

RESEARCH PAPER

Sodium and chloride exclusion and retention by non-grafted and grafted melon and *Cucurbita* plants

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Abstract

The effects of grafting on Na and Cl⁻ uptake and distribution in plant tissues were quantified in a greenhouse experiment using six combinations of melon (*Cucumis melo* L. cv. Arava) and pumpkin (*Cucurbita maxima* Duchesne × *Cucurbita moschata* Duchesne cv. TZ-148): non-grafted, self-grafted, melons grafted on pumpkins, and pumpkins grafted on melons. Total Na concentration in shoots of plants with pumpkin or melon rootstocks was <60 mmol kg⁻¹ and >400 mmol kg⁻¹, respectively, regardless of the scion. In contrast, shoot Cl⁻ concentrations were quite similar among the different scion–rootstock combinations. Na concentrations in exudates from cut stems of plants with a pumpkin rootstock were very low (<0.18 mM), whereas those in the exudates of plants with melon rootstocks ranged from 4.7 mM to 6.2 mM, and were quite similar to the Na concentration in the irrigation water. Root Na concentrations averaged 11.7 times those in the shoots of plants with pumpkin rootstocks, while in plants with melon rootstocks, values were similar. Two mechanisms could explain the decrease in shoot Na concentrations in plants with pumpkin rootstocks: (i) Na exclusion by the pumpkin roots; and (ii) Na retention and accumulation within the pumpkin rootstock. Quantitative analysis indicated that the pumpkin roots excluded ~74% of available Na, while there was nearly no Na exclusion by melon roots. Na retention by the pumpkin rootstocks decreased its amount in the shoot by an average 46.9% compared with uniform Na distribution throughout the plant. In contrast, no retention of Na could be found in plants grafted on melons.

Key words: Exudate, grafting, pumpkin.

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. For example, Yeo (1999) estimates that >20% of the irrigated land worldwide has been seriously affected by salinity. Attempts to increase salinity tolerance of sensitive crops by traditional breeding have not been very successful due to the physiological and genetic complexity of salt tolerance in plants (Flowers, 2004). In the last few decades, major attempts at dealing with this problem have included the generation of transgenic plants with novel introduced genes or altered expression levels of existing genes (Yamaguchi and Blumwald, 2005). While these efforts

await implementation under field conditions, it is important to develop more conventional methods, which will enable high crop production under saline conditions in the near future. One such method consists of grafting salt-sensitive species onto more tolerant rootstocks. In tree crops, grafting is a well-established and commonly used practice. For example in citrus, Cleopatra mandarin rootstock was found to be a highly salt-tolerant rootstock which could transmit its salt-tolerant traits to more sensitive species via grafting (Moya *et al.*, 2002). The chloride toxicity tolerance of Cleopatra mandarin rootstock was attributed to its exclusion by the roots (Banuls and Primo-Milo, 1991) and to reduction of transpiration and water and salt uptake by the plant

(Moya *et al.*, 2002). In addition, pears grafted on quince and irrigated with saline water accumulated a substantial amount of Cl^- in their leaves, while the absorbed Na was mostly stored in the roots (Musacchi *et al.*, 2006).

Recently, the grafting of vegetable plants has become more common, and various methods and machines for vegetable grafting have been developed. Although the main purpose of vegetable grafting has been to control soilborne diseases and nematodes, improved tolerance to environmental stresses such as low soil temperatures (Okimura *et al.*, 1986), flooding (Liao and Lin, 1996), salinity (Romero *et al.*, 1997), and high B concentrations (Edelstein *et al.*, 2005, 2007) has been shown.

Grafting tomato plants for increased salinity tolerance is the most common grafting practice among vegetables. Exposure of tomato cultivars Fanny and Goldmar grafted on tomato hybrid AR-9704 to high NaCl concentrations (up to 75 mM) resulted in lower Cl^- and Na concentrations in the leaves as compared with non-grafted plants (Fernandez-Garcia *et al.*, 2002, 2004). Santa-Cruz *et al.* (2001) also found an increase in fruit yield when a salt-sensitive tomato cultivar was grafted on a tolerant rootstock and irrigated with water containing 50 mM NaCl as compared with self-grafted plants. Here as well, the concentrations of Na and Cl^- in the leaves of the plants grafted on the tolerant rootstock were lower than in the self-grafted plants. Estan *et al.* (2006) and Martinez-Rodriguez *et al.* (2008) also showed that grafting of a commercial tomato hybrid onto rootstocks having the potential to exclude saline ions reduced the concentration of both Na and Cl^- in the leaves of the scion. Moreover, they observed a high negative correlation between fruit yield and leaf Na and Cl^- concentrations in salt-treated plants, suggesting that grafting could be effective for overcoming salinity stress in tomatoes.

