



The effectiveness of grafting to improve NaCl and CaCl₂ tolerance in cucumber



Giuseppe Colla ^{a,*}, Youssef Rousphael ^b, Rama Jawad ^b, Pradeep Kumar ^a, Elvira Rea ^c, Mariateresa Cardarelli ^c

^a Dipartimento di Scienze e Tecnologie per l'Agricoltura, le Foreste, la Natura e l'Energia Università della Tuscia, via S. C. De Lellis snc, 01100 Viterbo, Italy

^b Department of Crop Production, Faculty of Agricultural Engineering and Veterinary Medicine, Lebanese University, Dekwaneh-El Maten, Beirut, Lebanon

^c CRA-Centro di ricerca per lo studio delle relazioni tra pianta e suolo, Via della Navicella 2-4, 00184 Roma, Italy

ARTICLE INFO

Article history:

Received 13 May 2013

Received in revised form

12 September 2013

Accepted 13 September 2013

ABSTRACT

The aim of the current work was to determine whether grafting could improve salinity tolerance of cucumber using three different salt stressors such as CaCl₂, NaCl and their combination CaCl₂ + NaCl with equimolar concentrations, and to study the changes induced by the rootstocks in the shoot growth at agronomical and physiological levels. A greenhouse experiment was carried out to determine yield, growth, fruit quality, SPAD values, electrolyte leakage, chlorophyll fluorescence, CAT, APX, GPX activities and mineral composition and assimilate partitioning of cucumber plants (*Cucumis sativus* L. cv. 'Ekron'), either ungrafted or grafted onto two commercial rootstocks: 'Affyne' (*C. sativus* L.) and 'P360' (*Cucurbita maxima* Duch. × *Cucurbita moschata* Duch.) and cultured in quartziferous sand. Plants were supplied with four nutrient solutions: non-salt control, 20 mM CaCl₂, 30 mM NaCl or 10 mM CaCl₂ + 15 mM NaCl. Significant depression of yield, and shoot biomass production in response to an increase of salinity concentration in the nutrient solution was observed with more detrimental effects with CaCl₂. The three salt types improved fruit quality in both grafting combinations by increasing fruit dry matter and total soluble solids content by 8% and 6%, respectively. The lowest SPAD index and the lowest efficiency of the PSII in dark-adapted leaves, measured as the F_v/F_m ratio were recorded in ungrafted plants treated with CaCl₂. Moreover, at the three salt treatments the percentage of yield and biomass reduction in comparison to control was significantly lower in the plants grafted onto 'P360' and especially 'Affyne' than ungrafted plants, with the highest yield, and shoot reduction recorded with CaCl₂ in comparison to those recorded with NaCl and CaCl₂ + NaCl treatments. Grafted cucumber plants exposed to NaCl and CaCl₂ + NaCl were capable of maintaining higher chlorophyll content (SPAD index), higher photochemical activity of PSII, increased the capacity of antioxidant system by enhancing the GPX activity, a better nutritional status (higher N, K, and Ca, and lower Na) in the shoot tissues and a higher membrane selectivity in comparison with ungrafted ones. The higher crop performance of grafted (Ekron/Affyne) cucumber recorded with NaCl than with CaCl₂, was attributed to the limited capability of the rootstocks to restrict Cl⁻ shoot uptake, thus Cl⁻, which continues passing to the leaves, becomes the more significant toxic component of the saline solution.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Salinity continues to be a major factor in reduced crop productivity and profit in many arid and semi-arid regions, despite the advanced management techniques developed in recent decades (Edelstein et al., 2011). Salinity imposes two constraints on plants: an osmotic effect resulting from the lower soil water potential and an ionic effect resulting from the direct toxicity of saline ions and

the ion imbalance in the plants leading to several morphological and physiological changes (Munns and Tester, 2008).

The inhibition of plant growth under saline conditions often involves a decrease in their photosynthetic capacity (Liu et al., 2012). The reduced photosynthesis is caused not only by stomatal closure, but also by non-stomatal factors that reduced 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 the chloroplasts (Misra et al., 2001), as well as the PSII activity (Lu and Vonshak, 2002), and the quantum yield of PSII electron transport (Xia et al., 2004). Generally, plants possess an antioxidant system that includes antioxidant enzymes, such

* Corresponding author. Tel.: +39 0761 357536; fax: +39 0761 357453.

E-mail address: giucolla@unitus.it (G. Colla).

as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) (Noctor and Foyer, 1998). Superoxide dismutase, the first enzyme in the detoxifying process, converts $O_2^{\bullet-}$ radicals to H_2O_2 . Catalase and POD scavenge the accumulated H_2O_2 to non-toxic levels by conversion to H_2O and O_2 (Apel and Hirt, 2004).

Whether salt mainly exerts an osmotic or an ion-specific effect varies depending on the plant species and/or genotype in question. However, the two effects and plant responses cannot be fully separated. Salinity will always induce an osmotic effect, and its intensity will always be proportional to salt concentrations. The specific ion effect may be intense even at low concentrations in sensitive species, while others are less sensitive to the salt ions and will only be affected at higher concentrations (Pagter et al., 2009). If osmotic stress is the main consequence of plant exposure to salt stress, iso-osmotic solutions of different salinity types would be expected to exert the same influence on plant growth and physiology. Where direct ion specific effects occur, salinity type would be expected to be of major importance (Pagter et al., 2009). Most studies concerning salt tolerance of plant species have been based on experiments in which NaCl is the predominant salt. Relatively few studies have focused on the effects of CaCl₂ on plant growth and physiology (Tas et al., 2005; Trajkova et al., 2006; Yokas et al., 2008), even if CaCl₂ is present at higher concentrations than NaCl in the soils and groundwater in many areas of the world (Marschner, 1995).

Calcium plays an important role in plant survival of salinity stress by increasing the resistance to the stress (Greenway and Munns, 1980). Studies have shown that Ca²⁺ can reduce the negative effect of salinity on plants and alleviate the growth inhibition mainly by mitigating the ionic effect of salinity rather than the osmotic effect (Rengel, 1992). Supplemental Ca²⁺ can maintain membrane integrity in both roots and shoots by limiting Ca²⁺ displacement from the membrane by Na⁺ ions (Cramer et al., 1985). Sodium uptake can also be reduced and K⁺ uptake increased by the presence of Ca²⁺ in the soil solution (Cramer et al., 1985). However, the responses vary depending not only on plant species but also on the salts (NaCl, CaCl₂ or Na₂SO₄) and the source of Ca²⁺ ions (CaCl₂ or CaSO₄) (Volkmar et al., 1998; Caines and Shennan, 1999).

At present, many researchers have aimed toward molecular engineering in order to improve plant salt tolerance. However, there existed some limitations owing to the complexity of quantitative traits, insufficient genetic knowledge of tolerance components and lack of efficient selection criteria (Cuartero and Fernandez-Muñoz, 1999). According to recent studies, grafting was a useful technique to increase plant vigor and yield, induce 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; Lee et al., 2010; Rousphaei et al., 2008a,b; Savvas et al., 2010; Schwarz et al., 2010; Borgognone et al., 2013; Temperini et al., 2013) and also improve fruit quality (Proietti et al., 2008; Rousphaei et al., 2010a,b). In relation to salt tolerance, many studies have been carried out to determine the response of grafted plants to NaCl (Santa-Cruz et al., 2002; Fernández-García et al., 2002; Estañ et al., 2005; Colla et al., 2005, 2006a,b; Goreta et al., 2008; Martínez-Rodríguez et al., 2008; He et al., 2009; Huang et al., 2009a,b; Edelstein et al., 2011; Rousphaei et al., 2012a). However, investigations whether grafting would enhance the salt tolerance of vegetables to other types of salinity is needed as basic requirement for the continued success of grafting (Colla et al., 2010a, 2012).

Starting from the above considerations, the aim of this study was to investigate the different tolerance of grafted cucumber in terms of yield, growth, fruit quality, leaf chlorophyll content (SPAD value), leaf electrolyte leakage, leaf chlorophyll fluorescence, CAT, APX,

GPX activities of leaves and mineral composition and assimilate partitioning, to two different salt stressors such as NaCl, CaCl₂, and their combination (NaCl + CaCl₂). The study was performed in terms of equimolar concentrations of the three different chloride salts in order to evaluate the ion effects of the three salinity sources on the agronomical and physiological responses of greenhouse grafted and ungrafted cucumber.

2. Materials and methods

2.1. Plant material, growth conditions and treatments

The experiment was conducted in Spring–Summer 2012 in a 300 m² polymethylmethacrylate 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. The greenhouse was maintained at daily temperatures between 18 and 33 °C, and day/night relative humidities of 55/85%. *Cucumis sativus* L. cv. Ekron (Enza Zaden, Verona, Italy) was grafted onto two commercial rootstocks: 'P360' (*Cucurbita maxima* × *Cucurbita moschata*; Società Agricola Italiana Sementi, Cesena, Italy), and 'Affyne' (*C. sativus* L.; Rijk Zwaan Italia srl, Calderara di Reno, Bologna, Italy) using the 'tongue approach grafting' described by Lee (1994), whereas ungrafted 'Ekron' was used as a control plant. The *Cucurbita* and cucumber rootstocks were selected as the most representative commercial rootstocks used in Italy due to their compatibility with cucumber cultivars, and to their resistance to soilborne pathogens. At the two true-leaf stage (April 20), grafted and ungrafted plants were grown into pots (d 30 cm, h 30 cm) containing 17.7 l of quartziferous sand. Pots were disposed in double rows on the greenhouse floor. The space between plants within a row was 0.45 m and the distance between the centers of double rows was 1.5 m, resulting in a plant density of 3 plants m⁻². Plants were grown as vertical cordons where all laterals branches were pruned back as they developed until the plant reached the overhead support wire. There, the terminal bud was removed above the support cable and two lateral branches were trained over the cable and allowed to grow back downward (Hickman, 1998).

