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A Comparative Study of Salt Tolerance of Three Almond Rootstocks: Contribution of Organic and Inorganic Solutes to Osmotic Adjustment

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ABSTRACT

In this study, we assessed the relative contribution of organic and inorganic solutes to osmotic adjustment (OA) in three almond rootstocks subjected to four levels of soil salinity. The results showed that leaf water and osmotic potentials were affected by salinity in GF677 and Bitter almond, but less so in GN15, suggesting a higher selectivity for K^+ and Ca^{2+} against Na^+ in this latter rootstock. GN15 excluded Na^+ and accumulated Cl^-. Nevertheless, in this rootstock, Cl^- and Na^+ were the main osmolytes involved in OA, while the osmotic role of K^+, Ca^{2+} and Mg^{2+} was small. Proline had the highest relative contribution of organic solutes to OA in the leaves of GN15 and GF677, while in Bitter almond it was not effective. The role of soluble sugars was rather marginal in terms of OA in all three genotypes. All three rootstocks displayed a degree of OA in the presence of high NaCl concentrations in the growth medium, but used different osmolytes to achieve it. Therefore, breeders should be careful in choosing biochemical parameters to assess OA capability of *Prunus* genotypes.

Keywords: Essential cations, NaCl, Proline, *Prunus*, Soluble sugars.

INTRODUCTION

Salinity affects photosynthesis by reducing pigments' concentration (Lutts *et al*., 1996) and stomatal conductance (Brugnoli and Lauteri, 1991), by changing chloroplast ultra-structure (Geissler *et al*., 2009) and by altering the plant's water status (Gebre and Tschaplinski, 2000). Osmotic adjustment (OA) is a common reaction by plants to osmotic stress in order to maintain leaf turgor and protect the photosynthetic machinery from the effects of stress (Gebre and Tschaplinski, 2000). Osmotic adjustment can be accomplished through the synthesis of low molecular weight compatible solutes like amino-acids or soluble sugars and the uptake of ions such as Na^+ and K^+ or both from the growth medium (Hare *et al*., 1998; Mahouachi, 2009; Dichio *et al*., 2009; Schulze *et al*., 2002). It has been hypothesized that these compounds benefit stressed cells in two ways: (i) by acting as cytoplasmic osmolytes, thereby facilitating water uptake and retention (Hare *et al*., 1998), and (ii) by protecting and stabilizing macromolecules and structures (i.e. membranes, chloroplasts, and

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liposomes) from damage induced by stress conditions by replacing water molecules in their vicinity thus preventing the formation of intra-molecular hydrogen bonds that can cause irreversible structural disorder (Bohnert and Jensen, 1996; Chaves *et al*., 2003). This accumulation of solutes is also required for balancing the osmotic potential created by Na^+ and Cl^- in the vacuole where they are sequestered (Ashraf, 2004).

Species and varieties of crop plants differ greatly in respect to the type of solutes they accumulate and the relative contribution of these solutes to lowering the osmotic potential (Gagneul *et al*., 2007). Generally, the osmolyte that plays the major role in OA is species-dependent (Rhodes *et al*., 2002) whereas the degree of OA is influenced by several factors, such as the rate and duration of stress development (Jones and Rawson, 1979), the intensity of stress (Turner and Jones, 1980), the plant's genotype (Morgan, 1984), the age of the tissue and the stage of plant development (Ma *et al*., 2006). Osmotic adjustment also requires time to develop; therefore, fast reductions in plant water potential, such as on sandy soils, may not allow full expression of OA (Blum, 1996). Water, osmotic, and turgor potentials are inter-related in plant cells and are markedly affected when plants are exposed to salt stress (Wang *et al*., 2003).

Although it has been reported to accumulate proline in its leaves in response to increased soil salinity (Najafian *et al*., 2008), almond tree has been classified by several researchers as sensitive to salinity based on visible damage to its leaves (Ranjbarfordoei *et al*., 2002, 2006; Najafian *et al*., 2008). However, the physiological implications of salt stress for the tree have not been studied enough. In the present study, we investigated the degree of tolerance of three almond rootstocks to soil salinity induced by NaCl and assessed the significance of osmotic adjustment in the tissues of these widely used rootstocks. More specifically, we examined the contribution of ions, proline, and soluble sugars to OA in these genotypes.

MATERIALS AND METHODS

Plant Material and Experimental Design

The present study was performed on eight-months-old rooted cuttings of three almond rootstocks: Bitter almond (*Prunus amygdalus*) and two hybrid *Prunus* rootstocks, GF677 (*Prunus amygdalus*×*Prunus persica*) and Garnem GN×15 (Garfi×Nemared). The plants were about 40 cm in length when they were received from a commercial nursery. They were cultivated individually in 4-L plastic pots containing desert dune-sand in a growth chamber under controlled conditions (Temperature: 25±2°C; Photoperiod: 16-h light:8-h dark; Light intensity (PAR): 500–700 $\mu M\ m^{-2}\ s^{-1}$). Upon receiving them from the nursery, the plants were cultivated for one month in the growth chamber and were irrigated every 4 days with a complete nutrient solution (N, 1.8 mM; P, 0.35 mM; K, 0.64 mM; Ca, 1.0 mM; Mg, 0.35 mM; S, 0.35 mM; Fe, 0.03 mM; Zn, 0.4 μM, Mn, 5.0 μM; Cu, 0.1 μM and B, 0.02 mM). After this initial acclimation period, the plants were divided into four groups of four plants each; each group received a salinity treatment by increasing the concentration of NaCl in the nutrient solution to 0, 25, 50 or 75 mM. To avoid osmotic shock, NaCl concentrations were increased gradually, by 25 mM per day, until the desired concentration was reached. Every four days, the substrate in the pot was washed twice with deionized water to avoid salt build-up, then, 500 mL of the nutrient solution, enough to cause some drainage, was applied. The experimental design was a completely randomized block experiment with four replicates (each pot contained one plant being a replicate). The plants tissues were sampled four weeks after starting salinity treatments. At the end of the experiment, the four upper leaves of the main shoot of each tree were collected to measure leaf relative water content. Four mid-shoot leaves were also used to measure leaf water potential. The remaining tissues (leaves and roots) of each

