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POLYAMINES AND OTHER SECONDARY METABOLITES OF GREEN-LEAF AND RED-LEAF ALMOND ROOTSTOCKS TRIGGER IN RESPONSE TO SALINITY

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Abstract

Almond trees are very sensitive to salinity, and saline water is the only alternative for irrigation in many semiarid regions. Thus, the use of salt-tolerant rootstocks may allow an economically-feasible yield under saline irrigation. In this study, we evaluated the effects of chloride salts on plant secondary metabolites in red- and green-leaf almond biotypes to improve salt tolerance. one-year-old rooted cuttings of Bitter Almond (BA) and Garnem (GN15) rootstock seedlings were cultivated for 3 weeks under low-salinity water (control), or exposed to irrigation with $CaCl_2$ (10mM), KCl (10mM), and NaCl (75 mM), alone and in combination, for 4 weeks. In green leaves of BA, the supplementation of NaCl solutions with CaCl2 significantly increased anthocyanin, petunidin, and polyphenol concentration, indicating a possible involvement of these compounds in cell osmoregulation. In GN15 rootstock, spermidine increased significantly from control when $CaCl_2$ was added to control irrigation. However, the highest and most significant increase in both spermidine and putrescine in GN15 was caused by NaCl alone. The significant increase in polyamines, between control to NaCl treatment, in GN15 but not in BA rootstock, and the drop in antioxidant capacity in BA, but not in GN15, across treatments, suggest that GN15 may be more salt-tolerant than BA. Although the addition of $CaCl_2$ or KCl may not have any benefit in mitigating salinity in almond rootstocks, spermidine and putrescine may have a role in helping almond rootstocks cope with salinity.

Key words: Calcium, Potassium, Almond rootstocks, Cyanidin, Petunidin, Polyamine.

Abbreviations: Car, carotenoids; TAC, total antioxidant capacity; DPPH, (2,2-diphenyl-1-picrylhydrazyl).

Introduction

Almond trees have been described as sensitive to salinity (Ranjbartoreh *et al*., 2006; Najafian *et al*., 2008; Zrig *et al*., 2011). Therefore, the role of the rootstock is crucial in determining the performance of grafted trees under saline conditions (Reighard *et al*., 2008). In the Mediterranean region, Garnem (Garfi Almond × Nemared) peach hybrid with reddish leaves, has gained popularity among nurserymen for its tolerance to drought and calcareous soils (Zarrouk *et al*., 2005). In many countries including Tunisia, Bitter Almond (BA) is also still widely used as a rootstock for almond and peach because of its deep root system and tolerance to drought (Zrig *et al*., 2015). Previous studies reported that different almond rootstocks, such as the hybrid Garnem (GN15) and Bitter Almond (BA), are known for their distinct response to salinity (Zrig *et al*., 2011) and to fertilizers (Zrig *et al*., 2016). Solely based on the loss of leaf dry matter between GN15 and BA (57% and 39%, respectively), and under 75 mM NaCl, GN15 could be considered more susceptible to salinity than BA (Zrig *et al*., 2011). However, GN15 survived the highest salinity concentration (75 mM NaCl) because it had 70% higher root dry mass than BA and 56% higher root/shoot ratio than BA (Zrig *et al*., 2011), which made GN15 more efficient than BA in taking up water and nutrients from the soil, despite high salinity. Regarding their salinity-tolerance mechanisms, GN15 limited Na^+ and Cl^- uptake by its roots, while BA limited uptake of Na^+ by roots but had increased Cl^- in leaves as salinity increased (Zrig *et al*., 2015). GN15 plants also seemed to cope with salt toxicity using anthocyanins, more abundant in its red leaves than in the green leaves of BA (Zrig *et al*., 2011). In addition, GN15 was more resilient to the excess of toxic ions at high salinity. This tolerance may be due to the increase in free polyamines in response to stress (Zrig *et al*., 2011). In fact, high salinity decreased the photosynthetic assimilation rate and affected the total chlorophyll content of BA (Zrig *et al*., 2011). In addition, the improvement of the ionic balances and water status of BA trees in response to supplemental KCl or CaCl2 was apparently offset by a higher sensitivity to Cl^-, which caused some leaf abscission. Therefore, non-chloride salts should be the preferred form to provide Ca^+ and K+ for this rootstock (Zrig *et al*., 2016). A previous study reported that BA rootstocks are capable of adjusting osmotically to high salt environments. Indeed, the addition of CaCl2 to BA plants was followed by a large accumulation of proline and soluble sugars in BA rootstock leaves (Zrig *et al*., 2016).

