

# Effects of Exogenous Putrescine on Chlorophyll Fluorescence Imaging and Heat Dissipation Capacity in Cucumber (*Cucumis sativus* L.) Under Salt Stress

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**Abstract** The objective of this study was to identify the effects of exogenous putrescine on photosynthetic performance and heat dissipation capacity in cucumber seedlings under salt stress. The stress of 75 mM NaCl for 7 days caused a significant decrease in net photosynthetic rate ( $P_N$ ). The experiment employed a chlorophyll fluorescence imaging technique and demonstrated that the maximal quantum yield of photosystem II photochemistry ( $F_v/F_m$ ) and the actual photochemical efficiency of photosystem II ( $\Phi_{PSII}$ ) were reduced by salt stress. Moreover, salt stress markedly reduced

the photochemical quenching coefficient ( $q_P$ ) and non-photochemical quenching coefficient ( $q_N$ ), and significantly increased non-regulated heat dissipation ( $\Phi_{NO}$ ). However, stressed plants supplied with exogenous putrescine exhibited higher  $P_N$  and  $\Phi_{PSII}$ , which indicated that putrescine can alleviate the detrimental effects on photosynthesis induced by salt stress. Putrescine sprayed on stressed plants significantly enhanced the regulated energy dissipation ( $\Phi_{NPQ}$ ) and decreased  $\Phi_{NO}$ . Application of exogenous putrescine also changed the levels of xanthophyll cycle components and further enhanced the de-epoxidation state of xanthophyll cycle pigments under salt stress. Under control conditions, putrescine exerted little influence on the photosynthetic parameters in cucumber leaves. In conclusion, the application of exogenous putrescine may improve the heat dissipation capacity by promoting the xanthophyll cycle to reduce the damage caused by excess excitation energy, thus enhancing the salt tolerance of cucumber seedlings.

Yinghui Yuan and Sheng Shu contributed equally. Jin Sun is the first and major corresponding author.

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## Introduction

Soil salinity is a serious problem that threatens plant development and crop productivity worldwide. More than 800 million ha of land have been affected by salt throughout the world (FAO 2012). Soil salinity causes ion toxicity, nutrient (N, Ca, K, P, Fe, and Zn) deficiency, and osmotic/oxidative stress on plants; it thus limits plant productivity (Parida and Das 2005). In greenhouse cultivation, excessive fertilization, over-irrigation, lack of rainfall leaching, intensive farming, and strong evaporation

from the soil can lead to severe secondary soil salinization, which has been a limiting factor for greenhouse crop production in China (Yu and others 2005). Several physiological processes in plants are affected by salinity, among which photosynthesis is affected the most (Shu and others 2012a).

Light intensity sometimes exceeds what leaves are capable of using for photosynthesis, even when a plant is growing under conditions considered to be optimal (Hubbart and others 2012). Higher plants must dissipate excessive light energy to avoid molecular damage to pigments and proteins that comprise the photosynthetic apparatus (Gilmore 1997). Additional environmental stress, such as salinity, can further limit the use of absorbed light energy, thus leading to photoinhibition of photosystem II (PSII) (Lu and others 2002). Excessive chlorophyll excitation energy within the light-harvesting antennae of PSII (LHCII) is dissipated by non-photochemical quenching (NPQ), which is one of the most important photoprotection responses in higher plants (Külheim and others 2002). NPQ is a complex and finely regulated process that consists of three different components: (1) a rapid phase of energy- or  $\Delta$ pH-dependent quenching (qE); (2) a middle phase of state-transition quenching (qT); and (3) a slow phase of photoinhibitory quenching (qI). Nilkens and others (2010) identified a slowly inducible zeaxanthin-dependent component, qZ. The rapidly inducible and reversible component of NPQ, qE, is usually considered to be triggered by low lumenal pH, although the xanthophyll cycle and the PsbS protein are also necessary for this process (Johnson and others 2011). Amines are known stimulators of NPQ. Dibucaine, a tertiary amine, can induce qE via LHCII aggregation (Ioannidis and others 2011). It is reasonable to speculate that endogenous amines in chloroplasts (such as polyamines) are involved in the photosynthetic process in a similar manner to dibucaine.

Polyamines (PAs) are low-molecular-weight polycations implicated in a wide array of fundamental processes in all living organisms. Putrescine (Put), spermidine (Spd), and spermine (Spm) are the main PAs present in plants (Mendes and others 2011). Polyamines are often described as endogenous plant growth regulators or intracellular messengers that mediate physiological responses (Galston 1983; Denaxa and others 2013). Polyamines are well known for their positive effects on photosynthetic efficiency under various stress conditions because of their acid-neutralizing and antioxidant properties (Velikova and others 2000). The PA homeostasis regulated by genetic reprogramming of enzymes involved in PA metabolism may play important roles in assuring optimal photosynthetic efficiencies and optimal fitness under different environmental conditions (Ioannidis and others 2013). The reduction in the quantity of LHCII-associated Put due to

low temperature leads to an increase in the LHCII, which results in the inactivation of PSII reaction centers and an increase in dissipated energy (Sfakianaki and others 2006). Putrescine has been shown to increase light energy use by stimulating photophosphorylation (Ioannidis and others 2006) as well as ATP synthesis (Ioannidis and Kotzabasis 2007).

Polyamines may stimulate qE in *Nicotiana tabacum* (Ioannidis and Kotzabasis 2007). Spermidine and spermine up-regulate NPQ in isolated LHCII of *Scenedesmus obliquus*, whereas the effect of Put is marginal (Ioannidis and others 2011). Given that dissipation of excessive excitation energy is essential for higher plants, especially under stress conditions, we speculated that Put may play a role in NPQ induction of plants in a challenging environment. In addition, reports about the effects of PAs on energy dissipation of salt-stressed plants are rare. In this study, we examined the effects of Put on photosynthetic performance, NPQ induction, and xanthophyll cycle capacity in control and salt-stressed cucumber seedlings. The objective was to clarify the possible mechanism by which PAs protect the photosynthetic apparatus from salt-induced damage in cucumber plants.

