

## Improving cucumber photosynthetic capacity under NaCl stress by grafting onto two salt-tolerant pumpkin rootstocks

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### Abstract

Cucumber plants were either self-grafted or grafted onto two salt-tolerant pumpkin rootstocks Chaojiqianwang (*Cucurbita moschata* Duch), and Figleaf Gourd (*Cucurbita ficifolia* Bouche). Plants were grown hydroponically in 0, 30, 60, or 90 mM NaCl for 16 d in greenhouse. Salinity induced a smaller decrease in plant shoot dry mass, leaf area, net photosynthetic rate, and stomatal conductance in the two rootstock-grafted plants compared to the self-grafted plants. In addition, a significant increase in intercellular CO<sub>2</sub> concentration, as well as a significant decrease in the initial and total ribulose-1,5-bisphosphate carboxylase/oxygenase activities were observed only in the self-grafted plants under 90 mM NaCl treatment. These results suggest that the use of salt tolerant rootstock can improve cucumber photosynthetic capacity under salt stress through both stomatal and non-stomatal pathways.

*Additional key words:* *Cucumis sativus*, *Cucurbita ficifolia*, *Cucurbita moschata*, net photosynthetic rate, salinity, stomatal limitation.

### Introduction

Recently, the use of salt-tolerant rootstock has been demonstrated to be a valid strategy in increasing salt tolerance in plants, such as melon (Romero *et al.* 1997), tomato (Estañ *et al.* 2005, Martínez-Rodríguez *et al.* 2008, Albacete *et al.* 2009, He *et al.* 2009), and watermelon (Goreta *et al.* 2008). Previous studies have also suggested that cucumber is a salt-sensitive plant (Chartzoulakis 1994), and that the use of a salt-tolerant pumpkin rootstock could improve its adaptation to salt stress (Yang *et al.* 2008). However, little is known of the physiological response mechanisms of cucumber plants grafted onto different rootstocks under salt stress.

Growth inhibition observed in many plants subjected to salinity is often associated with a decrease in their photosynthetic capacity (Chartzoulakis 2005, Stepień and Kłobus 2006, Zuccarini 2008, He *et al.* 2009). The reduction in photosynthesis caused by increased salinity can be due to several factors, including lower stomatal conductance, depression in specific metabolic processes

in carbon uptake, or a combination of these factors (Yeo *et al.* 1985, Flexas *et al.* 2004, Zhang *et al.* 2009).

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco; EC 4.1.1.39) is an enzyme that incorporates CO<sub>2</sub> into plants during photosynthesis and investigation of its activity provided information on the limitations of carboxylation brought about by salinity (Delfine *et al.* 1998, Flexas *et al.* 2004).

*Cucurbita ficifolia* and *Cucurbita moschata* are usually used as rootstocks for cucumber (Lee 1994). Our previous study suggested that cucumber plants grafted onto *C. moschata* have an ability to exclude Na<sup>+</sup> but not Cl<sup>-</sup> in the leaves (Zhu *et al.* 2008). We also found that cucumber plants grafted onto *C. ficifolia* have an ability to exclude Na<sup>+</sup> and Cl<sup>-</sup> in the leaves (Huang *et al.* 2009). Both rootstocks can improve cucumber salt tolerance.

The present research was designed to examine the photosynthetic responses to salt stress in cucumber plants grafted onto two salt-tolerant pumpkin rootstocks.

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*Abbreviations:* c<sub>i</sub> - intercellular CO<sub>2</sub> concentration; d.m. - dry mass; f.m. - fresh mass; g<sub>s</sub> - stomatal conductance; P<sub>N</sub> - net photosynthetic rate; Rubisco - ribulose-1,5-bisphosphate carboxylase/oxygenase; RWC - relative water content.

