Grafting onto *Cucurbita moschata* rootstock alleviates salt stress in cucumber plants by delaying photoinhibition

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Abstract

To determine how the use of a given rootstock can influence the functioning of the photosynthetic apparatus of the scion under salt stress, the growth, gas exchange, photosystem II (PSII) efficiency, xanthophyll cycle, and chloroplast ultrastructure of nongrafted, self-grafted, and pumpkin-grafted (hereafter referred to as rootstock-grafted) cucumber (*Cucumis sativus* L.) plants were investigated at day 15 after being treated with 90 mM NaCl. The reductions in plant growth of the rootstock-grafted plants were lower than those of the nongrafted and self-grafted plants under 90 mM NaCl. The net photosynthetic rate, stomatal conductance, maximal and effective quantum yield of PSII photochemistry, photochemical quenching coefficient, and effective quantum-use efficiency of PSII in the light-adapted state of the nongrafted and self-grafted plants were significantly decreased under 90 mM NaCl. However, these reductions were alleviated when the cucumber plants were grafted onto the pumpkin (*Cucurbita moschata* Duch.) rootstock. The intercellular CO_2 concentrations were significantly increased in the nongrafted and self-grafted plants under 90 mM NaCl, whereas it was decreased in the rootstock-grafted plants. Nonphotochemical quenching (NPQ) and the de-epoxidation state of the xanthophyll cycle were significantly increased under 90 mM NaCl, particularly in the rootstock-grafted plants, suggesting the rootstock-grafted plants had higher potential to dissipate excess excitation energy and reduce the probability of photodamage to PSII. Under 90 mM NaCl, the number of grana was reduced, the thylakoids were swollen, and starch granules accumulated in all plants. However, the damage of chloroplast ultrastructure was alleviated in the rootstock-grafted plants. Taken together, the use of *C. moschata* rootstock alleviated salt stress in cucumber plants by delaying photoinhibition, probably due to a lower incidence of both stomatal and nonstomatal factors limiting photosynthesis.

Additional key words: chlorophyll fluorescence; *Cucumis sativus*; grafting; photosynthesis; salinity; xanthophyll cycle.

Introduction

Salinity is one of the major abiotic stresses that reduce the crop growth and productivity of many plants (Yamaguchi and Blumwald 2005, Shabala and Cuin 2008). The inhibition of plants growth under saline conditions often involves a decrease in their photosynthetic capacity (Yang *et al.* 2008, Zhang *et al.* 2009). The reduced photosynthesis is caused not only by stomatal closure, but also by nonstomatal factors that reduce PSII efficiency (Neves *et al.* 2008). The salt stress causing the reduced PSII efficiency is associated with the PSII complex, primary charge separation in PSII, and pigment-protein complexes of the thylakoid membranes of chloroplasts (Misra *et al.* 2001), as well as the PSII activity (Lu and Vonshak 2002), and the quantum yield

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Abbreviations: A – antheraxanthin; Chl – chlorophyll; C_i – intercellular CO_2 concentration; E – transpiration rate; F_m – maximal fluorescence of dark-adapted state; F_m' – maximal fluorescence of light-adapted state; F_o – minimal fluorescence of dark-adapted state; F_o' – minimal fluorescence of light-adapted state; F_s – steady-state fluorescence yield; F_v – maximal variable fluorescence; F_v/F_m – maximal quantum yield of PSII photochemistry; F_v'/F_m' – effective quantum use efficiency of PSII in the light-adapted state; g_s – stomatal conductance; NPQ – nonphotochemical quenching of chlorophyll fluorescence; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density; PSII – photosystem II; q_P – photochemical quenching coefficient; V – violaxanthin; Z – zeaxanthin; Φ_{PSII} – effective quantum yield of PSII photochemistry.

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of PSII electron transport (Xia *et al.* 2004). A consequence of the salinity-induced limitation of photosynthetic capacity is the exposure of plants to excess energy, which, if not safely dissipated, may be harmful to PSII because of an over-reduction of reaction centers (Demmig-Adams and Adams 1996, Foyer and Noctor 2005).

To protect the photosynthetic apparatus from photodamage under stress conditions, plants have evolved a variety of regulatory mechanisms to minimize the harmful effects of excess energy (Niyogi 1999, Ort and Baker 2002). The thermal dissipation of excess irradiance, measured as nonphotochemical quenching (NPQ), is of paramount importance in the protection of the photosynthetic apparatus against the deleterious effects of excess light (Jiang *et al.* 2006). Xanthophylls play a critical role in generating NPQ, and the extent of NPQ in leaves is correlated with the levels of zeaxanthin (Z) and antheraxanthin (A) (Demmig-Adams and Adams 1996), which are formed from violaxanthin (V) through the xanthophyll cycle (Pfündel and Bilger 1994). In this process, the excess energy is harmlessly dissipated as heat, thereby protecting the photosynthetic apparatus against photoinhibition. The ameliorative effects of the xanthophyll cycle on the photosynthesis of plants have been studied under salt stress (Qiu *et al.* 2003). However, few studies are concerned with the role of the xanthophyll cycle in grafted plants under salt stress.

