DOI: 10.1007/s11099-012-0002-1 PHOTOSYNTHETICA 50 (2): 180-188, 2012

Improving melon and cucumber photosynthetic activity, mineral composition, and growth performance under salinity stress by grafting onto *Cucurbita* hybrid rootstocks

Y. ROUPHAEL[*], M. CARDARELLI[**], E. REA[***], and G. COLLA[**+]

Department of Crop Production, Faculty of Agricultural Engineering and Veterinary Medicine, Lebanese University, Dekwaneh, Beirut, Lebanon[*]
Department of Agriculture, Forests, Nature and Energy (DAFNE), University of Tuscia, 01100 Viterbo, Italy[**]
Agricultural Research Council - Research Centre for the Soil-Plant System, 00184 Roma, Italy[***]

Abstract

The aim of the current work was to determine whether grafting could improve salinity tolerance of melon and cucumber, and whether possible induction of tolerance to salt stress was associated with the protection of the photosynthetic apparatus. Two greenhouse experiments were carried out to determine gas exchange, mineral composition, growth and yield of melon (*Cucumis melo* L. cv. Cyrano) and cucumber (*Cucumis sativus* L. cv. Akito) plants, either ungrafted or grafted onto the *Cucurbita* hybrid rootstocks (*Cucurbita maxima* Duch. × *Cucurbita moschata* Duch.), 'P360', and 'PS1313', respectively. Plants were grown hydroponically and supplied with two nutrient solutions – a nonsalinized control and a salinized solution which contained 40 mmol L^{-1} of NaCl. Salinity induced a smaller decrease in leaf area index (LAI), in grafted- compared to ungrafted plants. Similarly, the P_N and g_s reduction in NaCl treatment compared to control were significantly lower in grafted plants (34% and 34%, respectively, for melon and 14% and 15.5%, respectively, for cucumber) compared to ungrafted plants (42% and 40%, respectively, for melon and 30% and 21%, respectively, for cucumber). In all grafting combinations, negative correlations were recorded between Na^+ and Cl^- in the leaf tissue and P_N. Grafting reduced concentrations of sodium, but not chloride, in leaves. Under saline conditions a smaller reduction in melon and cucumber shoot biomass dry mass and fruit yield were recorded, with positive correlations between shoot biomass, yield and P_N. These results suggest that the use of salt tolerant *Cucurbita* rootstock can improve melon and cucumber photosynthetic capacity under salt stress and consequently crop performance.

Additional key words: *Cucumis melo*; *Cucumis sativus*; *Cucurbita maxima* × *Cucurbita moschata*; grafting; net photosynthetic rate; salinity.

Introduction

Grafting vegetable, including cucurbits (*e.g*. cucumber, melon, and watermelon), is a common practice in Japan, Korea, the Mediterranean basin, and several European countries. As with other vegetables, the main purpose of employing this technology in cucurbits is to control *Fusarium wilt* and other soilborne diseases (Crinò *et al.* 2007, Lee *et al.* 2010). Grafting restricts input of agro-chemicals against soilborne pathogens and is, therefore, considered an environment friendly cultivation technique, which is strongly recommended for integrated crop management systems (Rivard and Louws 2008). However, the impact of grafting on cucurbits includes not only a stronger resistance against pathogens but also a higher tolerance to abiotic stress conditions such as salinity, heavy metal, nutrient stress, thermal stress, water stress, organic pollutants, and alkalinity (Colla *et al*. 2010a,b,c, 2011; Rouphael *et al*. 2008 a,b; Savvas *et al*. 2009, 2010; Schwarz *et al*. 2010).

Salinity continues to be a major factor in reduced crop productivity and profit in many arid and semiarid regions, despite the advanced management techniques developed in recent decades (Edelstein *et al*. 2011). Salinity affects almost every aspect of the physiology and biochemistry of plants and significantly reduces growth and yield (Cuartero *et al.* 2006). The growth inhibition observed in many plants subjected to salinity is often combined with

Received 10 August 2011, *accepted* 9 November 2011.
[+]Corresponding author; fax: + 39 0761357453, e-mail: giucolla@unitus.it
Abbreviations: EC – electrical conductivity; LA – leaf area; LAI – leaf area index; g_s – stomatal conductance; P_N – net photosynthetic rate; R/S – root-to-shoot ratio.

a decrease in their photosynthetic capacity as a result of stomatal and/or nonstomatal limitation (Debez *et al*. 2008). The decrease in net photosynthetic rate induced by salinity depends also on the plant genotype. In general, net photosynthetic rate and stomatal conductance in salt-tolerant genotypes are less affected by salinity than in salt-sensitive genotypes (Naumann *et al.* 2007, Zhou *et al.* 2007, Lopez-Climent *et al.* 2008).

As a rapid alternative to breeding in combating problems caused by irrigation with saline water, grafting of existing elite, commercial cultivars onto selected rootstocks could be a promising tool (Colla *et al.* 2010c).

Previous studies on melon (*C. melo* L.) (Ruiz *et al*. 1997), and cucumber (*C. sativus* L.) (Huang *et al*. 2009a,b; Zhu *et al*. 2008) suggested that the enhanced salt tolerance of grafted vegetables has often been associated with lower Na^+ and/or Cl^- concentrations in the shoot. However, the above studies have been carried out using rootstocks such as *C. maxima* for melon, and *C. moschata*, *C. ficifolia*, and *Lagenaria siceraria* for cucumber, whereas limited information is available on the physiological response mechanisms of melon and cucumber grafted onto *Cucurbita* hybrid (*C. maxima* Duch. × *C. moschata* Duch.) rootstocks to salinity. The *Cucurbita* hybrid rootstocks are becoming very popular in Italy due to their higher compatibility with melon, cucumber and watermelon cultivars in comparison to the former ones (Nisini *et al*. 2002). Thus, investigation aimed at clarifying whether the *Cucurbita* hybrid rootstocks have the potential to improve the salt tolerance of melon and cucumber plants is a basic requirement for the continued success of grafting (Colla *et al.* 2010c).