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## Materials and methods

**Plants and treatments:** The experiment was conducted in a greenhouse located at the National Center of Vegetable Improvement, Huazhong Agricultural University, central China (latitude 30° 27' N, longitude 114° 20' E, and altitude 22 m above sea level). *Cucumis sativus* L. cv. Jinchun No. 2 (Tianjin Kerun Cucumber Institute, Tianjin, China) was grafted onto two salt-tolerant pumpkin rootstocks: Chaojiquanwang (*Cucurbita moschata* Duch., Tangshan Four Seasons Seed Industry, Hebei, China), and Figleaf Gourd (*Cucurbita ficifolia* Bouche, Chuxiong Lumiao Agricultural Technology Development Research Institute, Yunnan, China) (Zhu *et al.* 2008, Huang *et al.* 2009), using the procedure of insertion grafting described by Lee (1994). Self-grafted cucumber plants were used as control.

At the two true-leaf stage, the grafted plants were transferred to 20 dm<sup>3</sup> plastic containers (12 plants per container) containing full strength Hoagland's solution (Hoagland and Arnon 1950). The nutrient solutions were renewed at an interval of 5 d and aerated continuously by an air pump. After pre-culture for 5 d, the plants were subjected to salt stress induced by a range of NaCl concentrations (0, 30, 60, and 90 mM), with an increment of 30 mM per day until the desired concentrations were obtained; this process made the electrical conductivity (EC) of the nutrient solutions 1.98, 4.80, 7.46 and 9.84 dS m<sup>-1</sup>, respectively. The treatments were defined by factorial combinations of four salt levels (0, 30, 60, and 90 mM) and three rootstocks (Jinchun No. 2, Figleaf Gourd, and Chaojiquanwang). The 12 treatment combinations were replicated four times, with 12 plants in each replicate, and then arranged in a randomized complete block design. During the culture, plants were grown under a photon flux density of 400 - 800 μmol m<sup>-2</sup> s<sup>-1</sup>, day temperature between 16 - 34 °C, night temperature between 15 - 24 °C and relative humidity between 42 - 87 %. At day 16 after NaCl treatment, gas exchange parameters were measured non-invasively on the youngest fully developed leaf, after which samples (whole plant or the youngest fully developed leaf) were taken. After sampling, the leaf RWC was measured using fresh leaves. The other leaves were immediately frozen in liquid nitrogen and stored at -76 °C for later measurements of Rubisco activity as well as soluble protein and sugar contents.

**Determination of plant growth, leaf RWC and Na<sup>+</sup> and Cl<sup>-</sup> contents:** Four plants per treatment were harvested and rinsed in de-ionized water. They were then carefully blotted with tissue paper. The materials were divided into shoots (the part above the graft union) and roots (the part below the graft union). The leaf area was determined using a leaf area meter (*LiCOR 3100*; *Li-COR*, Lincoln, NE, USA). The dry mass of the shoot and root were determined after oven-drying at 75 °C for

3 d. The youngest fully expanded leaves from four plants per treatment were used for measuring leaf RWC in accordance with the method described by Weatherley (1950).

The youngest fully expanded leaves of the plants were used for the determination of Na<sup>+</sup> and Cl<sup>-</sup> content. Na<sup>+</sup> and Cl<sup>-</sup> extraction and determination were performed as described by Xu *et al.* (2006). The Na<sup>+</sup> content was analyzed using an atomic absorption spectrophotometer (*Varian spectra AA 220*, *Varian*, Palo Alto, CA, USA), and the Cl<sup>-</sup> content was determined by silver nitrate (AgNO<sub>3</sub>) titration.

**Leaf gas exchange measurements:** Leaf net photosynthetic rate (P<sub>N</sub>), stomatal conductance (g<sub>s</sub>), and intercellular CO<sub>2</sub> concentration (c<sub>i</sub>) were measured using a gas exchange system (*Li-6400*, *Li-COR*). The leaf chamber was controlled to maintain the leaf temperature at 25 °C, CO<sub>2</sub> concentration at 360 μmol mol<sup>-1</sup>, and photosynthetic photon-flux density at 800 μmol m<sup>-2</sup> s<sup>-1</sup>. Leaf gas exchange parameters were measured alternately between treatments to avoid any environmental variations that could affect the measurements. Four replicate plants per treatment were measured between 09:00 and 12:00 on sunny days. During the measurements, the air temperature was 27.5 ± 0.4 °C, air relative humidity was 70 ± 0.7 %, and photosynthetic active radiation was 737 ± 65 μmol m<sup>-2</sup> s<sup>-1</sup> in the greenhouse.