## Materials and Methods

### Plant Material and Experimental Conditions

Seeds of the salt-sensitive cucumber (*Cucumis sativus* L.) cv. Jinyou No. 4 were placed on filter paper moistened with sterile distilled water in petri dishes and germinated in the dark for 24 h at  $29 \pm 1$  °C. The germinated seeds were sown in washed quartz sand and transferred to a greenhouse in which the air temperature was maintained at  $28 \pm 1$  °C/ $19 \pm 1$  °C (day/night), with a maximum photosynthetic photon flux density (PPFD) of about  $1,200 \mu\text{mol m}^{-2} \text{s}^{-1}$  and relative humidity of 75–80 %. When the second true leaves were fully expanded, healthy seedlings of uniform size were selected and transplanted to plastic containers (the length, width and depth were 55, 40, and 10 cm, respectively) containing 20 L full-strength Hoagland solution, with 12 seedlings per container. The nutrient solution was aerated using an air pump with alternate on/off intervals of 20/10 min to maintain the dissolved oxygen concentration at  $8.0 \pm 0.2 \text{ mg L}^{-1}$ .

### Experimental Design

After pre-culture for 2 days, the cucumber seedlings were divided into four groups and treated as follows: (a) control, seedlings cultured in full-strength Hoagland nutrient solution (Cont); (b) seedlings cultured in full-strength

Hoagland nutrient solution with 8 mM Put applied as a foliar spray (Put); (c) salt stress-treatment, seedlings cultured in full-strength Hoagland nutrient solution with 75 mM NaCl (NaCl); (d) seedlings cultured in full-strength Hoagland nutrient solution with 75 mM NaCl and 8 mM Put applied as a foliar spray (NaCl + Put). The containers were arranged in a completely randomized block design with three replicates per treatment, comprising a total of 12 containers with 144 seedlings in the four treatments (36 seedlings per treatment). The concentrations of NaCl and Put were selected on the basis of the results of our previous experiment (Shu and others 2012a). The Put treatment in (b) and (d) was applied at 18:00 every day, and distilled water was applied as a foliar spray in the (a) and (c) treatments. The NaCl concentration in the nutrient solution was increased at the rate of 25 mM per day until a final concentration of 75 mM was obtained. The nutrient solutions were renewed every 2 days to maintain stable ion concentrations. Leaf and root samples from healthy cucumber seedlings were harvested at 7 days after the final concentration of NaCl was reached. Samples were frozen at  $-80\text{ }^{\circ}\text{C}$  until chemical analyses were performed.

#### Morphological Analyses

The height and stem diameter of the seedlings were determined using a ruler and vernier caliper, respectively. The length and volume of roots were measured with an EXPRESSION 1680 scanner (Epson, Sydney, Australia) and image analysis software (WinRHIZO; Regent Instruments, Montreal, Canada). For determination of fresh weight, the plants were washed with distilled water and weighed after wiping the water off. The plant samples were oven dried at  $75\text{ }^{\circ}\text{C}$  until a constant weight was attained and subsequently the dry weight was recorded.

#### Gas-Exchange Parameters Measurement

The net photosynthetic rate ( $P_N$ ), stomatal conductance ( $G_s$ ), transpiration rate ( $Tr$ ), and intercellular  $\text{CO}_2$  concentration ( $C_i$ ) of the third fully expanded leaf from the shoot tip were monitored using a portable photosynthesis system (LI-6400; LI-COR, Inc., Lincoln, NE, USA). Cuvette conditions were maintained at a PPF of  $1,000\text{ }\mu\text{mol photons m}^{-2}\text{ s}^{-1}$ , relative humidity of 70 %, leaf temperature of  $25\text{ }^{\circ}\text{C}$ , and external  $\text{CO}_2$  concentration of  $380 \pm 10\text{ }\mu\text{mol mol}^{-1}$ .

#### Chlorophyll Fluorescence Parameters Measurement

Chlorophyll fluorescence imaging of cucumber leaves was performed using an imaging-PAM fluorometer (Walz, Effeltrich, Germany). The third fully expanded leaf from the

shoot tip was placed in darkness for 30 min prior to measurement. The maximum quantum yield of PSII (Fv/Fm), actual photochemical efficiency of PSII ( $\Phi\text{PSII}$ ), photochemical quenching coefficient (qP), non-photochemical quenching coefficient (qN), quantum yield of regulated energy dissipation in PSII ( $\Phi\text{NPQ}$ ), and non-regulated energy dissipation in PSII ( $\Phi\text{NO}$ ) were measured or calculated in accordance with Lu and others (2003). The PAM-software selected areas in the fluorescence image for each plant. The mean of the corresponding area of all samples (obtained from 15 to 20 different plants) was calculated. Further information on chlorophyll fluorescence imaging measurements can be found in Calatayud and others (2006).

The PAM fluorometer (Walz) was used to obtain light response curves for NPQ and  $\Phi\text{PSII}$ . Leaves were illuminated with an internal halogen lamp of different intensities (0, 5, 70, 120, 200, 300, 470, 700, 1,120, 1,570, and  $2,310\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$ ). For each light intensity level, actinic light was irradiated for 20 s. The specific measurement procedure is described by Perkins and others (2006).

#### Xanthophyll Cycle Components Analysis

Violaxanthin (V), antheraxanthin (A), and zeaxanthin (Z) are xanthophyll cycle pigments. Leaf segments for determination of xanthophyll cycle components were ground in liquid nitrogen and then immersed in cold acetone in the dark for pigment extraction. After centrifugation, pigments in the supernatant were separated by gradient reversed-phase high-performance liquid chromatography using a spherisorb C18 column ( $4.6 \times 250\text{ mm}$ ,  $5\text{ }\mu\text{m}$  Kromasil) with a two-solvent system. The specific procedure followed the method of Shu and others (2012b). The de-epoxidation state of the xanthophyll cycle (DEPS) was calculated as:  $(Z + A)/(V + A + Z)$  (Peguero-Pina and others 2008).