The experiment was designed as a factorial combination of four nutrient solutions (non-salt control, 20 mM CaCl₂, 30 mM NaCl, or 10 mM CaCl₂ + 15 mM NaCl) and three grafting treatments (ungrafted Ekron; Ekron/Affyne or Ekron/P360). Each experimental unit consisted of twelve plants. The treatments were arranged in a randomized complete-block design with four replicates per treatment.

2.2. Nutrient solution management

The saline treatments were initialized 10 days after the transplanting. The basic (control) nutrient solution used in this experiment was a modified Hoagland and Arnon formulation. All chemicals used were of analytical grade, and composition of the basic nutrient solution was: 14.0 mM NO₃-N, 1.0 mM NH₄-N, 1.75 mM S, 1.5 mM P, 6.0 mM K, 4.5 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, with an electrical conductivity (EC) of 2.0 dS m⁻¹. The saline nutrient solutions had the same basic composition plus an additional 30 mM of NaCl, 20 mM of CaCl₂, or 15 mM of NaCl plus 10 mM of CaCl₂, giving EC values of 6.1, 6.5, and 6.3 dS m⁻¹, respectively. The pH of the nutrient solution for all treatments was 6.0 ± 0.3. All nutrient solutions were prepared using deionized water. Nutrient solution was pumped from independent tanks through a drip irrigation system, with one emitter per plant and an emitter flow rate of 21 h⁻¹. Irrigation scheduling was performed using electronic low-tension tensiometers (LT-Irrrometer,

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; Roush et al., 2004; Roush and Colla, 2005). Five to 17 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.

2.3. Yield and plant growth measurements

Fruits were harvested from May 18 to June 22. Fruits were harvested when they reached marketable size ($>20\text{ cm}$ of length), fruits that were deformed or badly misshapen were considered unmarketable (Roush et al., 2010a,b). The marketable yield, number of fruits and weight, were determined on eight plants per plot. At final harvest (June 22, 64 days after transplanting), eight plants per plot were separated into stems, leaves, and roots, and their tissues were dried in a forced-air oven at 80°C for 72 h for biomass determination. Shoot biomass was equal to the sum of aerial vegetative plant parts (leaves + stems). Root to shoot ratio was calculated by dividing root dry weight by the sum of leaf and stem dry weights. Leaf area (LA) was measured with an electronic area meter (Delta-T Devices Ltd., Cambridge, UK).

2.4. Fruit quality analysis

On 6 June (forty eight days after transplanting) ten representative fruits of each plot were analyzed for fruit quality parameters. Immediately after harvest, fruit shape index (SI), defined as the ratio of width to length was measured. Fruit firmness (N cm^{-2}) was determined using a penetrometer (Bertuzzi FT 011; Brugherio, Milan, Italy), fitted with an 8 mm-diameter round-head probe. The liquid extract obtained by liquefying the mesocarp of each fruit was used to determine the total soluble solids (TSS) contents by employing an Atago N1 refractometer (Atago Co. Ltd., Japan). Acidity was determined by potentiometric titration with 0.1 M NaOH up to pH 8.1 using 10 ml of juice, and the results were expressed as percentage of malic acid in the juice. Fruit juice pH was also measured with a pH meter (HI-9023; Hanna Instruments, Padova, Italy). Fruits were dried in a forced air oven at 80°C for 72 h and weighed to determine the fruit dry matter (DM).

2.5. Fluorescence measurements

At the termination of the experiment, modulated chlorophyll fluorescence was measured in dark adapted (for at least 15 min) leaves in the same leaf leaflet in 3 plants per graft combination, using a chlorophyll fluorometer Handy PEA (Hansatech Instruments Ltd, UK) with an excitation source intensity higher than $3000\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$ at the sample surface. The minimal fluorescence intensity (F_0) in a dark-adapted state was measured in the presence of a background far-red light to favor rapid oxidation of intersystem electron carriers. The maximal fluorescence intensities in the dark-adapted state (F_m) was measured by 0.8 s saturating pulses ($3000\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$). The maximum quantum yield of open photosystem II (PSII) (F_v/F_m) was calculated as $(F_m - F_0)/F_m$ (Maxwell and Johnson, 2000).

2.6. SPAD measurements

At the termination of the experiment, a chlorophyll meter (SPAD-502, Minolta Corporation, Ltd., Osaka, Japan) was adopted

to take dimensionless SPAD values from the fully expanded functional leaves (the fourth from the apex). Measurements were made at a central point on the leaflet between the midrib and the leaf margin. The meter was shielded from direct sunlight by the operator during each measurement. Fifteen leaves were measured randomly per plot and averaged to a single SPAD value for each treatment.

2.7. Determination of electrolyte leakage

Fifty five days after transplantation, the electrolyte leakage (EL) was determined as described by Lutts et al. (1995). Six randomly chosen plants per treatment (4 mature leaves per plant) were taken and cut into 1-cm segments. Leaf samples were washed 3 times with distilled water to remove surface contamination, and then placed in individual stoppered vials containing 10 mL of distilled water. The samples were incubated at room temperature (25°C) on a shaker (100 rpm) for 24 h. Electrical conductivity of the bathing solution (EC_1) was read after incubation. The same samples were then placed in an autoclave at 120°C for 20 min and a second reading of the EC (EC_2) was made after cooling the solution to room temperature. The EL was calculated as EC_1/EC_2 and expressed as percentage.

2.8. Assay of antioxidant enzyme activity

For the enzyme assays, the first fully expanded fresh leaves were harvested in each plot and immediately frozen in liquid nitrogen and stored at -80°C for later antioxidant enzyme activity analysis. Enzyme extractions were performed using a pre-chilled mortar and pestle with two volumes of an ice-cold extraction buffer (0.05 M potassium phosphate buffer, pH 7.0) containing 0.1% (w/v) ascorbic acid, 1% (w/v) polyvinylpyrrolidone, 1 mM $\text{Na}_2\text{-EDTA}$ and 0.1% (v/v) Triton X-100. After centrifugation ($15\,000 \times g$, 30 min, 4°C) the supernatant was set aside for the determination of the enzymes activity and protein content by a spectrophotometer (Perkin Elmer, Norwalk, CT, USA). Catalase (CAT, EC 1.11.1.6) activity was measured according to Havar and McHale (1989). Assay mixture (1 ml) contained 0.1 ml of 125 mM H_2O_2 and 20 μl of the crude extract in 0.05 M potassium phosphate buffer (pH 7.0). Enzyme activity was evaluated by following the decomposition of H_2O_2 at 240 nm for 1 min and calculated using the extinction coefficient ($0.036\text{ mM}^{-1}\text{ cm}^{-1}$). Activity of ascorbate peroxidase (APX, EC 1.11.1.11) was measured following the decrease of absorbance at 290 nm for 1 min (Nakano, 1981) corresponding to the oxidation of ascorbic acid. APX activity was calculated using its extinction coefficient ($2.8\text{ mM}^{-1}\text{ cm}^{-1}$). Activity of guaiacol peroxidase (GPX, EC 1.11.1.7) was measured in accordance with Chance and Maehly (1955). Assay mixture (1 ml) contained 0.1 ml of 90 mM guaiacol, 0.1 ml of 125 mM H_2O_2 and 50 μl of the crude extract in 0.05 M potassium phosphate buffer (pH 7.0). Enzyme activity was evaluated following the increase of absorbance at 470 nm for 40 s due to guaiacol oxidation and calculated using the extinction coefficient ($26.6\text{ mM}^{-1}\text{ cm}^{-1}$). The specific enzyme activity for all enzymes was expressed as $\text{mmol mg}^{-1}\text{ protein min}^{-1}$.

2.9. Mineral analysis

Dried biomass of plant tissues harvested at the end of the trial (leaf, stem, and root) and dry biomass of fruits harvested during the growing cycle 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, Ca, Mg, Na, Cl, Fe, Mn, and Zn. Nitrogen concentration in the plant tissues was determined after mineralization with sulfuric acid by "Kjeldahl method" (Bremner, 1965), P, K, Ca, Mg, Na, Fe, Mn, and Zn concentrations were determined by dry ashing at 400°C for 24 h,

dissolving the ash in 1:20 HNO₃, and assaying the solution obtained using an inductively coupled plasma emission spectrophotometer (ICP Iris; Thermo Optek, Milano, Italy) (Karla, 1998). Chloride ion concentrations were determined by titration with AgNO₃ in the presence of K₂CrO₄ (Eaton et al., 1995).

2.10. Statistical analysis

All data were statistically analyzed by ANOVA using the SPSS software package (SPSS 10 for Windows, 2001). Duncan's multiple range test was performed at $P=0.05$ on each of the significant variables measured.

3. Results

3.1. Fruit yield components

In grafted and ungrafted plants, the marketable yield, number of fruits and weight decreased in response to an increase of salinity concentration in the nutrient solution with more detrimental effects with CaCl₂ treatment (Table 1). The lowest yield observed on plants treated with CaCl₂, NaCl, and CaCl₂ + NaCl in comparison with control treatment was mainly attributed to a reduction in both the average fruit weight and the number of fruit per plant (Table 1). Moreover, with 20 mM CaCl₂, 30 mM NaCl, and 10 mM CaCl₂ + 15 mM NaCl concentration in the nutrient solution, the percentage of yield reduction in comparison to control was significantly lower in the plants grafted onto 'Affyne' (45%, 29%, and 22%, respectively) and 'P360' (43%, 40%, and 27%, respectively) than ungrafted plants (53%, 48%, and 33%, respectively) with the highest yield reduction recorded with CaCl₂, followed by NaCl in comparison to those recorded with CaCl₂ + NaCl treatment (Table 1). The low marketable yield of ungrafted plants was due to a reduction in the fruit mean weight and not to a change in the number of fruit per plant (Table 1).

Table 1

Effects of salt treatment in the nutrient solution and grafting combination on marketable fruit yield, marketable fruit mean weight and number of cucumber plants.