In the present study, we exposed ungrafted and rootstock grafted melon and cucumber plants to two different salinity levels of nutrient solutions (nonsalinized control, or 40 mM NaCl) and investigated whether grafting could improve tolerance to salinity, and whether the induced tolerance to salt stress was associated with a protective effect on the photosynthetic apparatus. Grafted and ungrafted plants were compared in terms of gas exchange, mineral composition, growth, and yield.

Materials and methods

Plant material, treatments and growth conditions: Two experiments were conducted, one on melon (Exp. 1) and one on cucumber (Exp. 2) in a polyethylene greenhouse situated on the Experimental Farm of Tuscia University, Central Italy (42°25'N; 12°08'E; 310 m a.s.l.). Plants were grown under natural light conditions. In both experiments, the greenhouse was maintained at daily temperatures between 18 and 33°C, and day/night relative humidities of 55/85%. In Exp. 1, *C. melo* L. cv. Cyrano (SAIS Seed Company, Cesena, Italy) was grafted onto the commercial rootstock 'P360' (*C. maxima* Duch. × *C. moschata* Duch.; SAIS Seed Company, Cesena, Italy), using the procedure of "cleft grafting" described by Lee (1994), while ungrafted cv. Cyrano was used as a control. In Exp. 2, *C. sativus* L. cv. Akito (Enza Zaden, Verona, Italy) was grafted onto the commercial 'PS1313' rootstock (*C. maxima* × *C. moschata*; *Peto Seeds*, California, USA) using the 'tongue approach grafting' described also by Lee (1994), whereas ungrafted 'Akito' was used as a control plant. The two *Cucurbita* rootstocks ('P360' and 'PS1313') were selected as the most representative commercial rootstocks used in Italy due to their compatibility with melon and cucumber cultivars, respectively, and their resistance to soilborne pathogens. Grafted and ungrafted melons and cucumbers were transplanted at the two true leaf stages into pots containing pumice (Exp. 1) and quartziferous sand (Exp. 2). In both experiments, pots were disposed in double rows on the greenhouse floor, with a plant density of 2.1 and 3 plants m^{-2} for melon and cucumber, respectively. Plants were trained vertically using plastic nets and nylon wires for melon and cucumber experiment, respectively.

The two experiments were designed as a factorial combination of two nutrient solutions (nonsalinized control, or 40 mM NaCl) and two grafting treatments (ungrafted or grafted). Each experimental unit included five and eight melon and cucumber, respectively. The treatments were arranged in a randomized complete block design with three replicates per treatment.

Nutrient solution management: In both experiments the saline treatments were initialized 10 days after transplanting. The basic (control) nutrient solution used in both experiments was a modified Hoagland and Arnon formulation. All chemicals used were of analytical grade, and composition of the melon basic nutrient solution was: 13.6 mM N-NO_3, 2.0 mM S, 1.4 mM P, 6.0 mM K, 5.2 mM Ca, 2.0 mM Mg, 1.0 mM Na, 1.0 mM Cl, 20 μM Fe, 9 μM Mn, 1.5 μM Cu, 3 μM Zn, 20 μM B and 0.3 μM Mo, whereas the composition of the basic nutrient solution in the cucumber experiment was: 14.0 mM NO_3–N, 1.0 mM NH_4–N, 1.5 mM S, 1.5 mM P, 6.0 mM K, 5.0 mM Ca, 1.5 mM Mg, 1.0 mM Na, 1.0 mM Cl, 20 μM Fe, 9 μM Mn, 0.3 μM Cu, 1.6 μM Zn, 20 μM B, and 0.3 μM Mo. Both basic nutrient solutions had an electrical conductivity (EC) of about 2.0 dS m^{-1}. The saline nutrient solutions had the same basic composition plus an additional 39 mM of NaCl, giving an average EC value of 6.3 dS m^{-1}. The pH of the nutrient solution for all treatments was 6.0 ± 0.3. All nutrient solutions were prepared by using deionized water.

In both experiments, nutrient solution was provided from independent tanks to each experimental unit through a drip irrigation system, with one emitter per plant and an

emitter flow rate of 2 L h^{-1}. Irrigation scheduling was performed using electronic low tension tensiometers (*LT Irrometer*; Riverside, CA, USA) that controlled irrigation based on substrate matric potential (Norrie *et al*. 1994). In each treatment, four tensiometers were installed and located in different pots to provide representative readings of the moisture tension. Tensiometers were connected to an electronic programmer that controlled the beginning (–5 kPa) and end (–1 kPa) of irrigation, which correspond to the high and low tension set points for the major part of the media (Kiehl *et al.* 1992). Five to seventeen fertigations were applied per day, each of 1–3 min duration. Timing of the irrigations was increased to have at least 35% of the nutrient solution draining from the pots.

Yield and growth measurements: In both experiments, fully mature fruits were harvested from 3 June to 21 July (Exp. 1), and from 4 April to 1 June (Exp. 2), and the number of fruits, mean fruit mass, and total yield were determined for each experimental plot. At final harvest 122 and 64 days after transplanting in Exps. 1 and 2, respectively, plants were separated into stems, leaves, and roots and their tissues were dried in a forced-air oven at 80°C for 72 h. Shoot biomass was equal to the sum of aerial vegetative plant parts (leaves + stems). Root-to-shoot ratio (R/S) was calculated by dividing root dry mass by the sum of leaf and stem dry masses. Leaf area (LA) was measured using an electronic area meter (*LI-COR Model 3100, Delta-T Devices Ltd*., Cambridge, UK). LAI was computed as green leaf area divided by ground area.

