the modified method of Flores and Galston (1982). In brief, an aliquot of the supernatants was mixed with 2 ml of 2 N NaOH and 15 µl of benzoyl chloride. The mixture was vortexed vigorously and incubated for 30 min at 37°C. The reaction was terminated by adding 2 ml of the saturated NaCl solution. The benzoyl polyamines were extracted with 2 ml of cold diethyl ether. Finally, 1 ml of the ether phase was evaporated to dryness and redissolved in 100 µl of methanol. Polyamines were assayed by HPLC using a C18 reverse phase column (4.6 by 250 mm, 5 µm Kromasil) and a two solvent system including a methanol gradient (36 to 64%, v/v) at a flow rate of 0.8 ml·min^{-1}.

Statistical analysis

All data were statistically analyzed with SAS software (SAS Institute, Cary, NC) using Duncan's multiple range test at the $P < 0.05$ level of significance.

RESULTS

Leaf growth

After 75 mM NaCl treatment for 10 days, growth of cucumber seedlings was significantly inhibited. The symptoms

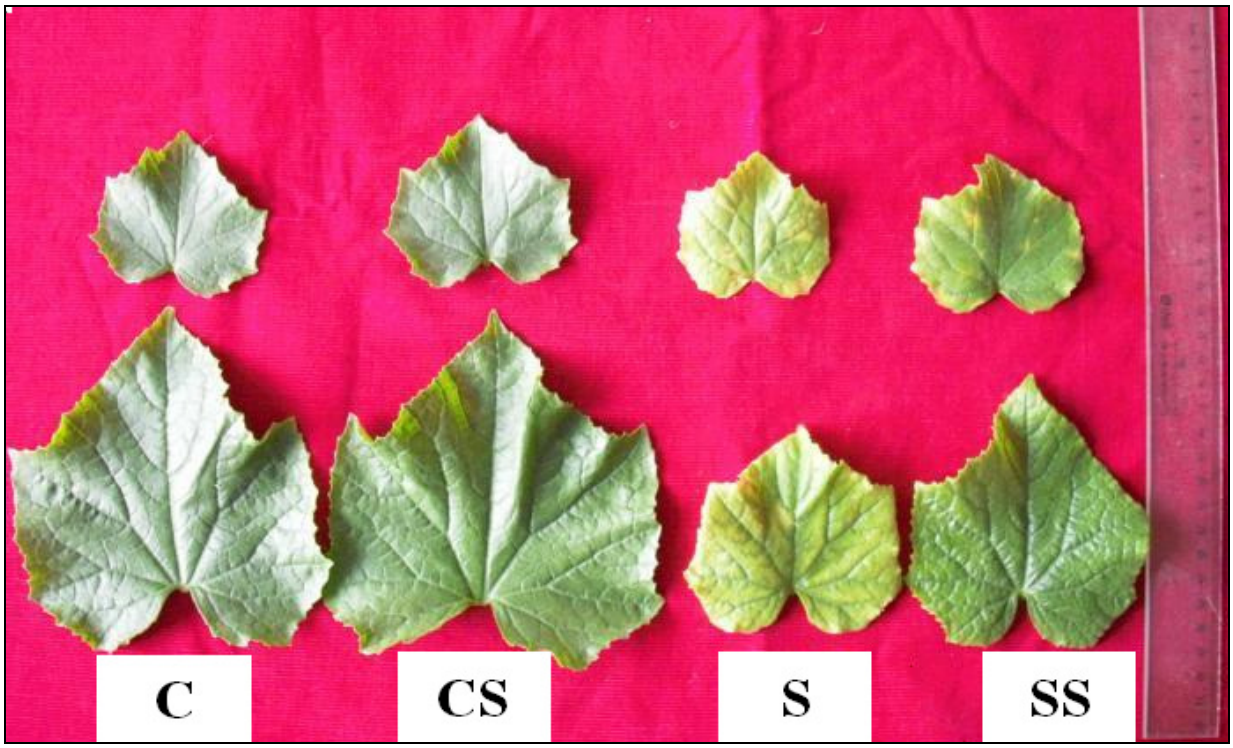

Figure 1. Effects of exogenous Spd on leaf morphology of cucumber seedlings under salt stress. The upper was the second true leaf, the lower layer was the third true leaf numbered basipetally, after seven days of the final concentrationof salt treatment. C; 0 mM NaCl+0 mM Spd; (b) CS; 0 mM NaCl+1 mM Spd; (c) S; 75 mM NaCl+0 mM Spd; (d) SS; 75 mM NaCl+1 mM Spd.

Table 1. Effects of exogenous Spd on chlorophyll contents and leaf areas in leaves of cucumber seedlings under salt stress.

Treatment	Chl a (μg·cm^{-2})	Chl b (μg ·cm^{-2})	Chl a/ b	Car (μg·cm^{-2})	Total Chl (μg·cm^{-2})	Area per leaf (cm^{-2})
C	38.9±0.3^{a}	12.3±0.1^{a}	3.2±0.5^{a}	11.9±0.05^{a}	51.2±0.3^{a}	146.6±4.9^{a}
CS	38.9±1.0^{a}	12.2±0.1^{a}	3.2±0.7^{a}	11.2±0.04^{ab}	51.1±1.0^{a}	148.1±7.4^{a}
S	30.7±1.1^{c}	10.9±0.6^{c}	2.8±0.5^{b}	10.9±0.02^{c}	41.6±0.6^{c}	67.5±2.2^{c}
SS	36.9±0.4^{b}	11.4±0.2^{b}	3.2±0.3^{a}	11.2±0.02^{b}	48.2±0.4^{b}	100.1±6.3^{b}

Each value is the mean ± SE of three independent experiments (n=3). Different letters indicate significant differences between treatments ($P < 0.05$). C; 0 mM NaCl+0 mM Spd; (b) CS; 0 mM NaCl+1 mM Spd; (c) S; 75 mM NaCl+0 mM Spd; (d) SS; 75 mM NaCl+1 mM Spd.

of salinity-induced damage on cucumber leaves were leaf chlorosis and yellow color, accompanied with dry dead spots (Figure 1). However, exogenous Spd alleviated the growth inhibition induced by salt stress. Under control conditions, Spd exerted little influence on leaf morphology in cucumber seedlings.