Zrig *et al*., (2016) reported that the addition of Ca^{2+} or K^+ mitigates differently the effect of high salinity on photosynthetic parameters, having a better effect in the red-leaf than in the green-leaf phenotype. Understanding the differential response of almond rootstocks to salinity is not just a matter of measuring leaf and root biomass, but may also depend on characterizing leaf biochemical composition. In fact, GN15 accumulates more anthocyanins then BA (Zrig *et al*., 2011). Anthocyanins

help plants cope with stresses such as drought (Basu *et al*., 2010; Sperdouli & Moustakas, 2012), cold (Crifò *et al*., 2011), and high salinity (Zrig *et al*., 2011; Eryılmaz, 2006). The accumulation of anthocyanin depends on leaf phenotype and it was reported that the red-leaf phenotype (like GN15) was, on average, five-times more effective neutralizing the oxidizer radical DPPH (2,2-diphenyl-1-picrylhydrazyl) than the green-leaf phenotype (like BA) (Steyn *et al*., 2002).

Regarding polyamine metabolism, different studies reported the important role of Ca^{2+} and K^{+} on the biosynthesis and transport of free polyamines. Polyamine metabolism in higher plants is also modulated by osmotic stress (Aziz *et al*., 1997). The accumulation of putrescine (Put) in leaves of K+-deficient barley was first reported by Richards and Coleman (1952), and subsequent studies have established that polyamines have a specific role in maintaining the cation-anion balance in plant cells (Reggiani *et al*., 1993). These studies suggested that, under high salinity, polyamines might contribute indirectly to osmotic adjustment and adaptation to excess toxic ions (such as Na^{+} and Cl-) by protecting cell membrane integrity. The accumulation of Put in salt-sensitive cultivars could result from K^{+} and Ca^{2+} deficiencies under saline conditions and the addition of these macronutrients could increase plant tolerance to salinity.

In the present study, our goals were: 1) to assess if the addition of Ca^{2+} and K+ to unstressed and NaCl-stressed plants could affect anthocyanin and polyamine production in both red- (GN15) and green-leaf (BA) phenotypes, and 2) to compare the response of secondary metabolites between GN15 and BA rootstocks in response to salinity.

Materials and Methods

Plant material and culture: The present investigation was performed on one-year-old rooted cuttings of two almond rootstocks: Bitter Almond (BA) (*Prunus amygdalus*) and one hybrid Garnem GN15 (hybrid Garfi x Nemared) (GN15). The two almond rootstocks were cultivated in plastic pots, with four replicates (each pot was a replicate) containing desert dune sand under controlled conditions (average PPFD of 700 mmol m-2 s-1; temperatures: 25 ±2 C; relative air humidity: 60%). The complete nutrient solution was prepared with municipal water with added salts to achieve an initial total ion concentration of 4.5 mM and a water electrical conductivity (ECw) of 2.97 dS m-1. Potted plants were watered with complete nutrient solution (consisting of 1.8 mM N, 0.35 mM P, 0.64 mM K, 1.0 mM Ca, 0.35 mM Mg, 0.35 mM S, 0.03 mM Fe, 0.4 µM Zn, 5 µM Mn, 0.1 µM Cu, and 0.023 mM B) during the first 3 weeks of cultivation. Each four potted plants of both rootstocks were subjected to one of the six different treatments, with values for ECw presented in Table 1. Four weeks after applying the treatments to the plants, fully expanded leaves from each plant were harvested, frozen in liquid nitrogen and stored in -80°C for further biochemical analyses.

Table 1. Irrigation solutions, their salt compositions, and water electrical conductivity (ECw) expressed in deciSiemens per meter (dS m^{-1}).

Treatment	Solution composition	ECw (dS m^{-1})
Control (C)	Basic nutriment solution : control (0)	2.97
C+$CaCl_2$	C + 10 mM $CaCl_2$	4.85
C+KCl	C + 10 mM KCl	4.14
NaCl (S)	C + 75 mM NaCl	12.45
S+$CaCl_2$	C + 75 mM NaCl + 10 mM $CaCl_2$	14.33
S+KCl	C + 75 mM NaCl + 10 mM KCl	13.19

Leaf osmotic potential (Ψπ): Leaf potential (Ψπ) was measured from 10 µL extracted leaf sap using a vapour pressure osmometer (Wescor 5520, Logan, UT, USA), and applying the van't Hoff equation: $\Psi\pi = -CRT$ where C is the concentration of solutes, T is the temperature in degrees k, and R the gas constant (Nobel, 1992).

Anthocyanin and carotenoids content: The total anthocyanin was calculated from four replicates using a standard cyanidin 3-glucoside (molar absorption coefficient of 23,900 L cm^{-1} mol^{-1} and molecular weight of 449.2 g mol^{-1}) and the results, expressed as mg of anthocyanin per kg fresh weight. The content of cyanidin-3,5-glucoside and petunidin-3-glucoside was quantified by high performance liquid chromatography coupled with a diode array detector (HPLC-DAD) according to a validated method (Serrano *et al*., 2005). The anthocyanin standards were provided by García-Viguera *et al*., (1999). Briefly, total carotenoids were extracted from leaves with acetone, mixed with ethyl ether and 10% NaCl, and partitioned until all carotenoids were transferred to the ethyl ether phase. Detailed extraction and analytical procedure were performed according to Minguez-Mosquera & Hornero-Méndez (1993).