Recently, the use of salt-tolerant rootstock was demonstrated to be a valid strategy in increasing the salt tolerance of fruit bearing vegetables, such as tomato (Estañ *et al.* 2005), eggplant (Bai *et al.* 2005), cucumber (Zhu *et al.* 2008a), watermelon (Yetisir and Uygur 2010), and melon (Edelstein *et al.* 2011). The increased salt tolerance could be attributed to the reduced transport of Na^+ and/or Cl^- to the scion (Estañ *et al.* 2005, Zhu *et al.* 2008a, Edelstein *et al.* 2011). It was also suggested that the improved salt tolerance of grafted plants was related with the enhancement of antioxidant enzymes activity, organic solutes accumulation, and gas-exchange capacity (He *et al.* 2009, Huang *et al.* 2009a, Zhen *et al.* 2010). However, data on the effects of functioning of the photosynthetic apparatus in plants exposed to salinity were limited.

The current study aims to determine how the use of a given rootstock can influence the functioning of the photosynthetic apparatus of the scion under salt stress, by measuring the changes in growth, net photosynthetic rate (P_N), PSII efficiency, the de-epoxidation state of the xanthophyll cycle, and the chloroplast ultrastructure of non-, self-, and rootstock-grafted cucumber plants under 0 and 90 mM NaCl treatments.

Materials and methods

Plant material and growth conditions: The present study was conducted in a greenhouse from September to November 2009 at Huazhong Agricultural University. During the experimental period, the air temperature was 18–30°C, the relative humidity was 60–70%, and the photon flux density (PFD) average was 293 μmol m^{-2} s^{-1} with a photoperiod of 13 h d^{-1}. The salt-sensitive cucumber (*Cucumis sativus* L.) cv. Jinchun No. 2 (Tianjin Kernel Cucumber Research Institute, Tianjin, China) was grafted onto a salt-tolerant pumpkin (*Cucurbita moschata* Duch.) cv. Chaojiquanwang (Tangshan Four Seasons Seed Industry, Hebei, China) (hereafter referred to as rootstock-grafted plants). The nongrafted and self-grafted cucumber plants were used as controls.

The seeds of the rootstock Jinchun No. 2 (for self-grafting) and Chaojiquanwang were sown on September 2 and 8 in 50-cell seedling plug trays filled with a 2:1 (v/v) mixture of peat and perlite, respectively. The seeds of the scion and nongrafted plants were both sown on September 12. The insert grafting method of Lee (1994) was performed five days after the cucumber scion seeds were sown, and then, the grafted plants were cultivated as described by Zhu *et al.* (2008a).

After the graft had been established, uniform seedlings that had two expanded leaves were transferred to plastic containers (63 cm × 36.5 cm × 19 cm, 8 plants per container) filled with 25 L of half-strength Hoagland solution (Hoagland and Arnon 1950). Nine days after transplantation, the seedlings were exposed to 0 mM or 90 mM NaCl for 15 days. The salt concentrations in the nutrient solution were increased at 30 mM d^{-1} increments until the final concentration (90 mM NaCl) was achieved. Each treatment was replicated six times with one container having 8 plants each per replicate, and all treatments were arranged in a completely randomized block design. The management of the nutrient solution during the experimental period was the same as described by Zhu *et al.* (2008b).

Plant growth parameters: After 15 days of salt treatment, six plants per treatment were harvested, and the roots were rinsed in deionized water and carefully blotted with tissue paper. After the plant height and stem diameter were measured, the plants were divided into leaves, stems, and roots. The leaf area was determined using a leaf area meter (*LiCOR-3100*, *LI-COR Inc.*, Lincoln, NE, USA). The shoots (leaves and stems) and roots were dried at 70°C for 48 h to constant mass, and their dry masses were recorded.

Gas-exchange measurements: P_N, stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and transpiration rate (E) were measured from the youngest fully expanded leaves using a portable *CIRAS-2* photosynthesis system (*PP-Systems*, Hitchin, UK) (Li *et al.* 2009). The CO_2 concentration, relative air humidity, PFD, and leaf temperature were maintained at 350 μmol(CO_2) mol^{-1}, 80–90%, 800 μmol(photon) m^{-2} s^{-1}, and 25°C, respectively.

Chlorophyll (Chl) fluorescence was measured on the same leaves used for gas-exchange determinations using a *FMS-2* fluorometer system (*Hansatech*, King's Lynn, UK). The minimal fluorescence (F_o) was measured by exposing the leaves of dark-adapted (30 min) plants to modulated red light. The maximal fluorescence values of the dark-adapted (F_m) and light-adapted (F_m') states were measured by 0.8-s saturating pulses (3,000 μmol m^{-2} s^{-1}). After the F_m' measurement, the actinic light (400 μmol m^{-2} s^{-1}) was switched off and the far-red light was turned on for 3 s to measure the minimal fluorescence of the light-adapted state (F_o'). The steady-state fluorescence level during exposure to natural illumination (F_s) was also measured. Using these parameters, the following ratios were calculated: the maximal quantum yield of PSII photochemistry, $F_v/F_m = (F_m - F_o)/F_m$; the effective quantum-use efficiency of PSII in the light-adapted state, $F_v'/F_m' = (F_m' - F_o')/F_m'$; the quantum yield of PSII photochemistry, $\Phi_{PSII} = (F_m' - F_s)/F_m'$; the photochemical quenching, $q_P = (F_m' - F_s)/(F_m' - F_o')$; and nonphotochemical quenching, $NPQ = (F_m - F_m')/F_m'$.