**Determination of Rubisco activity:** Total Rubisco activity was measured spectrophotometrically by coupling 3-phosphoglyceric acid formation with NADH oxidation at 25 °C according to a process described in Lilley and Walker (1974) with some modifications (Nakano *et al.* 2000). The total activity was assayed after the crude extract was activated in 0.1 cm<sup>3</sup> activation mixture containing 33 mM Tris-HCl (pH 7.5), 0.67 mM EDTA, 33 mM MgCl<sub>2</sub>, and 10 mM NaHCO<sub>3</sub> for 15 min. Initial Rubisco activity measurements were performed in a 0.1 cm<sup>3</sup> reaction medium containing 5 mM HEPES-NaOH (pH 8.0), 1 mM NaHCO<sub>3</sub>, 2 mM MgCl<sub>2</sub>, 0.25 mM dithiothreitol, 0.1 mM EDTA, 1 U creatine phosphokinase, 1 U 3-phosphoglyceric phosphokinase, 1 U glyceraldehydes 3-phosphate dehydrogenase, 0.5 mM ATP, 0.015 mM NADH<sub>2</sub>, 0.5 mM phosphor-creatine, 0.06 mM RuBP, and 0.01 cm<sup>3</sup> extract. The change in absorbance at 340 nm was monitored for 90 s.

**Determination of soluble protein and sugar contents:** The youngest fully expanded leaves from the four sample plants per treatment were used. The soluble protein content was determined according to the process described by Bradford (1976) using bovine serum albumin as standard. The soluble sugar content was determined using the anthrone method (Spiro 1966).

**Statistical analysis:** The data were presented as means  $\pm$  SE of four replicates. Differences between NaCl concentrations of the respective rootstock-grafted plants were compared using Tukey's least significant difference (LSD) test at  $P < 0.05$ . A two-factorial analysis of variance (ANOVA) was also performed to study the

effects of rootstock, NaCl stress, and their interactions on the plants. Linear correlation analysis was conducted between the parameters of the grafted cucumber seedlings. Statistical analyses were performed using SAS statistical software (version 8.0, SAS Institute, Cary, NC, USA).

## Results

Plant shoot dry mass and leaf area decreased progressively with an increase in NaCl concentration. However, plants grafted onto Figleaf Gourd and especially the ones grafted onto Chaojiqianwang showed smaller reductions compared to the self-grafted plants (Table 1). Compared to the unstressed plants, 90 mM NaCl decreased the shoot dry mass of the self-grafted, Chaojiqianwang-grafted, and Figleaf Gourd-grafted plants by 78, 51, and 66 %, respectively. Meanwhile, the root dry mass of the self-grafted and Figleaf Gourd-grafted plants decreased gradually with an increase in NaCl concentrations. However, no significant decrease was observed in the Chaojiqianwang-grafted plants (Table 1). Increasing NaCl concentrations gradually decreased the leaf RWC of all plants. However, a lower reduction was observed in the plants grafted onto Chaojiqianwang and especially Figleaf Gourd under 90 mM NaCl stress, compared to the self-grafted plants (Table 1). Although the leaf  $\text{Na}^+$  content increased in all plants, there was a smaller increase in the Chaojiqianwang-grafted and Figleaf Gourd-grafted plants (Table 1). The Figleaf Gourd-grafted plants showed an apparent  $\text{Cl}^-$  exclusion from the leaves, but no

obvious difference was observed between the Chaojiqianwang-grafted and self-grafted plants (Table 1).

Higher concentrations of NaCl (60 mM and 90 mM) obviously decreased  $P_N$  and  $g_s$  in all plants. However, the Figleaf Gourd-grafted and especially Chaojiqianwang-grafted showed a smaller decrease compared to the self-grafted plants (Table 2). Moreover,  $c_i$  was significantly affected by the rootstock, NaCl stress, and their interactions (Table 2). Compared with the unstressed plants, the self-grafted plants showed an obvious increase in  $c_i$  under 90 mM NaCl, while the opposite trend was observed in the Chaojiqianwang-grafted and Figleaf Gourd-grafted plants (Table 2).