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plant were harvested separately in the morning (between 9 to 11 am local time), weighed and divided into two batches. One was frozen in liquid nitrogen and then stored at -80°C for biochemical analyses. The other was briefly rinsed in de-ionized water, dried at 80°C for 48 hours, then weighed again and ground into a fine powder to pass through a 60-mesh screen for ion analyses.

Growth Parameters

Before the start of salt treatments, the tip of the main shoot of each plant was marked to be able later to measure shoot elongation during the period of exposure to salt. The number of leaves was also recorded for each plant.

Mineral Analyses

At the end of the trial, sub-samples of dried leaf and root tissues were stored for Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^- analyses. The tissues were milled into a fine powder to pass a 60-mesh screen, then, 20 mg of the powder was extracted with 20 mL of 0.1M HNO_3. After filtration, Na^+, K^+, Ca^{2+}, Mg^{2+} contents were determined with an atomic absorption spectrometer (Avanta, GBC, Australia). Cl content was determined with a chloride analyzer (Corning M926 chloride analyzer, Halstead, Essex, UK).

Leaf Relative Water Content, Water and Osmotic Potentials

Percent leaf relative water content (% RWC) was measured by using the method described by Kramer and Brix (1965) and calculated according to the following equation:

$$\% \text{ RWC} = 100 \times [(FW - DW)/(TW - DW)]$$

Where, *FW* is fresh weight, *DW* is dry weight, and *TW* is turgid weight determined after soaking the leaf samples in distilled water for 24 hours at 4°C in a refrigerator. Dry weight was measured after oven-drying the samples for 48 hours at 80°C. The *RWC* was measured on four leaves for each plant. Pre-dawn leaf water potential (Ψ_w) was measured on four median leaves with a Scholander pressure chamber (PMS, Albany, OR, USA) using a standard methodology (Gucci *et al*., 1997). The osmolality of the expressed sap of these same leaves after being frozen and thawed was measured with a vapour pressure osmometer (Wescor 5520, Logan, UT, USA), the osmolality values were converted to osmotic potential ($\Psi\pi$) by the van't Hoff equation: $\Psi\pi = -c_i RT$, (Nobel, 1992). Turgor potential (Ψp) was calculated as the difference between osmotic potential (Ψ_π) and water potential (Ψ_w) values ($\Psi p = \Psi w - \Psi\pi$).

Total OA was calculated as the difference in osmotic potential at full turgor between the control and salt-stressed plants (Martinez-Ballesta *et al*., 2004). The osmotic concentrations of solutes were calculated by the van't Hoff Equation: $\Psi si = -0.002479\ (RDW)\ C$. Where Ψsi indicates the contribution (in %) of solutes (individual Ψs); *RDW* is the dry mass relative to saturation (kg m^{-3}): $RDW = DW/TW\text{-}DW$; *C* is the molar concentration of solute (mol kg^{-1}); and 0.002479 m^3 MPa mol^{-1} RT is the amount at 25 °C. It was assumed that the osmotic solutes exhibit ideal behaviour (Alarcon *et al*., 1993).

Gas Exchange Measurements

Gas exchange measurements were carried out after four weeks of salt treatment. Net photosynthetic rate (A), transpiration rate (E), and stomatal conductance (Gs) of upper mature leaves were measured with a portable photosynthesis analysis system (Lcp *pro*+, ADC Systems Ltd, UK) under ambient conditions (PAR was 500-700 µmol m^{-2} s^{-1} and air temperature was 25±2°C).

Total Chlorophyll

Total chlorophyll (chl) concentration was determined by the method of Shabala *et al*. (1998) using 95.5% acetone. Chl

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concentrations were calculated from absorbance values of the extract at 644 and 662 nm measured with a spectrophotometer (Shimadzu, Japan).

Soluble Sugars Concentration

Total soluble sugars (TSS) in the tissue extract were determined according to the method of Robyt and White (1987). Plant material (0.2 g) was extracted in 80% methanol solution. The absorbance of the extract was read at 645 nm with a spectrophotometer (Shimadzu, Japan).

Proline Content

Frozen leaves (0.2 g) were homogenized with 5 mL of 3% aqueous sulfosalicylic acid and centrifuged at 8,000×g for 15 minutes. Two millilitres of acid-ninhydrin and 2 mL of glacial acetic acid were added to 2 mL of the homogenate in a test tube. The mixture was then incubated at 100°C and the organic toluene phase containing the chromophore was used to quantify the amount of proline, as described by Bates *et al*. (1973), by reading its absorbance at 520 nm with a spectrophotometer.

Statistical Analysis

Data were subjected to analysis of variance using Proc GLM of SAS statistical software version 6.12 (SAS Institute, Cary, NC, USA). A completely randomized design with four replicates was used. Where applicable, means were separated by Duncan's Multiple Range Test with a level of significance $P \leq 0.05$.

RESULTS

Effect of NaCl on Growth

There were clear differences among genotypes in plant growth under salinity conditions (Table 1). In fact, GN15 showed the highest reduction (43%) in shoot growth as compared to the control trees, while Bitter

Table 1. Growth parameters of almond rootstock plants fed with increasing concentrations of NaCl.