Xanthophyll pigment analysis: The leaves were immersed in liquid nitrogen immediately after Chl fluorescence determination and stored at –80°C until use. Photosynthetic pigments were extracted from leaves with 80% ice-cold acetone. The xanthophyll pigments (V, A, and Z) were separated and qualified essentially following the method of Guo *et al.* (2006). The extracts were centrifugated at 10,000 × *g* for 3 min and the supernatants were filtered through a 0.45 μm membrane filter before injection into high-performance liquid chromatography (*ProStar 230*, *Varian Inc.*, CA, USA). The de-epoxidation form for the xanthophyll cycle was expressed as (A + Z)/(V + A + Z).

Electron microscopy: The leaf blade segments (2 mm × 2 mm) were fixed for 2 h with 2.5% (v/v) glutaraldehyde in 0.1 M of phosphate buffer (pH 7.2) followed by 2 h in 2% (w/v) osmic acid. After dehydration in a graded ethanol series up to 100% ethanol, the samples were embedded *via* propylene oxide in low-viscosity Spurr's resin. The ultrastructure was evaluated on transverse ultrathin sections of embedded objects contrasted with a saturated solution of uranyl acetate in 70% (v/v) aqueous ethanol, followed by a lead citrate solution treatment using a transmission electron microscope (*H-7650*, *Hitachi Ltd.*, Tokyo, Japan) at an operating voltage of 70 kV (Reynolds 1963).

Statistical analysis: All of the parameters were measured with six replicates per treatment from different containers ($n = 6$). Statistical analysis was performed using the *SAS 9.1* software (*SAS Institute*, Cary, NC, USA). The differences between the means were established using *Duncan*'s multiple range test ($p<0.05$), the linear correlation analysis was subjected to a correlation between the (A + Z)/(V + A + Z) ratio and NPQ was assayed using the *SAS 9.1* software.

Results

Plant growth: No significant difference was observed in shoot dry mass, shoot/root ratio, and leaf area between the nongrafted and self-grafted treatments (Table 1). Although plant height, stem diameter, shoot dry mass, and leaf area were significantly reduced for all plants under the 90 mM NaCl treatment, the reduction in the nongrafted and self-grafted plants was more severe than those in the rootstock-grafted plants. Compared with those under the 0 mM NaCl treatment, the plant height, stem diameter, shoot dry mass, and leaf area of the self-grafted plants under the 90 mM NaCl treatment decreased by 32%, 21%, 64%, and 67%, respectively. However, the

Table 1. Plant growth of the nongrafted, self-grafted, and rootstock-grafted cucumber seedlings under 0 and 90 mM NaCl stress. Measurements were performed at day 15 after NaCl treatment. Data are the mean ± SE ($n = 6$). Values in each column followed by *the same letters* are not significantly different ($p>0.05$) according to *Duncan*'s multiple range test.

Treatment	Plant height [cm]	Stem diameter [cm]	Shoot dry mass [g]	Root dry mass [g]	Shoot/root ratio	Leaf area [cm^2]
0 mM						
Nongrafted	127.75 ± 2.82^{a}	0.61 ± 0.02^{ab}	9.74 ± 0.77^{b}	1.23 ± 0.12^{a}	7.99 ± 0.32^{bc}	$2{,}039.54 \pm 195.57^{c}$
Self-grafted	128.77 ± 4.16^{a}	0.62 ± 0.02^{ab}	10.87 ± 1.02^{b}	1.30 ± 0.15^{a}	8.62 ± 0.70^{b}	$2{,}789.82 \pm 172.50^{b}$
Rootstock-grafted	135.63 ± 6.22^{a}	0.65 ± 0.05^{a}	15.71 ± 1.99^{a}	1.43 ± 0.20^{a}	11.06 ± 0.39^{a}	$3{,}810.39 \pm 347.67^{a}$
90 mM						
Nongrafted	82.80 ± 3.33^{c}	0.48 ± 0.03^{c}	3.85 ± 0.24^{c}	0.49 ± 0.06^{b}	8.26 ± 0.82^{b}	868.04 ± 66.62^{d}
Self-grafted	86.92 ± 4.35^{c}	0.49 ± 0.02^{c}	3.86 ± 0.36^{c}	0.51 ± 0.02^{b}	7.60 ± 0.74^{bc}	917.53 ± 88.17^{d}
Rootstock-grafted	106.13 ± 3.66^{b}	0.53 ± 0.04^{bc}	7.73 ± 0.82^{b}	1.25 ± 0.15^{a}	6.26 ± 0.22^{c}	$1{,}774.50 \pm 104.40^{c}$