The initial and total Rubisco activities increased significantly under 90 mM NaCl stress for the Chaojiqianwang-grafted and Figleaf Gourd-grafted plants. In contrast, a significant decrease in the initial and total Rubisco activities was observed in the self-grafted plants (Table 2).

NaCl stress induced an obvious increase in the soluble protein content of the Figleaf Gourd-grafted plants, and the Chaojiqianwang-grafted plants showed a stable soluble protein content under NaCl stress (Table 2). The

Table 1. Shoot dry mass, root dry mass, leaf area, leaf relative water content (RWC),  $\text{Na}^+$  and  $\text{Cl}^-$  contents of cucumber grafted onto Jinchun No. 2 (self-grafted), Chaojiqianwang, and Figleaf Gourd under 0, 30, 60, or 90 mM NaCl stress for 16 d. Data are mean  $\pm$  SE ( $n = 4$ ). Different letters between the NaCl concentrations of the respective rootstock-grafted plants indicate significant differences according to Tukey's LSD test. \*\*\* represent  $P \leq 0.001$  and ns - not significant.

Rootstock	NaCl conc. [mM]	Shoot dry mass [g]	Root dry mass [g]	Leaf area [ $\text{cm}^2$ ]	Leaf RWC [%]	$\text{Na}^+$ content [ $\text{mg g}^{-1}(\text{d.m.})$ ]	$\text{Cl}^-$ content [ $\text{mg g}^{-1}(\text{d.m.})$ ]
Jinchun No. 2	0	7.47 $\pm$ 0.28a	1.32 $\pm$ 0.08a	1422 $\pm$ 74a	78.11 $\pm$ 0.97a	0.93 $\pm$ 0.33d	5.35 $\pm$ 0.75d
	30	5.05 $\pm$ 0.41b	0.95 $\pm$ 0.16b	1073 $\pm$ 111b	81.06 $\pm$ 0.65a	4.47 $\pm$ 0.67c	26.82 $\pm$ 6.73c
	60	2.93 $\pm$ 0.69c	0.55 $\pm$ 0.02c	487 $\pm$ 114c	71.11 $\pm$ 1.69b	10.14 $\pm$ 0.91b	69.01 $\pm$ 0.99b
	90	1.68 $\pm$ 0.07c	0.47 $\pm$ 0.04c	312 $\pm$ 32c	62.36 $\pm$ 3.89c	14.81 $\pm$ 0.67a	89.01 $\pm$ 5.54a
Chaojiqianwang	0	11.86 $\pm$ 1.71a	1.70 $\pm$ 0.26a	2034 $\pm$ 332a	80.79 $\pm$ 0.80a	0.62 $\pm$ 0.23b	4.85 $\pm$ 0.67d
	30	11.28 $\pm$ 0.93ab	1.67 $\pm$ 0.18a	2029 $\pm$ 175a	81.82 $\pm$ 0.50a	0.90 $\pm$ 0.19b	36.82 $\pm$ 2.98c
	60	8.11 $\pm$ 0.94bc	1.18 $\pm$ 0.18a	1563 $\pm$ 170ab	77.42 $\pm$ 0.56b	3.77 $\pm$ 0.70a	60.92 $\pm$ 7.30b
	90	5.82 $\pm$ 0.22c	1.36 $\pm$ 0.19a	941 $\pm$ 67b	76.23 $\pm$ 0.96b	2.84 $\pm$ 0.55a	93.47 $\pm$ 8.41a
Figleaf Gourd	0	12.08 $\pm$ 0.85a	1.34 $\pm$ 0.17a	2098 $\pm$ 258a	82.70 $\pm$ 0.85a	0.81 $\pm$ 0.24b	4.39 $\pm$ 0.77c
	30	9.26 $\pm$ 0.61b	0.79 $\pm$ 0.09b	1725 $\pm$ 141a	81.51 $\pm$ 0.85ab	1.85 $\pm$ 0.50ab	28.31 $\pm$ 3.96b
	60	6.77 $\pm$ 0.27c	0.64 $\pm$ 0.08b	1224 $\pm$ 83b	80.78 $\pm$ 0.36ab	3.08 $\pm$ 0.57a	35.22 $\pm$ 3.71b
	90	4.09 $\pm$ 0.39d	0.53 $\pm$ 0.09b	667 $\pm$ 39c	79.97 $\pm$ 0.54b	3.09 $\pm$ 0.52a	51.99 $\pm$ 5.69a
$F_{\text{Rootstock}}$		47.92***	27.14***	28.74***	16.78***	199.42***	19.70***
$F_{\text{NaCl}}$		44.63***	14.73***	35.03***	10.62***	120.31***	131.08***
$F_{\text{Rootstock} \times F_{\text{NaCl}}}$		0.82ns	0.96ns	0.79ns	5.10***	42.21***	6.00***