Ruiz *et al.* (2006) found that grafting salt-sensitive tobacco cultivars on a more tolerant cultivar increases biomass production and yield quality of the grafted plants under conditions of saline stress. Although the mechanism of salt tolerance transmission in the grafted tobacco plants was not known, Ruiz *et al.* (2006) showed that the foliar concentrations of Na and Cl^- were lower in the grafted compared with the non-grafted cultivars. Similar results were found by Romero *et al.* (1997), Colla *et al.* (2005, 2006b), and Edelstein *et al.* (2005) in melon and watermelon plants that were grafted on salt-tolerant cucurbit rootstocks, resulting in higher fruit yield in the grafted plants.

Several factors may be responsible for the salt sensitivity of non-halophytes, one being the uptake and accumulation of salt ions in the plant tissue. Chloride influx may require energy, and is probably catalysed by a $\text{Cl}^-/2\text{H}^+$ symporter (Munns and Tester, 2008). However, it is mostly taken up freely with water, and may thus accumulate in the leaves according to the transpiration rate (Wahome, 2003).

Na uptake by root cells has not been fully elucidated. Na can be absorbed by the root cell symplast or loaded into the xylem by two main transport mechanisms: (i) passive diffusion through the lipid bilayer (Harvey, 1985;

Davenport *et al.*, 1997); or (ii) passage through proteinaceous channels in the cell membrane (Plett and Moller, 2010). The latter mechanism depends on metabolic energy, as demonstrated by Dannel *et al.* (2000) and Dordas and Brown (2000). For example, Na permeability coefficients of purified plasma membrane vesicles obtained from *Cucurbita pepo* L. (squash) roots were six times greater than those of microsomal vesicles. Na uptake by intact *C. pepo* plants was reduced by 40–90% following administration of channel transport inhibitors, and other non-electrolytes similar in molecular size to boric acid competed with B, thereby decreasing its uptake rate (Dordas and Brown, 2000). The membrane potentials of other channels may, however, be depolarized and less selective, thereby allowing Na to enter (Maser *et al.*, 2002). Na may also enter cells via the KUP/HAK/KT potassium transporters, glutamate-activated channels, LCT transporters and cyclic nucleotide-gated channels (Maser *et al.*, 2002; Tester and Davenport, 2003).

Na uptake by the plant does not ensure its further transport and distribution, as it may be retained in the roots or lower plant parts. Blom-Zanstra *et al.* (1998) showed a gradient in Na concentration along the xylem and pith cells of sweet pepper plants, such that Na was mainly located in the basal pith cells. The long-distance transport of Na in the plant takes place mainly in the xylem, similar to other alkaline ions (Zeller and Feller, 2000; Plaut *et al.*, 2004), and thus may depend, to some extent, on transpiration rate.

As mentioned above, grafting of vegetable plants markedly decreases leaf Na concentration in leaves of grafted plants (Colla *et al.*, 2006a); however, the mechanism responsible for low Na concentration in the leaves of grafted plants is not completely known. Mechanisms that could potentially decrease Na concentration in the leaves of grafted vegetable plants are: (i) the graft itself acting as a barrier that limits Na transport from the rootstock to the scion; (ii) the rootstock excluding Na, which, in turn, decreases its uptake rate by the plant; and (iii) the rootstock retaining and accumulating Na in its tissues, and consequently decreasing its movement to the plant shoot. The objective of the present work was to study and quantify the effects of grafting salt-sensitive melon plants on salt-tolerant cucurbita rootstocks on the plants' uptake of Na and Cl^- and their distribution within the plant organs.

Materials and methods

Experimental set-up

Melon (*Cucumis melo* L., cv. Arava) and commercial *Cucurbita maxima* Duchesne \times *Cucurbita moschata* Duchesne rootstock 'TZ-148' (pumpkin) plants were used in this study. The melon and pumpkin plants were grown as non-grafted plants, self-grafted plants, melon grafted on pumpkin, and pumpkin grafted on melon. All of the plants were planted on 30 January 2006 in 10.0 l pots containing Perlite no. 2 (Agrical, Habonim, Israel), one seedling per pot, in a heated greenhouse at the Newe Ya'ar Research Center of the ARO, in northern Israel. The plants were irrigated to excess with fresh water (Table 1) five times a day by

a drip system so that ~35% of the irrigation water drained as leachate to allow leaching of excess salts from the pot. Plants were fertilized with Shefer (Fertilizers & Chemicals, Haifa, Israel) containing N, P, K (7:1.4:5.8, weight percentages), and microelements (Fe, Mn, Zn, Cu, and Mo). The fertilizer was applied via the irrigation system, with a concentration in the irrigation water of 0.2% (v/v). The experiment was conducted in a completely randomized design, with four replicates (four pots) for each treatment (plant type).