#### Statistical Analysis

Every experiment other than the morphological analyses was conducted using three biological replicates. Morphological analyses were performed with 15 biological replicates. All data were statistically analyzed with SAS software (SAS Institute, Inc., Cary, NC, USA) using the Duncan's multiple range test at the  $\alpha = 0.05$  level of significance.

## Results

#### Morphological Parameters

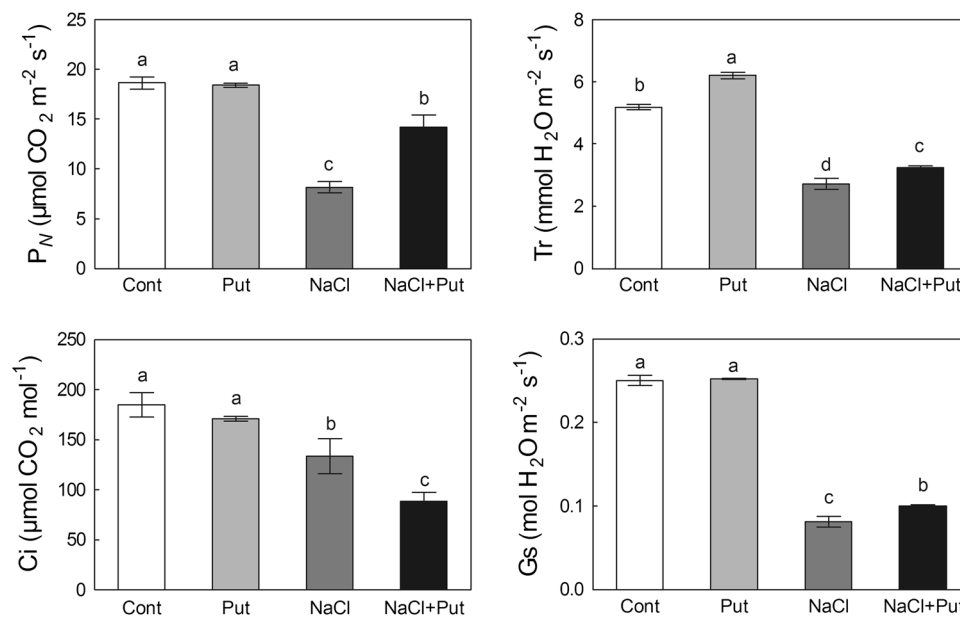
As shown in Table 1, plant height, stem diameter, fresh and dry weight, and root length and volume of cucumber seedlings were markedly decreased by NaCl stress. The

**Table 1** Effects of exogenous putrescine (Put) on growth parameters of cucumber seedlings exposed to 75 mM NaCl

Treatments	Plant height (cm)	Stem diameter (mm)	Fresh weight (g plant <sup>-1</sup> )	Dry weight (g plant <sup>-1</sup> )	Root length (m)	Root volume (cm <sup>3</sup> plant <sup>-1</sup> )
Cont	24.5 ± 0.15 <sup>a</sup>	6.19 ± 0.10 <sup>a</sup>	34.2 ± 3.40 <sup>b</sup>	3.13 ± 0.40 <sup>a</sup>	20.6 ± 1.33 <sup>a</sup>	18.9 ± 0.85 <sup>a</sup>
Put	25.9 ± 0.22 <sup>a</sup>	6.53 ± 0.21 <sup>a</sup>	41.5 ± 3.98 <sup>a</sup>	3.23 ± 0.66 <sup>a</sup>	20.7 ± 0.31 <sup>a</sup>	18.7 ± 0.53 <sup>a</sup>
NaCl	10.1 ± 0.19 <sup>c</sup>	4.27 ± 0.16 <sup>c</sup>	12.3 ± 0.93 <sup>d</sup>	1.01 ± 0.12 <sup>c</sup>	9.65 ± 0.95 <sup>c</sup>	5.25 ± 0.41 <sup>c</sup>
NaCl + Put	17.6 ± 0.53 <sup>b</sup>	4.71 ± 0.19 <sup>b</sup>	21.6 ± 0.59 <sup>c</sup>	1.64 ± 0.05 <sup>b</sup>	13.6 ± 0.54 <sup>b</sup>	10.8 ± 0.69 <sup>b</sup>

Each value is the mean ± SE of 15 seedlings. Different letters indicate significant differences at  $P < 0.05$  according to Duncan's multiple range test

*Cont* control, seedlings cultured in full-strength Hoagland nutrient solution, *Put* seedlings cultured in control nutrient solution with 8 mM Put sprayed on leaves, *NaCl* salt stress, seedlings cultured in nutrient solution supplemented with 75 mM NaCl, *NaCl + Put* combination of NaCl and Put treatments



**Fig. 1** Effects of exogenous putrescine (Put) on net photosynthetic rate ( $P_N$ ), transpiration rate (Tr), intercellular  $\text{CO}_2$  concentration (Ci), and stomatal conductance (Gs) in leaves of cucumber seedlings under NaCl stress. The parameters were measured on the third fully expanded leaf, numbered basipetally. The data represent the mean ± SE ( $n = 3$ ). Different letters indicate significant differences

at  $P < 0.05$  according to Duncan's multiple range test. *Cont* control, seedlings cultured in full-strength Hoagland nutrient solution, *Put* seedlings cultured in control nutrient solution with 8 mM Put sprayed on leaves, *NaCl* salt stress, seedlings cultured in nutrient solution supplemented with 75 mM NaCl, *NaCl + Put* combination of NaCl and Put treatments

values of these parameters were reduced by 58.8, 31.0, 64.0, 67.7, 53.2, and 72.2 %, respectively, compared with those of the control plants. However, exogenous Put alleviated salt stress-induced inhibition of growth. The values of the above parameters showed increases of 1.74-fold, 1.10-fold, 1.76-fold, 1.62-fold, 1.41-fold, and 2.06-fold, respectively, compared with those of non-Put-treated salt-stressed plants. Under non-stress conditions, Put exerted no effects on these parameters.