Salt treatment	Graft combination	Yield (kg plant ⁻¹)	Fruit	
			Number (n. plant ⁻¹)	Mean weight (g fruit ⁻¹)
Control	Ekron	3.26	12.7	257.5
	Ekron/Affyne	3.22	11.8	272.7
	Ekron/P360	3.23	12.5	259.3
	Mean	3.24 a	12.3 a	263.2 a
CaCl ₂	Ekron	1.52 (53)	7.5	202.7
	Ekron/Affyne	1.76 (45)	8.8	202.3
	Ekron/P360	1.85 (43)	8.4	220.0
	Mean	1.71 d	8.2 b	208.4 c
NaCl	Ekron	1.71 (48)	7.6	227.0
	Ekron/Affyne	2.30 (29)	9.6	244.3
	Ekron/P360	1.95 (40)	7.8	249.2
	Mean	1.99 c	8.3 b	240.2 b
CaCl ₂ + NaCl	Ekron	2.17 (33)	8.6	252.9
	Ekron/Affyne	2.51 (22)	9.8	258.4
	Ekron/P360	2.35 (27)	8.6	274.5
	Mean	2.34 b	9.0 b	261.9 a
Significance				
Salt (S)		***		***
Graft combination (G)		**	NS	*
S × G		NS	NS	NS

Values are the means of four replicate samples. The percentage of reduction in enriched-salt treatments with respect to the control is reported in parenthesis.

Means within columns separated using Duncan's multiple range test $P=0.05$.

NS, nonsignificant.

* Significant at $P<0.05$.

** Significant at $P<0.01$.

*** Significant at $P<0.001$.

3.2. Biomass production and partitioning

Similarly to fruit yield components, the shoot and root biomass in both grafted and ungrafted plants decreased in response to an increase of salinity in the nutrient solution especially with CaCl₂ and NaCl treatments, whereas an opposite trend was observed for the root-to-shoot (R/S) ratio (Table 2). Moreover, at the three salt treatments (20 mM CaCl₂, 30 mM NaCl, and 10 mM CaCl₂ + 15 mM NaCl), the biomass weight reductions of the shoot in comparison to control were clearly lower in both grafting combinations (Ekron/Affyne and Ekron/P360) with respect to those of the ungrafted plants, with the highest biomass reductions recorded with CaCl₂, and NaCl compared to CaCl₂ + NaCl treatment (Table 2). The R/S ratio increased from 0.07 to 0.11 in response to nutrient solution salinity. Finally, the leaf injuries caused by salt stressors are presented in Fig. 1.

3.3. Fruit quality

No significant differences among treatments were observed for fruit firmness (avg. 1.66 N cm⁻²) and fruit juice pH (avg. 5.7). When averaged over grafting combination, the highest fruit dry matter (DM) and total soluble solids (TSS) content were recorded in plants treated with CaCl₂, NaCl, and CaCl₂ + NaCl with no significant difference observed between the three salt types (Table 3). Moreover, when averaged over salt treatment, the shape index (SI) in grafted plants (Ekron/Affyne and Ekron/P360) was significantly higher by 4% compared to ungrafted plants, whereas the highest titratable acidity (TA) was recorded in Ekron and Ekron/P360 (avg. 0.095%) compared to Ekron/Affyne (0.084%).

3.4. Leaf area, chlorophyll fluorescence, SPAD index, and electrolyte leakage

When averaged over grafting combination, the final leaf area (LA) was highest in non saline nutrient solution, followed by plants treated with CaCl₂ + NaCl, and plants with NaCl, and finally on

Table 2

Effects of salt treatment in the nutrient solution and grafting combination on shoot and root biomass dry weight, and on the root-to-shoot ratio (R/S) of cucumber plants.

Salt treatment	Graft combination	Shoot (g plant ⁻¹)	Root (g plant ⁻¹)	R/S
Control	Ekron	91.2	5.1	0.06
	Ekron/Affyne	90.6	6.2	0.07
	Ekron/P360	94.5	7.0	0.07
	Mean	92.1 a	6.1 a	0.07 b
CaCl ₂	Ekron	32.6 (64)	3.3	0.10
	Ekron/Affyne	41.6 (54)	4.2	0.10
	Ekron/P360	42.8 (55)	5.1	0.12
	Mean	39.0 d	4.2 ab	0.11 a
NaCl	Ekron	44.2 (52)	3.4	0.08
	Ekron/Affyne	54.4 (40)	4.0	0.07
	Ekron/P360	47.8 (48)	4.6	0.10
	Mean	48.8 c	4.0 b	0.08 b
CaCl ₂ + NaCl	Ekron	63.0 (31)	5.0	0.08
	Ekron/Affyne	72.6 (20)	5.9	0.08
	Ekron/P360	69.5 (24)	5.8	0.07
	Mean	68.4 b	5.6 ab	0.08 b
Significance				
Salt (S)		***	**	**
Graft combination (G)		**	NS	**
S × G		NS	NS	NS

Values are the means of four replicate samples. The percentage of reduction in enriched-salt treatments with respect to the control is reported in parenthesis.

Means within columns separated using Duncan's multiple range test, $P=0.05$.

NS, nonsignificant.

** Significant at $P<0.01$.

*** Significant at $P<0.001$.



Fig. 1. The leaf injuries caused by 10 mM CaCl₂ + 15 mM NaCl, 30 mM NaCl, and 20 mM CaCl₂ in cucumber plants fifty days after transplanting.

Table 3

Effects of salt treatment in the nutrient solution and grafting combination on fruit shape index (SI), firmness, fruit dry matter (DM), total soluble solids (TSS) content, juice pH and titratable acidity (TA) of cucumber fruits.

Salt treatment	Graft combination	SI	Firmness (N cm ⁻²)	DM (%)	TSS (°brix)	pH	TA (%)
Control	Ekron	0.25	1.66	3.26	3.13	5.67	0.10
	Ekron/Affyne	0.26	1.73	3.36	3.30	5.73	0.09
	Ekron/P360	0.25	1.71	3.44	3.46	5.74	0.10
	Mean	0.25	1.70	3.35 b	3.30 b	5.71	0.10
CaCl ₂	Ekron	0.26	1.66	3.51	3.44	5.68	0.09
	Ekron/Affyne	0.28	1.59	3.74	3.48	5.65	0.08
	Ekron/P360	0.24	1.56	3.66	3.59	5.69	0.09
	Mean	0.26	1.60	3.64 a	3.50 a	5.67	0.09
NaCl	Ekron	0.25	1.77	3.51	3.50	5.72	0.09
	Ekron/Affyne	0.28	1.71	3.58	3.47	5.81	0.08
	Ekron/P360	0.27	1.64	3.57	3.44	5.65	0.10
	Mean	0.27	1.71	3.55 a	3.47 ab	5.73	0.09
CaCl ₂ + NaCl	Ekron	0.25	1.63	3.63	3.57	5.66	0.09
	Ekron/Affyne	0.27	1.69	3.82	3.65	5.71	0.08
	Ekron/P360	0.27	1.62	3.58	3.42	5.63	0.10
	Mean	0.26	1.64	3.68 a	3.55 a	5.67	0.09
Significance							
Salt (S)		NS	NS	***	**	NS	NS
Graft combination (G)		*	NS	NS	NS	NS	***
S × G		NS	NS	NS	NS	NS	NS

Values are the means of four replicate samples.

Means within columns separated using Duncan's multiple range test, $P=0.05$.

NS, nonsignificant.

* Significant at $P<0.05$.

** Significant at $P<0.01$.

*** Significant at $P<0.001$.

Table 4

Effects of salt treatment in the nutrient solution and grafting combination on final leaf area, maximum quantum use efficiency of PSII in dark-adapted state, SPAD index, and leaf electrolyte leakage of cucumber plants.

Salt treatment	Graft combination	Leaf area ($\text{m}^2 \text{ plant}^{-1}$)	Maximum quantum use efficiency of PSII (F_v/F_m)	SPAD index	Electrolyte leakage (%)
Control	Ekron	2.00	0.83 a	66.1 a	45.3
	Ekron/Affyne	1.91	0.83 a	67.9 a	43.2
	Ekron/P360	2.03	0.83 a	69.4 a	41.8
	Mean	1.98 a	0.83 a	67.8 a	43.4 d
CaCl_2	Ekron	1.23	0.68 c	23.2 e	73.9
	Ekron/Affyne	1.40	0.76 b	46.7 c	63.1
	Ekron/P360	1.43	0.78 ab	40.7 d	65.9
	Mean	1.35 d	0.74 b	36.9 d	67.6 a
NaCl	Ekron	1.40	0.75 b	47.0 c	63.3
	Ekron/Affyne	1.62	0.83 a	52.7 bc	56.7
	Ekron/P360	1.52	0.82 a	51.4 bc	60.1
	Mean	1.51 c	0.80 a	50.4 c	60.0 b
$\text{CaCl}_2 + \text{NaCl}$	Ekron	1.59	0.82 a	49.4 bc	57.9
	Ekron/Affyne	1.84	0.83 a	57.9 ab	52.0
	Ekron/P360	1.79	0.83 a	54.5 b	53.6
	Mean	1.74 b	0.83 a	53.9 b	54.5 c
Significance					
Salt (S)		***	***	***	***
Graft combination (G)		**	***	***	**
$S \times G$		NS	***	**	NS

Values are the means of four replicate samples.

Means within columns separated using Duncan's multiple range test, $P=0.05$.

NS, nonsignificant.

** Significant at $P<0.01$.

*** Significant at $P<0.001$.

plants treated with CaCl_2 (Table 4). The highest electrolyte leakage (EL) values were observed with plants treated with CaCl_2 , while the lowest values were recorded in the non saline treatment (Table 4). Moreover, when averaged over salt treatment the final LA was significantly higher by 9% in both grafting combination (avg. $1.69 \text{ m}^2 \text{ plant}^{-1}$) than ungrafted (avg. $1.55 \text{ m}^2 \text{ plant}^{-1}$) cucumber plants, whereas the lowest EL value was recorded in the plants grafted onto 'Affyne' rootstock (avg. 58.3%). Finally, the lowest SPAD index and the lowest efficiency of the PSII in dark-adapted leaves, measured as the F_v/F_m ratio were recorded in ungrafted plants (Ekron) treated with CaCl_2 (Table 4).