Net photosynthetic rate and stomatal conductance measurements: One week before the final harvest, leaf net photosynthetic rate (P_N) and stomatal conductance (g_s) were determined using portable photosynthesis systems (*LI-6200; LI-COR Inc., Lincoln*, NE, USA). Those measurements were made on the most recent fully expanded leaves between 10:00 and 12:00 h on sunny days, using six replicate leaves per treatment. The *LI-6200* was equipped with a well stirred 2.5×10^{-5} m^3 leaf chamber with constant area inserts (1.2×10^{-3} m^2) and fitted with a variable intensity red source (*Model QB1205LI-670, Quantum Devices Inc*. Barneveld, WI, USA) (Tennessen *et al*. 1994). Leaf temperature within the chamber was 30 ± 2°C, vapour pressure difference between the leaf and air was 2.6 ± 0.3 kPa, and CO_2 concentration was 365 ± 10 μl L^{-1}. During the measurements, the air temperature was 27.1 ± 0.5 °C, air relative humidity was 70 ± 1.0 %, and photosynthetic active radiation was 689 ± 68 μmol m^{-2} s^{-1} in the greenhouse.

Mineral analysis: Dried plant tissues (leaf and root) were ground separately in a Wiley mill to pass through a 20-mesh screen, then 0.5 g of the dried plant tissues were analyzed for the following macro and micronutrients: N, P, K, Na, and Cl. N concentration in the plant tissues was determined after mineralization with sulfuric acid according to the Kjeldahl method (Bremner 1965); P, K, and Na concentrations were determined by dry ashing at 400°C for 24 h, dissolving the ash in 1:20 HNO_3, and assaying the solution obtained by using of an inductively coupled plasma emission spectrophotometer (*ICP Iris; Thermo Optek*, Milano, Italy) (Isaac and Johnson 1998). Chloride ion concentrations were also determined by dry ashing at 400°C for 24 h, dissolving the ash in 1:20 HNO_3, and by titration with $AgNO_3$ in the presence of K_2CrO_4 (Eaton *et al.* 1995).

Statistical analysis: All data were statistically analyzed by applying *ANOVA* using the *SPSS* software package (*SPSS 10 for Windows*, 2001, Chicago, IL, USA). *Duncan*'s multiple range test was performed at p=0.05 on each of the measured significant variables. In both experiments, regression analysis was conducted to identify relationships between the measured parameters (P_N, Na^+ and Cl^- concentration in leaf tissue, shoot biomass, dry mass, and yield).

Results

Leaf gas exchange: In the melon experiment, LAI was significantly affected by salinity; the highest values were recorded in the nonsaline treatment in comparison to plants treated with NaCl, whereas, in the cucumber experiment the highest LAI was observed in both grafting combinations receiving non saline nutrient solution, followed by grafted plants treated with NaCl, and finally on ungrafted plants treated with NaCl (Table 1). Under normal growth conditions, grafting did not have any significant effect on leaf gas exchange of melon and cucumber, as similar P_N and g_s were found in ungrafted and grafted plants, while, high concentrations of NaCl (40 mM) decreased the P_N and g_s in both grafting combinations. However, the grafted plants showed a smaller decrease compared to the ungrafted plants. The relative reduction of P_N and g_s in the NaCl treatment, with reference to control, were significantly lower in grafted plants (34% and 34%, respectively, for melon and 14% and 15.5%, respectively, for cucumber) than in ungrafted plants (42% and 40%, respectively, for melon and 30% and 21%, respectively, for cucumber). Finally, the data showed an inverse linear relationship between P_N and leaf Na^+ and leaf Cl^- concentrations for both melon and cucumber plants (Fig. 1).

Mineral composition: The data indicating the impact of grafting combination and salinity on the macro- and microelements concentration of melon and cucumber

Table 1. Effects of NaCl concentration and grafting combination on leaf area index (LAI), net photosynthetic rate (P_N), and stomatal conductance (g_s) of melon and cucumber plants. Values are the means of three replicate samples. *Different letters* within a column indicate significant differences (p<0.05, LSD test). NS, *, **, *** – nonsignificant or significant at p<0.05, 0.01 or 0.001, respectively.

NaCl [mM]	Grafting combination	Melon LAI	Melon P_N [μmol(CO_2) m^{-2} s^{-1}]	Melon g_s [mmol m^{-2} s^{-1}]	Cucumber LAI	Cucumber P_N [μmol(CO_2) m^{-2} s^{-1}]	Cucumber g_s [mmol m^{-2} s^{-1}]
0	Ungrafted	2.21	22.4[a]	211.0[a]	2.67[a]	23.0[a]	197.4
	Grafted	2.33	23.7[a]	228.2[a]	2.55[a]	23.9[a]	202.4
	Mean	2.2 [a]	23.1	219.6	2.61	23.5	199.9[a]
40	Ungrafted	1.19	12.8[c]	127.3[c]	1.44[c]	16.1[c]	155.4
	Grafted	1.23	15.6[b]	149.9[b]	1.59[b]	20.5[b]	170.9
	Mean	1.21[b]	14.2	138.6	1.52	18.3	163.2[b]
Significance							
NaCl concentration (S)		***	**	***	**	**	***
Graft combination (G)		NS	*	**	NS	*	NS
S × G		NS	*	*	*	*	*