The shoot (stems and leaves) and roots of each plant were harvested separately immediately after the last irrigation event at 30 d after planting, before bearing fruits. After the harvest, the shoot and roots of each plant were weighed, washed gently in deionized water, dried at 60 °C for 48 h, reweighed, and stored pending chemical analysis. At the time of harvest, the stem of each plant was cut ~5 cm above the substrate surface in the pot, ~1 cm below the graft in the grafted plants. The xylem sap exudates from the cut stem were collected for 2 h into plastic tubes; their quantity was recorded, and they were then immediately frozen at -20 °C until chemical analysis.

Chemical analysis

In three of the four replications of each treatment, the total concentration of Na and soluble Cl⁻ in the plant shoot and root tissues was determined. For this chemical analysis, the whole dry shoot and root system of each plant were separately ground to 2 mm mesh, and representative subsamples of the ground material were analysed. The Na content was determined by ashing 0.25 g of each subsample in a furnace at 600 °C for 5 h, and then adding 5 ml of 1 M HCl to the cooled ash; the solution was filtered after 15 min and analysed. The concentration of Na was determined by flame photometry (Lachica *et al.*, 1973). The concentration of soluble Cl⁻ in the plant organs was analysed after aqueous extraction of 0.25 g of the plant subsamples in 25 ml of distilled water. The Cl⁻ concentration in the infiltrated solution was measured by titration with AgNO₃ according to Koltoff and Kuroda (1951). The concentrations of Na and Cl⁻ in the xylem sap exudates were determined similarly after the exudates had reached room temperature.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) (SAS Institute, Cary, NC, USA). Separation of means was subjected to Tukey's honestly significant difference test, with a significance level of 0.05.

Results and Discussion

The dry weights of the whole plant, shoot, and roots of the six different grafting combinations: non-grafted pumpkin, non-grafted melon, melon or pumpkin grafted on their own rootstocks, melon grafted on pumpkin, and pumpkin grafted on melon, are presented in Table 2. In some cases, the grafting had effects on the development and growth of the scions, rootstocks, and whole plant, as evidenced by the dry weight of their biomass (Table 2). For example, the dry

Table 1. Values of pH and electrical conductivity (EC) and concentrations of Cl⁻, Na, and K in the water used for irrigation

pH	EC (dS m ⁻¹)	Cl ⁻ (mM)	Na (mM)	K (mM)
7.0	1.9	7.0	5.9	1.9

weight of the roots of melons grafted on pumpkin rootstocks (M/P) was significantly lower than those of the non-grafted pumpkins (P) or pumpkins grafted on their own rootstocks (P/P) (Table 2). Moreover, the dry weight of the roots of pumpkin grafted on melon rootstock (P/M) was significantly higher than those of the non-grafted melon (M) or melon grafted on its own rootstock (M/M) (Table 2). These effects of grafting on the development and growth of the scions and rootstocks were probably the result of physiological relationships existing between the scions and rootstocks in the different grafting plants (Oda, 2002).

Total Na and soluble Cl⁻ concentrations in the shoots of plants from the six different grafting combinations are presented in Fig. 1A and B, respectively. A high concentration of Na, ranging from 390 mmol kg⁻¹ to 414 mmol kg⁻¹, was found in the shoots of plants with melon rootstocks, regardless of the scion type (Fig. 1A). In contrast, very low Na concentrations (41–62 mmol kg⁻¹) were found in the shoots of plants with pumpkin rootstocks, with either melon or pumpkin scions (Fig. 1A). The shoot Cl⁻ concentrations were generally quite similar among the six different grafting combinations (Fig. 1B).