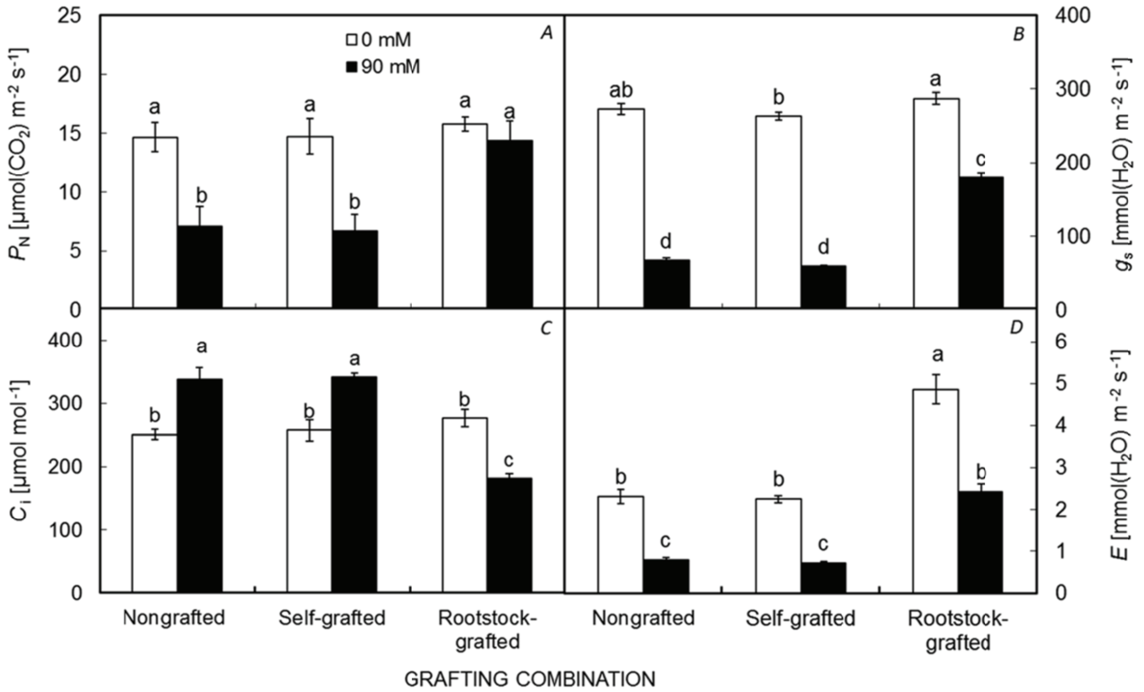

Fig. 1. Changes in the net photosynthetic rate (P_N, *A*), stomatal conductance (g_s, *B*), intercellular CO$_2$ concentration (C_i, *C*), and transpiration rate (E, *D*) in the leaves of the nongrafted, self-grafted, and rootstock-grafted cucumber seedlings under 0 and 90 mM NaCl stress. Measurements were performed at day 15 after NaCl treatment. Data are the mean ± SE ($n = 6$). Bars with *the same letters* are not significantly different ($p>0.05$) according to *Duncan*'s multiple range test.

inhibitory effects were mitigated by rootstock-grafting, as indicated by 22%, 18%, 51%, and 53% reduction in plant height, stem diameter, shoot dry mass, and leaf area, respectively (Table 1). No significant difference was also observed in the root dry mass of the rootstock-grafted plants between the control (unstressed) and 90 mM NaCl-treated plants (Table 1). However, the root dry mass of the nongrafted and self-grafted plants significantly decreased in the 90 mM NaCl treatment. The shoot/root ratios of the nongrafted and self-grafted plants were unaffected by the 90 mM NaCl treatment, but decreased significantly in the rootstock-grafted plants (Table 1).

Gas-exchange parameters: As shown in Fig. 1*A*, compared with that in the 0 mM NaCl treatment, P_N was dramatically reduced in the nongrafted and self-grafted plants under the 90 mM NaCl treatment. However, this reduction was alleviated by rootstock grafting. No significant difference was observed in the P_N values of all plants under the 0 mM NaCl treatment. However, the P_N values of the rootstock-grafted plants were significantly higher than those of the nongrafted and self-grafted plants under the 90 mM NaCl treatment (Fig. 1*A*). As shown in Fig. 1*B*,*D*, g_s and E of the rootstock-grafted plants were significantly higher than those of the nongrafted and self-grafted plants whether or not under the 90 mM NaCl treatment. Compared with those of the controls, the C_i values of the non- and self-grafted plants under the 90 mM NaCl treatment significantly increased ($p<0.05$). However, C_i decreased in rootstock-grafted plants (Fig. 1*C*).

PSII photochemistry: Compared with those under the 0 mM NaCl treatment, the F_v/F_m values significantly decreased ($p<0.05$) in the nongrafted and self-grafted plants under the 90 mM NaCl treatment. However, the reduction in F_v/F_m in the rootstock-grafted plants was not significant (Fig. 2*A*). In the non- and self-grafted plants, q_P, F_v'/F_m', and Φ_{PSII} were significantly decreased under the 90 mM NaCl treatment (Fig. 2*B,C,D*). However, these parameters were less affected in the rootstock-grafted plants. As shown in Fig. 2*E*, NPQ was significantly increased in all plants under the 90 mM NaCl treatment, particularly in the rootstock-grafted plants. Compared with the plants under the 0 mM NaCl treatment, the NPQ of the nongrafted, the self-grafted, and the rootstock-grafted plants in the 90 mM NaCl treatment was increased by 16%, 24%, and 72%, respectively.