3.5. Antioxidant enzyme activities

No significant differences among treatments were observed for catalase ($237.9 \text{ mmol mg}^{-1} \text{ protein min}^{-1}$) and ascorbate peroxidase ($0.50 \text{ mmol mg}^{-1} \text{ protein min}^{-1}$). Guaiacol peroxidase (GPX) was significantly affected by salinity ($P<0.001$) and grafting combination ($P<0.01$) but not by salinity \times grafting interaction (data not shown). When averaged over grafting combinations, the highest GPX activity was recorded in plants treated with CaCl_2 ($0.37 \text{ mmol mg}^{-1} \text{ protein min}^{-1}$). Finally, when averaged over salinity treatments, the plants grafted onto 'Affyne' showed the highest GPX activity ($0.212 \text{ mmol mg}^{-1} \text{ protein min}^{-1}$) than plants ungrafted and grafted onto P360 (avg. $0.097 \text{ mmol mg}^{-1} \text{ protein min}^{-1}$).

3.6. Mineral composition and partitioning

The macro and micro elements concentration and distribution in cucumber plants as a function of the grafting combination and salinity treatments are displayed in Tables 5 and 6. The concentration of N in leaves and stems was significantly affected by salinity \times grafting interaction where the lowest values were recorded in ungrafted (Ekron) plants treated with CaCl_2 . The concentration of N in fruits was highly influenced by salinity with the lowest values recorded with the three salt treatments (Table 5). The concentration of P in leaves, stems and roots was significantly

affected by grafting combination with values recorded for grafted plants (avg. 7.2 , 12.9 and 16.8 mg g^{-1} , respectively) being higher than ungrafted plants (avg. 6.7 , 9.9 and 11.9 mg g^{-1} , respectively). Moreover, the concentration of P in leaves, stems and fruits was significantly affected by salinity with the lowest values recorded with plants treated with CaCl_2 , NaCl, and $\text{CaCl}_2 + \text{NaCl}$ with no significant difference observed between the three salt types (Table 5).

The lowest concentration of K in leaves, stems, fruits and roots was observed in plants treated with NaCl. Moreover, the concentration of K in roots was significantly affected by grafting, with the highest values observed on Ekron/P360 combination (18.9 mg g^{-1}). Similarly, the lowest concentration of Ca in leaves, stems, fruits and roots was recorded in plants treated with NaCl (Table 5).

The concentration of Mg in leaves, fruits and roots was significantly affected by salinity with the lowest values recorded with plants treated with CaCl_2 and $\text{CaCl}_2 + \text{NaCl}$ treatments, whereas no significant difference among treatments was observed on Mg concentration in stems (Table 5). The concentration of Mg in leaves and roots was significantly affected by grafting combination with the highest values recorded for Ekron and Ekron/P360 combination.

Concentration of Na^+ in all plant tissue increased as the NaCl and $\text{CaCl}_2 + \text{NaCl}$ level in the nutrient solution increased (Table 6). The accumulation of Na^+ in leaf tissue of plants treated with NaCl and $\text{CaCl}_2 + \text{NaCl}$, with respect to control, was significantly lower in grafted plants (1446%, and 153%, respectively for Ekron/Affyne; and 5055% and 1240%, respectively for Ekron/P360) in comparison to that of ungrafted plants (3289% and 1433% respectively).

The concentration of Cl^- in leaves was significantly affected by salinity, with the highest values observed in plants treated with CaCl_2 , followed by $\text{CaCl}_2 + \text{NaCl}$, and NaCl, treatments, with the lowest valued recorded in the control (Table 6), whereas no significant difference among treatments was observed on Cl^- concentration in fruits and roots. Moreover, the concentration of Cl^- in leaves was significantly affected by grafting combination, with the highest values recorded on ungrafted (avg. 60.1 mg g^{-1} DW) in comparison to grafted plants (avg. 50.7 mg g^{-1} DW; Table 6).

The effect of salinity on leaves, stems and fruits micronutrient concentrations (Mn and Zn) was highly significant (Table 6). The

Table 5

Effects of salt treatment in the nutrient solution and grafting combination on macronutrient composition of leaves, stems, fruits, and roots of cucumber plants.

Salt treatment	Graft combination	Macronutrients (g kg^{-1} DW)																			
		N				P				K				Ca				Mg			
		Leaves	Stems	Fruits	Roots	Leaves	Stems	Fruits	Roots	Leaves	Stems	Fruits	Roots	Leaves	Stems	Fruits	Roots	Leaves	Stems	Fruits	Roots
Control	Ekron	49.8 a	40.1 a	39.4	34.3	7.8	13.4	16.6	15.9	26.1	29.8	36.3	24.2	45.3 d	8.2	4.7	11.2	7.3	3.3	2.8 a	2.3
	Ekron/Affyne	42.6 bc	28.8 b	36.9	33.3	8.2	17.5	15.7	19.3	24.1	26.9	30.3	21.6	48.1 d	8.7	3.9	7.7	6.9	3.9	2.2 b	1.6
	Ekron/P360	45.9 b	29.1 b	43.8	31.8	8.5	15.5	16.1	16.4	31.2	31.4	34.5	22.6	46.7 d	8.8	4.9	10.4	7.2	3.3	2.7 a	2.0
	Mean	46.0 a	32.6 a	40.0 a	33.1 a	8.1 a	15.5 a	16.1 a	17.2	27.1 a	29.3 a	33.7 a	22.8 a	46.6 b	8.6 b	4.4 b	9.7 a	7.1 a	3.4	2.5 a	1.9 a
CaCl_2	Ekron	31.9 e	22.9 d	34.8	20.6	7.1	7.3	12.9	8.3	17.8	19.8	30.8	10.1	68.0 a	15.3	6.4	12.1	3.7	3.0	1.7 cd	1.3
	Ekron/Affyne	37.8 cd	28.4 b	31.1	22.9	6.7	11.4	12.1	15.9	20.3	24.1	29.1	18.9	51.1 cd	15.0	5.4	9.9	2.0	2.9	1.5 d	1.4
	Ekron/P360	35.6 d	26.8 b	35.6	20.5	7.7	10.4	13.9	13.7	18.5	21.0	31.4	15.4	60.9 b	16.5	5.4	9.9	3.0	2.9	2.0 b	1.2
	Mean	35.0 c	26.0 c	33.8 b	21.3 c	7.1 ab	9.7 b	13.0 b	12.6	18.8 b	21.6 b	30.4 bc	14.8 b	60.0 a	15.6 a	5.7 a	10.6 a	2.9 c	2.9	1.7 d	1.3 b
NaCl	Ekron	40.5 c	27.7 b	34.0	29.5	5.7	10.1	14.8	12.4	15.1	17.5	28.5	15.0	31.8 e	7.2	3.4	6.6	5.2	3.1	2.2 b	2.2
	Ekron/Affyne	44.1 b	29.3 b	30.5	34.3	5.9	13.7	13.0	19.7	15.9	19.9	30.1	8.3	32.4 e	6.8	3.8	6.3	5.1	3.6	2.2 b	1.4
	Ekron/P360	45.8 b	28.8 b	34.0	32.6	7.3	11.4	15.7	15.3	15.6	15.3	26.8	16.7	24.9 f	6.6	3.2	8.1	4.5	3.1	2.0 b	2.5
	Mean	43.4 a	28.6 b	32.8 b	32.1 a	6.3 b	11.7 b	14.5 ab	15.8	15.5 c	17.5 c	28.4 c	13.3 b	29.7 c	6.9 b	3.4 c	7.0 b	4.9 b	3.2	2.1 b	2.0 a
$\text{CaCl}_2 + \text{NaCl}$	Ekron	36.8 cd	25.0 cd	31.9	19.5	6.3	8.8	13.6	11.0	13.4	19.0	31.6	15.1	55.8 bc	13.3	5.3	10.2	3.4	2.9	1.9 bc	1.6
	Ekron/Affyne	40.0 c	26.5 bc	33.4	28.8	6.0	12.6	12.6	19.5	16.9	19.1	29.8	11.4	44.4 d	11.8	5.2	8.9	2.5	3.3	1.8 bc	1.2
	Ekron/P360	43.5 bc	26.9 bc	33.5	28.3	7.6	10.9	13.2	14.8	16.1	20.9	33.4	20.9	53.5 c	16.0	5.4	10.7	3.3	3.0	2.1 bc	1.5
	Mean	40.1 b	26.1 c	32.9 b	25.5 b	6.6 b	10.7 b	13.1 b	15.0	15.4 c	19.6 bc	31.5 ab	15.7 b	51.2 b	13.7 a	5.3 a	9.9 a	3.0 c	3.0	1.9 c	1.4 b
Significance		***	***	***	***	**	*	*	NS	***	***	***	***	***	***	***	***	NS	***	***	
Salt (S)		NS	*	*	**	*	*	*	NS	***	***	***	***	***	***	***	***	NS	***	***	
Graft combination (G)		NS	*	*	**	*	*	*	NS	**	NS	NS	NS	*	*	*	NS	NS	**	***	
S × G		*	***	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	*	NS	

Values are the means of four replicate samples.

Means within columns separated using Duncan's multiple range test, $P=0.05$.

NS, nonsignificant.

* Significant at $P<0.05$.** Significant at $P<0.01$.*** Significant at $P<0.001$.

Table 6

Effects of salt treatment in the nutrient solution and grafting combination on sodium, chloride, iron, manganese, and zinc composition of leaves, stems, fruits, and roots of cucumber plants.