Similar results were found by Edelstein *et al.* (2005) in non-grafted melons (cv. Arava) and melons grafted on pumpkin rootstocks (TZ-148) irrigated with fresh water (EC 1.8 dS m⁻¹, 5.1 mM Na, 7.3 mM Cl⁻) or saline water (EC 4.5 dS m⁻¹, 15.5 mM Na, 34 mM Cl⁻). In that study, the total Na and soluble Cl⁻ concentrations were determined in old leaves in the lower third of the plant after fruit harvest. The Na concentrations in the leaves of the melon plants irrigated with fresh or saline water were 610 mmol kg⁻¹ and 740 mmol kg⁻¹, respectively, in non-grafted plants and 200 mmol kg⁻¹ and 270 mmol kg⁻¹, respectively, in the grafted plants (Edelstein *et al.*, 2005). In contrast, the Cl⁻ concentrations in the leaves of the melon plants that were irrigated with fresh or saline water were 570 mmol kg⁻¹ and 1510 mmol kg⁻¹, respectively, in non-grafted plants, and 670 mmol kg⁻¹ and 1380 mmol kg⁻¹, respectively, in grafted plants. Those results indicated that the relative

Table 2. Dry weight of whole plant, shoot, and root of the six different plant combinations: non-grafted pumpkin (P), self-grafted pumpkin (P/P), melon grafted on pumpkin rootstock (M/P), non-grafted melon (M), self-grafted melon (M/M), and pumpkin grafted on melon rootstock (P/M)

Plant types	Dry weight (kg plant ⁻¹)		
	Whole plant	Shoot	Roots
P	0.1711 a ^a	0.1553 a	0.0158 a
P/P	0.1811 a	0.1688 a	0.0123 a
M/P	0.0822 b	0.0777 b	0.0045 b
M	0.0465 b	0.0428 b	0.0037 b
M/M	0.0641 b	0.0594 b	0.0047 b
P/M	0.1511 a	0.1396 a	0.0115 a

^a For each column, different letters indicate statistically significant (at the 0.05% level) differences between the plant types.

effect of grafting melons on pumpkin rootstocks on the decrease of Na accumulation in the plant shoots was similar under irrigation with low- and high-salinity water. Similar results were also found by Colla *et al.* (2006a, b) with non-grafted melon and watermelon plants and their counterparts grafted on *Cucurbita* rootstocks. The plants were irrigated with water at various ECs, ranging from 2 dS m⁻¹ to 9.7 dS m⁻¹. In both melon and watermelon plants, grafting reduced the concentration of Na, but not Cl⁻, in the leaves

The results in Fig. 1 and those in Edelstein *et al.* (2005) and Colla *et al.* (2006a, b) indicate that the presence of a pumpkin rootstock leads to a reduction in Na accumulation in the plant shoot, while Cl⁻ accumulation in the plant shoot is not affected. The low concentrations of Na in the shoot of plants with pumpkin rootstocks (Fig. 1A) could result from three main mechanisms: (i) the graft itself acts as a selective barrier that limits Na movement from the rootstock to the scion; (ii) various rootstocks have different selectivities for Na absorption, and the pumpkin rootstock excludes Na; or (iii) accumulation and binding of Na in the tissues of pumpkin rootstock, which would limit its movement toward the shoot, as reported for some fruit trees (Elmotaium *et al.*, 1994; Papadakis *et al.*, 2004).

The Na concentrations in the shoots of the non-grafted and self-grafted pumpkins were similar (Fig. 1A). Similarly, the Na concentrations in the shoots of the non-grafted and self-grafted melons were similar (Fig. 1A). These results suggested that the graft itself does not act as a selective

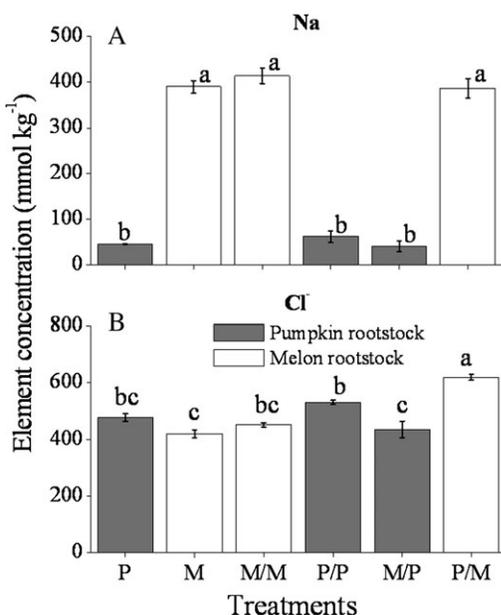


Fig. 1. Total Na (A) and soluble Cl⁻ (B) concentrations in the shoots of non-grafted pumpkins (P), non-grafted melons (M), self-grafted melons (M/M), self-grafted pumpkins (P/P), melons grafted on pumpkins (M/P), and pumpkins grafted on melons (P/M). Bars indicate two standard errors. Different letters indicate statistically significant (at the 0.05% level) differences between plant types for each element.

barrier limiting Na movement from the rootstock toward the scion in the grafted plants.