Chlorophyll contents

As shown in Table 1, under saline condition, Chl a, Chl b, Chl a/b and total Chl content were decreased by 21.1, 11.0, 11.4 and 18.7% as compared to the controls, respectively, and leaf area of cucumber seedlings was also significantly reduced by 53.9%. However, application of exogenous Spd increased significantly the contents of chlorophyll (Chl a, Chl b, Chl a+b and Car) and leaf area in cucumber plants. As a result, exogenous Spd can alleviate the reduction of photosynthetic pigments content and improve the growth of cucumber seedlings. There were no remarkable differences in these parameters between control plants and those sprayed with Spd.

Photosynthetic gas-exchange parameters

Photosynthetic gas-change parameters showed no obvious influence by treatments with exogenous Spd in the control plants (Figure 2). Under saline condition, net photosynthetic rate (P_N) and stomatal conductance (Gs)

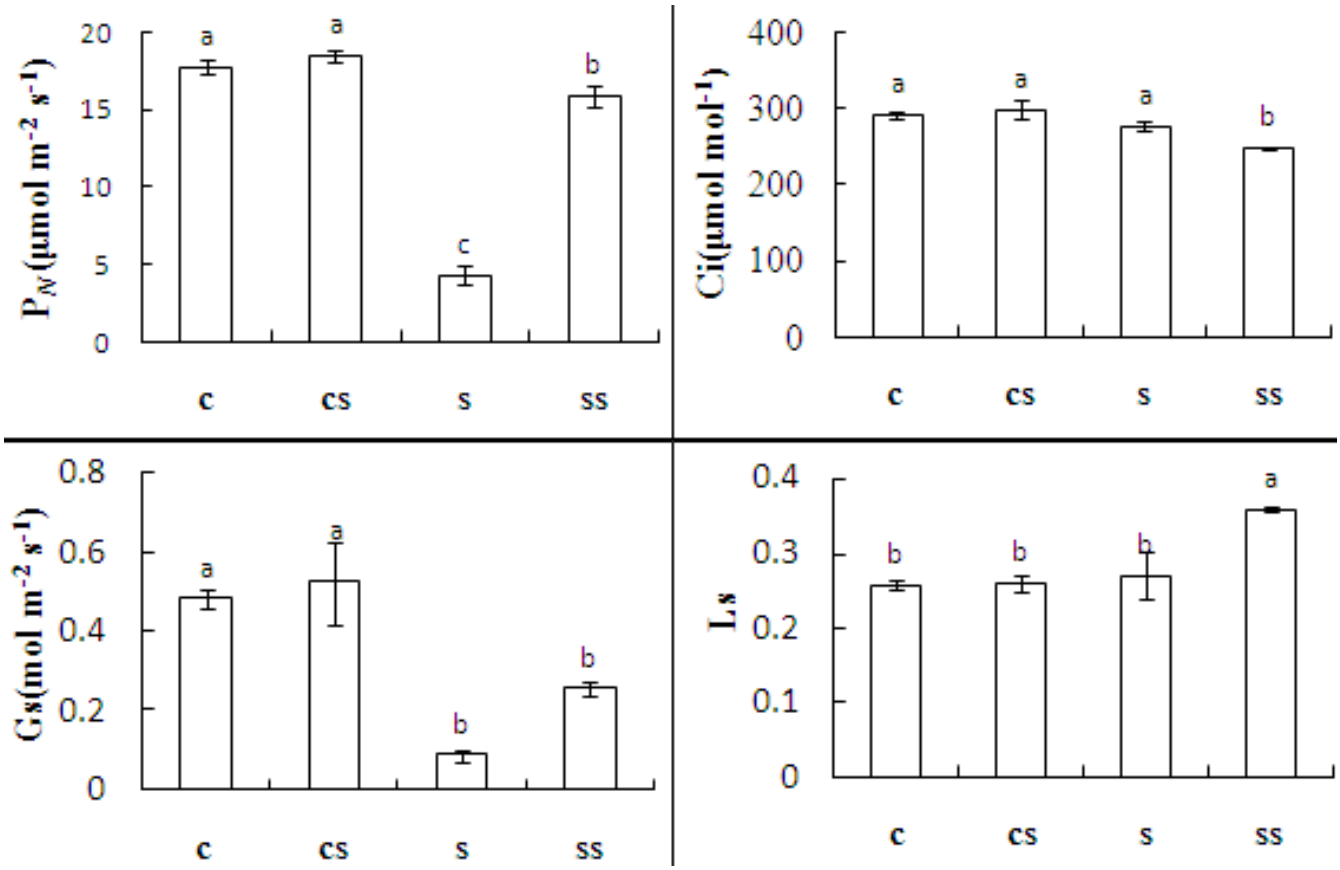

Figure 2. Effects of exogenous Spd on gas exchanges parameters in leaves of cucumber seedlings under salt stress. The data were taken on the third leaves, numbered basipetally, after seven days of the final concentration of salt treatment. Each histogram represents a mean±SE of three independent experiments (n=3). Different letters indicate significant differences between treatments (P <0.05). C; 0 mM NaCl+0 mM Spd; (b) CS; 0 mM NaCl+1 mM Spd,;(c) S; 75 mM NaCl+0 mM Spd; (d) SS; 75 mM NaCl+1 mM Spd.

were significantly reduced by 75.8 and 82.0% as compared with the control, respectively, but the intercellular CO_2 (Ci) and stomatal limitation (Ls) showed no influence by salt stress. Application of exogenous Spd increased P_N and gs in leaves of cucumber seedlings with NaCl by 72.9 and 194.9%, respectively, and also significantly decreased Ci. However, it is interesting that Spd induced an increase in the Ls under salt stress. It was showed that Spd alleviated non-stomatal limitation induced by salt stress might be responsible for improving photosynthetic efficiency of cucumber seedlings.