Salt treatment	Graft combination	Elements (g kg^{-1} DW)																			
		Na				Cl				Fe				Mn				Zn			
		Leaves	Stems	Fruits	Roots	Leaves	Stems	Fruits	Roots	Leaves	Stems	Fruits	Roots	Leaves	Stems	Fruits	Roots	Leaves	Stems	Fruits	Roots
Control	Ekron	0.33 d	0.61 d	0.50 d	1.59 e	43.2	26.4	33.9	47.5	0.038	0.024	0.033 a	0.076	0.107	0.027	0.020	0.083	0.019	0.031	0.032	0.045
	Ekron/Affyne	0.13 d	0.13 d	0.19 d	2.08 e	40.4	28.2	41.3	45.6	0.035	0.032	0.029 b	0.063	0.133	0.043	0.020	0.054	0.016	0.049	0.026	0.042
	Ekron/P360	0.20 d	0.47 d	0.87 d	1.76 e	41.2	30.3	27.0	42.5	0.039	0.025	0.034 a	0.099	0.118	0.032	0.020	0.083	0.021	0.037	0.031	0.043
	Mean	0.21 c	0.40 c	0.52 c	1.81 c	41.6 c	28.3 b	34.0	45.2	0.037	0.027	0.032 a	0.079	0.119 a	0.034 a	0.020 a	0.073 a	0.018 a	0.039 a	0.030 a	0.043
CaCl ₂	Ekron	0.52 d	1.13 d	1.02 d	2.12 e	75.8	52.6	43.3	47.3	0.032	0.022	0.026 bc	0.064	0.066	0.016	0.015	0.025	0.016	0.015	0.024	0.022
	Ekron/Affyne	0.16 d	0.16 d	0.22 d	4.12 d	53.8	41.2	44.8	49.4	0.028	0.029	0.028 b	0.050	0.071	0.033	0.019	0.029	0.013	0.038	0.027	0.038
	Ekron/P360	0.30 d	0.84 d	0.98 d	2.55 e	60.4	47.1	35.9	48.2	0.040	0.023	0.030 b	0.082	0.070	0.025	0.016	0.032	0.017	0.023	0.027	0.040
	Mean	0.32 c	0.71 c	0.66 c	2.93 c	63.3 a	46.9 a	41.3	48.3	0.033	0.025	0.028 b	0.066	0.069 b	0.024 b	0.017 b	0.028 b	0.015 b	0.025 c	0.026 b	0.033
NaCl	Ekron	11.17 a	13.43 a	9.10 a	11.83 b	58.2	53.8	39.6	51.0	0.039	0.028	0.023 c	0.067	0.074	0.020	0.010	0.042	0.016	0.023	0.023	0.035
	Ekron/Affyne	2.01 c	5.59 c	2.91 c	23.14 a	51.6	38.0	35.7	45.3	0.032	0.035	0.030 b	0.062	0.094	0.028	0.015	0.035	0.016	0.043	0.027	0.036
	Ekron/P360	10.31 a	14.76 a	6.93 b	11.16 b	51.3	49.4	36.6	48.5	0.040	0.028	0.025 c	0.066	0.064	0.023	0.011	0.040	0.019	0.032	0.023	0.038
	Mean	7.83 a	11.26 a	6.31 a	15.37 a	53.7 b	47.0 a	37.3	48.2	0.036	0.030	0.026 b	0.064	0.077 b	0.024 b	0.012 c	0.039 b	0.017 b	0.033 b	0.024 b	0.036
CaCl ₂ + NaCl	Ekron	5.06 b	8.27 b	2.46 c	6.84 c	63.1	51.4	47.7	49.0	0.039	0.028	0.025 c	0.069	0.071	0.022	0.014	0.043	0.018	0.016	0.024	0.041
	Ekron/Affyne	0.33 d	1.51 d	0.42 d	19.59 a	48.7	38.6	40.8	49.3	0.029	0.032	0.027 bc	0.059	0.072	0.030	0.018	0.034	0.014	0.038	0.025	0.033
	Ekron/P360	2.68 c	5.85 c	2.23 c	5.24 cd	58.6	52.5	39.6	50.3	0.039	0.026	0.028 bc	0.083	0.072	0.027	0.016	0.043	0.019	0.027	0.027	0.039
	Mean	2.68 b	5.20 b	1.70 b	10.55 b	56.8 b	47.4 a	42.6	49.5	0.036	0.029	0.027 b	0.070	0.071 b	0.026 b	0.016 b	0.040 b	0.017 b	0.027 c	0.025 b	0.038
Significance		***	***	***	***	***	***	NS	NS	NS	NS	***	NS	***	***	***	**	***	***	NS	
Salt (S)		***	***	***	***	***	***	NS	NS	NS	NS	***	NS	***	***	***	**	***	***	NS	
Graft combination (G)		***	***	***	***	**	*	NS	NS	NS	NS	*	NS	NS	*	*	NS	***	***	NS	
S × G		***	***	***	***	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	

Values are the means of four replicate samples.

Means within columns separated using Duncan's multiple range test, $P=0.05$.

NS, nonsignificant.

* Significant at $P<0.05$.** Significant at $P<0.01$.*** Significant at $P<0.001$.

tissue concentrations of these elements declined as the external salinity concentration increased.

4. Discussion

4.1. Agronomical response

In the present study, salt stress had a negative influence on yield and biomass production of cucumber as has also been demonstrated in other studies on melon (Colla et al., 2006a), tomato (Dorais et al., 2001), watermelon (Colla et al., 2006b), zucchini squash (Rouphael et al., 2006), and cucumber (Huang et al., 2009a,b; Colla et al., 2012) grown hydroponically. According to Munns (2005) plants display a two-phase growth response to salinity. The first phase appears quickly and is due to osmotic stress caused by salt outside the plants. The second phase takes time to develop, and results from the toxic effect of salt inside the plant, as the ability of the cells to compartmentalize salt in the vacuole is exceeded. Our results revealed a higher susceptibility of cucumber marketable yield to CaCl_2 compared to NaCl salinity, indicating that cucumber has entered the second growth response phase as CaCl_2 inhibited marketable yield more than NaCl . The greater yield reduction in CaCl_2 than NaCl was caused by toxic effects of Cl^- as observed in other plants (Chavan and Karadge, 1980; Bhivare et al., 1988; Colla et al., 2012). In contrast Trajkova et al. (2006), have reported a higher susceptibility of both vegetative growth and fruit yield of hydroponic cucumber to NaCl compared to CaCl_2 . Explanations for this disagreement could be the different molarity of the two salinity treatments used in the former experiment (24 mM NaCl vs. 12 mM CaCl_2), and consequently the two effects (osmotic and ionic) on plant responses cannot be fully separated. Besides, Trajkova et al. (2006) used the same molarity of chloride so it was expected a higher detrimental effect on cucumber growth and yield with NaCl (due to sodium toxicity) compared to CaCl_2 . Plants tested with both $\text{CaCl}_2 + \text{NaCl}$ showed higher yield and shoot biomass, than those treated with CaCl_2 or NaCl separately confirming the mitigation of Na^+ toxicity by supplemental Ca^{2+} (Wu and Wang, 2012). Moreover, under equimolar concentrations of the three different chloride salts the percentage of yield, and shoot biomass weight reductions in comparison to control were significantly lower in the plants grafted onto 'Affyne' than those grafted onto 'P360' and the ungrafted plants with NaCl and $\text{CaCl}_2 + \text{NaCl}$ treatments, whereas the lowest reduction in yield and shoot biomass was observed in both grafting combinations (Ekron/Affyne and Ekron/P360) than ungrafted plants with the CaCl_2 treatment. The higher yield of cucumber from grafted plants observed in this study has been reported earlier on tomato (Fernández-García et al., 2002; Estañ et al., 2005), melon (Colla et al., 2006a), and watermelon (Colla et al., 2006b). It was demonstrated that grafting directly affects plant yield (Neilsen and Kappel, 1996; Rivero et al., 2003) by interactions of the following processes: increase of water and nutrient uptake resulting from the vigorous root system of the rootstock (Lee, 1994; Colla et al., 2008; Rouphael et al., 2008b), enhanced production of endogenous hormones (Zijlstra et al., 1994), or enhancement of scion vigor (Leoni et al., 1990). The joint action of some or all of these processes could explain the higher yield in cucumber from grafted plants observed in the current study.

In general, salinity reduces the yield of vegetable crops but, in many cases, improves their quality as observed in plants grown in both soil and soilless culture (Francois and Maas, 1994; Rouphael et al., 2012b). In the present study, the aspects of grafted and ungrafted cucumber fruit quality most affected by the three salt types were also those which are particularly important for consumer satisfaction [i.e. taste (fruit DM, and TSS contents)]. The three salinity types improved fruit quality by increasing fruit DM and TSS

contents. The increases in fruit DM, and TSS contents observed in the current work agreed with previous results on cucumber (Huang et al., 2009a). A similar positive effect of salinity on fruit DM, sugar and organic acids was also found in tomato (Petersen et al., 1998; and references cited therein), in pepper (Navarro et al., 2002), in melon (Colla et al., 2006a), in watermelon (Colla et al., 2006b), and zucchini squash (Rouphael et al., 2006) grown in soilless culture. Salt tolerance in plants requires a net increase in the quantity of osmotically-active solutes in tissues (Gorham et al., 1985). The increase in TSS contents of cucumber fruits due to salinity may reflect an osmotic adjustment by enhanced synthesis of sugars in plant tissues (Greenway and Munns, 1980). Nevertheless, Mitchell et al. (1991) indicated that salt stress influenced osmotic potential and solute content in tomato fruit because it reduced water accumulation. There are several conflicting reports on changes in fruit quality due to grafting and whether grafting effects are advantageous or deleterious (Proietti et al., 2008; Rouphael et al., 2010a,b). In the present study, the nutritional quality of grafted cucumber were similar (DM and TSS) or higher (TA) to that of the plants grown on their own roots. Therefore, the use of grafted cucumber under saline conditions would be a potential strategy in increasing total yield and taking advantage of the quality effect of saline water.