Xanthophyll cycle: No significant differences were observed in the changes of xanthophyll cycle pigments and de-epoxidation state in the xanthophyll cycle for all plants grown under the 0 mM NaCl treatment (Fig. 3). However, the 90 mM NaCl treatment resulted in a significant increase in Z content, as well as in a significant

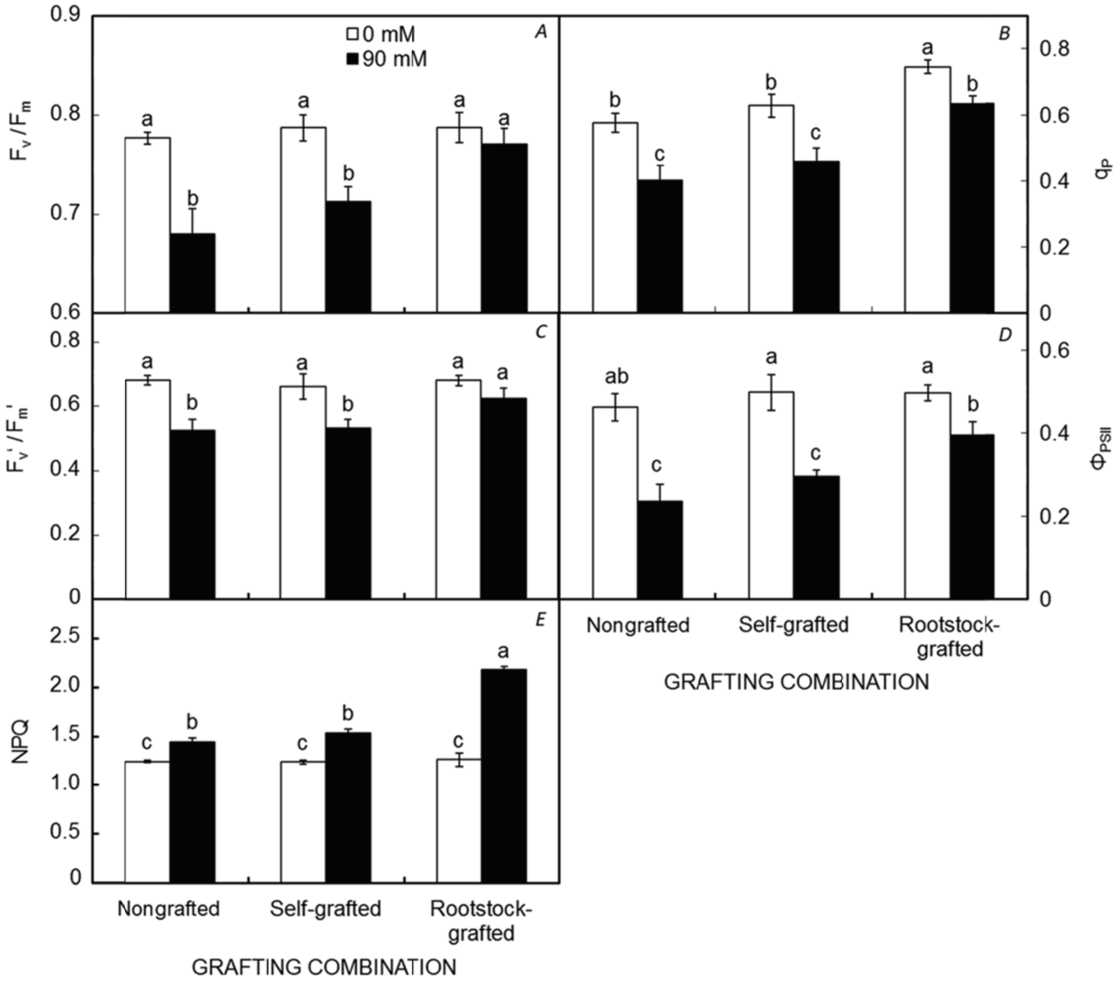

Fig. 2. Changes in the maximal quantum yield of PSII photochemistry (F_v/F_m, *A*), photochemical quenching (q_P, *B*), effective quantum-use efficiency of PSII in the light-adapted state (F_v'/F_m', *C*), quantum yield of PSII photochemistry (Φ_{PSII}, *D*), and nonphotochemical quenching of chlorophyll fluorescence (NPQ, *E*) in the nongrafted, self-grafted, and rootstock-grafted cucumber seedlings under 0 and 90 mM NaCl stress. Measurements were performed at day 15 after NaCl treatment. Data are the mean ± SE ($n = 6$). Bars with *the same letters* are not significantly different ($p>0.05$) according to *Duncan*'s multiple range test.

decrease in V content, in the three different plant combinations (Fig. 3*A,B*). On the other hand, the increase in A was only observed in the rootstock-grafted cucumber leaves (Fig. 3*C*). Thus, the de-epoxidation state of the xanthophyll cycle was improved for the three different plant combinations, particularly for the rootstock-grafted plants (Fig. 3*E*). The ratio of (A + Z)/(V + A + Z) was positively correlated with the NPQ ($R^2 = 0.9945^{***}$, $p<0.001$) (Fig. 4).