Table 2. Net photosynthetic rate ( $P_N$ ), stomatal conductance ( $g_s$ ), intercellular  $CO_2$  concentration ( $c_i$ ), initial Rubisco activity, total Rubisco activity, soluble protein, and soluble sugar contents of cucumber grafted onto Jinchun No. 2 (self-grafted), Chaojiqianwang, and Figleaf Gourd under 0, 30, 60, or 90 mM NaCl stress for 16 d. Data are mean  $\pm$  SE ( $n = 4$ ). Different letters between the NaCl concentrations of the respective rootstock-grafted plants indicate significant differences according to Tukey's LSD test. \*, \*\*, \*\*\* represent  $P \leq 0.05$ , 0.01 and 0.001, respectively, ns - not significant.

Rootstock	NaCl [mM]	$P_N$ [ $\mu\text{mol m}^{-2}\text{s}^{-1}$ ]	$g_s$ [ $\text{mmol m}^{-2}\text{s}^{-1}$ ]	$c_i$ [ $\mu\text{mol mol}^{-1}$ ]	Initial Rubisco activity [ $\mu\text{mol m}^{-2}\text{s}^{-1}$ ]	Total Rubisco activity [ $\mu\text{mol m}^{-2}\text{s}^{-1}$ ]	Soluble protein content [ $\text{mg g}^{-1}$ (f.m.)]	Soluble sugar content [ $\text{mg g}^{-1}$ (f.m.)]
Jinchun No. 2	0	15.2 $\pm$ 0.6a	315.6 $\pm$ 33.1a	270.6 $\pm$ 12.7b	9.1 $\pm$ 1.6a	21.9 $\pm$ 2.8a	23.3 $\pm$ 0.6a	14.2 $\pm$ 1.1c
	30	14.5 $\pm$ 1.3a	236.6 $\pm$ 18.8b	232.3 $\pm$ 17.8c	9.9 $\pm$ 1.9a	18.6 $\pm$ 2.0ab	24.2 $\pm$ 1.3a	15.2 $\pm$ 0.2bc
	60	10.9 $\pm$ 0.9b	127.9 $\pm$ 26.5c	221.7 $\pm$ 17.3c	9.5 $\pm$ 2.3a	19.3 $\pm$ 3.9ab	22.7 $\pm$ 0.8a	19.0 $\pm$ 0.9ab
	90	6.1 $\pm$ 1.0c	69.5 $\pm$ 12.4d	338.3 $\pm$ 13.5a	5.0 $\pm$ 0.5b	8.7 $\pm$ 1.7b	13.7 $\pm$ 1.7b	20.0 $\pm$ 2.3a
Chaojiqianwang	0	15.8 $\pm$ 0.8a	302.2 $\pm$ 26.9a	268.8 $\pm$ 2.5a	6.1 $\pm$ 0.5b	17.4 $\pm$ 1.6b	21.4 $\pm$ 1.1a	16.1 $\pm$ 2.4c
	30	15.6 $\pm$ 0.4a	309.9 $\pm$ 32.7a	264.0 $\pm$ 7.7a	6.2 $\pm$ 1.0b	16.6 $\pm$ 1.2b	20.1 $\pm$ 1.4a	17.3 $\pm$ 0.4bc
	60	16.6 $\pm$ 0.3a	287.9 $\pm$ 22.5a	247.6 $\pm$ 8.3a	8.4 $\pm$ 1.6b	16.1 $\pm$ 0.9b	20.4 $\pm$ 0.6a	22.1 $\pm$ 1.7ab
	90	14.0 $\pm$ 0.8b	191.6 $\pm$ 29.3b	218.3 $\pm$ 13.5b	13.6 $\pm$ 0.5a	23.4 $\pm$ 4.0a	19.8 $\pm$ 1.4a	25.9 $\pm$ 0.1a
Figleaf Gourd	0	15.6 $\pm$ 0.5a	342.5 $\pm$ 51.3a	271.3 $\pm$ 8.2a	8.9 $\pm$ 1.4c	17.3 $\pm$ 1.8c	17.3 $\pm$ 1.5b	16.6 $\pm$ 2.5c
	30	15.9 $\pm$ 0.5a	299.0 $\pm$ 28.9a	256.7 $\pm$ 7.7a	12.1 $\pm$ 1.4bc	20.8 $\pm$ 1.5c	22.2 $\pm$ 1.0a	23.5 $\pm$ 1.1b