4.2. Physiological response

Salinity interferes with several aspects of plant biochemistry, including photosynthesis, pigment synthesis, antioxidant defense system and membrane integrity (Colla et al., 2010a). Since the response to CaCl_2 , NaCl , and their combination depends on the plant ability to counteract the different effects induced by the three salt types, the different changes induced by the rootstocks ('Affyne' and 'P360') in the shoot growth should be also shown at physiological level. This was indeed the case, since the main physiological changes induced by the rootstocks were observed in the leaves of the genotype 'Ekron'. The lower yield and shoot reductions in grafted plants treated with both NaCl and CaCl_2 seems to be related to the better functioning of the photosynthetic apparatus. The reduction of photochemical activity is considered to be one of the non-stomatal factors that limit photosynthesis (Souza et al., 2004). Thus the activity of PSII was investigated in the present study by using chlorophyll fluorescence technology. F_v/F_m , the maximum quantum efficiency of PSII, is frequently used as an indicator of the photoinhibition or of stress damage to the PSII (Calatayud and Barreno, 2004). In the present study, F_v/F_m was significantly reduced in the ungrafted plants under 30 mM NaCl and especially under 20 mM CaCl_2 , suggesting the occurrence of photoinhibition, and this could be a consequence of damage to PSII (Demmig-Adams and Adams, 1992). However, no change was detected in the rootstock-grafted plants especially with NaCl . This result suggests that grafted plants can delay photoinhibition under salt stress. Similar results were observed in tomatoes under sever rather slight salt stress (He et al., 2009), and also in cucumber grafted onto *C. moschata* rootstock under saline conditions (Liu et al., 2012). In addition to reduced chlorophyll fluorescence, leaf area decreased in response to salinity in particular with CaCl_2 and in ungrafted rather with grafted plants. 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. A reduction in leaf area with excess of salt in particular NaCl has been reported for melon (Colla et al., 2006a), watermelon (Colla et al., 2006b), and zucchini squash (Rouphael et al., 2006). Pérez-Alfocea et al. (2010) proposed that plants have a genetic capacity to exclude Na^+ or any other major toxic ions such as Cl^- (depending on salt source and plant species) from shoots by maintaining energy consuming root toxic ion efflux. However, when salinity induced growth inhibition of both vegetative and fruit tissues a decreasing of sink activity

is observed leading to carbohydrate accumulation and subsequent metabolic inhibitions of photosynthesis additional to the stomatal limitations; the inhibitions of photosynthetic apparatus causes premature senescence and thus compromises assimilate supply to the roots (and other sink organs) for maintaining the original capacity for toxic ion efflux. As a consequence, more toxic ion is transported and accumulated in leaf tissues provoking ion-specific damage with additional negative effects on leaf senescence, photosynthesis and growth.

Another common damage under saline conditions is the accumulation of excessive ROS (Asada, 2006), which occurs when the reduction in photosynthesis is much higher than the extent of the reduction in effective quantum yield, suggesting electrons flowing to oxygen molecules rather than being used for carbon assimilation. Multiple antioxidant enzymes systems are believed to play an important role in the scavenging of ROS, and thus protect cells from oxidative damage. The antioxidant enzymes respond differently under saline conditions. The activities of CAT and APX were unaffected by salinity treatment, whereas activity of GPX was enhanced under salt stress especially with CaCl_2 , and more considerably with plants grafted onto Affyne than with ungrafted plants. Similar results were observed in grafted tomato and cucumber grown under saline conditions (He et al., 2009; Zhen et al., 2010). Therefore, we conclude that salt-tolerant rootstock (e.g. Affyne) play an important role in alleviating the oxidative damage caused by NaCl and especially CaCl_2 by enhancing the antioxidant capacity.

In many crops, cell membrane stability has been widely used as a criterion to differentiate stress tolerant and susceptible cultivars and in some cases higher membrane stability could be correlated with abiotic stress tolerance (Premchandra et al., 1992). The current study showed that the salt treatment $\text{CaCl}_2 + \text{NaCl}$ reduced the amount of ion leakage. Calcium is an important factor in the maintenance of membrane integrity (Lauchli and Epstein, 1970). It has been reported that an increase in Ca^{2+} concentration associated with membranes produced a reduction in their leakiness (Leopold and Willing, 1984). These findings indicate that if Ca^{2+} protects the membranes from the adverse effects of Na^+ , then in cucumber root membranes a reduction in Ca^{2+} concentration could decrease the membrane integrity, thus affecting water transport. The present study also showed that the grafting combination Ekron/Affyne reduced the amount of ion leakage in salinity stressed cucumber plants, especially those treated with NaCl and $\text{CaCl}_2 + \text{NaCl}$ indicating that grafting has facilitated the maintenance of membrane functions (i.e. semi-permeability). Finally, salinity toxicity in particular with CaCl_2 resulted in a significant loss in chlorophyll content (SPAD index) especially with ungrafted plants, since the SPAD index was significantly higher in grafted than ungrafted cucumber plants.

4.3. Nutrient uptake, translocation and accumulation

High ion concentrations of Na^+ , and Cl^- in the soil or water may depress nutrient-ion activities and produce extreme ratios of $\text{Na}^+/\text{Ca}^{2+}$, Na^+/K^+ , $\text{Ca}^{2+}/\text{Mg}^{2+}$, $\text{Cl}^-/\text{NO}_3^-$ (Grattan and Grieve, 1999). As a result, the plant becomes susceptible to specific-ion injury as well as to nutritional disorders that may result in reduced growth and yield (Grattan and Grieve, 1999). Using equimolar concentrations of salts allowed us to assess relative sensitivities of cucumber to Ca^{2+} , Cl^- and Na^+ . When averaged over grafting combination, the concentration of Cl^- was higher in leaves of CaCl_2 than NaCl -treated plants. In fact, the cucumber treated with CaCl_2 exhibited lowest yield and biomass production compared with plants exposed to NaCl , suggesting that Cl^- contributed to salt toxicity. In some plant species, Cl^- uptake and transport appeared to be less controlled than that of Na^+ (Alaoui-Sosse et al., 1998), this has been observed in the present study where Cl^- concentration was higher than Na^+ concentration in shoot tissues.

In common with previous studies concerning hydroponically lettuce (Tas et al., 2005) and cucumber (Trajkova et al., 2006) tissue concentrations of cationic nutrients, were generally reduced in salt treated plants and often more in NaCl than CaCl_2 -treated plants. The reduced tissue concentration of K, and Ca in NaCl rather than CaCl_2 -treated plants may be due to interference with their uptake by Na^+ (Hu and Schmidhalter, 2005). In addition, the anion level in particular NO_3^- was reduced more by CaCl_2 than by NaCl supporting the well known phenomena that an increase in Cl^- uptake and accumulation is often accompanied by a decrease in shoot-N concentration in horticultural crops (Grattan and Grieve, 1999). Moreover, mitigation of Na^+ toxicity by supplemental Ca^{2+} ($\text{CaCl}_2 + \text{NaCl}$ treatment) was also observed in the current experiment. The mitigating effect of supplemental Ca^{2+} on the toxicity of Na^+ is probably due to the replacement of displaced Ca^{2+} , thus restoring cell wall stability and plasma membrane integrity, facilitating higher K^+/Na^+ selectivity, and so improving plant salt tolerance (Zhang et al., 2010).

In the present study, the concentration of Na^+ in the aerial parts was less in grafted than in ungrafted plants in particular with Ekron/Affyne combination, suggesting that grafted Ekron/Affyne plants excluded more Na^+ than ungrafted plants and, as a result, limit Na^+ concentrations in leaves leading to a higher yield and biomass production. The sodium exclusion hypothesis was supported by Fernández-García et al., 2002, Colla et al. (2006a), Colla et al. (2006b), and Huang et al. (2009a) who observed Na^+ ion exclusion in grafted tomato, melon, watermelon, and cucumber, respectively. The possible exclusion or reduction in the concentration of Cl^- was not observed in grafted plants, since chloride has been found to accumulate to a level much higher into aerial part especially with CaCl_2 than with NaCl and had a greater negative effect on plant development. Based on the nutrient composition of plant tissues, grafted and ungrafted plants responded differently to salinity stress, as has been observed previously for yield and growth parameters. For ungrafted plants, the presence of CaCl_2 , NaCl , $\text{CaCl}_2 + \text{NaCl}$ caused significant decrease in macronutrient leaf concentration compared to grafted plants. The improved crop performance of grafted plants in particular the Ekron/Affyne combination treated with salt solution in comparison to ungrafted plants was attributed to their strong capacity to inhibit the Na^+ (for NaCl and $\text{CaCl}_2 + \text{NaCl}$ treatments) accumulation in the aerial parts and to maintain a better plant nutritional status.

5. Conclusions

In conclusion, our results demonstrate that in both grafted and ungrafted plants, the reduced yield and biomass production was due to both osmotic and ion specific effects, with CaCl_2 being more phytotoxic than NaCl and $\text{CaCl}_2 + \text{NaCl}$. The present study reveals substantial differences in the agronomical and physiological responses between grafted and ungrafted plants in response to salt type. The percentage of yield and biomass reduction in comparison to control was significantly lower in the plants grafted onto 'P360' and especially 'Affyne' than ungrafted plants. Grafted cucumber plants exposed to NaCl and $\text{CaCl}_2 + \text{NaCl}$ were capable of maintaining higher chlorophyll content (SPAD index), higher photochemical activity of PSII, increased the capacity of antioxidant system by enhancing the GPX activity, a better nutritional status (higher N, K, and Ca, and lower Na) in the shoot tissues and higher a membrane selectivity in comparison with ungrafted ones. The concentration of Na^+ in the aerial parts was less in grafted than in ungrafted plants in particular with Ekron/Affyne combination. Finally, the higher crop performance of grafted cucumber plants recorded with NaCl than with CaCl_2 , was attributed to the limited capability of the rootstocks (Affyne and P360) to restrict Cl^- shoot

uptake, thus Cl^- , which continues passing to the leaves, becomes the more significant toxic component of the saline solution.