	Salinity (mM NaCl)	Shoot extension (cm)	Number of leaves
Bitter almond	Control	24.0±2.0 a[a]	110.3±5.4 a
	25	19.7±1.5 ab	78.0±6.9 b
	50	17.0±1.3 b	74.0±4.0 b
	75	15.5±0.3 b	69.2±6.1 b
GF677	Control	40.7±1.7 a	136.0±7.0 a
	25	36.5±2.7 a	98.0±7.6 b
	50	29.5±2.1 b	79.0±1.3 bc
	75	28.0±0.7 b	74.0±2.51 c
GN15	Control	32.3±0.7 a	41.3±0.6 a
	25	27.5±1.7 b	32.7±0.3 b
	50	21.0±0.8 c	22.0±0.8 c
	75	18.3±1.0 c	21.5±0.9 c
Analysis of variance			
Salinity		**[b]	**
Rootstock		**	**
Salinity x rootstock		ns	*

[a] Values are the means ± SE of four replicates. Different letters indicate significant differences between treatments within columns (Duncan test). [b] ns. *. **: non-significant or significant at $P<0.05$ or $P<0.01$ respectively.

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almond (35%) and GF677 (31%) were less affected.

Effect of NaCl on Nutrient Partitioning

The three rootstocks showed significant ($P< 0.05$) differences in the accumulation of Na^+ in their roots with increased soil salinity, whereas, Ca^{2+} and Mg^{2+} concentrations decreased in all three rootstocks (Table 2). As for K^+, its concentration decreased in the roots of Bitter almond but not in the roots of GF677 and GN15, except when NaCl concentration in the medium was increased to 75 mM. In the leaves of all rootstocks, adding NaCl to the culture medium decreased significantly K^+ concentration after four weeks of treatment. Indeed, adding 75 mM NaCl, decreased K^+ concentrations by 40, 38, and 32% in GF677, bitter almond, and GN15, respectively. Leaf Na^+ content in salt stressed plants of the three almond rootstocks increased with the medium salinity. After four weeks of treatment with 75 mM NaCl, the highest increase in Na^+ was recorded in the leaves of GN15 (68%) as compared to bitter almond (56%) and GF677 (57%). However, GN15's leaves still contained less Na^+ (in terms of concentration) than the other two genotypes (Table 2). Leaf Na^+ concentration was about four times higher in GF677 and bitter almond compared to GN15. The addition of salt to the growth medium increased Cl^- concentration in the leaves but not in the roots. The largest accumulation of Cl^- was recorded in the leaves of bitter almond (60%) and GF677 (50%) as compared to GN15 (31%) (Table 2). Salinity decreased leaf and root Ca^{2+}/Na^+ and Mg^{2+}/Na^+ ratios regardless of genotype. Nevertheless, GN15 maintained the highest ratios at all salinity levels (Figure 1).

Leaf Water Relation

Leaf *RWC* was stable at around 90% for GN15 plants, but it decreased significantly in

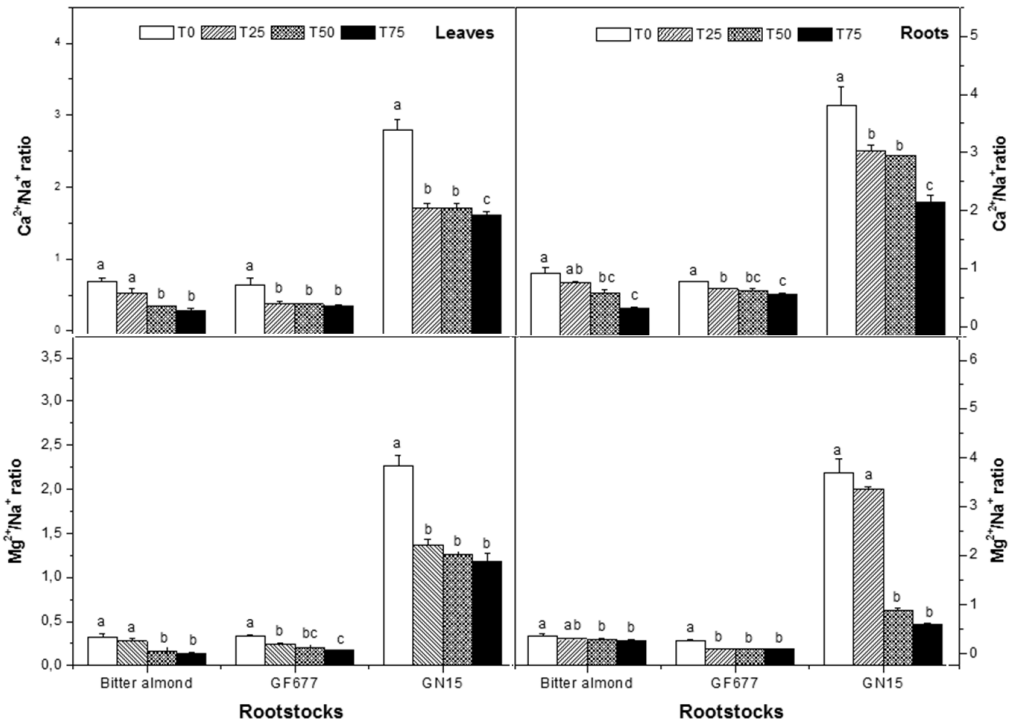

Figure. 1. Effect of NaCl on Ca^{2+}/Na^+ and Mg^{2+}/Na^+ ratios in the leaves and roots of three almond rootstocks. Values are the means ± SE of four replicates. Different letters indicate significant differences between treatments (Duncan test, $P\leq 0.05$).

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Table 2. Ion concentrations in the leaves and roots of almond rootstock plants fed with increasing concentrations of NaCl.