#### Gas-Exchange Parameters

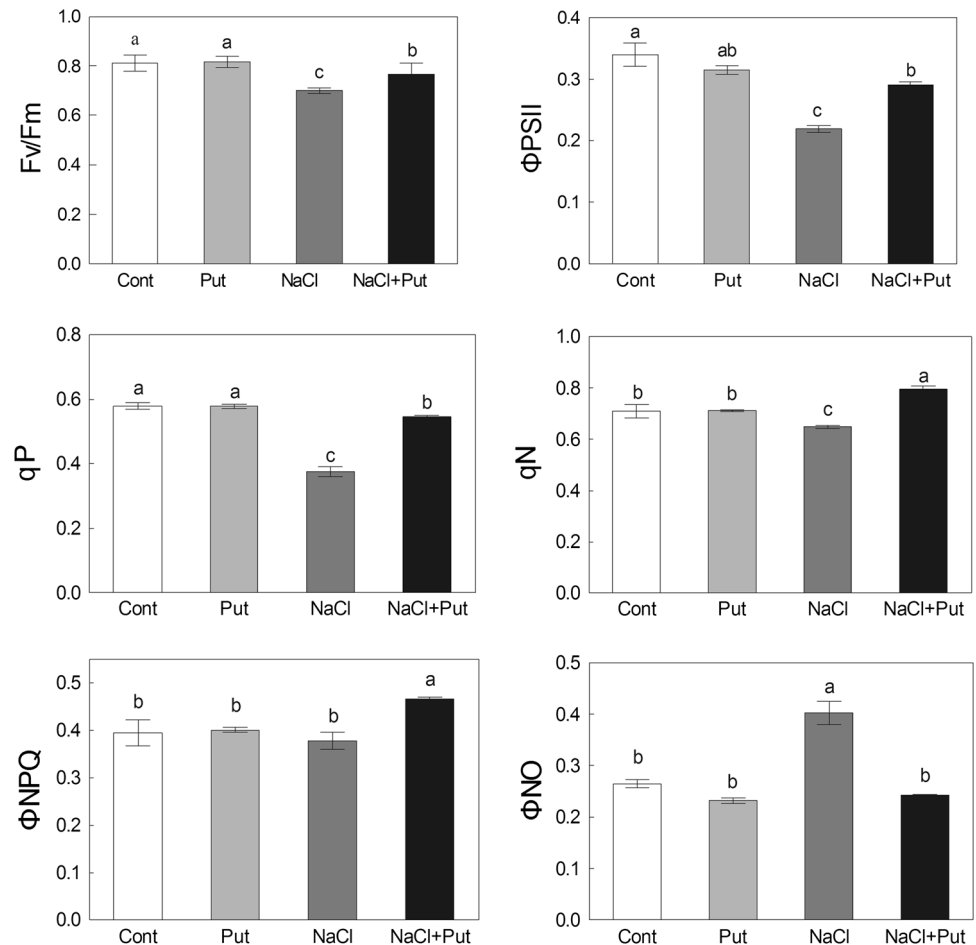
Application of 8 mM Put as a foliar spray did not affect gas-exchange parameters under the control conditions

(Fig. 1). Salt stress reduced  $P_N$ , Tr, Ci, and Gs to 43.9, 52.6, 72.1, and 32.3 % of the control levels, respectively. However, salt-induced negative effects were significantly diminished by exogenous Put, which increased  $P_N$ , Tr, and Gs by 73.3, 18.6, and 23.4 %. The changing pattern of Tr was consistent with Gs, whereas Ci was further decreased by Put application in stressed plants.

#### Chlorophyll Fluorescence

Compared with the control, 75 mM NaCl stress significantly decreased Fv/Fm,  $\Phi\text{PSII}$ , qP, and qN by 13.7, 35.5, 35.2, and 8.6 %, respectively (Fig. 2). In contrast, application of Put increased these parameters compared with NaCl-only

**Fig. 2** Effects of exogenous putrescine (Put) on maximum quantum yield of PSII (Fv/Fm), actual photochemical efficiency of PSII ( $\Phi$ PSII), photochemical quenching coefficient (qP), non-photochemical quenching coefficient (qN), quantum yield of regulated energy dissipation in PSII ( $\Phi$ NPQ), and non-regulated energy dissipation in PSII ( $\Phi$ NO) in leaves of cucumber seedlings under NaCl stress. The parameters were measured on the third fully expanded leaf, numbered basipetally. The data represent the mean  $\pm$  SE ( $n = 3$ ). Different letters indicate significant differences at  $P < 0.05$  according to Duncan's multiple range test. *Cont* control, seedlings cultured in full-strength Hoagland nutrient solution, *Put* seedlings cultured in control nutrient solution with 8 mM Put sprayed on leaves, *NaCl* salt stress, seedlings cultured in nutrient solution supplemented with 75 mM NaCl, *NaCl + Put* combination of NaCl and Put treatments



treatments. Respective pseudo-color images of leaves indicated the status of the six parameters under different treatments (Fig. 3). The values of Fv/Fm,  $\Phi$ PSII, and qP across the leaf surface decreased unevenly under salt stress. Spots on the leaf margins indicated areas damaged by NaCl. Salt stress caused a decline in photosynthetic capacity across the whole leaf, and the inhibition in localized areas was particularly severe. The inhibition due to salinity began from the leaf margin. Photosynthetically active and inactive areas within the leaf showed great spatial heterogeneity. Apart from the suppressed spots on the leaf margin, under stress qN near the veins decreased significantly compared with the control. Exogenous Put significantly enhanced Fv/Fm and  $\Phi$ PSII in salt-stressed plants. Treatment with Put also greatly enhanced  $\Phi$ NPQ and decreased  $\Phi$ NO of salt-stressed plants. In addition, it was found that Put and salt stress can change the energy distribution ratio (Fig. 4). Under normal conditions, approximately one-third of the light energy absorbed by PSII was dissipated by the photochemical process. When subjected to salt stress, the proportion decreased to 22 %, while more energy was consumed through non-regulated energy dissipative processes (about 40 % compared with 26 % in Cont). These results showed that exogenous Put