References

- Alaoui-Sosse, B.L., Sehmer, P., Barnola, P., Dizengremel, P., 1998. Effect of NaCl salinity on growth and mineral partitioning in *Quercus robur* L., a rhythmically growing species. *Trees* 12, 424–430.
- Apel, K., Hirt, H., 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.* 55, 373–399.
- Asada, K., 2006. Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant Physiol.* 141, 391–396.
- Bhivare, V.N., Nimbalkar, J.D., Chavan, P.D., 1988. Photosynthetic carbon metabolism in French bean leaves under saline conditions. *Environ. Exp. Bot.* 28, 117–121.
- Borgognone, D., Colla, G., Rousphael, Y., Cardarelli, M., Rea, E., Schwarz, D., 2013. Effect of nitrogen form and nutrient solution pH on growth and mineral composition of self-grafted and grafted tomatoes. *Sci. Hortic.* 149, 61–69.
- Bremner, J.M., 1965. Total nitrogen. In: Black, C.A., Evans, D.D., White, I.L., Ensminger, L.E., Clark, F.E. (Eds.), *Methods of Soil Analysis*. Agronomy Monograph 9, Part 2. American Society of Agronomy, Inc., Madison Wisconsin, USA, pp. 1149–1178.
- Caines, A.M., Shennan, C., 1999. Interactive effects of Ca^{2+} and NaCl salinity on the growth of two tomato genotypes differing in Ca^{2+} use efficiency. *Plant Physiol. Biochem.* 37, 569–576.
- Calatayud, A., Barreno, E., 2004. Response to ozone in two lettuce varieties on chlorophyll a fluorescence, photosynthetic pigments, and lipid peroxidation. *Plant Physiol. Biochem.* 42, 549–555.
- Chance, B., Maehly, A.C., 1955. Assay of catalase and peroxidase. *Methods Enzymol.* 2, 764–775.
- Chavan, P.D., Karadge, B.A., 1980. Influence of sodium chloride and sodium sulfate salinization on photosynthetic carbon assimilation in peanut. *Plant Soil* 56, 201–207.
- Colla, G., Fanasca, S., Cardarelli, M., Rousphael, Y., Saccardo, F., Graifenberg, A., Curadi, M., 2005. Evaluation of salt tolerance in rootstocks of Cucurbitaceae. *Acta Hortic.* 697, 469–474.
- Colla, G., Rousphael, Y., Cardarelli, M., Massa, D., Salerno, A., Rea, E., 2006a. Yield, fruit quality and mineral composition of grafted melon plants grown under saline conditions. *J. Hortiv. Sci. Biotechnol.* 81, 146–152.
- Colla, G., Rousphael, Y., Cardarelli, M., Rea, E., 2006b. Effect of salinity on yield, fruit quality, leaf gas exchange, and mineral composition of grafted watermelon plants. *HortScience* 41, 622–627.
- Colla, G., Rousphael, Y., Cardarelli, M., Temperini, O., Rea, E., Salerno, A., Pierandrei, F., 2008. Influence of grafting on yield and fruit quality of pepper (*Capsicum annuum* L.) grown under greenhouse conditions. *Acta Hortic.* 782, 359–363.
- Colla, G., Rousphael, Y., Leopardi, C., Bie, Z., 2010a. Role of grafting in vegetable crops grown under saline conditions. *Sci. Hortic.* 127, 147–155.
- Colla, G., Rousphael, Y., Cardarelli, M., Salerno, A., Rea, E., 2010b. The effectiveness of grafting to improve alkalinity tolerance in watermelon. *Environ. Exp. Bot.* 68, 283–291.
- Colla, G., Suarez, C.M.C., Cardarelli, M., Rousphael, Y., 2010c. Improving nitrogen use efficiency in melon by grafting. *HortScience* 45, 559–565.
- Colla, G., Rousphael, Y., Mirabelli, C., Cardarelli, M., 2011. Nitrogen-use efficiency traits of mini-watermelon in response to grafting and nitrogen-fertilization doses. *J. Plant Nutr. Soil Sci.* 174, 933–941.
- Colla, G., Rousphael, Y., Rea, E., Cardarelli, M., 2012. Grafting cucumber plants enhance tolerance to sodium chloride and sulfate salinization. *Sci. Hortic.* 135, 177–185.
- Cramer, G.R., Läuchli, A., Polito, V.S., 1985. Displacement of Ca^{2+} by Na^+ from the plasmalemma of root cells. A primary response to salt stress? *Plant Physiol.* 79, 207–211.
- Cartero, J., Fernandez-Muñoz, R., 1999. Tomato and salinity. *Sci. Hortic. (Amsterdam)* 78, 83–125.
- Demmig-Adams, B., Adams III, W.W., 1992. Photoprotection and other responses of plants to high light stress. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 43, 599–626.
- Doris, M., Papadopoulos, A.P., Gosselin, A., 2001. Influence of electric conductivity management on greenhouse tomato yield and fruit quality. *Agronomie* 21, 367–383.
- Eaton, A.D., Clesceri, L.S., Greenberg, A.E., 1995. *Standard Methods for the Examination of Water and Wastewater*, 19th ed. Am. Public Health Assoc., Washington, USA.
- Edelstein, M., Plaut, Z., Ben-Hur, M., 2011. Sodium and chloride exclusion and retention by non-grafted and grafted melon and Cucurbita plants. *J. Exp. Bot.* 62, 177–184.
- Estañ, M.T., Martinez-Rodriguez, M.M., Perez-Alfocea, F., Flowers, T.J., Bolarin, M.C., 2005. Grafting raises the salt tolerance of tomato through limiting the transport of sodium and chloride to the shoot. *J. Exp. Bot.* 56, 703–712.
- Fernández-García, N., Martínez, V., Cerdá, A., Carvajal, M., 2002. Water and nutrient uptake of grafted tomato plants grown under saline conditions. *J. Plant Physiol.* 159, 899–905.
- Francois, L.E., Maas, E.V., 1994. Crop response and management of salt-affected soils. In: Pessarakli, M. (Ed.), *Handbook of Plant and Crop Stress*. Marcel Dekker, New York, pp. 343–449.
- Goreta, S., Bucevic-Popovic, V., Selak, G.V., Pavela-Vrancic, M., Perica, S., 2008. Vegetative growth, superoxide dismutase activity and ion concentration of salt-stressed watermelon as influenced by rootstock. *J. Agric. Sci.* 146, 695–704.
- Grattan, S.R., Grieve, C.M., 1999. Salinity-mineral nutrient relations in horticultural crops. *Sci. Hortic.* 78, 127–157.
- Greenway, H., Munns, R., 1980. Mechanisms of salt tolerance in nonhalophytes. *Annu. Rev. Plant Physiol.* 31, 149–190.
- Gorham, J., Wyn Jones, R.G., McDonnell, E., 1985. Some mechanisms of salt tolerance in crop plants. *Plant Soil* 89, 15–40.
- Havir, E.A., McHale, N.A., 1989. Enhanced-peroxidatic activity in specific catalase isozymes of tobacco, barley and maize. *Plant. Physiol.* 91, 15–812.
- He, Y., Zhu, Z.J., Yang, J., Ni, X.L., Zhu, B., 2009. Grafting increases the salt tolerance of tomato by improvement of photosynthesis and enhancement of antioxidant enzymes activity. *Environ. Exp. Bot.* 66, 270–278.
- Hickman, G.V., 1998. *Commercial Greenhouse Vegetable Handbook*. University of California, Division of Agriculture and Natural Resources Communication Services – Publications, Oakland, CA, pp. 15.
- Hu, Y., Schmidhalter, U., 2005. Drought and salinity: a comparison of their effects on mineral nutrition of plants. *J. Plant Nutr. Soil Sci.* 168, 541–549.
- Huang, Y., Zhu, J., Zhen, A., Chen, L., Bie, Z.L., 2009a. 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.
- Huang, Y., Tang, R., Cao, Q.L., Bie, Z.L., 2009b. Improving the fruit yield and quality of cucumber by grafting onto the salt tolerant rootstock under NaCl stress. *Sci. Hortic.* 122, 26–31.
- Karla, Y.P., 1998. *Handbook of Reference Methods for Plant Analysis*. CRC Press Inc., Boca Raton, FL, pp. 165–170.
- Kiehl, P.A., Lieth, J.H., Burger, D.W., 1992. Growth response of chrysanthemum to various container medium moisture tension levels. *J. Am. Soc. Hortic. Sci.* 114, 87–392.
- Lauchli, A., Epstein, E., 1970. Transport of potassium and rubidium in plant roots: the significance of calcium. *Plant Physiol.* 45, 639–641.
- Lee, J.M., 1994. Cultivation of grafted vegetables I. Current status, grafting methods, and benefits. *HortScience* 29, 235–239.
- Lee, J.M., Kubota, C., Tsao, S.J., Bie, Z., Hoyos Echevarria, P., Morra, L., Oda, M., 2010. Current status of vegetable grafting: diffusion, grafting techniques, automation. *Sci. Hortic.* 127, 93–105.
- Leoni, S., Grudina, R., Cadinu, M., Madeddu, B., Carletti, M.G., 1990. The influence of four rootstocks on some melon hybrids and a cultivar in greenhouse. *Acta Hortic.* 287, 127–134.
- Leopold, A.C., Willing, R.P., 1984. Evidence for toxicity effects of salt on membranes. In: Staples, R.C., Toennissen, D.H. (Eds.), *Salinity Tolerance in Plants: Strategies for Crop Improvement*. Wiley, New York, USA, pp. 67–76.
- Liu, Z.X., Bie, Z.L., Huang, Y., Zhen, A., Lei, B., Zhang, H.Y., 2012. Grafting onto Cucurbita moschata rootstock alleviates salt stress in cucumber plants by delaying photoinhibition. *Photosynthetica* 50, 152–160.
- Lu, C.M., Vonshak, A., 2002. Effects of salinity stress on photosystem II function in cyanobacterial *Spirulina platensis* cells. *Physiol. Plant.* 114, 405–413.
- Luettts, S., Kinet, J.M., Bouharmont, J., 1995. Changes in plant response to NaCl during development of rice varieties differing in salinity resistance. *J. Exp. Bot.* 46, 1843–1852.
- Marschner, H., 1995. *Mineral Nutrition of Higher Plants*, 2nd ed. Academic Press Limited, San Diego, CA.
- Martinez-Rodriguez, M.M., Estañ, M.T., Moyano, E., Abellán-García, J.O., Flores, F.B., Campos, J.F., Al-Azzawi, M.J., Flowers, T.J., Bolarín, M.C., 2008. The effectiveness of grafting to improve salt tolerance in tomato when an 'excluder' genotype is used as scion. *Environ. Exp. Bot.* 63, 392–401.
- Maxwell, K., Johnson, G.N., 2000. *Chlorophyll fluorescence – a practical guide*. *J. Exp. Bot.* 51, 659–668.
- Misra, A.N., Srivastava, A., Strasser, R.J., 2001. Utilization of fast chlorophyll a fluorescence technique in assessing the salt/ion sensitivity of mung bean and Brassica seedlings. *J. Plant Physiol.* 158, 1173–1181.
- Mitchell, J.P., Shennan, C., Grattan, S.R., 1991. Developmental changes in tomato fruit composition in response to water deficit and salinity. *Physiol. Plant.* 83, 177–185.
- Munns, R., 2005. Genes and salt tolerance: bringing them together. *New Phytol.* 167, 645–663.
- Munns, R., Tester, M., 2008. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* 59, 651–681.
- Nakano, Y., Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.* 22, 867–880.
- Navarro, J.M., Garrido, C., Carvajal, M., Martinez, V., 2002. Yield and fruit quality of pepper plants under sulphate and chloride salinity. *J. Hortic. Sci. Biotechnol.* 77, 52–57.
- Neilsen, G., Kappel, F., 1996. 'Bing' sweet cherry leaf nutrition is affected by rootstock. *HortScience* 31, 1169–1172.
- Neves, J.P.C., Ferreira, L.F.P., Vaz, M.M., Gazarini, L.C., 2008. Gas exchange in the salt marsh species *Atriplex portulacoides* L. and *Limoniastrum monopetalum* L. in Southern Portugal. *Acta Physiol. Plant.* 30, 91–97.
- Noctor, G., Foyer, C.H., 1998. Ascorbate and glutathione: keeping active oxygen under control. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49, 249–279.
- Norrie, J., Graham, M.E.D., Gosselin, A., 1994. Potential evapotranspiration as a means of predicting irrigation timing in greenhouse tomatoes grown in peat bags. *J. Am. Soc. Hortic. Sci.* 119, 163–168.
- Pagter, M., Bragato, C., Malagoli, M., Brix, H., 2009. Osmotic and ionic effects of NaCl and Na_2SO_4 salinity on *Phragmites australis*. *Aquat. Bot.* 90, 43–51.
- Petersen, K.K., Willumsen, J., Kaack, K., 1998. Composition and taste of tomatoes as affected by increased salinity and different salinity sources. *J. Hortic. Sci. Biotechnol.* 73, 205–215.