		Leaves			Roots		
	Treatment	Bitter almond	GF677	GN15	Bitter almond	GF677	GN15
	0	895.20±21.6b	800.87±34.9b	188.55±43.0b	687.98±83.7a	712.28±54.9a	150.90±4.3b
Na	25	965.07±9.2b	1168.12±86.8a	302.71±4.8a	770.67±17.5a	729.77±9.0a	174.17±1.5ab
(µeq.g-1DW)	50	1327.51±86.4a	1228.17±17.3a	314.82±2.7a	812.02±57.5a	732.31±39.2a	184.14±7.4a
	75	1394.47±31.5a	1260.55±17.6a	317.42±3.6a	820.46±36.6a	761.51±44.4a	194.95±9.2a
Salinity			***		ns		
Rootstock			***		***		
Salinity x rootstock			***		ns		
	0	420.84±8.1a	869.00±22.1a	984.74±19.0a	160.05±0.0a	207.75±9.0a	112.59±7.9a
K	25	339.69±13.0ab	736.06±28.5b	705.75±51.9b	152.17±1.4a	181.67±16.7ab	92.66±1.6ab
(µeq.g-1DW)	50	300.50±83.1b	731.40±28.2b	705.37±21.3b	96.68±0.9b	170.59±23.0ab	91.66±9.2ab
	75	259.50±14.9b	520.15±7.5b	666.38±63.6b	91.82±5.8b	148.08±13.7b	74.20±4.5b
Salinity			***			***	
Rootstock			***			**	
Salinity x rootstock			**			ns	
	0	653.19±3.6a	610.08±72.4a	592.33±13.6a	602.07±33.0a	503.08±8.6a	551.56±37.7a
Ca	25	604.00±3.2a	483.50±48.5b	522.59±10.5ab	587.89±32.1a	470.51±0.8ab	542.27± 22.6a
(µeq.g-1DW)	50	451.38±1.8b	458.14±3.4b	505.95±0.4bc	454.46±44.6b	443.39±13.2bc	541.88± 20.4a
	75	340.00±1.6c	413.40±8.6b	484.09±22.8c	248.08±12.3c	431.87±2.8c	421.97± 28.3b
Salinity			***			***	
Rootstock			ns			ns	
Salinity x rootstock			ns			*	
	0	286.49± 28.7a	274.18± 11.2a	445.00± 32.5a	234.16± 19.3a	181.28± 10.0a	419.00± 28.1a
Mg	25	260.96± 25.2ab	258.75± 12.0 ab	404.00± 17.8a	227.85± 14.2a	67.49± 0.9b	407.00± 18.6a
(µeq.g-1DW)	50	231.28± 35.8b	251.85± 5.2ab	390.00± 65.5a	218.72± 18.7a	62.55± 5.5b	161.00± 7.4b
	75	202.88± 12.6b	221.81± 4.8b	374.00± 29.4a	220.17± 5.3a	57.20± 7.8b	98.00± 15.6b
Salinity			***			***	
Rootstock			***			***	
Salinity x rootstock			***			***	
	0	408.20±47.5b	360.91±19.1b	1016.76±4.4b	413.18±19.3a	511.49±16.7a	856.22±7.0b
Cl	25	626.40±82.9ab	444.29±16.7b	1368.96±8.8a	416.91±2.6a	560.03±8.8a	954.54±4.4ab
(µeq.g-1DW)	50	808.93±174.2a	552.56±7.0a	1390.11±2.6a	431.84±0.8a	583.67±9.6a	1075.25±51.0a
	75	1041.65±143.4a	688.21±29.0a	1474.74±154.0a	461.3±35.0a	596.53±11.8a	1115.08±47.5a
Salinity			***			***	
Rootstock			***			***	
Salinity x rootstock			***			***	

Values are the means ± SE of four replicates. Different letters indicate significant differences between treatments within columns (Duncan test). Non-significant (**ns**) or significant at $P<0.05$ (*) or $P<0.01$ (**) *or* $P\leq 0.001$ (***).

bitter almond and GF677 with 75 mM NaCl treatment (Figure 2). GN15 under NaCl-stress conditions did not show any significant change in leaf sap $\Psi\pi$; however, in GF677, leaf sap $\Psi\pi$ decreased sharply with increasing salinity (Table 3). Water potentials (Ψw) were relatively higher in GN15 than in the other two genotypes (Table 3). Indeed, water potentials in Bitter almond and GF677 significantly decreased as salt stress intensified.

Our results also show that during the period of salt stress, OA increased in the three genotypes especially at 75 mM NaCl. GN15 displayed a higher ability to osmotically adjust to increasing growth medium salinity compared to Bitter almond and GF677 (Table 3).

Gas exchange Measurements

At the end of the experimental period, leaf gas exchange parameters decreased with increasing stress in all the three rootstocks (Figure 3). In the presence of 75mM NaCl, *A* decreased by 37 and 30% in GF677 and Bitter almond, respectively, while in GN15, *A* was less affected (25%). Stomatal conductance (Gs) and *E* decreased significantly in all three rootstocks with increasing NaCl concentrations in the growth medium. Nevertheless, GN15 was least affected compared to Bitter almond and GF677.

Chlorophyll Content

Salinity induced a decline in chl concentration in the leaves of Bitter almond and GF677 by 25 and 34%, respectively, in the presence of 75 mM NaCl (Figure 4). The reduction was lower in GN15.