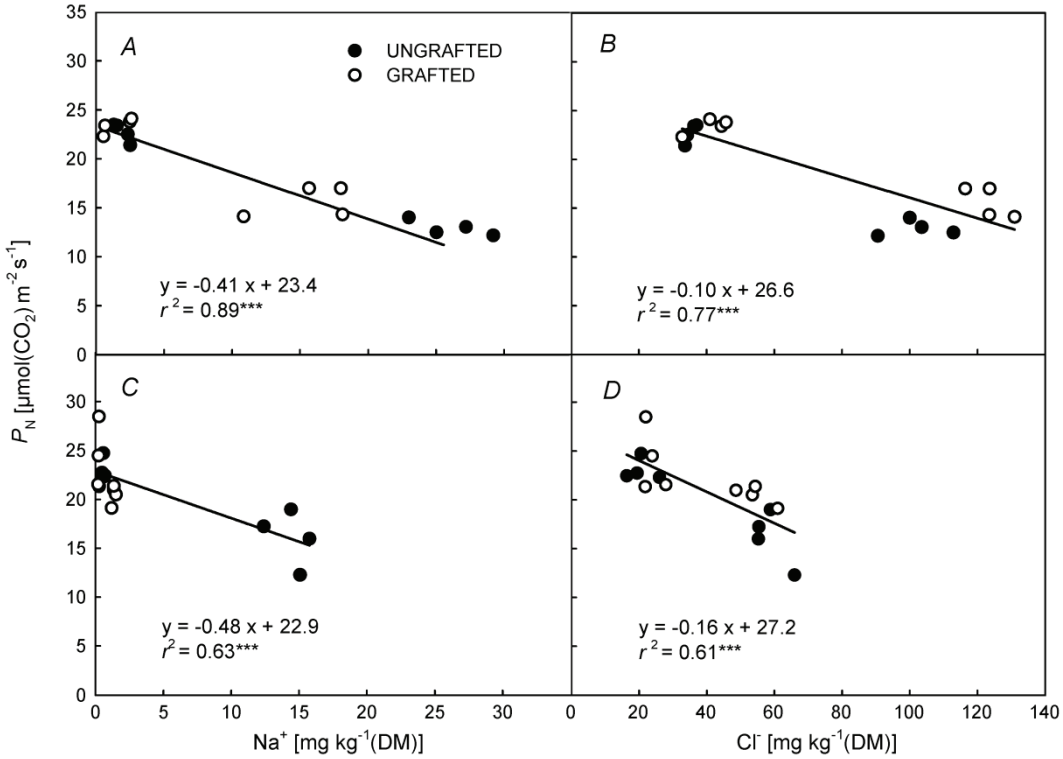

Fig. 1. Relationship between net photosynthetic rate (P_N) and (*A*) leaf Na^+ concentration, and (*B*) leaf Cl^- concentration in melon grafting combinations, and (*C*) leaf Na^+ concentration, (*D*) leaf Cl^- concentration in cucumber grafting combinations.

leaves and roots are displayed in Tables 2 and 3, respectively. In both experiments, the concentrations of N, P, and K in leaves and roots were significantly affected by salinity where the highest values were recorded in the nonsalinized treatment in comparison to plants treated with NaCl. The K concentrations in melon and cucumber leaves exhibited highly significant differences by salinity × grafting combination interactions with values recorded for grafted plants (22.1 and 25.8 g kg^{-1} for melon and cucumber, respectively) being higher than ungrafted plants (16.3 and 16.2 g kg^{-1} for melon and cucumber, respectively) under saline conditions (Tables 2, 3).

The Na^+ concentrations in melon and cucumber leaves and roots increased as the salinity level in the nutrient solution was raised (Tables 2, 3). The relative accumulation of Na^+ in melon and cucumber leaf tissues of plants treated with NaCl, with reference to control, were significantly lower in grafted plants (981%, and

Table 2. Effects of NaCl concentration and grafting combination on mineral concentration of leaves and roots in melon plants. Values are the means of three replicate samples. *Different letters* within a column indicate significant differences (p<0.05, LSD test). NS, *, **, *** – nonsignificant or significant at p<0.05, 0.01 or 0.001, respectively.

NaCl [mM]	Grafting combination	N [g kg^{-1}(DM)]		P [g kg^{-1}(DM)]		K [g kg^{-1}(DM)]		Na [g kg^{-1}(DM)]		Cl [g kg^{-1}(DM)]	
		Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots
0	Ungrafted	36.7	24.5	4.7	6.6	35.2^{a}	26.1	1.9^{c}	14.7	35.3	34.9
	Grafted	37.1	23.1	4.4	6.5	38.6^{a}	24.2	1.6^{c}	16.2	40.9	37.0
	Mean	36.9^{a}	23.8^{a}	4.5^{a}	6.5^{a}	36.9	25.2^{a}	1.8	15.4^{b}	38.1^{b}	36.0^{b}
40	Ungrafted	28.9	12.8	4.1	4.6	16.3^{c}	13.3	26.1^{a}	32.6	101.7	85.3
	Grafted	29.5	15.0	3.1	5.3	22.1^{b}	17.4	15.7^{b}	35.0	123.6	81.9
	Mean	29.2^{b}	13.9b	3.6^{b}	4.9^{b}	19.2	15.4^{b}	20.9	33.8^{a}	112.7^{a}	83.6^{a}
Significance											
NaCl concentration (S)		***	**	*	*	***	*	***	***	***	***
Graft combination (G)		NS	NS	NS	NS	NS	NS	**	*	NS	NS
S × G		NS	NS	NS	NS	*	NS	*	NS	NS	NS

Table 3. Effects of NaCl concentration and grafting combination on mineral concentration of leaves and roots in cucumber plants. Values are the means of three replicate samples. *Different letters* within a column indicate significant differences (p<0.05, LSD test). NS, *, **, *** – nonsignificant or significant at p<0.05, 0.01 or 0.001, respectively.

NaCl [mM]	Grafting combination	N [g kg^{-1}(DM)]		P [g kg^{-1}(DM)]		K [g kg^{-1}(DM)]		Na [g kg^{-1}(DM)]		Cl [g kg^{-1}(DM)]	
		Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots
0	Ungrafted	41.6	26.3	6.7	11.7	34.6^{a}	16.7	0.6^{c}	1.5^{c}	20.6	15.2
	Grafted	40.6	28.2	6.3	18.5	32.2^{a}	24.2	0.5^{c}	3.9^{c}	23.9	10.8
	Mean	41.1^{a}	27.2a	6.5^{a}	15.1^{a}	33.4	20.4^{a}	0.4	2.7	22.3^{b}	13.0^{b}
40	Ungrafted	33.1	13.6	5.4	9.4	16.2^{c}	10.4	14.4^{a}	8.8^{b}	58.8	45.6
	Grafted	35.5	14.1	5.7	14.9	25.8^{b}	11.0	4.3^{b}	20.2^{a}	54.3	42.5
	Mean	34.3^{b}	14.3c	5.5^{b}	12.2^{b}	21.0	10.7^{b}	9.4	14.5	56.6^{a}	44.0^{a}
Significance											
NaCl concentration (S)		*	**	**	*	**	***	***	***	***	***
Graft combination (G)		NS	NS	NS	***	***	**	***	***	NS	NS
S × G		NS	NS	NS	NS	**	NS	***	***	NS	NS

860%, respectively) in comparison with that estimated for ungrafted plants (1,374%, and 2,400%, respectively). Finally, the concentration of Cl^- in melon and cucumber tissues was significantly affected by salinity, but not by the grafting combination (Tables 2, 3). The concentration of Cl^- increased as the salinity level in the nutrient solution was raised. The highest Cl^- concentrations were observed in the leaves (Tables 2, 3).