The effects of grafting on the absorption of Na and Cl⁻ by the rootstock and their movement toward the shoot were also studied by analysing the exudates from a cut made in the shoot. The steady flow rates of the exudates from the stems of the six different combinations of melon and pumpkin rootstocks and scions during 120 min of sampling ranged from an average 9.18 ml h⁻¹ in self-grafted melons to 24.84 ml h⁻¹ in non-grafted pumpkins (Fig. 2). These exudate flow rates may represent the flow rate of the solutes through the xylem of the different plants.

The concentrations of Na and Cl⁻ in the exudates and their amounts exuding from the cut stem per unit time for the six different melon and pumpkin combinations are presented in Fig. 3. While the concentration of Na in the exudates of plants with pumpkin rootstocks was very low (<0.18 mM) (Fig. 3A), its concentration in the exudates of plants with melon rootstocks was high, ranging from 4.7 mM to 6.3 mM, and quite similar to its concentration in the irrigation water (Fig. 3A and Table 1). Although the steady flow rates of the exudates from the plants with pumpkin rootstocks were nearly twice as high as those from the plants with melon rootstocks (Fig. 2), the amount of Na flowing out of the xylem per unit time in the latter plants was >30 times higher on average than that from plants with pumpkin rootstocks (Fig. 3B). Since the shoot was cut for exudate collection below the graft in the grafted plants, the results in Figs. 2, 3A, B indicate that the reason for the low Na concentration in the shoots of plants with pumpkin rootstocks is related mainly to the behaviour of the rootstock and not of the graft itself or the plant shoots.

The concentrations of Cl⁻ in the exudates of plants with pumpkin rootstocks were somewhat lower than those in plants with melon rootstocks (Fig. 3C). The amounts of Cl⁻ exuded from the xylem through the cut stem per unit time (Fig. 3D) were similar, in general, among the plants irrespective of scion or rootstock, except for melons grafted on pumpkin rootstocks (Fig. 3D). The higher exudate flow

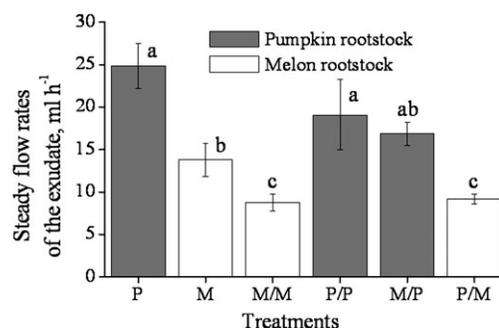


Fig. 2. Average steady-flow rates of exudates during 120 min of sampling from non-grafted pumpkins (P), non-grafted melons (M), self-grafted melons (M/M), self-grafted pumpkins (P/P), melons grafted on pumpkins (M/P), and pumpkins grafted on melons (P/M). Bars indicate two standard errors. Different letters indicate statistically significant (at the 0.05% level) differences between plant types.

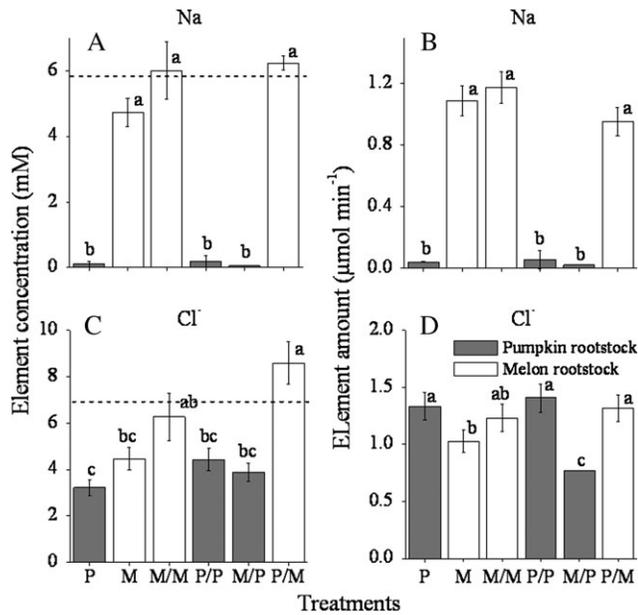


Fig. 3. Concentrations of Na (A) and Cl⁻ (C) in the exudates and the amounts of Na (B) and Cl⁻ (D) flowing out from the cut stem per unit time for non-grafted pumpkins (P), non-grafted melons (M), self-grafted melons (M/M), self-grafted pumpkins (P/P), melons grafted on pumpkins (M/P), and pumpkins grafted on melons (P/M). Bars indicate two standard errors. Different letters indicate statistically significant (at the 0.05% level) differences between plant types for each panel. The lateral, dashed lines in A and C indicate the average elements concentration in the irrigation water.