	60	13.4 $\pm$ 0.4b	181.1 $\pm$ 23.1b	191.7 $\pm$ 5.9b	14.2 $\pm$ 1.7b	29.9 $\pm$ 1.9b	24.6 $\pm$ 1.2a	28.5 $\pm$ 0.8a
	90	12.2 $\pm$ 0.5c	99.3 $\pm$ 8.2c	154.5 $\pm$ 11.4c	20.4 $\pm$ 1.1a	41.7 $\pm$ 1.0a	24.9 $\pm$ 0.1a	31.2 $\pm$ 0.8a
$F_{\text{Rootstock}}$		19.01***	14.00***	6.72***	6.51***	10.03***	2.77ns	30.44***
$F_{\text{NaCl}}$		13.36***	23.88***	4.07**	3.23*	3.38*	4.65**	29.13***
$F_{\text{Rootstock} \times F_{\text{NaCl}}}$		3.87**	2.96*	10.67***	2.33ns	5.59***	11.80***	2.08ns

soluble sugar content of all plants increased progressively with an increase in NaCl concentrations, and the plants grafted onto Chaojiqianwang and especially Figleaf

Gourd showed a bigger increase in soluble sugar content compared to the self-grafted plants (Table 2).

## Discussion

In the present study, the Chaojiqianwang-grafted plants showed higher salt tolerance than the plants grafted onto Figleaf Gourd. This suggests that the salt tolerance of cucumber plants differed with rootstocks. Compared with the shoot, the root growth of all grafted plants was less affected by salt stress, which may be attributed to the less sensitivity of the root growth than shoot growth to salinity (Nomura *et al.* 1998, Munns and Tester 2008).

Stomatal and non-stomatal limitations to photosynthesis under salt or water stress are common phenomena (Steduto *et al.* 2000, Flexas *et al.* 2004, Santos *et al.* 2009, Cai *et al.* 2010). A previous study suggested that mild to severe salt stress levels predominantly affect the diffusion of  $CO_2$  in leaves through a decrease of stomatal and mesophyll conductances, but it does not affect the biochemical capacity to assimilate  $CO_2$  (Flexas *et al.* 2004). In the present study, stomatal limitation is suggested to play an important role in the reduction of photosynthetic rate in the Figleaf Gourd-grafted and Chaojiqianwang-grafted plants under NaCl stress. This is supported by the concurrent decrease of  $g_s$  and  $c_i$  under NaCl treatment. In contrast, the decrease of photosynthetic rate in the self-grafted plants could be attributed to both stomatal and

non-stomatal limitations after treatment with 90 mM NaCl. This could be confirmed by a decrease in  $g_s$  and an accompanying increase in  $c_i$ .