Chlorophyll fluorescence parameters

Application of exogenous Spd exerted no significant influence on each of the chlorophyll fluorescence of seedlings without NaCl (Figure 3). Under saline condition, the maximum quantum efficiency of PSII (F_v/F_m), efficiency of open PSII centers (F_v'/F_m'), photochemical fluorescence quenching coefficient (qP), actual PSII efficiency (ΦPSII) and relative electron transport rate (rETR) of cucumber seedlings were significantly decreased by 3.34, 5.21, 40.3, 42.1 and 42.1% as compared with the control, respectively. However, non-regulated non-photochemical energy loss in PSII (ΦNO) was significantly increased by 55.6%. It is interesting that q_N was markedly reduced by salinity, but regulated non-photochemical energy loss in PSII (ΦNPQ) showed no significant influence. Application of exogenous Spd significantly enhanced the F_v/F_m, F_v'/F_m', qP, ΦPSⅡ and rETR of plants with NaCl. Moreover, Spd further increased the q_N and ΦNPQ and reduced the ΦNO in leaves of salt-stressed plants. These results indicate that Spd could improve the decrease in photochemical efficiency through the protection of thermal dissipation pathway the against damage of PSII in cucumber seedlings with NaCl.

Xanthophyll cycle components and de-epoxidation state

Under non-saline condition, there were no significant changes in the content of V, A and Z, size of VAZ and de-epoxidation state of the xanthophyll cycle ((Z+A)/(V+Z+A), DEPS) of cucumber leaves by foliar spray of exogenous

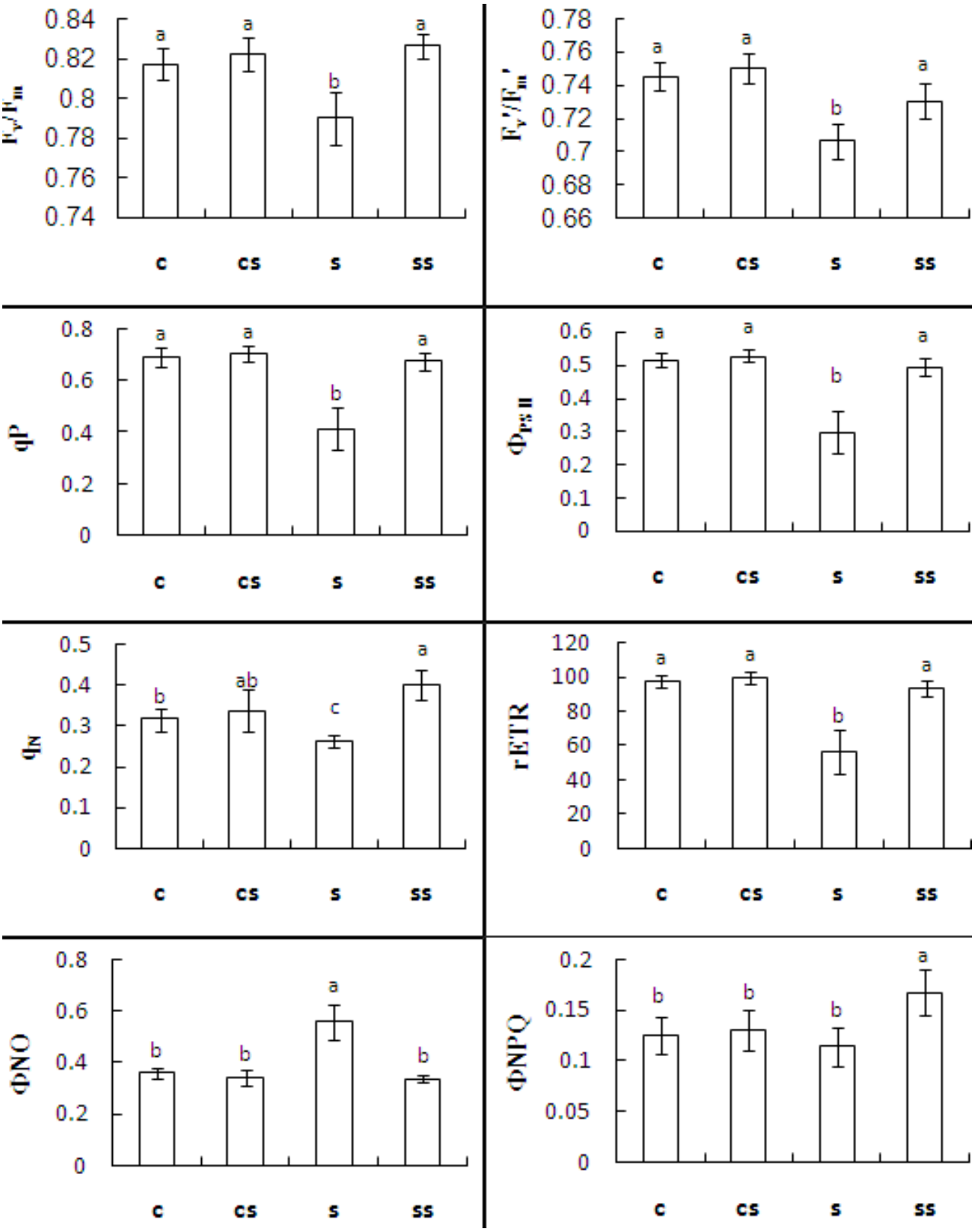

Figure 3. Effects of exogenous Spd on chlorophyll fluorescence parameters in leaves of cucumber seedlings under salt stress. The data were taken on the third leaves, numbered basipetally, after seven days of the final concentration salt treatment. Each histogram represents a mean±SE of three independent experiments (n=3). Different letters indicate significant differences between treatments ($P < 0.05$). C; 0 mM NaCl+0 mM Spd; (b) CS; 0 mM NaCl+1 mM Spd; (c) S; 75 mM NaCl+0 mM Spd; (d) SS; 75 mM NaCl+1 mM Spd.