rates from the plants with pumpkin rootstocks relative to those with melon rootstocks (Fig. 2) compensated for the lower Cl⁻ concentrations in the former's exudates (Fig. 3C). Thus similar Cl⁻ amounts were exuded from the xylem per unit time (Fig. 3D), and Cl⁻ concentrations were similar in the plant shoots of all plant types (Fig. 1B).

Elements that are absorbed by the plant roots can accumulate to higher levels in the root compared with shoot tissues, be distributed uniformly throughout the plant, or accumulate to higher levels in the shoot compared with root tissues, mainly as a result of transpiration. Thus, another mechanism that could decrease the concentration of Na in the shoots of plants with pumpkin rootstocks (Fig. 1A) might be its retention by the root tissue. The total Na and soluble Cl⁻ concentrations in the roots of the six different plant combinations are presented in Fig. 4. In the plants with the pumpkin rootstocks, root Na concentrations were significantly higher than in the shoot (compare Figs 1A and 4A), by on average 11.7-fold. In contrast, in the plants with melon rootstocks, the Na concentrations in the roots and shoots were similar (compare Figs 1A and 4A). The Cl⁻ concentration in the roots was 1.4 and 0.74 times higher on average than in the shoot for plants with pumpkin and melon rootstocks, respectively (compare Figs 1B and 4B). These results indicate 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

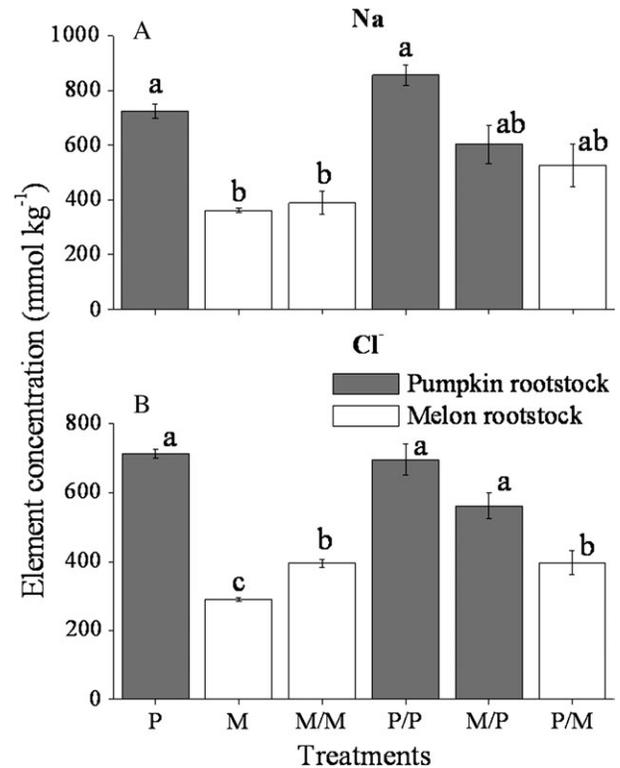


Fig. 4. The total Na and soluble Cl⁻ concentrations in the roots of non-grafted pumpkins (P), non-grafted melons (M), self-grafted melons (M/M), self-grafted pumpkins (P/P), melons grafted on pumpkins (M/P), and pumpkins grafted on melons (P/M). Bars indicate two standard errors. Different letters indicate statistically significant (at the 0.05% level) differences between plant types for each element.

rootstocks was much more uniform, despite a slight retention of Cl⁻ in the roots.

Two possible mechanisms could explain the decrease in leaf Na concentrations in the plants grafted on pumpkin rootstocks (Fig 1A): (i) Na exclusion by the pumpkin rootstock; and (ii) Na retention and accumulation in the tissue of the pumpkin rootstock.

All of the plants in this study were grown in pots filled with Perlite, an inert material. As irrigation was in excess and with high frequency (five times a day), it is highly likely that the Cl⁻ and Na concentrations in the irrigation water were similar to their concentrations in the solution near the roots. Although chloride uptake is probably catalysed by a Cl⁻/2H⁺ symporter (Munns and Tester, 2008), the high irrigation frequency most probably caused a constant external Cl⁻ concentration and a fixed point in the Michaelis–Menten curve, and therefore, the Cl⁻ uptake rate can be considered as a constant.