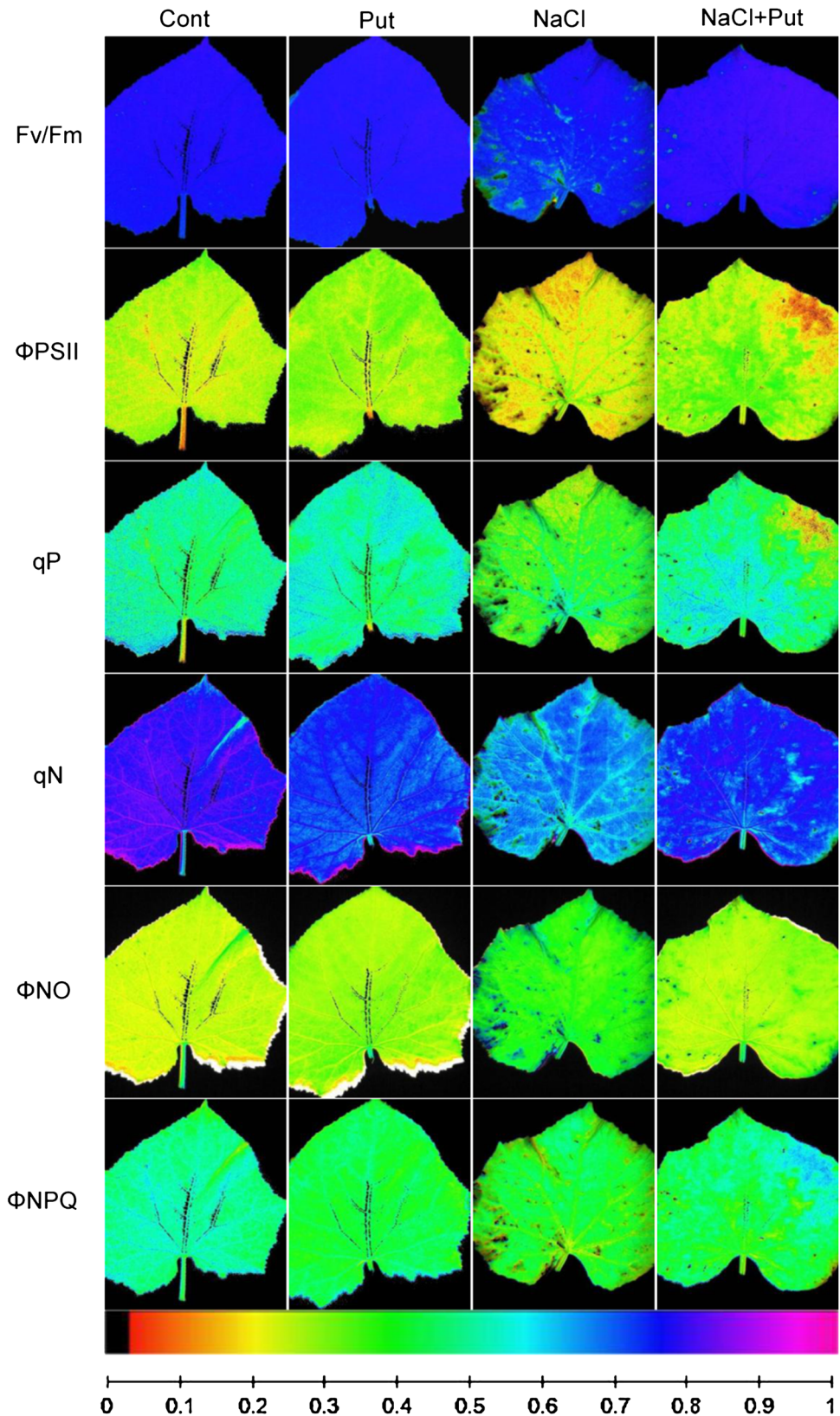
could alleviate the decrease in photochemical efficiency of cucumber through regulating the heat dissipation capacity. Under the control conditions, foliar application of Put exerted no significant influence on chlorophyll fluorescence parameters.

#### NPQ and $\Phi$ PSII Light Response Curves

In all four treatments, NPQ was induced rapidly with increasing light level (Fig. 5). When the light increased to a certain intensity level, the increasing rate of NPQ slowed down and gradually reached a stable value. In low-light conditions ( $<200 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), the differences in NPQ among the treatments were not significant. At a light intensity level higher than  $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ , the NPQ value was significantly decreased by NaCl compared with that of the control. Foliar application of Put increased NPQ in salt-stressed leaves under different light intensities ( $200\text{--}2,300 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Compared with the control, exogenous Put alone exerted no significant influence on the NPQ light response curve.

In contrast to NPQ,  $\Phi$ PSII decreased with the increase in light intensity (Fig. 5). In low-light conditions ( $<200 \mu\text{mol m}^{-2} \text{s}^{-1}$ ),  $\Phi$ PSII decreased rapidly. As the