Table 2. Effect of salt stress with or without exogenous Spd on the content of endogenous free, bound and conjugated polyamines in leaves of cucumber.

Chemical form	Treatment	Put (nmol·g^{-1} FW)	Spd (nmol·g^{-1} FW)	Spm (nmol·g^{-1} FW)	Put/ Spd+Spm
Free	C	191.27±0.83^{ab}	491.07±1.55^{b}	66.35±0.69^{c}	0.343^{a}
	CS	171.43±1.18^{b}	427.52±1.51^{b}	57.88±0.12^{c}	0.353^{a}
	S	125.05±0.58^{c}	363.70±1.50^{c}	122.99±0.08^{b}	0.257^{b}
	SS	218.11±0.62^{a}	621.66±1.15^{a}	162.04±0.12^{a}	0.278^{b}
Bound	C	1.71±0.57^{c}	16.80±0.62^{c}	2.93±0.13^{b}	0.086^{b}
	CS	1.92±0.59^{c}	15.07±1.36^{c}	2.40±0.04^{b}	0.110^{b}
	S	8.11±0.14^{b}	34.45±0.95^{b}	1.61±0.03^{c}	0.225^{a}
	SS	11.03±0.36^{a}	46.39±1.26^{a}	3.71±0.16^{a}	0.220^{a}
Conjugated	C	3.27±0.15^{c}	24.18±0.48^{b}	2.02±0.05^{b}	0.125^{c}
	CS	3.13±0.17^{c}	25.23±1.22^{b}	1.96±0.14^{b}	0.115^{c}
	S	4.66±0.22^{b}	20.87±0.84^{c}	1.22±0.05^{c}	0.211^{a}
	SS	5.99±0.08^{a}	32.13±0.26^{a}	3.17±0.01^{a}	0.170^{b}

Each value is the mean ± SE of three independent experiments (n=3). Different letters indicate significant differences between treatments ($P < 0.05$). C; 0 mM NaCl+0 mM Spd; (b) CS; 0 mM NaCl+1 mM Spd; (c) S; 75 mM NaCl+0 mM Spd; (d) SS; 75 mM NaCl+1 mM Spd.

Spd. Salt stress caused a remarked decrease in the V and Z and an increase in the content of A. The size of VAZ was decreased, but the ratio of DEPS was significantly increased by NaCl. In contrast, application of exogenous Spd increased contents of the V, Z and VAZ by 29.1, 102.9 and 37.3% in the leaves of salt-stressed plants, respectively, and further enhanced content of the antheraxanthin (A). The ratio of DEPS was also significantly enhanced by 29.3% with the treatment of Spd as compared to salt stress. These results suggest that Spd alleviated salt-induced the inhibition of photochemical efficiency via regulating xanthophyll components and accelerating de-epoxidation state of the xanthophyll cycle.

Endogenous polyamines levels

As shown in Table 2, Spd was at the highest concentration in the leaves of the cucumber plants, followed by Put and then by Spm (Put > Spm), and all of those polyamines existed in the free, conjugated and bound forms, of which the free polyamine was the most abundant. In the non-saline conditions, exogenous Spd had no influence on free, bound and conjugated polyamines in the leaves of cucumber seedlings as compared with the control (at the $P < 0.05$ level of significance). Under saline condition, the content of free Put and Spd were significantly decreased while Spm content increased, and there was an opposite trend in the bound polyamines. Salt stress significantly increased conjugated Put content but decreased Spd and Spm. Moreover, the free Put/(Spd+Spm) ratio was decreased by NaCl, but increased for bound and conjugated Put/ (Spd+Spm) ratio. However, application of exogenous Spd diminished the reduction of endogenous polyamines and enhanced the contents of free, bound and conjugated polyamines in the leaves of cucumber seedlings under salt stress. Compared with salinity, Spd induced an increase in the free Put and Spd by 74.4 and 70.9% respectively, with a great increase in the bound and conjugated Spm by 130.4 and 159.8% respectively. In addition, Spd also increased the free Put/(Spd+Spm) ratio and decreased bound and conjugated Put/(Spd+Spm) ratio in the leaves of salt-stress plants. These results suggest that exogenous Spd treatment could alleviate the salt-induced damage by regulating levels of the endogenous polyamines.

DISCUSSION

In this contribution, we studied the effects of exogenous Spd on the plant growth, chlorophyll contents, photosynthesis, xanthophyll cycle and endogenous polyamine levels of cucumber seedling exposed to NaCl stress. It was shown that Spd could ameliorate the inhibition of photochemical efficiency induced by salt stress.

Cucumber seedlings with Spd grew better than seedlings without Spd under salinity (Figure 1). This result agrees with a previous study by Duan et al. (2008). Moreover, in this study, we found that Spd is also involved in the protection of photosynthetic pigments content under salt stress. Spd induced an increase in the amount of both total Chl and Chl a/b ratios as compared with non-Spd treated salt-stressed plants (Table 1). This phenolmenon enhanced by Spd was similar to the results obtained by Chattopadhayay et al. (2002). They demonstrated that at physiological concentrations, Spd and Spm

significantly prevented chlorophyll loss and down-regulated chloroplast-encoded genes, such as *psb*A, *psb*B, *psb*E and *rbc*L. Similarly, it was reported that Spd mitigated the degradation of chlorophyll in salt-stressed rice (Roychoudhury et al., 2011). Therefore, this result indicated that Spd could protect the integrity of PSII from salinity-induced damage.