Under the present experimental conditions, for an element that is taken up freely by the plant and is not excluded by the plant rootstock, such as Cl⁻ (Wahome, 2003; Colla *et al.*, 2006a, b), the relationship between the average concentration of Cl⁻ in the irrigation water and in the tissue of the whole plant is defined by Equation 1:

$$T \cdot C_{fw} = C_{fp} \cdot M \quad (1)$$

where T is the total transpiration of the plant during the growth period, C_{fw} (mM) and C_{fp} (mmol kg⁻¹) are the average concentrations of Cl⁻ in the irrigation water and in the tissues of the whole plant, respectively, and M (kg) is the total dry biomass of the plant.

Equation 1 can be converted to Equation 2.

$$\frac{C_{fp} \cdot M}{T \cdot C_{fw}} = 1 \quad (2)$$

Under the same conditions, for an element that is excluded by the plant rootstock, such as Na, the relationship between the element's concentration in the irrigation water (C_{ew}) (mM) and in the tissues of the whole plant (C_{ep}) (mmol kg⁻¹) is defined in Equation 3

$$T \cdot C_{ew} > C_{ep} \cdot M \quad (3)$$

and this equation can be converted to Equation 4

$$\frac{C_{ep} \cdot M}{T \cdot C_{ew}} < 1 \quad (4)$$

The absorption factor (AF) of an excluded element for a given plant, as a percentage of the absorption of a non-excluded element by the same plant under the same growing conditions for the same period, can be calculated by Equation 5, which is a combination of Equations 2 and 4:

$$AF = \left(\frac{C_{ep} \cdot C_{fw}}{C_{ew} \cdot C_{fp}} \right) \cdot 100 \quad (5)$$

The AF values of Na, given as percentages of the non-excluded Cl⁻ and calculated by Equation 5 for the various melon and pumpkin grafting combinations, are presented in Table 3. The C_{fp} and C_{fw} values for Cl⁻, and the C_{ep} and C_{ew} values for Na which were used to calculate the AF values are presented in Tables 1–3. The AF values of Na in the plants with pumpkin rootstocks averaged 25.9%, while the AF values of Na in the plants with melon rootstocks averaged 98.6% (Table 3). These results indicate that the pumpkin rootstock excludes on average ~74% of the potential amount of Na available for absorption, while there is nearly no Na exclusion by the melon rootstock.

It is well accepted that nearly all of the Na which is transported to the foliage accumulates there, because its circulation from the foliage to the roots via the phloem is negligible (Munns and Tester, 2008). Therefore, the accumulation of Na in the shoot is a result of its transport via the xylem; accordingly, restriction of its accumulation to the roots, as found here in the plants grafted on pumpkin rootstocks (Figs 1, 4), should be due to a decrease in its transfer to the xylem. This transfer depends on Na influx

Table 3. Average concentration of total Na and soluble Cl⁻ in the whole plant and values of Na absorption (AF) and retention (RF) factors for the six different plant combinations: non-grafted pumpkin (P), self-grafted pumpkin (P/P), melon grafted on pumpkin rootstock (M/P), non-grafted melon (M), self-grafted melon (M/M), and pumpkin grafted on melon rootstock (P/M)

Plant types	Average concentration in the whole plant (mmol kg ⁻¹)		AF (%)	RF (%)
	Na	Cl ⁻		
P	105.2 b ^a	493.1 b	25.1	59.6
P/P	128.4 b	537.8 b,c	31.5	41.4
M/P	77.0 b	395.2 c	21.0	39.6
M	455.8 a	411.2 c	106.3	~ 0
M/M	390.1 a	439.7 b,c	99.1	~ 0
P/M	471.5 a	602.9 a	90.5	~ 0

^a For each column, different letters indicate statistically significant (at the 0.05% level) differences between the plant types.

into the root epidermis and cortex cells, and its efflux back into the soil solution, the combination of these two processes being defined as exclusion in this study. It has been suggested that the initial entry of Na from the soil solution into the root cortex cytoplasm via non-selective cation channels is passive, and is favoured by differences in concentration and voltage (Cheeseman, 1982). The rate of this influx must be high, as Na accumulation may reach an external concentration of 50 mM within a matter of minutes (Tester and Davenport, 2003).