Rubisco is one of the most abundant proteins in plants. It is widely accepted as the ultimate rate-limiting step in photosynthetic carbon fixation (Jensen 2000). In the present study, both the initial and total Rubisco activities decreased in the self-grafted plants under 90 mM NaCl stress. In contrast, both the initial and total Rubisco activities increased under some circumstances (*e.g.*, in the Chaojiqianwang-grafted and Figleaf Gourd-grafted plants under 90 mM NaCl). Similarly, previous studies found that the Rubisco activity of spinach was not affected by up to 20 d of exposure to 1 % (m/v) NaCl solution (Delfine *et al.* 1998). The initial and total Rubisco activities in fully expanded tomato leaflets increased as a result of treatment with 50 or 100 mM NaCl (Mäkelä *et al.* 2000). The soluble protein content also increased, indicating that it may have an important role in plant adaptation to a high NaCl content (Takabe *et al.* 1988).

With the onset of salinity stress, the reduced rate of photosynthesis is not the only cause of growth reduction because there is also a rapid change in leaf expansion

rates (Cramer and Bowman 1991, Passioura and Munns 2000, Fricke *et al.* 2004). In this study, the shoot dry mass was significantly correlated with the net photosynthetic rate ( $r = 0.81$ ,  $P < 0.001$ ) and the leaf area ( $r = 0.99$ ,  $P < 0.001$ ). This suggests that salinity influences cucumber growth through a reduction in both photosynthetic rate and leaf area.

NaCl stress can induce a change in leaf water status, leading to a decrease in stomatal conductance and the perturbation of the Calvin cycle (Flexas *et al.* 2004, Kholová *et al.* 2009). Moreover, excessive accumulation of  $\text{Na}^+$  and  $\text{Cl}^-$  is toxic and may disrupt the integrity of the photosynthetic apparatus (Yeo *et al.* 1985, Bethke and Drew 1992, Aghaleh *et al.* 2009). Lower  $\text{Na}^+$  accumulation and higher relative water content were observed in both the Chaojiqianwang-grafted and Figleaf Gourd-grafted plants, which may partly explain why these rootstock-grafted plants had higher photosynthetic capacities.

In our study, Chaojiqianwang was more effective than Figleaf Gourd in increasing the gas exchange capacity of cucumber under NaCl stress. Compared to the Chaojiqianwang-grafted plants, the Figleaf Gourd-grafted plants had a higher soluble sugar content. However, a higher accumulation of soluble sugar means more energy cost, even though it can help absorb water from the salt solution by decreasing leaf water osmotic

potential. It can also help act as a compatible compound for protecting large molecules, such as enzymes from damage (Murakeozy *et al.* 2003). In addition, a higher accumulation of soluble sugar may also result in a feedback to plant photosynthesis (Paul and Foyer 2000, Iglesias *et al.* 2002). In contrast, Chaojiqianwang-grafted plants accumulated more  $\text{Cl}^-$  than soluble sugar for osmotic adjustment with less energy cost. Rather than  $\text{Cl}^-$ ,  $\text{Na}^+$  has been reported as the primary cause of salt damage in cucumber. This appears to be the reason for the accumulation of a large amount of  $\text{Cl}^-$  in Chaojiqianwang, while showing a lower degree of growth inhibition (Trajkova *et al.* 2006). The present study demonstrated the importance of osmotic components, while taking into consideration the salt tolerance mechanism of grafted plants.

In conclusion, Chaojiqianwang-grafted and Figleaf Gourd-grafted plants improved the photosynthetic capacity of cucumber plants by increasing the gas exchange capacity and/or the Rubisco activity under NaCl stress, particularly under 90 mM NaCl. The higher photosynthetic capacity of the Chaojiqianwang-grafted and Figleaf Gourd-grafted plants could be partly attributed to the lower  $\text{Na}^+$  content and higher RWC in the leaves. In addition, Chaojiqianwang was more effective than Figleaf Gourd in increasing the photosynthetic capacity of cucumber plants.

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