The decrease in the photosynthetic capacity was mainly ascribed to stomatal and non-stomatal limitation. It has been proposed that whether stomatal or non-stomatal factors are the main cause of the reduced P_N can be judged by the change trend of Gs and Ci (Farquhar and Sharkey, 1982). If both Ci and Gs decreased simultaneously, P_N is mainly limited by stomatal conductance. In contrast, if gs decreased but Ci was not change or showed an increase, the decrease of P_N can be ascribed to the non-stomatal factors which included the limitation of carbon assimilation or performance of photosynthetic apparatus. In this study, salt stress decreased P_N and Gs of cucumber seedlings, but had no influence on Ci and Ls. Thus, the decrease of P_N in the salt-treated plants was mainly attributed to the non-stomatal limitation. It has been reported that the decline in photosynthesis was mostly due to the stomotal factors in the NaCl-treated plants of both cotton cultivars (Meloni et al., 2003), while Silva et al. (2011) ascribed the reduction in the P_N to the non-stomatal limitation. Application of exogenous Spd treatment increased P_N and Gs with a reduction of Ci (Figure 2). These results suggest that Spd improved photosynthetic efficiency by alleviating inhibition of non-stomatal limitation rather than regulation of stomatal factor.

Photosystem II (PSII) is an important component of photosynthetic structure and plays a novel role in light energy conversion and electron transport process. Previous study showed that PSII was one of the primary damage sites on photosynthetic apparatus by environmental stress (Baker, 1991). Chlorophyll fluorescence is routinely used as a rapid, sensitive and non-destructive test for the assessment of the salinity tolerance of plants (Glynn et al., 2003). The maximum quantum yield of PSII photochemistry (F_v/F_m) is frequently used as an indicator of photoinhibition (Adams III et al., 1990). Our results suggest that salt stress treatments led to a decrease in maximal efficiency of PSII photochemistry (F_v/F_m) in dark-adapted leaves (Figure 3). Moreover, the photochemical quenching coefficient (qP) and actual PSII efficiency (ΦPSII) were also dropped by salinity in light adapted leaves. A decrease in qP values indicates an increase in the fraction of reduced Q_A (Q_A^-) of PSII, which means there is an increase in susceptibility to photoinhibition (Lu and Lu, 2004). On the other hand, the increased level of ΦNO suggests the inability of a plant to protect itself against damage by excess energy, which eventually will lead to photodamage. However, Spd treated seedlings with NaCl showed an increase in F_v/F_m, ΦPSII, rETR and qP, which indicate that exogenous Spd alleviated the damage of the photoinhibition and improved the photochemical efficiency of cucumber seedlings. In addition, the increase in q_N of Spd on plants with NaCl reflected a reduced demand for the production of electron transport and, hence, increased heat dissipation. Exogenous Spd reduced ΦNO and increased the level of ΦNPQ in plants with NaCl. ΦNPQ corresponds to the fraction of energy dissipated in form of heat via the regulated photoprotective NPQ-mechanism (Kramer et al., 2004). This result showed that Spd protected photosynthetic activation centers against salt-induced photoinhibition damage by enhancing energy dissipation process, namely, non-photochemical quenching progress.

Xanthophyll cycle plays an important mechanism in decreasing the transfer of captured excitation energy to the PSII reaction centers and thus limiting the amount of photodamage to the photosynthetic apparatus (Demmig-Adams and Adams III, 1992). The xanthophyll cycle pigments zeaxanthin and antheraxanthin are formed from violaxanthin under conditions of excess excitation energy and are both thought to be involved in the photoprotective dissipation process (Gilmore, 1997; Demming-Adams, 2006). High salinity had no significant effect on zeaxanthin and violaxanthin of the halophyte *Artimisia anethifolia*, even high irradiance conditions (Lu et al., 2003). In this study, our results showed that salinity resulted in a significant loss in V and Z and the decrease was accompanied with a significant decline in VAZ. These results are similar to those of Abadía et al. (1999). However, the application of exogenous Spd enhanced the content of V, Z and VAZ and profoundly increased the ratio of DEPS in salt-stressed leaves (Figure 4). Both the xanthophyll cycle pool size and the conversion of V to Z and A correspond well with the thermal dissipation capacity as indicated by efficiency of excitation transfer (F_v'/F_m'). Thus, the results suggest that Spd improved the conversion of xanthophyll pigments and played an important role in dissipating excitation pressure to avoid possible photodamage to PSII. Moreover, Spd enhanced the content of xanthophyll components to stabilize the LHC complexes in the thylakoid membrane against the damage of excess energy. It was supported by the increase of ΦNPQ. These results are consistent with those of Demetriou et al. (2007) who showed that polyamines have a significant role in the modulation of the functional antenna size.