Proline Content

Proline content was much higher in the leaves than in the roots of the control plants of the three almond rootstocks. Salinity had a significant effect on proline content in the roots and more so in the leaves (Figure 5). Proline content substantially increased when NaCl concentration in the growth medium increased. In the presence of 75 mM NaCl, proline concentration in the leaves of GN15 and GF677 increased two-folds. In Bitter almond, leaf proline concentration increased in the presence of 50 mM NaCl, then it decreased with the higher level of salinity. Proline content of root tissue increased considerably in response to increased salt concentration for GN15 and GF677 compared to their controls, whereas in Bitter almond proline concentration was unaffected by the salinity of the medium.

Total Soluble Sugars (TSS)

Overall, salt stress did not induce an increase of leaf TSS concentration, except in GF677 with 75 mM NaCl and in Bitter almond in the presence of 25 mM NaCl (Figure 5). However, in the roots, there was a significant accumulation of TSS in GF677 and GN15 in the presence of 25 mM NaCl; for higher salinity levels, TSS declined. In Bitter almond, the concentration of TSS decreased with increasing salinity stress.

Osmotic Adjustment

The contribution of inorganic solutes to leaf osmolality is shown in Figure 6. K^+, Ca^{2+}, and Mg^{2+} did not contribute to OA in the three rootstocks, whereas Na^+ contributed 6% and 19% to OA in GN15 and Bitter almond, respectively, under 75 mM NaCl treatment. Furthermore, Cl^- ions accounted for most OA in the leaves of GN15 and Bitter almond (40 and 17%, respectively). Its contribution to OA in GF677 was small.

Proline and TSS displayed different accumulation patterns among the

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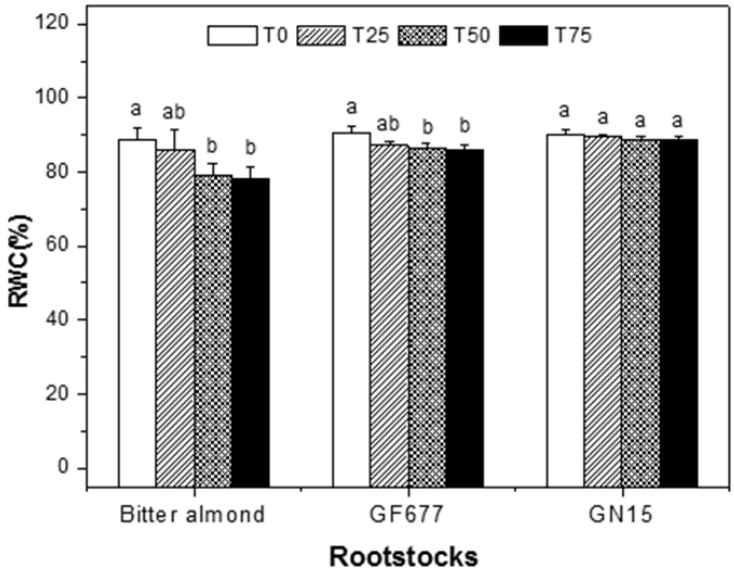

Figure 2. Effects of NaCl on leaf RWC of three almond rootstocks. Values are the means ± SE of four replicates. Different letters indicate significant differences between treatments (Duncan test, $P \leq 0.05$).

Table 3. Water, osmotic and turgor potential and osmotic adjustment of almond rootstock plants fed with increasing concentrations of NaCl.

Varieties	NaCl (mM)	Ψ_π (MPa)	Ψ_p (MPa)	Ψ_w (MPa)	OA
Bitter almond	0	-3,42±0.01 a [a]	2,37±0.06 b	-1,05±0.07 a	
	25	-4,23±0.07 b	2,98±0.02 a	-1,25±0.02 a	0,81±0.06 b
	50	-4,21±0.05 b	2,46±0.06 b	-1,75±0.02 b	0,79±0.081 b
	75	-4,91±0.07 c	2,96±0.07 a	-1,95±0.02 b	1,49±0.10 a
GF677	0	-1,11±0.05 a	0,14±0.05 c	-1,25±0.07 a	
	25	-3,99±0.17 b	2,47±0.16 b	-1,53±0.03 ab	2,88±0.14 b
	50	-5,43±0.00 c	3,56±0.03 a	-1,88±0.03 bc	4,32±0.05 a
	75	-5,92±0.17 c	3,97±0.14 a	-1,95±0.02 c	4,81±0.13 a
GN15					
	0	-1,20±0.00 a	2,15±0.03 b	-0,95±0.02 a	
	25	-1,16±0.07 ab	2,11±0.16 b	-0,95±0.02 a	0,06±0.00 b
	50	-1,51±0.08 bc	2,73±0.03 a	-1,23±0.06 a	0,58±0.11 a
	75	-1,62±0.18 c	2,90±0.14 a	-1,28±0.03 a	0,75±0.19 a

[a] Values are the means ± SE of four replicates. Different letters indicate significant differences between treatments within columns (Duncan test, $P \leq 0.05$).

rootstocks in the presence of NaCl. Their contribution to OA was small (Figure 7). Proline accounted for 0.121% of total osmolality in GN15 leaves and 0.185% in GF677 in the presence of 75 mM NaCl. This contribution may be actually much larger if one would consider only the volume of the cytosol which represents but a small fraction of the volume of a mature cell. The contribution of TSS to leaf OA was less important, especially in GF677.