Electron microscopy: As shown in Fig. 5, the chloroplasts were affected by the 90 mM NaCl treatment and more severe damage was observed in the nongrafted and self-grafted plant leaves. For the rootstock-grafted plants, slight signs of alterations were observed in the chloroplasts that have disordered lamellar systems with grana (Fig. 5*F*). These alterations were more pronounced in the chloroplasts from the leaves of the nongrafted and self-grafted plants under the 90 mM NaCl treatment (Fig. 5*E,D*). In addition, a decrease in the degree of grana stacking was observed. The screening of various electron micrographs revealed an increase in the starch content of the chloroplasts of leaves from the 90 mM NaCl-treated plants when compared with those from the 0 mM NaCl treatments. Furthermore, the starch content greatly increased in nongrafted and self-grafted plants.

Discussion

In the present study, grafting using a salt-tolerant rootstock counteracts the deleterious effects of salinity stress on the plant growth (Table 1). This is in agreement with earlier findings that grafting cucumber plants onto

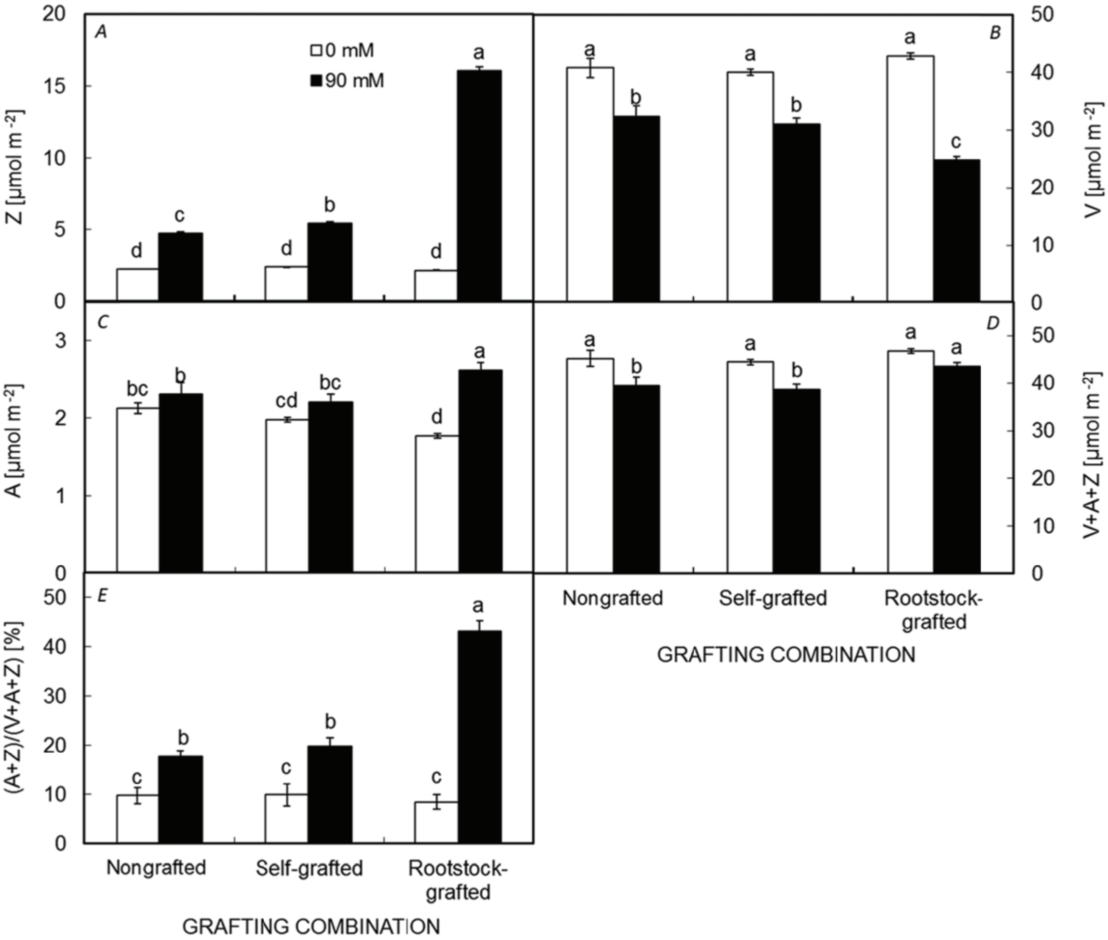

Fig. 3. Changes in xanthophyll cycle pigments (zeaxanthin Z, violaxanthin V, and antheraxanthin A) and de-epoxidation state of the xanthophyll cycle pigments [(A+Z)/(V+A+Z)] in the leaves of the nongrafted, self-grafted, and rootstock-grafted cucumber seedlings under 0 and 90 mM NaCl stress. Measurements were performed at day 15 after NaCl treatment. Data are the mean ± SE ($n = 6$). Bars with *the same letters* are not significantly different ($p>0.05$) according to *Duncan*'s multiple range test.