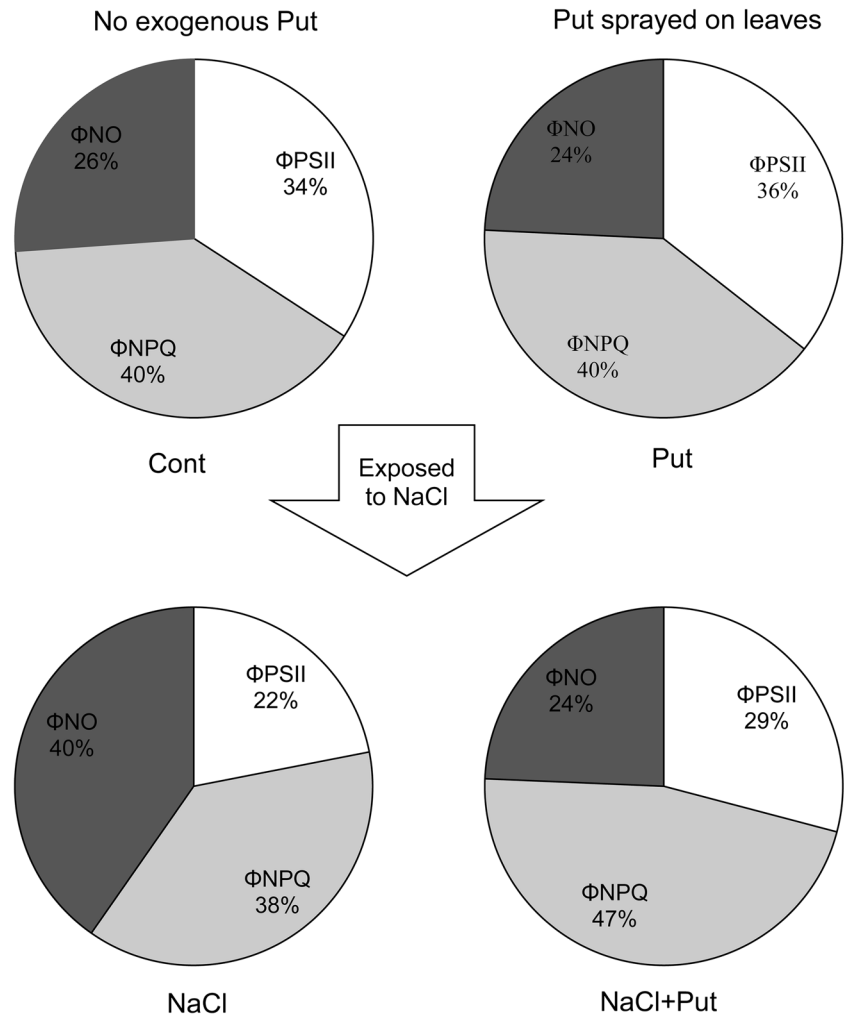
**Fig. 3** Detection of the effects of exogenous putrescine (Put) on cucumber leaves under NaCl stress using a chlorophyll fluorescence imaging technique. Images of Fv/Fm,  $\Phi$ PSII, qP, qN,  $\Phi$ NPQ, and  $\Phi$ NO with actinic illumination of  $450 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  are shown. Each image in the same column represents the same leaf. From left to right: Cont (control, seedlings cultured in full-strength Hoagland nutrient solution), Put (seedlings cultured in control nutrient solution with 8 mM Put sprayed on leaves), NaCl (salt stress, seedlings cultured in nutrient solution supplemented with 75 mM NaCl), and NaCl + Put (combination of Put and NaCl treatments). The color scale at the bottom indicates values from 0 (black) to 1 (pink) (Color figure online)



light intensity increased, the decline gradually slowed. Compared with the control, salt stress reduced the  $\Phi$ PSII value in cucumber leaves. Under high-light conditions

(>600  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), the difference in  $\Phi$ PSII between control and stressed plants was significant. Foliar application of Put to NaCl-treated plants alleviated the decline,

**Fig. 4** Effects of exogenous putrescine (Put) on energy dissipation via photochemical energy conversion ( $\Phi$ PSII, white), regulated ( $\Phi$ NPQ, light gray), and non-regulated mechanisms ( $\Phi$ NO, dark gray) of PSII in cucumber leaves under NaCl stress. The parameters were measured using an imaging-PAM fluorometer and calculated with the equation:  $\Phi$ PSII +  $\Phi$ NPQ +  $\Phi$ NO = 1 (Kramer and others 2004). *Cont* control, seedlings cultured in full-strength Hoagland nutrient solution, *Put* seedlings cultured in control nutrient solution with 8 mM Put sprayed on leaves, *NaCl* salt stress, seedlings cultured in nutrient solution supplemented with 75 mM NaCl, *NaCl + Put* combination of NaCl and Put treatments



and the effect was particularly significant under high-light conditions. Similar to NPQ, Put application to non-salt-stressed plants did not influence the  $\Phi$ PSII light response curve significantly.

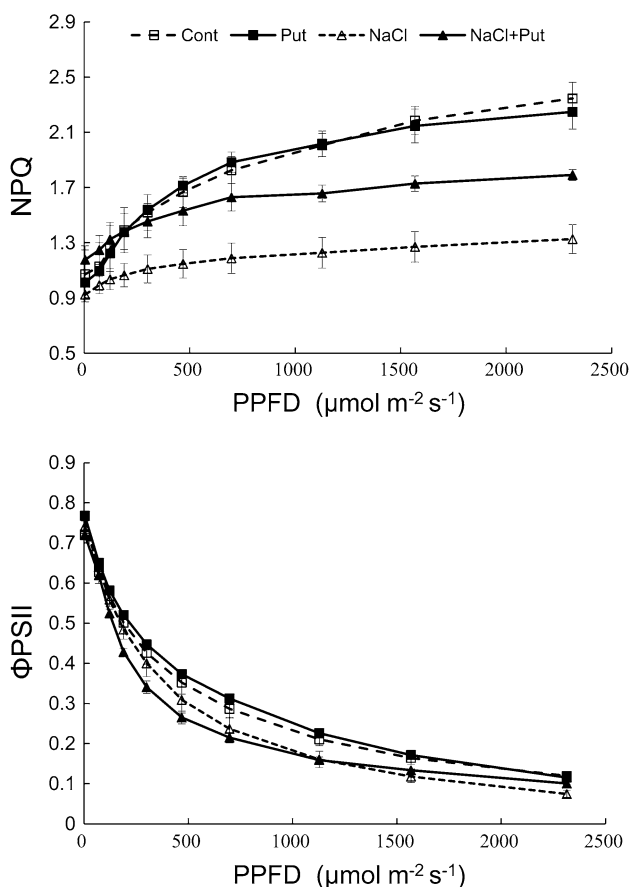
#### Xanthophyll Cycle Components and De-Epoxidation State

Xanthophyll cycle components and their de-epoxidation state in cucumber leaves were modified by treatments with NaCl and/or Put. The contents of violaxanthin, zeaxanthin, and total xanthophyll cycle pigments were reduced by NaCl, but the reduction was alleviated by foliar spray of exogenous Put, which increased the above contents by 27.7, 132.9, and 30.7 %, respectively, in the leaves of salt-stressed plants (Fig. 6). In contrast, the content of antheraxanthin was significantly increased by NaCl, but was not responsive to exogenous Put application. Furthermore, Put enhanced the DEPS in salt-stressed plants but had no effect on the xanthophyll cycle components of non-salt-stressed plants. These results suggested that Put alleviated salt-

induced inhibition of photochemical efficiency via regulation of xanthophyll components and acceleration of the de-epoxidation state of the xanthophyll cycle.