Growth and yield: In the melon experiment, the shoot dry mass decreased (avg. from 1.67 to 0.98 t ha^{-1}) and R/S increased (avg. from 0.07 to 0.11) in response of nutrient solution salinity, the shoot dry mass was significantly higher in grafted (avg. 1.44 t ha^{-1}) than ungrafted melon plants (avg. 1.20 t ha^{-1}). In the cucumber experiment, the highest shoot dry mass was observed in both grafting combinations receiving nonsaline nutrient solution (avg. 2.80 t ha^{-1}), followed by grafted plants treated with NaCl (avg. 1.73 t ha^{-1}), and finally on ungrafted plants treated with NaCl (avg. 1.50 t ha^{-1}). When averaged over grafting combinations the root dry mass decreased (avg. from 0.25 to 0.20 t ha^{-1}) and R/S increased (avg. from 0.09 to 0.13) in response to nutrient solution salinity.

In grafted and ungrafted plants, total yield, fruit mean mass, and number of melon and cucumber fruit decreased in response to an increase of salinity concentration in the nutrient solution (data not shown). The lowest yield observed in melon and cucumber plants treated with NaCl as compared with the control treatment was mainly attributed to a reduction in both the average fruit mass and the number of fruit per plant (data not shown). In the melon experiment, the increase of the nutrient solution salinity significantly reduced the total yield (avg. from 48.2 to 26.8 t ha^{-1}). Moreover, the total melon yield was significantly higher by 13% in grafted (avg. 39.7 t ha^{-1}) than in ungrafted plants (avg. 35.2 t ha^{-1}).

In the cucumber experiment the highest total yield was observed in both grafting combinations receiving nonsalinized nutrient solution (avg. 114.1 t ha^{-1}), followed by the grafted plants treated with NaCl (avg. 72.4 t ha^{-1}), and finally on ungrafted plants treated with NaCl

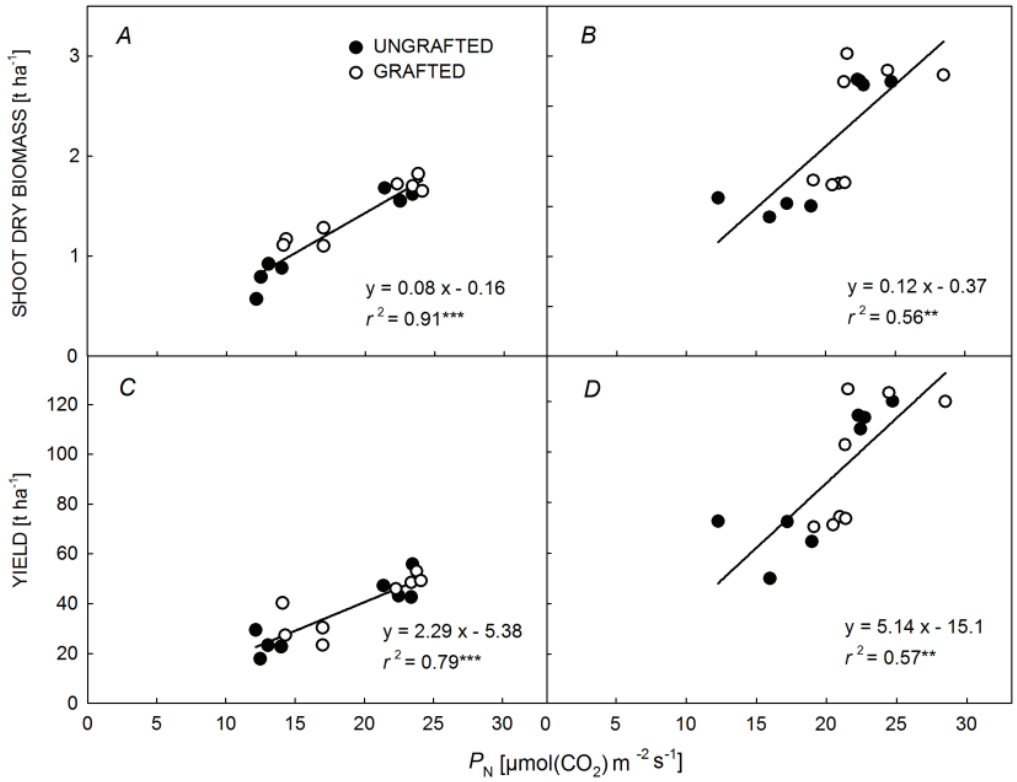

Fig. 2. Relationship between net photosynthetic rate (P_N) and (*A*) shoot biomass, and (*C*) fruit yield in melon grafting combinations, and (*B*) shoot biomass, and (*D*) fruit yield in cucumber grafting combinations.

(avg. 64.9 t ha^{-1}). Finally, the current data showed a positive linear relationship between P_N and shoot biomass dry mass or total yield for both melon and cucumber plants (Fig. 2).