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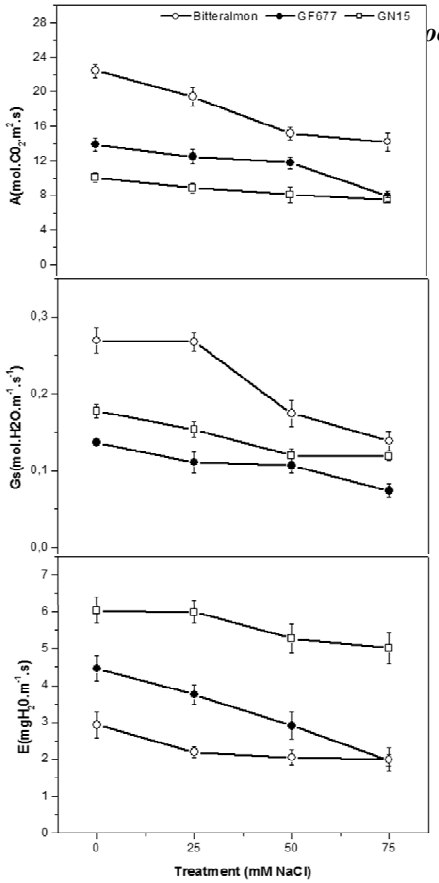

Figure 3. Effect of NaCl on leaf gas exchange of three almond rootstocks. Values are the means ± SE of four replicates.

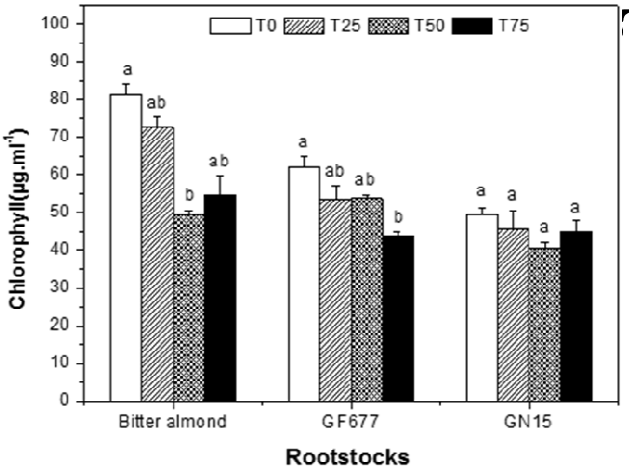

Figure 4. Effect of NaCl on total chlorophyll content in the leaves of three almond rootstocks. Values are the means ± SE of four replicates. Different letters indicate significant differences between treatments (Duncan test, $P \leq 0.05$).

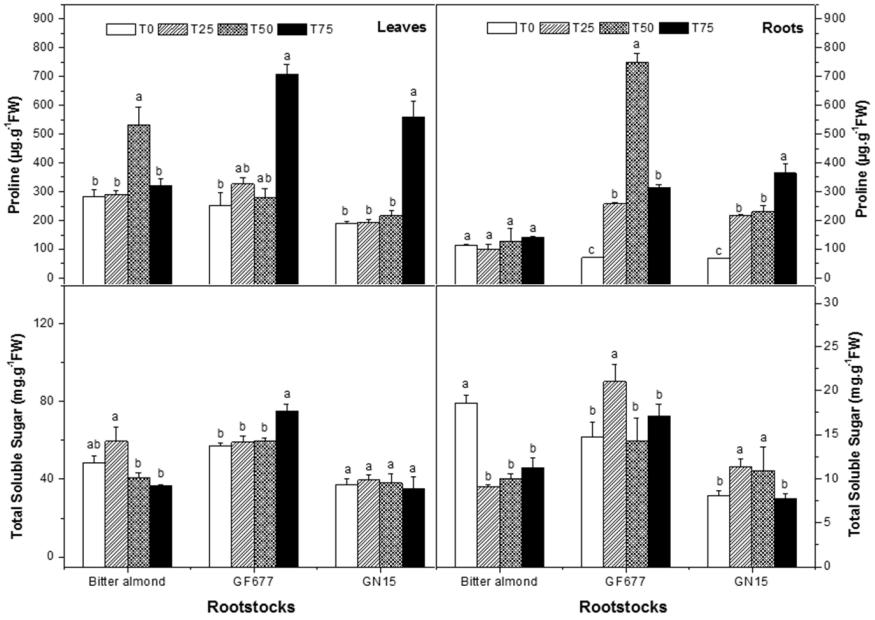

Figure 5. Effect of NaCl on proline and soluble sugars concentrations in the leaves and roots of three

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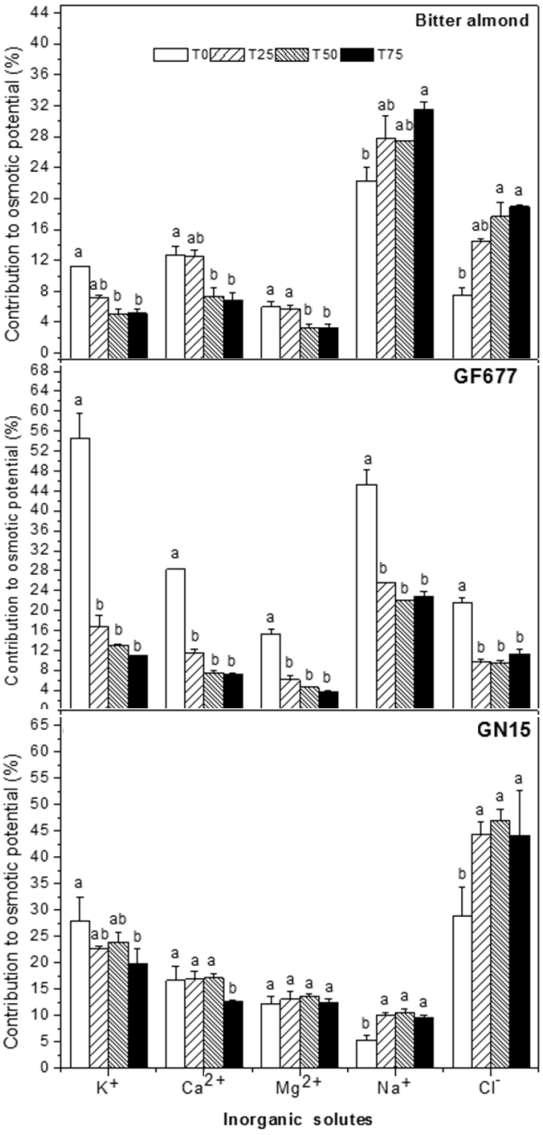

Figure 6. Relative contribution of inorganic solutes to leaf osmolality in three almond rootstocks exposed to different NaCl concentrations during four weeks. Values are the means ± SE of four replicates. Different letters indicate significant differences between treatments (Duncan test, $P \leq 0.05$).