It has been suggested that PAs are associated with plant responses under stress conditions. Indeed, it has been observed that plants significantly accumulate PAs under environmental stresses. This accumulation constitutes a mechanism that may confer adaptive and protective functions under abiotic stresses. Accumulation of PAs result in presumed protective effects, acting as free radical scavengers, stabilizing cellular membranes and maintain cellular ionic balance under salinity (Tang and Newton, 2005; Duan et al., 2008; Janicka-Russak et al., 2010). In our study, exogenous application of Spd caused a significant increase in endogenous PAs levels

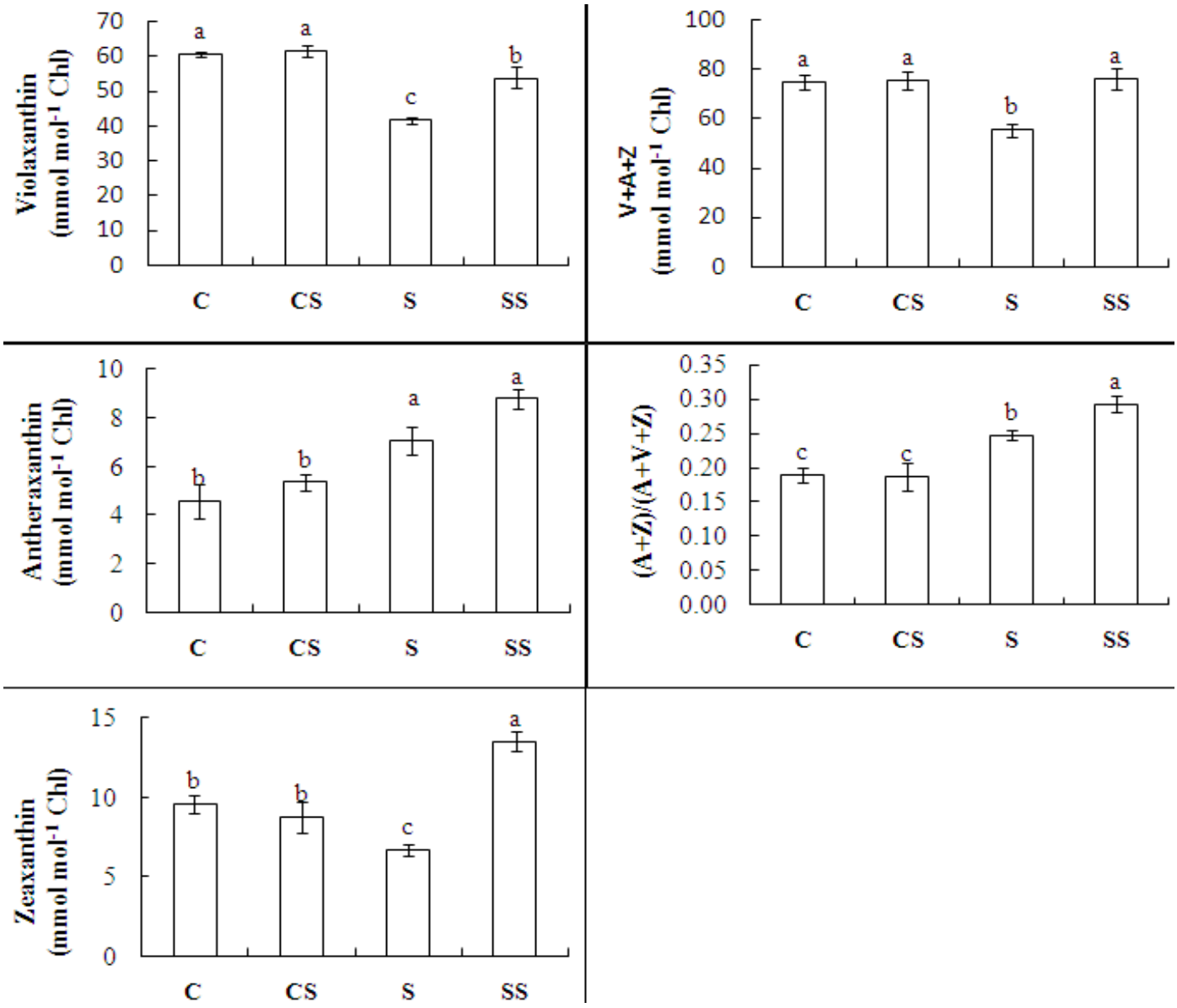

Figure 4. Effects of exogenous Spd on xanthophyll cycle components and de-epoxidation state of xanthophyll cycle (DEPS) in leaves of cucumber seedlings under salt stress. The data were taken on the third leaves, numbered basipetally, after seven days of the final concentration of salt treatment. Each histogram represents a mean±SE of three independent experiments (n=3). Different letters indicate significant differences between treatments ($P < 0.05$). C; 0 mM NaCl+0 mM Spd; (b) CS; 0 mM NaCl+1 mM Spd; (c) S; 75 mM NaCl+0 mM Spd; (d) SS; 75 mM NaCl+1 mM Spd.

in leaves of the salt-stressed cucumber plants. We observed that free Put and Spd induced by exogenous Spd were in higher concentrations (Table 2). There are several published literatures suggesting that the free Put and Spd could serve as a valuable protection counter-measure against membrane deterioration in the control of plant tolerance to salinity (Benavides et al., 1997; Roussos and Pontikis, 2007). These results indicate that the accumulation of free Put may be beneficial for growth and development of cucumber plants.

In recent years, several studies have indicated that bound and conjugated-polyamines participate in the structure and function of the photosynthetic apparatus as one of the main regulatory factors under environmental conditions, such as UV-B radiation (Lütz et al., 2005), low temperature (Sfakianaki et al., 2006) and salinity (Demetriou et al., 2007). The proposed mechanism is based on polyamines inducing the reorganization of the photosynthetic apparatus and bounding to the photo-synthetic complexes, like thylakoids, the extrinsic proteins, LHCII antenna complex and D1 and D2 proteins (Hamdani et al., 2011). In this work, we found that

exogenous Spd induced an increase in bound and conjugated-polyamines of the salt-stressed cucumber leaves, especially conjugated Spd and Spm. These polyamines may serve as membrane surface stabilizers through interaction with phospholipids or other negatively charged groups of photosynthetic protein. Thus PAs improved the photosynthetic capacity of plants by increasing the level of the photochemical efficiency of PSII. Moreover, an increased endogenous conjugated polyamines levels might stabilize xanthophyll components and promote the conversion of V to Z and), which activated violaxanthin de-epoxidation within the xanthophyll cycle and increased energy dissipation, a fact that is supported by the increment of regulated non-photochemical energy loss in PSII (ΦNPQ, Figure 3). Therefore it might be possible that the observed Spd induced an increase in the photochemical efficiency of the salt stressed leaves are at least partly due to improved process of energy dissipation.