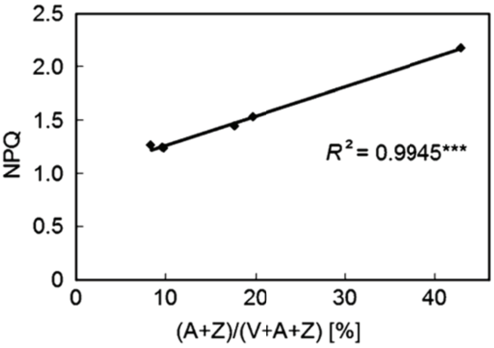

Fig. 4. Correlation analysis between the de-epoxidation state of the xanthophyll cycle (A+Z)/(V+A+Z) and non-photochemical quenching (NPQ) in the leaves of cucumber seedlings. Measurements were performed at day 15 after NaCl treatment ($n = 6$). $^{***}p<0.001$.

salt-tolerant pumpkin plants improve salt tolerance (Zhu *et al.* 2008a, Huang *et al.* 2009a, Zhen *et al.* 2010). Moreover, the fruit yield and quality of cucumber are improved by grafting onto the salt-tolerant rootstock under NaCl stress (Huang *et al.* 2009b). These results suggested that the use of salt-tolerant rootstock is an effective way to increase cucumber performance under salinity. The different performances between the rootstock-grafted and nongrafted (self-grafted) plants reflect the effect of the *C. moschata* root system on shoot growth, which may be due to hormone (such as ABA, cytokinins, and ethylene) biosynthesis, the delivery of hormones from the roots to the shoots *via* the xylem, and then the regulation of shoot physiology and senescence (Albacete *et al.* 2009).

Large differences in the performance of the photosynthetic apparatus were observed between the rootstock-grafted plants and the nongrafted or self-grafted plants. The salt-tolerant rootstock-grafted plants had higher P_N values than the nongrafted and self-grafted cucumber plants (Fig. 1*A*). The inhibitory effect of NaCl on g_s was significant in the present experiment, but the inhibitory effect on the rootstock-grafted plants was much milder than those on the nongrafted and self-grafted plants (Fig. 1*B*). Meanwhile, C_i was dramatically

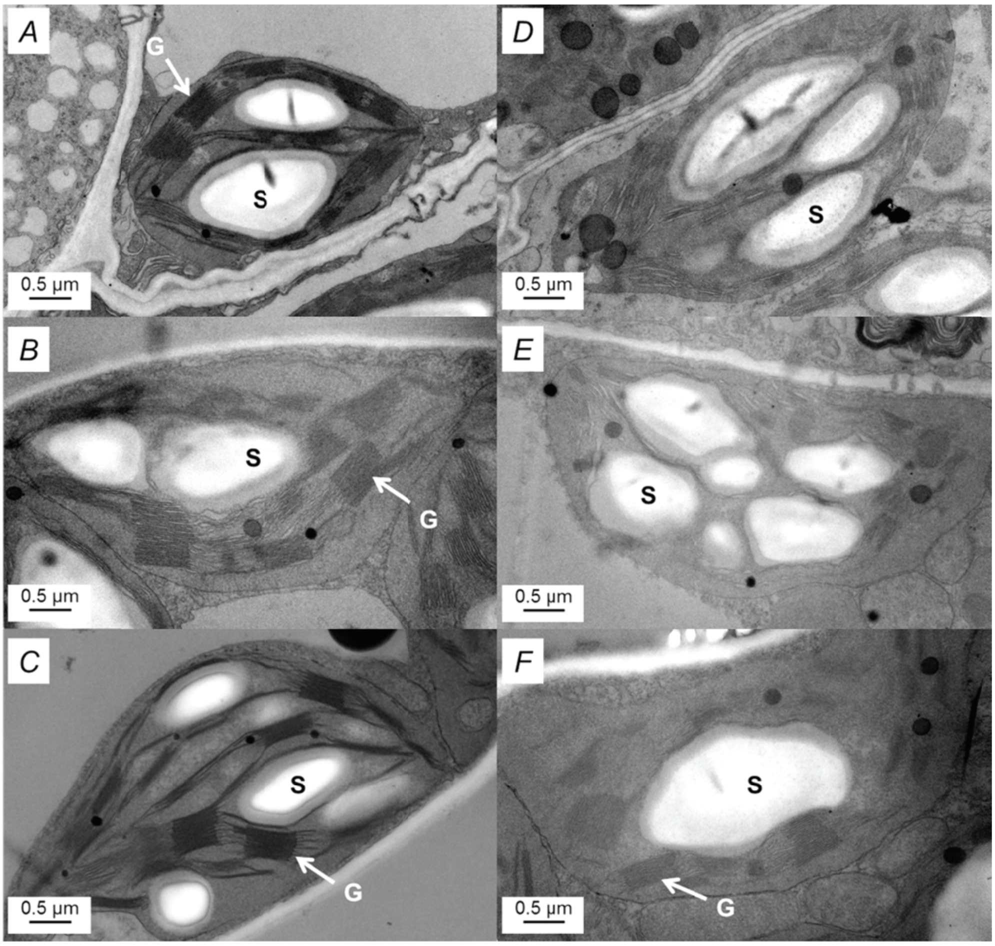

Fig. 5. Transmission electron micrographs of chloroplasts from leaves of cucumber under the different treatments: (*A*) nongrafted, 0 mM NaCl, (*B*) self-grafted, 0 mM NaCl, (*C*) rootstock-grafted, 0 mM NaCl, (*D*) nongrafted, 90 mM NaCl, (*E*) self-grafted, 90 mM NaCl, and (*F*) rootstock-grafted, 90 mM NaCl. The leaf segments were cut at day 15 after NaCl treatment. S – starch granule; G – grana.