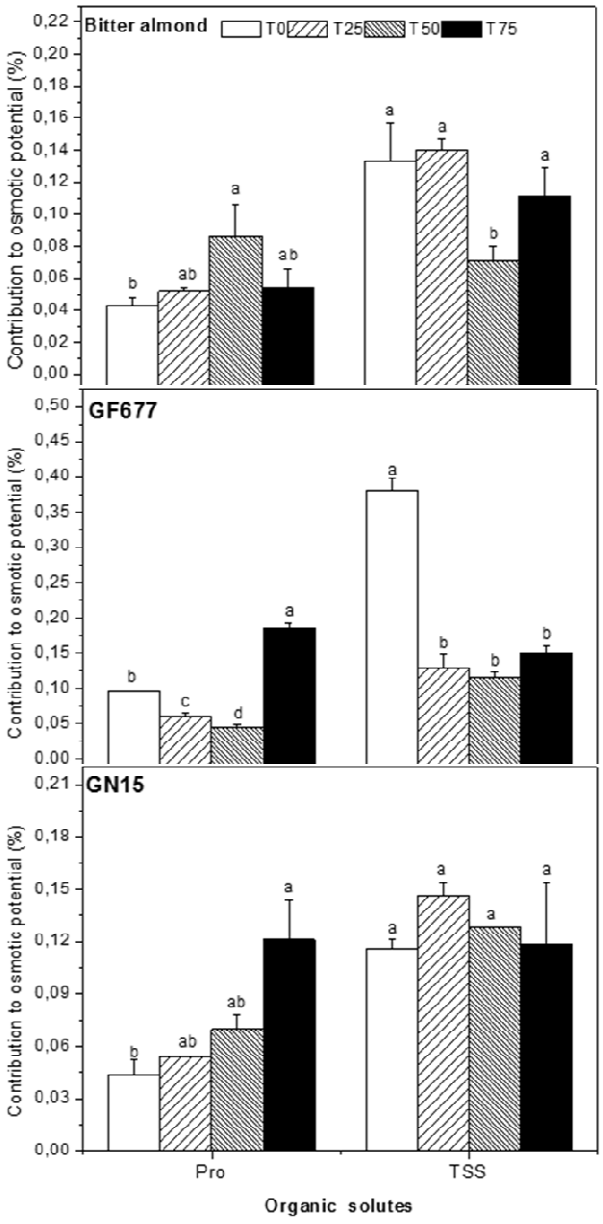

Figure 7. Relative contribution of proline (Pro) and soluble sugars (TSS) to leaf osmolality in three almond rootstocks exposed to different NaCl concentrations during four weeks. Values are the means ± SE of four replicates. Different letters indicate significant differences between treatments (Duncan test, $P \leq 0.05$).

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DISCUSSION

Plants have developed various mechanisms to deal with the deleterious effects of salt stress. Among these, OA is one of the ubiquitous strategies of defence against excessive soil salinity. The results obtained in the present study suggest that GN15 and GF677 rootstocks were more tolerant to salt stress than Bitter almond. In fact, GN15 and GF677 maintained some shoot growth and leafing at all NaCl concentrations tested. The *RWC* and Ψ_w of Bitter almond and GF677 were decreased by salt stress throughout the experiment, but the effect was more pronounced in the former rootstock. This may indicate a less effective stomatal control (Bartels and Sunkar, 2005). Indeed, a good correlation is often observed between water potential and *Gs* (Guerfel *et al*., 2008), thus indicating that leaf water status interacts with *Gs* and *E* under water stress. In the present study, *Gs* and *E* decreased with increasing salinity; the effect was more acute in Bitter almond and GF677 than in GN15. The capacity of GN15 to maintain higher leaf *RWC* and osmotic potential than the other genotypes under salt stress may be attributed to its ability to postpone dehydration. The differences in Ψ_π indicate different degrees of OA among the three rootstocks. The high Ψ_p in GF677 reflects a greater capacity for cell turgor maintenance essentially through OA, which helped to reduce Ψ_π and thus Ψ_w as salt concentration in the medium increased. It has been hypothesized that OA helps the plant maintain turgor so that continued growth can occur, albeit at a reduced rate, resulting in an overall decrease in biomass accumulation (Gonzalez and Ayerbe, 2011). After four weeks of salinity treatment, there were no differences in *RWC* among treatments in GN15 (Table 3), thus indicating that the leaves were able to maintain cell turgor regardless of soil salinity level. The concentrations of K^+, Ca^{2+}, and Mg^{2+} in GN15 leaves were less affected by increasing soil salinity compared to Bitter almond and GF677 leaves (Table 2). Na^+ concentration in GN15 leaves increased with soil salinity, but remained far lower than in GF677 and Bitter almond leaves suggesting a restriction on the uptake of this cation by GN15 roots. This was not the case for Cl^- which accumulated in both roots and leaves of GN15 and contributed significantly to OA. The restriction on Na^+ uptake helped maintain high Ca/Na and Mg/Na ratios in GN15 tissues. Furthermore, the higher leaf K^+, Ca^{2+}, and Mg^{2+} concentrations could have also alleviated the negative effect of Na^+ and Cl^-, thus, giving a degree of tolerance to GN15. For NaCl concentrations less than 75 mM, the three cations appear to have also contributed effectively to OA in the leaves of GN15, but not in GF677 and Bitter almond.