- Pérez-Alfocea, F., Albacete, A., Ghanem, M.E., Dodd, I.C., 2010. Hormonal regulation of source–sink relations to maintain crop productivity under salinity: a case study of root-to-shoot signalling in tomato. *Funct. Plant Biol.* 37, 592–603.
- Premchandra, G.S., Saneoka, H., Fujita, K., Ogata, S., 1992. Leaf water relations, osmotic adjustment, cell membrane stability, epicuticular wax load and growth as affected by increasing water deficits in Sorghum. *J. Exp. Bot.* 43, 1569–1576.
- Proietti, S., Rousphae, Y., Colla, G., Cardarelli, M., De Agazio, M., Zucchini, M., Moscatello, S., Battistelli, A., 2008. Fruit quality of mini-watermelon as affected by grafting and irrigation regimes. *J. Sci. Food Agric.* 88, 1107–1114.
- Rengel, Z., 1992. The role of calcium in salt toxicity. *Plant Cell Environ.* 15, 625–632.
- Rivero, R.M., Ruiz, J.M., Romero, L., 2003. Role of grafting in horticultural plants under stress conditions. *J. Food Agric. Environ.* 1, 70–74.
- Rousphae, Y., Colla, G., Battistelli, A., Moscatello, S., Proietti, S., Rea, E., 2004. Yield, water requirement, nutrient uptake and fruit quality of zucchini squash grown in soil and closed soilless culture. *J. Hortic. Sci. Biotechnol.* 79, 423–430.
- Rousphae, Y., Colla, G., 2005. Radiation and water use efficiencies of greenhouse zucchini squash in relation to different climatic parameters. *Eur. J. Agron.* 23, 183–194.
- Rousphae, Y., Cardarelli, M., Rea, E., Battistelli, A., Colla, G., 2006. Comparison of the subirrigation and drip-irrigation systems for greenhouse zucchini squash production using saline and non-saline nutrient solutions. *Agric. Water Manage.* 82, 99–117.
- Rousphae, Y., Cardarelli, M., Rea, E., Colla, G., 2008a. Grafting of cucumber as a means to minimize copper toxicity. *Environ. Exp. Bot.* 63, 49–58.
- Rousphae, Y., Cardarelli, M., Colla, G., Rea, E., 2008b. Yield, mineral composition, water relations, and water use efficiency of grafted mini-watermelon plants under deficit irrigation. *HortScience* 43, 730–736.
- Rousphae, Y., Cardarelli, M., Di Mattia, E., Tullio, M., Rea, E., Colla, G., 2010a. Enhancement of alkalinity tolerance in two cucumber genotypes inoculated with an arbuscular mycorrhizal biofertilizer containing *Glomus intraradices*. *Biol. Fertil. Soils* 46, 499–509.
- Rousphae, Y., Schwarz, D., Krumbein, A., Colla, G., 2010b. Impact of grafting on product quality of fruit vegetables. *Sci. Hortic.* 127, 172–179.
- Rousphae, Y., Cardarelli, M., Rea, E., Colla, G., 2012a. Improving melon and cucumber photosynthetic activity, mineral composition, and growth performance under salinity stress by grafting onto *Cucurbita* hybrid rootstocks. *Photosynthetica* 50, 180–188.
- Rousphae, Y., Cardarelli, M., Bassal, A., Leonardi, C., Giuffrida, F., Colla, G., 2012b. Vegetable quality as affected by genetic, agronomic and environmental factors. *J. Food Agric. Environ.* 10, 680–688.
- Santa-Cruz, M.M., Martinez-Rodriguez, F., Perez-Alfocea, R., Romero-Aranda, R., Bolarin, M.C., 2002. The rootstock effect on the tomato salinity response depends on the shoot genotype. *Plant Sci.* 162, 825–831.
- Savvas, D., Colla, G., Rousphae, Y., Schwarz, D., 2010. Amelioration of heavy metal and nutrient stress in fruit vegetables by grafting. *Sci. Hortic.* 127, 156–161.
- Schwarz, D., Rousphae, Y., Colla, G., Venema, J.H., 2010. Grafting as a tool to improve tolerance of vegetables to abiotic stresses: thermal stress, water stress and organic pollutants. *Sci. Hortic.* 127, 162–171.
- Souza, R.P., Machado, E.C., Silva, J.A.B., Lagoa, A., Silveira, J.A.G., 2004. Photosynthetic gas exchange, chlorophyll fluorescence and some associated metabolic changes in cowpea (*Vigna unguiculata*) during water stress and recovery. *Environ. Exp. Bot.* 51, 45–56.
- Tas, G., Papadandonakis, N., Savvas, D., 2005. Responses of lettuce (*Lactuca sativa* L. var. *longifolia*) grown in closed hydroponic system to NaCl or CaCl₂ salinity. *J. Appl. Bot. Food Qual.* 79, 136–140.
- Temperini, O., Calabrese, N., Temperini, A., Rousphae, Y., Tesi, R., Lenzi, A., Carito, A., Colla, G., 2013. Grafting artichoke into cardoon rootstocks: graft compatibility, yield and verticillium wilt incidence. *Sci. Hortic.* 149, 22–27.
- Trajkova, F., Papadandonakis, N., Savvas, D., 2006. Comparative effects of NaCl and CaCl₂ salinity on cucumber grown in a closed hydroponic system. *HortScience* 41, 437–441.
- Volkmar, K.M., Hu, Y., Steppuhn, H., 1998. Physiological responses of plants to salinity: a review. *Can. J. Plant Sci.* 78, 19–27.
- Wu, G.Q., Wang, S.M., 2012. Calcium regulates K⁺/Na⁺ homeostasis in rice (*Oryza sativa* L.) under saline conditions. *Plant Soil Environ.* 58, 121–127.
- Xia, J.R., Li, Y.J., Zou, D.H., 2004. Effects of salinity stress on PSII in *Ulva lactuca* as probed by chlorophyll fluorescence measurements. *Aquat. Bot.* 80, 129–137.
- Yokas, I., Tuna, A.L., Burun, B., Altunlu, H., Altan, F., Kaya, C., 2008. Responses of the tomato (*Lycopersicon esculentum* Mill.) plant to exposure to different salt forms and rates. *Turk. J. Agric. For.* 32, 319–329.
- Zhang, J.L., Flowers, T.J., Wang, S.M., 2010. Mechanisms of sodium uptake by roots of higher plants. *Plant Soil* 326, 45–60.
- Zhen, A., Bie, Z.L., Huang, Y., Liu, Z.X., Li, Q., 2010. Effects of scion and rootstock genotypes on the anti-oxidant defense systems of grafted cucumber seedlings under NaCl stress. *Soil Sci. Plant Nutr.* 56, 263–271.
- Zijlstra, S., Groot, S.P.C., Jansen, J., 1994. Genotypic variation of rootstocks for growth and production in cucumber; possibilities for improving the root system by plant breeding. *Sci. Hortic.* 56, 185–196.