decreased in the rootstock-grafted plants, but significantly increased in the nongrafted and self-grafted plants. This result might imply that the use of rootstock alleviates salt stress in cucumber plants, probably because of a lower incidence of both stomatal and nonstomatal factors that limit photosynthesis. This low incidence is probably due to better root-to-shoot hormonal and ionic transportation movement in the rootstock-grafted plants under salt stress, which in turn maintains both growth and assimilates production, as well as the original capacity for Na^+ efflux in the scion under saline conditions (Pérez-Alfocea *et al*. 2010).

Given that the decrease in F_v/F_m can be due to the development of slowly relaxing quenching processes and photodamage to the PSII reaction center, F_v/F_m is widely used to detect stress-induced perturbations in the photosynthetic apparatus and as an indicator of leaf senescence (Baker 2008, Pérez-Alfocea *et al*. 2010). In the present study, F_v/F_m was significantly reduced in the nongrafted and self-grafted plants under the 90 mM NaCl treatment (Fig. 2*A*), suggesting that photoinhibition occurred, which could be a consequence of damage to PSII (Demmig-Adams and Adams 1992). However, no change was detected in the rootstock-grafted plants (Fig. 2*A*). This result suggests that rootstock-grafted plants can delay the photoinhibition under salt stress. Similar results were observed in tomatoes under severe rather than slight salt stress (He *et al*. 2009). A decreased Φ_{PSII} implies lower electron transport for carbon fixation, thereby decreasing the net CO_2 assimilation rate (Maxwell and Johnson 2000). In this study, the higher Φ_{PSII} values in the rootstock-grafted plants were well correlated with the better photosynthetic performance under the 90 mM NaCl treatment. In the rootstock-grafted plants under the 90 mM NaCl treatment, Φ_{PSII} was significantly decreased with no decrease in the F_v/F_m. This result might be due to an enhancement in NPQ, which suggests that more energy is dissipated safely as heat in PSII (Fig. 2*E*). Therefore, photoinhibition may be more active in the nongrafted and self-grafted plants, whereas source and sink activities remain more active in the rootstock-grafted ones and NPQ still protects the photosynthetic apparatus from excess of energy and partial stomatal closure (Ghanem *et al*. 2008). On the other hand, in the nongrafted and self-grafted plants, these mechanisms were less effective at this time point, probably because of more stomatal closure and growth impairment in the plants. These data agree with the hypothesis recently stated in tomato (Pérez-Alfocea *et al.* 2010).

The xanthophyll cycle-dependent nonconstitutive thermal dissipation is an important component of NPQ, which is a vital photoprotective process during various adverse environmental stresses (Qiu *et al.* 2003, Jiang *et al.* 2006, Zhou *et al*. 2006). In the current study, the Z level was significantly increased by the de-epoxidation of V under salt stress, particularly in the rootstock-grafted plants (Fig. 3*A*,*B*). The rootstock-grafted cucumber plants showed higher NPQ levels and xanthophyll cycle capacities, which suggests that thermal dissipation safely and effectively removes excess excitation energy when the cucumber plant is grafted onto a salt-tolerant rootstock, thereby delaying photoinhibition (Fig. 2*A*).

Electron microscopy showed that chloroplasts were significantly affected in the leaves of the NaCl-treated plants. Under the 90 mM NaCl treatment, the thylakoid membranes were swelled and the starch content was increased (Fig. 5). This response was similar with the ultrastructural observations of NaCl-treated wheat cultivars (Salama *et al.* 1994). The starch accumulation may be attributed to the higher rate of assimilate synthesis than the rate of assimilate export from the leaf (He *et al*. 1994). Compared with those of the rootstock-grafted plants, the larger starch grains of the nongrafted and self-grafted plants were a reflection of the inability of salinity-affected cells to mobilize starch. The immobilization could be due to either membrane disorders, resulting in an increase in membrane permeability, or to destruction of enzymes, which could decrease photosynthesis (Strid *et al.* 1990). When cucumber plants were grafted onto salt-tolerant rootstocks, the deleterious effect was mitigated. The ultrastructure alteration of the chloroplast in the nongrafted and self-grafted plants was at least partially caused by excess excitation energy that was not dissipated effectively.

In conclusion, the use of *C. moschata* rootstock alleviates salt stress in cucumber plants by delaying photoinhibition during the osmotic phase of salinity (15 days of salinity), probably due to a lower incidence of both stomatal and nonstomatal factors that limit photosynthesis. Hormonal and nutritional signals inherent to the rootstock affecting source and sink relations could be the basis for better regulation of scion processes under saline conditions. Furthermore, the similar responses of the nongrafted and self-grafted cucumber plants to salt stress suggest that the improved salt tolerance of the rootstock-grafted plants was more affected by the rootstock than the grafting itself.

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