There was an increase in leaf Cl^- concentration in the stressed plants of all three genotypes in comparison with the controls. Na^+ concentrations increased too in the presence of NaCl especially in GN15 leaves (+40%). It appears that Cl^- and Na^+ ions contributed also to OA in the leaves of stressed GN15 plants. Araujo *et al.* (2006) found that the main water potential gradient between growing regions of the shoot and the xylem in this rootstock was achieved through osmotic gradients generated by Na^+ and Cl^- accumulated in shoot tissues. However, this mechanism of leaf turgor maintenance by the accumulation of inorganic solutes, especially Cl^-, can have deleterious effects on the plant. Perez-Perez *et al*. (2007) observed that seedlings pre-conditioned by salinity were able to maintain their *RWC* under drought, but high accumulation of Cl^- damaged the leaves. In the present investigation, it appeared that the high accumulation of Cl^- in leaves of GN15 may have been responsible for the death of older leaves.

The contribution of soluble sugars and proline to OA in the tissues of stressed plants was minimal. Nevertheless, NaCl caused proline to accumulate in the leaves of all three rootstocks and in the roots of

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GF677 and GN15 (Figure 5). This indicates that proline plays a role in almond rootstocks' tolerance to salinity stress. Indeed, the larger accumulation of proline in the leaves and roots of GN15 and GF677 rootstocks was associated with a relatively better tolerance of salinity compared with Bitter almond. In response to drought or salinity stress in plants, proline accumulation normally occurs in the cytosol (small volume compared to the rest of the cell) where it contributes substantially to the cytoplasmic osmotic adjustment. Furthermore, and in addition to its role as a compatible osmolyte, proline provides protection against photoinhibition under adverse conditions by restoring the pool of the terminal electron acceptor of the photosynthetic electron transport chain (Lawlor and Cornic, 2002; Szabados and Savoure, 2009). Our data suggest that proline has protected the photosynthetic apparatus in GN15 leaves as indicated by the stability of Chl content and helped maintain cell turgor, which is required to keep stomata open for gas exchange. Proline may also play the role of a secondary signal under stress (Van den Ende and El-Esawe, 2013). The accumulation of proline was not universal here; indeed, unlike the other two rootstocks, Bitter almond did not appear to accumulate proline (nor TSS) when soil salinity increased.

In conclusion, this study demonstrates that OA does occur in the tissues of almond rootstock plants when challenged with elevated levels of salt in the growth medium. The three genotypes relied mainly on inorganic ions to achieve OA but the ions differed. Cl contributed the most to OA in GN15; K was next. In Bitter almond and GF677, Na contributed the most to OA; Cl and K were next.

The three genotypes did accumulate proline in the presence of NaCl but maybe mainly for osmoprotection of enzymes and cellular structures rather than osmoregulation (Dichio *et al*., 2006). Soluble sugars did not seem to be important for OA in all the studied rootstocks. The three rootstocks displayed a degree of OA in the presence of high NaCl concentrations in the growth medium, but used different osmolytes to achieve it. Therefore, breeders should be careful in choosing biochemical parameters to assess OA capability of *Prunus* genotypes.

Abbreviations

Ψ_w: Water potential; Ψ_π: Osmotic potential; Ψ_p: Turgor potential; A: Photosynthetic assimilation rate; *Gs*: Stomatal conductance; E: Transpiration rate; TSS: Total Soluble Sugars, OA: Osmotic Adjustment.

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مطالعه تطبیقی تحمل شوری در سه پایه بادام: نقش مواد محلول آلی و غیر آلی در تنظیم اسمزی

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چکیده

در این پژوهش، نقش مواد محلول آلی و غیر آلی در تنظیم اسمزی در سه ژنوتیپ پایه بادام تحت ۴ سطح شوری خاک ارزیابی شد. نتایج نشان داد که در بادام تلخ و ژنوتیپ GF677، پتانسیل آب و

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پتانسیل اسمزی در برگ تحت تاثیر شوری قرار گرفت ولی این تاثیر در ژنوتیپGN15 کمتر بود. این نتایج اشاره داشت که در ژنوتیپ اخیر، جذب انتخابی K^+ و Ca^{2+}بیشتر از Na^+بود. ژنوتیپ GN15، یون سدیم را دفع (رد) میکرد ولی یون Cl^- را می انباشت. با این وجود، در این ژنوتیپ پایه، یون های سدیم و کلر عمده ترین اسمولیت های (osmolytes) فعال در تنظیم اسمزی بودند در حالیکه نقش K^+ و Ca^{2+} و Mg^{2+}در این فرایند کم بود. همچنین، در تنظیم اسمزی در برگ های GN15 و GF677، به طور نسبی، بیشترین نقش مواد آلی محلول را پرولین داشت ولی در بادام تلخ این ماده موثر نبود. در همه این سه ژنوتیپ پایه بادام ، نقش قند های محلول در تنظیم اسمزی چشمگیر نبود. هر سه آنها در حضور غلظت های بالای NaCl در محیط رشد درجاتی از تنظیم اسمزی را نشان دادند ولی برای آن از اسمولیت های متفاوتی استفاده کردند. بنا بر این، برای ارزیابی تنظیم اسمزی در ژنوتیپ های *Prunus*، بهنژادگران باید در انتخاب پارامترهای بیوشیمیایی دقت داشته باشند.

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