Conclusion

Application of exogenous Spd improved the photosynthetic capacity of the salt stressed cucumber seedlings. The positive effect of Spd on photosynthesis might be involved in exogenous Spd regulating endogenous PAs changes and stabilizing xanthophyll components to protect LHCII reaction centers against salinity-induced oxidative damage. The further interests of our research group are to study the physiological effects of exogenous polyamines on the structure and function of the photosynthetic apparatus in more details.

ACKNOWLEDGEMENTS

This work was funded by the National Basic Research Program of China (973 Program, No.2009CB119000) and National Natural Science Foundation of China (No. 30900995; No. 31071831) and was supported by the China Earmarked Fund for Modern Agro-industry Technology Research System (CARS-25-C-03) and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD) and sponsored for scientific innovation research of college graduate in Jangsu province (CXZZ11-0666).

REFERENCES

Abadía A, Belkhodja R, Morales F, Abadía J (1999). Effects of salinity on the photosynthetic pigment composition of barley (*Hordeum vulgare* L.) grown under a triple-line-source sprinkler system in the field. J. Plant Physiol.154: 392-400.

Adams III WW, Demmig-Adams B, Rosenstiel TN, Ebbert V (2001). Dependence of photosynthesis and energy dissipation activity upon growth form and light environment during the winter. Photosyn. Res. 67: 51-62.

Adams III WW, Demmig-Adams B, Winter K, Schreiber U (1990). The ratio of variable to maximum chlorophyll fluorescence from photosystem II, measured in leaves at ambient temperature and at 77K, as an indicator of the photon yield of photosynthesis. Planta. 180: 166-174.

Allakhverdiev SI, Sakamoto A, Nishiyama Y, Murata N (2000). Inactivation of photosystems I and II in response to osmotic stress in *Synechococcus*. Contribution of water channels. Plant Physiol. 122: 1201-1208.

Aranjuelo I, Erice G, Nogués S, Morales F, Irigoyen JJ, Sánchez-Díaz M (2008). The mechanisms involved in the photoprotection of PSII at elevated CO_2 in nodulated alfalfa plants. Environ. Exp. Bot. 64: 295-306.

Bais HP, Ravishankar GA (2002). Role of polyamines in the ontogeny of plants and their biotechnological applications. Plant Cell, Tissue and Organ Culture. 69: 1-34.

Baker NR (1991). A possible role for photosystem II in environmental perturbations of photosynthesis. Physiol. Plant. 81: 563-570.

Benavides MP, Aizencang G, Tomaro ML (1997). Polyamines in *Helianthus annuus* L. during germination under salt stress. J. Plant Growth Regul.16: 205-211.

Brugnoli E, Björkman O (1992). Growth of cotton under continuous salinity stress: influence on allocation pattern, stomatal and non-stomatal components of photosynthesis and dissipation of excess light energy. Planta. 187: 335-347.

Chattopadhayay MK, Tiwari BS, Chattopadhyay G, Bose A, Sengupta DN, Ghosh B (2002). Protective role of exogenous polyamines on salinity-stressed rice (*Oryza sativa*) plants. Physiol. Plant. 116: 192-199.

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: 272-280.

Demmig-Adamas B, Adamas III WW (1992). Photoprotection and other responses of plants to high light stress. Annu. Rev. Plant Physiol. Plant Mol. Biol. 43: 599-626.

Demmig-Adams B, Adams III WW (1996). The role of xanthophyll cycle carotenoids in the protection of photosynthesis. Trend Plant Sci. 1: 21-26.

Demmig-Adams B, Adams III WW (2006). Photoprotection in an ecological context: the remarkable complexity of thermal energy dissipation. New Phytol. 172: 11-21.

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.

Eskling M, Arvidsson PO, Akerlind HE (1997). The xanthophyll cycle, its regulation and components. Plant Physiol. 100: 806-816.

Farquhar GD, Sharkey TD (1982). Stomatal conductance and photosynthesis. Ann. Rev. Plant Physiol. 33: 317-345.

Flores HE, Galston AW (1982). Analysis of polyamines in higher plants by high performance liquid chromatography. Plant Physiol. 69: 701-706.

Genty B, Briantais JM, Baker NR (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta. 990: 87-92.

Gilmore AM (1997). Mechanistic aspects of xanthophyll cycle-dependant photoprotection in higher plant chloroplast and leaves. Physiol. Plant. 99: 197-209.

Glynn CP, Gillian AF, Gavin O (2003). Foliar salt tolerance of acer genotypes using chlorophyll fluorescence. J. Arbori-cult. 29: 61-65.

Hamdani S, Yaakoubi H, Carpentier R (2011). Polyamines interaction with thylakoid proteins during stress. J. Photochem. Photobiol. B: Biol, 10.1016/j.jphotobiol.2011.02.007.

Holden M (1965). Chlorophylls. In: Goodwin, T.W. (Ed.), Chemistry and biochemistry of plant pigments, Academic Press, London, pp. 461-488.

Ioannidis NE, Kotzabasis K (2007). Effects of polyamines on the functionality of photosynthetic membrane in vivo and in vitro. Biochim. Biophys. Acta. 1767: 1372-1382.