Even if the rate of such unidirectional influx were much lower, giving the presently observed external concentration of 5.9 mM, it is highly feasible that most of the Na⁺ which enters the outer part of the root is pumped back via the plasma membrane by Na⁺/H⁺ antiporters, rather than by passive transport (Tester and Davenport, 2003). Efflux of Na could potentially also take place to some extent by Na-translocating ATPases (Mennen *et al.*, 1990). However, the thermodynamic cost of moving Na from the cytosol to the soil solution by this mechanism would be very high, namely 1 ATP per 1 Na extruded. Tester and Davenport (2003) claimed that the energy cost for moving Na⁺ to the external solution is much lower and depends on the Na⁺ concentration in the external solution. Some genes encoding Na⁺ efflux proteins have been detected (Pardo *et al.*, 2006), mainly in *Arabidopsis*. Although no such information is available on cucurbit plants, it is possible that the activity of Na⁺/H⁺ antiporters in pumpkin roots is encoded to a lesser extent or that they are less active than in melon, resulting in lower exclusion of Na⁺. Several cation channels have a higher selectivity for K⁺ than for Na⁺, such as the potassium inward-rectifying channel, which selectively accumulates K⁺ over Na⁺ ions. Another possibility is the histidine kinase transporter (HKT), which has a low affinity for Na⁺ transporter and blocks Na⁺ entry into the cytosol (Tuteja, 2007). These mechanisms may also be operating to

increase the exclusion of Na in pumpkins, although direct evidence is not available. The limited Na⁺ accumulation in plant shoots grafted on pumpkins was clearly reflected in its low concentration in the exudate, indicating that the concentration of Na in the xylem sap must also be low. This implies that transport of Na⁺ from the root symplast into the xylem of pumpkin roots was reduced, or that there was a marked retrieval of Na⁺ back from the xylem before it could be transported by the transpiration stream. The possibility that ions in the cytosol may not be loaded into the xylem, but rather retained in the roots, extruded, and sequestered in vacuoles of root cells, has been raised by several investigators (Blumwald and Poole, 1987; Gaxiola *et al.*, 2001). This may restore ionic homeostasis and keep the cytosol nearly free of Na⁺; it would also allow growth of young cells (Hasegawa *et al.*, 2000). The sequestration of Na⁺ within vacuoles is achieved by tonoplast Na⁺/H⁺ antiporters similar to those of the Na⁺/H⁺ exchanger, which allows coupling of H⁺ movement inside the cytosol along an electrochemical gradient and retrieval of Na⁺ out of the cytosol (Tuteja, 2007).

The effect of element retention in the roots of a particular plant on reducing its concentration in the plant shoots can be quantified by a retention factor (*RF*), defined in Equation 6. In this equation, the accumulation and retention of the element in the roots of the plant are related to the concentration of the element in the entire plant, provided its distribution in the tissues of the whole plant is uniform.

$$RF = \left[\frac{(C_r - C_a) \cdot M_r}{C_a \cdot M_f} \right] \cdot 100 \quad (6)$$

in which C_r is the element's concentration (mmol kg⁻¹) in the root system, C_a is the average concentration (mmol kg⁻¹) of the element in the whole plant, and M_r and M_f are the dry weights (kg) of the roots and shoot of the plant, respectively. The *RF* values for Na in the different plant combinations, calculated by Equation 6, are presented in Table 3. The C_r , C_a , M_r , and M_f values used to calculate the *RF* values were taken from Tables 2 and 3 and Fig. 4. The *RF* values for Na in the plants grafted on pumpkins averaged 46.9% (Table 3), indicating that from the total amount of Na absorbed by the plants with pumpkin rootstocks, the retention of Na in the pumpkin rootstock decreased the amount of Na in the shoot by 46.9% as compared with its amount when uniformly distributed in the whole plant. In contrast, the *RF* values for Na in the plants grafted on melons were ~0 (Table 3), suggesting that, in practice, there is no retention of Na⁺ by melon roots.

Na ions that are available for xylem loading will move through the symplast, cross the endodermis, enter the apoplast of the stele, and move into the xylem. This loading is active, namely driven by a pump, rather than being a passive leak (Tester and Davenport, 2003). The gene *SOS1* encoding the Na⁺/H⁺ antiporter has been reported to

be expressed at the xylem–symplast boundary in *Arabidopsis* (Shi *et al.*, 2002); however, it is not known whether such a system exists in the plants used in the present study. The Na concentration in the exudate was 0.1–0.2 mM in pumpkins and 4.7–6.3 mM in melons. Provided the exudate indeed represents the xylem sap, this indicates that either xylem loading of Na was inhibited in pumpkin roots, or that its concentration in the cytosol had already been reduced by its sequestration in vacuoles. Both cases would result in Na retention in the roots rather than its moving to the foliage.

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