Discussion

Growth inhibition observed in many plants subjected to salinity is often associated with a decrease in their photosynthetic capacity (Chartzoulakis 2005, Stępień 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 and depression in specific metabolic processes linked to carbon uptake, or a combination of these factors (Yeo *et al.* 1985, Flexas *et al.* 2004, Zhang *et al.* 2009). P_N of both grafted and ungrafted plants decreased with the increase of salt concentration, but the extent of the decrease was larger in ungrafted than in grafted plants. These observations suggest that melon and cucumber plants grafted onto the *Cucurbita* hybrid rootstocks 'P360' and 'PS1313', maintained a higher production potential by sustaining photosynthetic process *via* better management of stomatal parameters (Table 1). This is in agreement with previous findings that rootstock grafting improves the photosynthesis performance of plants under salinity (Moya *et al.* 2002, Massai *et al.* 2004, Colla *et al.* 2006, He *et al.* 2009). 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 seemed also to play an important role in the reduction of photosynthetic rate in both grafted and ungrafted plants under NaCl stress. This is indicated by the decrease of g_s under NaCl salinity conditions. Moreover, in both grafting combinations, P_N significantly correlated with both the dry shoot biomass and the fruit yield (Fig. 2), thereby indicating that plant growth is directly linked to photosynthesis (Sharma *et al.* 2005).

Excessive accumulation of Na^+ and Cl^- is toxic and may disrupt the integrity of the photosynthetic apparatus (Yeo *et al.* 1985, Bethke and Drew 1992, Aghaleh *et al.* 2009). It has been proposed that the reduction of leaf gas exchange in response to salinity is due to increase in leaf Na^+ concentration (Garcia-Legaz *et al.* 1993, Walker *et al.* 1993). However, other authors ascribed reductions in photosynthetic capacity to high tissue concentrations of Cl^- (Bañuls *et al.* 1997, Garcia-Sanchez *et al.* 2002). In

our experiments, the highly significant correlation between P_N and foliar concentrations of Na^+ and Cl^- suggests that toxic effects of the accumulated ions could be involved in the reduction of photosynthesis and stomatal conductance since significant positive correlations existed between P_N and g_s (Ma *et al.* 2006). This is in agreement with the findings of Martinez-Ballesta *et al.* (2004) and Colla *et al.* (2006) in pepper and watermelon, respectively. In addition to the reduction of net photosynthetic rates, LAI decreased in response to salinity especially in ungrafted plants treated with NaCl (Table 1). Restriction of leaf area may be the result of the suppressed net photosynthetic rates; since the latter effect reduces the available assimilates for leaf growth.

It is well known that high ion concentrations of Na^+ and Cl^- in the soil or water may depress nutrient ion activities and produce extreme ratios of Na^+/Ca^{2+}, Na^+/K^+, Ca^{2+}/Mg^{2+}, Cl^-/NO_3^-, and SO_4^{2-}/Mg^{2+}. As a result, the plant becomes susceptible to specific-ion (*e.g.,* Na^+, Cl^-) injury as well as to nutritional disorders that may result in reduced growth and yield (Grattan and Grieve 1999). In the present study, the concentration of Na^+ in the aerial parts was leser in grafted than in ungrafted plants, suggesting that grafting of melon and cucumber plants onto *Cucurbita* rootstocks limits Na^+ concentrations in leaves (Tables 2, 3). Two mechanisms could explain the decrease in shoot Na^+ concentrations in plants grafted onto *Cucurbita* hybrid rootstocks: (*1*) Na^+ exclusion by the *Cucurbita* roots; and (*2*) Na^+ retention and accumulation within the *Cucurbita* hybrid rootstock as reported by Edelstein *et al.* (2011). The sodium exclusion hypothesis was supported by Romero *et al.* (1997), Fernandez-Garcia *et al.* (2002), Colla *et al.* (2006), and Huang *et al.* (2009b) who observed Na^+ ion exclusion in grafted tomato, melon, watermelon, and cucumber, respectively. The concentration of Na^+ in leaves of grafted and ungrafted cucumber exposed to high external NaCl levels was lower [4.3 and 14.4 g kg^{-1}(DM), respectively] than those recorded in melon [15.7 and 26.1 g kg^{-1}(DM), respectively]. These results point to significant differences between the two *Cucurbita* hybrid rootstocks 'PS1313' and 'P360' in their ability to exclude or retain Na^+.

Reductions in the concentration of Cl^- were not observed between grafted and ungrafted plants indicating that the tested *Cucurbita* hybrid rootstocks were not able to exclude and/or retain Cl^- in the roots; thus, Cl^-, the translocation of which to the leaves is not limited by grafting, becomes the most harmful toxic component of the saline solution. Our results are in line with those of Edelstein *et al.* (2005) in melon who observed that pumpkin rootstocks have a high capacity to retain Na^+ in their tissues, while the distribution of Cl^- between roots and shoots in the plants with pumpkin rootstocks was much more uniform, despite a slight retention of Cl^- in the roots.

The NaCl salinity reduced the leaf concentration of N, P, and K in both grafting combinations of melon and cucumber but the decrease of the leaf K concentration was less pronounced in grafted plants. The improved crop performance of melon and cucumber plants grafted onto *Cucurbita* rootstocks and treated with salt solution in comparison to ungrafted plants exposed to identical salinity levels was attributed to their strong capacity to inhibit the Na translocation to the aerial parts and to maintain a better plant nutritional status with higher K concentration.

Conclusion: Our investigation suggests that melon and cucumber plants grafted onto *Cucurbita* rootstocks and ungrafted ones behaved differently under saline conditions. Grafting onto salt tolerant rootstocks improved the photosynthesis performance due to maintenance of higher stomatal conductance. The higher photosynthetic capacity of grafted melon and cucumber plants could be partly attributed to restriction of the Na^+ transport to aerial part and the better nutritional status in leaves, which mitigated the salinity imposed suppression of the plant growth and yield.

References

Aghaleh, M., Niknam, V., Ebrahimzadeh, H., Razavi, K.: Salt stress effects on growth, pigments, proteins and lipid peroxidation in *Salicornia persica* and *S. europaea*. – Biol. Plant. **53**: 243-248, 2009.

Bañuls, J., Serna, M.D., Legaz, F., Primo-Millo, E.: Growth and gas exchange parameters of *Citrus* plants stressed with different salts. – J. Plant Physiol. **50**: 194-199, 1997.

Bethke, P.C., Drew, M.C.: Stomatal and nonstomatal components to inhibition of photosynthesis in leaves of *Capsicum annuum* during progressive exposure to NaCl salinity. – Plant Physiol. **99**: 219-226, 1992.