Janicka-Russak M, Kabała K, Młodzińska E, Kłobus G (2010). The role of polyamines in the regulation of the plasma membrane and the tonoplast proton pumps under salt stress. J. Plant Physiol. 167: 261-

269.
Kalaji HM, Bosa GK, Kościelniak J, Żuk-Gołaszewska K (2010). Effects of salt stress on photosystem II efficiency and CO_2 assimilation of two Syrian barley landraces. Environ. Exp. Bot. doi: 10.1016/j.envexpbot. 2010.10.009.
Khan MA, Ungar IA (1997). Effect of thermoperiod on recovery of seed germination of halophytes from saline conditions. Am. J. Bot. 84: 279-283.
Kotzabasis K, Fotinou C, Roubelakis-Angelaki KA, Ghanotakis D (1993). Polyamines in the photosynthetic apparatus. Photosynth Res. 38: 83-88.
Kramer DM, Johnson G, Kiirats O, Edwards GE (2004). New fluorescence parameters for the determination of Q_A redox state and excitation energy fluxes. Photosynth Res. 79: 209-218.
Lakshmi A, Ramanjulu S, Veeranjaneyulu K, Sudhakar C (1996). Effect of NaCl on photosynthesis parameters in two cultivars of mulberry. Photosynthetica, 32: 285-289.
Logothetis K, Dakanali S, Ioannidis N, Kotzabasis K (2004). The impact of high CO_2 concentrations on the structure and function of the photosynthetic apparatus and the role of polyamines. J. Plant Physiol.161: 715-724.
Lu C, Vonshak A (2002). Effects of salinity stress on photosystem II function in cyanobacterial *Spirulina platensis* cells. Physiol. Plant. 114: 405-413.
Lu CM, Jiang GM, Wang BS, Kuang TY (2003). Photosystem II photochemistry and photosynthetic pigment composition in salt-adapted halophyte *Artimisia anethifolia* grown under outdoor conditions. J. Plant Physiol. 160: 403-408.
Lu QT, Lu CM (2004). Photosynthetic pigment composition and photosystem II photochemistry of wheat ears. Plant Physiol. Biochem. 42: 395-402.
Lütz C, Navakoudis E, Seidlitz HK, Kotzabasis K (2005). Simulated solar irradiation with enhanced UV-B adjust plastid-and thylakoid-associated polyamine changes for UV-B protection. Biochim. Biophys. Acta. 1710: 24-33.
Megdiche M, Hessini K, Gharbi F, Jaleel CA, Ksouri R, Abdelly C (2008). Photosynthesis and photosystem 2 efficiency of two salt-adapted halophytic seashore *Cakile maritima* ecotypes. Photosynthetica. 46: 410-419.
Mehta P, Jajoo A, Mathur S, Bharti S (2010). Chlorophyll α fluorescence study revealing effects of high salt stress on photosystem II in wheat leaves. Plant Physiol. Biochem. 48: 16-20.
Meloni DA, Oliva MA, Martinez CA, Cambraia J (2003). Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. Environ. Exp. Bot. 49: 69-76.
Misra AJ, Sahu SM, Misra M, Singh P, Meera I, Das N, Kar M, Shau P (1997). Sodium chloride induced changes in leaf growth, and pigment and protein contents in two rice cultivars. Biol. Plant. 39: 257-262.
Misra AN, Srivastava A, Strasser RJ (2001). Utilization of fast chlorophyll *a* fluorescence technique in assessing the salt/ion sensitivity of mung bean and brassica seedlings. J. Plant Physiol. 158: 1173-1181.
Munns R, Tester M (2008). Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 59:651-81.
Navakoudis E, Lütz C, Langebartels C, Lütz-Meindl U, Kotzabasis K (2003). Ozone impact on the photosynthetic apparatus and the protective role of polyamines. Biochim. Biophys. Acta. 1621: 160-169.
Roussos PA, Pontikis CA (2007). Changes of free, soluble conjugated and bound polyamine titers of jojoba explants under sodium chloride salinity in vitro. J. Plant Physiol. 164: 895-903.
Roychoudhury A, Basu S, Sengupta DN (2011). Amelioration of salinity stress by exogenously applied spermidine or spermine in three varieties of indica rice differing in their level of salt tolerance. J. Plant Physiol. 168: 317-328.
Sfakianaki M, Sfichi L, Kotzabasis K (2006). The involvement of LHCII-associated polyamines in the response of the photosynthetic apparatus to low temperature. J. Photochem. Photobiol. B: Biol. 84: 181-188.
Silva EN, Ribeiro RV, Ferreira-Silva SL, Viégas RA, Silveire JAG (2011). Salt stress induced damages on the photosynthesis of physic nut young plants. Plant Physiol. Biochem. doi: 10.1590/S0103–90162011000100010.
Sudhir P, Murthy SDS (2004). Effects of salt stress on basic processes of photosynthesis. Photosynthetica, 42: 481-486.
Tang W, Newton RJ (2005). Polyamines reduce salt-induced oxidative damage by increasing the activities of antioxidant enzymes and decreasing lipid peroxidation in Virginia pine. Plant Growth Regul. 46: 31-43.
Xia JR, Li YJ, Zou DH (2004). Effects of salinity stress on PSII in *Ulva lactuca* as probed by chlorophyll fluorescence measurements. Aquat. Bot. 80: 129-137.
Zapata PJ, Serrano M, Pretel MT, Botella MA (2008). Changes in free polyamine concentration induced by salt stress in seedlings of different species. Plant Growth Regul. 56: 167-177.
Zhang RH, Li J, Guo SR, Tezuka T (2009). Effects of exogenous putrescine on gas-exchange characteristics and chlorophyll fluorescence of NaCl-stressed cucumber seedlings. Photosynth Res. 100: 155-162.
Ziska LH, Seemann JR, DeJong TM (1990). Salinity induced limitations on photosynthesis in *Prunus salicina*, a deciduous tree species. Plant Physiol. 93: 864-870.