#### Discussion

It has been well documented that metabolic activity decreased in plants subjected to stressful environments (Fariduddin and others 2013; Parida and Das 2005). Plants may reduce their growth as an avoidance strategy in response to stress (Rollins and others 2013). Sharma and others (2011) found that Put may regulate the absorption and accumulation of ions and improve antioxidant enzyme activities, thus improving the tolerance of the salt-susceptible *Karna khatta*. In the present study, NaCl stress significantly suppressed the growth of cucumber seedlings, whereas exogenous Put enhanced the growth and photosynthetic characteristics of salt-stressed plants. Putrescine might be involved in improving heat dissipation capacity



**Fig. 5** Light response curves for non-photochemical quenching (NPQ) and actual photochemical efficiency of PSII ( $\Phi\text{PSII}$ ) of cucumber leaves exposed to putrescine (Put) and/or NaCl treatments. Leaves were illuminated with an internal halogen lamp of different intensities (0, 5, 70, 120, 200, 300, 470, 700, 1,120, 1,570, and 2,310  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). For each light intensity level, actinic light was irradiated for 20 s. The data represent the mean  $\pm$  SE ( $n = 3$ ). *Cont* control, seedlings cultured in full-strength Hoagland nutrient solution, *Put* seedlings cultured in control nutrient solution with 8 mM Put sprayed on leaves, *NaCl* salt stress, seedlings cultured in nutrient solution supplemented with 75 mM NaCl, *NaCl + Put* combination of NaCl and Put treatments

and regulating the de-epoxidation state of the xanthophyll cycle.

The reduction of  $\text{CO}_2$  assimilation is a common response to stress conditions as a result of stomatal closure and further damage to the photosynthetic apparatus. Interestingly, whether a stomatal or non-stomatal factor is the main cause of the reduced  $P_N$  can be judged by the trends of changes in  $G_s$  and  $C_i$  (Long and Bernacchi 2003). In the current study, NaCl stress decreased  $P_N$  mainly because of deterioration of stomatal conditions, which can be deduced by the simultaneous decreases in  $C_i$  and  $G_s$  (Fig. 1). The decrease in  $C_i$  was probably caused by stomatal limitation. Partial closure of stomata also suppressed transpiration, which led to a decrease in the absorption and use of water and nutrients in cucumber (Yuan and others

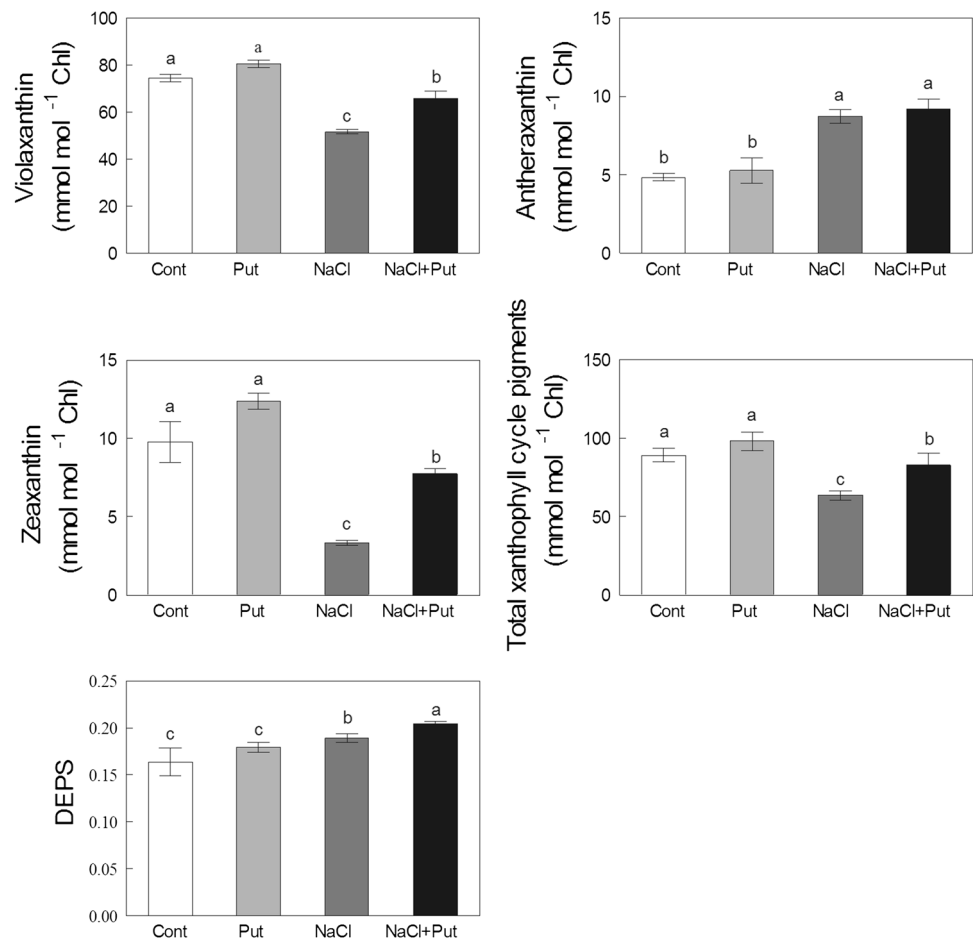
2012). Application of exogenous Put increased  $G_s$  but aggravated the reduction of  $C_i$ , probably because Put enhanced the photosynthetic capacity of mesophyll cells, which thus consume higher quantities of  $\text{CO}_2$  (Yang and Lu 2005). Wang and others (2008) found that a significant positive correlation exists between  $G_s$  and  $\text{Tr}$ . Similarly, enhancement of  $\text{Tr}$  was directly related with the increase in  $G_s$  induced by Put in the present study. In light of this result, we speculated that Put improved the photosynthetic capacity by enhancing the carbon assimilation capacity in leaves as well as regulating stomatal movement under salt stress.