Bremner, J.M.: Total nitrogen. – In: Black, C.A., Evans, D.D., White, I.L., Ensminger, L.E., Clark, F.E. (ed.): Methods of Soil Analysis. Agronomy Monograph **9**: 1149-1178, 1965.

Chartzoulakis, K.S.: Salinity and olive: growth, salt tolerance, photosynthesis and yield. – Agron. Water Manage. **78**: 108-121, 2005.

Colla, G., Rouphael, Y., Cardarelli, M., Rea, E.: Effect of salinity on yield, fruit quality, leaf gas exchange, and mineral composition of grafted watermelon plants. – HortSci. **41**: 622-627, 2006.

Colla, G., Suãrez, C.M.C., Cardarelli, M., Rouphael, Y.: Improving nitrogen use efficiency in melon by grafting. – HortScience **45**: 559-565, 2010a.

Colla, G., Rouphael, Y., Cardarelli, M., Salerno, A., Rea, E.: The effectiveness of grafting to improve alkalinity tolerance in watermelon. – Environ. Exp. Bot. **68**: 283-291, 2010b.

Colla, G., Rouphael, Y., Leopardi, C., Bie, Z.: Role of grafting in vegetable crops grown under saline conditions. – Sci. Hort. **127**: 147-155, 2010c.

Colla, G., Rouphael, Y., Mirabelli, C., Cardarelli, M.: Nitrogen use efficiency traits of mini-watermelon in response to grafting and nitrogen fertilization doses. – J. Plant Nutr. Soil Sci. **174**: 933-941, 2011.

Crinò, P., Lo Bianco, C., Rouphael, Y., Colla, G., Saccardo, F., Paratore, A.: Evaluation of rootstock resistance to fusarium wilt and gummy stem blight and effect on yield and quality of a grafted 'Inodorus' melon. – HortSci. **42**: 521-525, 2007.

Cuartero, J., Bolarín, M.C., Asíns, M.J., Moreno, V.: Increasing salt tolerance in the tomato. – J. Exp. Bot. **57**: 1045-1058, 2006.

Debez, A., Koyro, H.W., Grignon, C., Abdelly, C., Huchzermeyer, B.: Relationship between the photosynthetic activity and the performance of Cakile maritime after long-term salt treatment. – Physiol. Plant. **133**: 373-385, 2008.

Eaton, A.D., Clesceri, L.S., Greenberg, A.E.: Standard Methods for the Examination of Water and Wastewater, 19th Ed. – Am. Public Health Assoc. ,Washington 1995.

Edelstein, M., Ben-Hur, M., Cohen, R., Burger, Y., Ravina, I.: Boron and salinity effects on grafted and non-grafted melon plants. – Plant Soil **269**: 273-284, 2005.

Edelstein, M., Plaut, Z., Ben-Hur, M.: Sodium and chloride exclusion and retention by non-grafted and grafted melon and *Cucurbita* plants. – J. Exp. Bot. **62**: 177-184, 2011.

Fernández-García, N., Martínez, V., Cerdá, A., Carvajal, M.: Water and nutrient uptake of grafted tomato plants grown under saline conditions. – J. Plant Physiol. **159**: 899-905, 2002.

Flexas, J., Bota, J., Loreto, F., Cornic, G., Sharkey, T.D.: Diffusive and metabolic limitations to photosynthesis under drought and salinity in C_3 plants. – Plant Biol. **6**: 269-279, 2004.

García-Legaz, M.F., Ortiz, J.M., García-Lidón, A.G., Cerdá, A.: Effect of salinity and on growth, ion content and CO_2 assimilation rate in lemon varieties on different rootstock. – Physiol. Plant. **89**: 427-432, 1993.

García-Sánchez, F., Jifon, J.L., Carvajal, M., Syvertsen, J.P.: Gas exchange, chlorophyll and nutrient contents in relation to Na^+ and Cl^- accumulation in 'Sunburst' mandarin grafted on different rootstocks. – Plant Sci. **162**: 705-712, 2002.

Grattan, S.R., Grieve, C.M.: Salinity-mineral nutrient relations in horticultural crops. – Sci. Hort. **78**: 127-157, 1999.

He, Y., Zhu, Z.J., Yang, J., Ni, X.L., Zhu, B.: Grafting increases the salt tolerance of tomato by improvement of photosynthesis and enhancement of antioxidant enzymes activity. – Environ. Exp. Bot. **66**: 270-278, 2009.

Huang, Y., Zhu J., Zhen A., Chen L., Bie, Z.L.: Organic and inorganic solutes accumulation in the leaves and roots of grafted and ungrafted cucumber plants in response to NaCl stress. – J. Food Agric. Environ. **7**: 703-708, 2009a.

Huang, Y., Tang, R., Cao, Q.L., Bie, Z.L.: Improving the fruit yield and quality of cucumber by grafting onto the salt tolerant rootstock under NaCl stress. – Sci. Hortic. **122**: 26-31, 2009b.

Isaac R.A., Johnson W.C: Elemental determination by inductively coupled plasma atomic emission spectrometry. – In: Kalra, Y.P. (ed.): Handbook of Reference Methods for Plant Analysis. Pp. 165-170. CRC Press, Boca Raton 1998.

Kiehl, P.A., Lieth, J.H., Burger, D.W.: Growth response of chrysanthemum to various container medium moisture tension levels. – J. Amer. Soc. Hort. Sci. **114**: 387-392, 1992.

Lee, J.M.: Cultivation of grafted vegetables I. Current status, grafting methods, and benefits. – HortSci. **29**: 235-239, 1994.

Lee, J.M., Kubota, C., Tsao, S.J., Bie, Z., Hoyos Echevarria, P., Morra, L., Oda, M.: Current status of vegetable grafting: Diffusion, grafting techniques, automation. – Sci. Hort. **127**: 93-105, 2010.

Lopez-Climent, M.F., Arbona, V., Perez-Clemente, R.M., Gomez-Cadenas, A.: Relationship between salt tolerance and photosynthetic machinery performance in citrus. – Environ. Exp. Bot. **62**: 176-184, 2008.

Ma, Q.Q., Wang, W., Li, Y.H., Li, D.Q., Zou, Q.: Alleviation of photoinhibition in drought-stressed wheat (*Triticum aestivum* L.) by foliar-applied glycinebetaine. − J. Plant Physiol. **163**: 165-175, 2006.

Martinez-Ballesta, M.C., Martinez, V., Carvajal, C.: Osmotic adjustment, water relations and gas exchange in pepper plants grown under NaCl or KCl. – Environ. Exp. Bot. **52**: 161-174, 2004.

Massai, R., Remorini, D., Tattini, M.: Gas exchange, water relations and osmotic adjustment in two scion/rootstock combinations of prunus under various salinity concentrations. – Plant Soil **259**: 153-162, 2004.

Moya, J.L., Tadeo, F.R., Gomez-Cadenas, A., Primo-Millo, E., Talon, M.: Transmissible salt tolerance traits identified through reciprocal grafts between sensitive Carrizo and tolerant Cleopatra citrus genotypes. – J. Plant Physiol. **159**: 991-998, 2002.

Naumann, J.C., Young, D.R., Anderson, J.E.: Linking leaf chlorophyll fluorescence properties to physiological responses for detection of salt and drought stress in coastal plant species. – Physiol. Plant **131**: 422-433, 2007.

Nisini, P.T., Colla, G., Granati, E., Temperini, O., Crinò, P., Saccardo, F.: Rootstock resistance to fusarium wilt and effect on fruit yield and quality of two muskmelon cultivars. – Sci. Hort. **93**: 281-288, 2002.

Norrie, J., Graham, M.E.D., Gosselin, A.: Potential evapotranspiration as a means of predicting irrigation timing in greenhouse tomatoes grown in peat bags. – J. Amer. Soc. Hort. Sci. **119**: 163-168, 1994.

Rivard, C.L., Louws, F.J.: Grafting to manage soilborne diseases in heirloom tomato production. – HortSci. **43**: 2104-2111, 2008.

Romero, L., Belakbir, A., Ragala, L., Ruiz, J.M.: Response of plant yield and leaf pigments to saline conditions: effectiveness of different rootstocks in melon plants (*Cucumis melo* L.). – Soil Sci. Plant Nutr. **43**: 855-862, 1997.

Rouphael, Y., Cardarelli, M., Rea, E., Colla, G.: Grafting of cucumber as a means to minimize copper toxicity. – Environ. Exp. Bot. **63**: 49-58, 2008a.

Rouphael, Y., Cardarelli, M., Colla, G., Rea, E.: Yield, mineral composition, water relations, and water use efficiency of grafted mini-watermelon plants under deficit irrigation. – HortSci. **43**: 730-736, 2008b.

Ruiz, J.M., Belakbir, A., López-Cantarero, I., Romero, L.: Leaf-macronutrient content and yield in grafted melon plants. A model to evaluate the influence of rootstock genotype. – Sci. Hort. **71**: 227-234, 1997.

Savvas, D., Papastavrou, D. Ntatsi, G., Ropokis, A., Olympios, C., Hartmann, H., Schwarz, D.: Interactive effects of grafting and Mn-supply level on growth, yield and nutrient uptake by tomato. – HortSci. **44**: 1978-1982, 2009.

Savvas, D., Colla, G., Rouphael, Y., Schwarz, D.: Amelioration of heavy metal and nutrient stress in fruit vegetables by grafting. – Sci. Hort. **127**: 156-161, 2010.

Schwarz, D., Rouphael, Y., Colla, G., Venema, J.K.: Grafting as

a tool to improve tolerance of vegetables to abiotic stresses: thermal stress, water stress and organic pollutants. – Sci. Hort. **127**: 162-171, 2010.

Sharma, N., Gupta, N.K., Gupta, S., Hasegawa, H.: Effect of NaCl salinity on photosynthetic rate, transpiration rate, and oxidative stress tolerance in contrasting wheat genotypes. – Photosynthetica **43**: 609-613, 2005.

Stępień, P., Kłobus, G.: Water relations and photosynthesis in *Cucumis sativus* L. leaves under salt stress. – Biol. Plant. **50**: 610-616, 2006.

Tennessen, D.J., Singsaas, E.L., Sharkey, T.D.: Light-emitting diodes as a light source of photosynthesis research. – Photosynth. Res. **39**: 85-92, 1994.

Walker, R.R., Blackmore, D.H., Sung, Q.: Carbon dioxide assimilation and foliar ion concentration in leaves of lemon (*Citrus limon* L.) trees irrigated with NaCl or Na_2SO_4. – Aust. J. Plant Physiol. **20**: 173-185, 1993.

Yeo, A.R., Capron, S.J.M., Flowers, T.J.: The effect of salinity upon photosynthesis in rice (*Oryza sativa* L.): gas exchange by individual leaves relation to their salt content. – J. Exp. Bot. **36**: 1240-1248, 1985.

Zhang, R.H., Li, J., Guo, S.R., Tezuka, T.: Effects of exogenous putrescine on gas-exchange characteristics and chlorophyll fluorescence of NaCl-stressed cucumber seedlings. – Photosynth. Res. **100**: 155-162, 2009.

Zhou, Y.H., Huang, L.F., Zhang, Y.L., Shi, K., Yu, J.Q., Nogues, S.: Chill-induced decrease in capacity of RuBP carboxylation and associated H_2O_2 accumulation in cucumber leaves are alleviated by grafting onto figleaf gourd. – Ann. Bot. **100**: 839-848, 2007.

Zhu J., Bie Z.L., Huang Y., Han, X.Y.: Effect of grafting on the growth and ion contents of cucumber seedlings under NaCl stress. – Soil Sci. Plant Nutr. **54**: 895-902, 2008.

Zuccarini, P.: Effects of silicon on photosynthesis, water relations and nutrient uptake of *Phaseolus vulgaris* under NaCl stress. – Biol. Plant. **52**: 157-160, 2008.