Photosynthetic activity over the leaf surface is usually heterogeneous. This fact makes conventional chlorophyll fluorescence analysis based on point measurements highly error prone. Chlorophyll fluorescence imaging solves this problem and presents an instantaneous overview of the fluorescence emission pattern of the whole leaf surface (Gorbe and Calatayud 2012). The heterogeneous decrease in  $F_v/F_m$  and  $\Phi\text{PSII}$  (Fig. 3) in the present study suggested that salt stress caused a decline in photosynthetic capacity across the whole leaf, and that inhibition in localized areas was particularly severe.

In the present study, exogenous Put played a role in protecting PSII against excessive energy through improving the thermal dissipation of the excitation energy. To our knowledge, fluorescence yield is the result of competition for excitation energy among several decay pathways (Rascher and others 2000). The relationship among dissipative processes is described by the equation  $\Phi\text{PSII} + \Phi\text{NPQ} + \Phi\text{NO} = 1$  (Kramer and others 2004). The decrease in  $\Phi\text{PSII}$  indicated that the radiant energy dissipation process was affected by NaCl stress. The decrease in  $F_v/F_m$  implied that NaCl stress-induced photoinhibition fluorescence quenching (Xia and others 2009). Krause and Weis (1991) considered that lower  $F_v/F_m$  values indicated that excessive energy is dissipated as heat in the antenna complex, thus resulting in a decreased quantum yield. However, our present results indicated that the decrease in  $\Phi\text{PSII}$  was mainly ascribed to the substantial increase in energy dissipation via a non-regulated mechanism,  $\Phi\text{NO}$  (Fig. 4). Exogenous Put largely improved photoprotective down-regulation of PSII (as estimated by the  $\Phi\text{NPQ}$  value) and inhibited the non-regulated energy dissipative process in salt-stressed plants, thus enhancing the photoprotective capacity of the photosynthetic apparatus. This finding is consistent with the study by Ioannidis and Kotzabasis (2007), who found that PAs can increase NPQ and activate photo-protection. Putrescine also increased the proportion of energy used in photochemical reactions ( $\Phi\text{PSII}$ ), resulting in the improved photosynthetic activity (Fig. 1). We inferred that exogenous Put may be involved in alleviating the photoinhibition of  $\Phi\text{PSII}$  and enhancing the



**Fig. 6** Effects of exogenous putrescine (Put) on xanthophyll cycle components and de-epoxidation state of xanthophyll cycle (DEPS) in cucumber leaves under NaCl stress. The parameters were measured on the third fully expanded leaf, numbered basipetally. The data represent the mean  $\pm$  SE ( $n = 3$ ). Different letters indicate significant differences at  $P < 0.05$  according to Duncan's multiple range test. *Cont* control, seedlings cultured in full-strength Hoagland nutrient solution, *Put* seedlings cultured in control nutrient solution with 8 mM Put sprayed on leaves, *NaCl* salt stress, seedlings cultured in nutrient solution supplemented with 75 mM NaCl, *NaCl + Put* combination of NaCl and Put treatments



photochemical quenching process as well as thermal dissipation, thus protecting leaves from damage caused by excessive energy excitation in the reaction centers. This deduction agrees with the results of He and others (2002) who showed that Spd can alleviate the low-temperature photoinhibition of isolated thylakoids.

Non-photochemical quenching processes quench singlet-excited chlorophylls and harmlessly dissipate excessive excitation energy as heat, thus helping to regulate and protect photosynthesis in response to excess light energy (Müller and others 2001). Recent studies demonstrated that Spm can increase NPQ under low-light conditions both in higher plants and green algae (Ioannidis and others 2011; Ioannidis and Kotzabasis 2007). In the current research, we found that Put also stimulated NPQ in cucumber seedlings under saline conditions. Trimeric LHCII is a putative site of qE, a rapid phase of NPQ (Pascal and others 2005). Positively charged PAs can neutralize the negative charges of LHCII so that repulsion between different complexes is minimized (Navakoudis and others 2007). Thus, putrescine may induce aggregation of LHCII by screening the surface charge, which in turn increases energy dissipation. In addition to the conformational changes of LHCII,

zeaxanthin is essential for qE formation (Demmig-Adams and others 1990). Violaxanthin, antheraxanthin, and zeaxanthin are xanthophyll cycle pigments located in the protein complexes of the thylakoid membrane. Their main function is to increase thermal dissipation of excess excitation energy and protect chloroplasts from high-light stress. Violaxanthin de-epoxidase catalyzes the de-epoxidation of violaxanthin to zeaxanthin via the intermediate antheraxanthin as part of the xanthophyll cycle (Bukhov and others 2001; Demmig-Adams and others 1996). Zeaxanthin may either directly dissipate the excess excitation energy as heat (Horton and others 2005) or interact with protonated chlorophyll-binding proteins to induce NPQ (Gilmore 1997). In the present work, the content of zeaxanthin in salt-stressed plants was drastically increased by exogenous Put, most likely because of enhanced de-epoxidation of violaxanthin. Stressed plants treated with exogenous Put exhibited not only an increased xanthophyll cycle pool but also a higher xanthophyll de-epoxidation state, thereby enhancing the capacity for thermal energy dissipation. These results indicated that exogenous Put can initiate xanthophyll cycle-dependent NPQ, and dissipate excessive excitation energy as heat to protect the

photosynthetic structures from photoinhibition damage caused by salt stress.

In conclusion, our experimental results suggested that exogenous Put promoted the growth of cucumber seedlings subjected to salt stress through improving photosynthetic performance. Putrescine enhanced the thermal dissipation capacity by accelerating the de-epoxidation state of the xanthophyll cycle and regulating xanthophyll components, thus protecting the reaction centers of PSII from damage caused by excess excitation energy. Overall, exogenous Put improved the salt tolerance of the photosynthetic apparatus, which further enhanced plant salt tolerance in general.

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