Total antioxidant capacity (TAC): One gram of frozen leaves was homogenized in 5 ml of 50 mM phosphate buffer (pH 7.8) with 3 ml of ethyl acetate. The homogenate was centrifuged at 15,000 rpm for 15 min at 4◦C. the lower fraction of two obtained fraction was used for the quantification of total antioxidant capacity (TAC) (Serrano *et al*., 2009). The results are expressed as the mean± SE in mg of Trolox equivalent per 100g fresh weight.

Phenolic compounds contents: Phenolic compounds were determined using a mixture of MeOH (2:8) containing 2 mM NaF. The extract was quantified by the Folin-Ciocalteu reagent. The results were expressed as mg of gallic acid equivalents per 100mg fresh weight (Tomas-Barberan *et al*.; 2001).

Free polyamine contents: One gram frozen leaves from each sample was extracted with 10 mL of 5% cold perchloric acid with 1.6 hexanediamine (100 nmol g−1 of tissue). After the centrifugation (30 min at 15.000 rpm), 2 mL aliquot of the supernatant was used to determine free polyamines by HPLC with absorbance at 254 nm (Serrano *et al*., 2003). The calibration curves were y = 10.66x + 170.00, r2 = 0.94 for putrescine (Put), y = 10.19x−39.96 r2 = 0.96 for spermidine (Spd), and y = 11.52x−4.32, r2 = 0.90 for spermine (Spm).

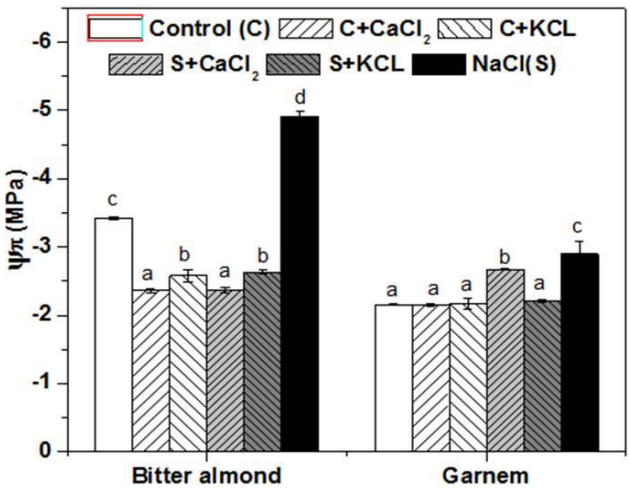

Fig. 1. Water relation: Osmotic potential (Ψπ) in leaves of two almond rootstocks irrigated with low-salinity water (control) and with waters containing 75 mM NaCl (NaCl, S), 10 mM KCl or 10 mM $CaCl_2$ alone or combined with 75 mM NaCl. Each point represents the mean (± SE) of three replicates; values marked by different small letters are significantly different at p≤5%.

Statistical analyses: Variance of data was analyzed with GLM procedure of SAS software (SAS Institute, Cary, NC, USA. 1996) for a Complete Randomized Block design with four replicates. Where applicable, means were separated by Duncan's Multiple Range Test (p≤0.05).

Results

Effects of ionic interactions on osmotic potential: 'Bitter Almond' leaf Ψπ decreased significantly, and by 28%, in plants treated with NaCl only (ECw = 10 dS m^{-1}), but not between the control and the other salt treatments, regardless the addition of either $CaCl_2$ or KCl, while 'Garnem' leaf Ψπ decreased significantly with both NaCl + $CaCl_2$ (ECw = 14.3 dS m^{-1}) and with NaCl alone (ECw = 12.4 dS m^{-1}) by 19% and 25%, respectively (Fig. 1).

Ionic interactions on anthocyanin contents: The addition of $CaCl_2$ to NaCl treatments increased total anthocyanins significantly by 43% in 'Bitter Almond' compared to both control plants and salt-treated plants. In 'Garnem', total anthocyanins decreased in all treatments compared to the control (Fig. 2). Results were similar for petunidine-3-glucoside content for both 'Bitter Almond' and 'Garnem'. Cyanidin-3,5-glucoside was much lower in 'Bitter Almond' than in 'Garnem', with no treatment effect on the former, but with a significant decrease in 'Garnem' (Fig. 2).

Effects of ionic interactions on carotenoids contents: The presence of NaCl in the culture medium decreased the total carotenoids in leaves of both rootstocks (Fig. 2). The addition of $CaCl_2$ alone increased leaf carotenoids content in Bitter Almond, compared to control and the other treatments. Under the NaCl + KCl treatment, carotenoids were at their lowest concentration mainly in Garnem (Fig. 2).

Effect of ionic interactions on polyphenols: The addition of all salts to the culture medium decreased polyphenols significantly in 'Garnem'. In 'Bitter Almond' polyphenols were slightly, but significantly, higher than in control plants when $CaCl_2$ was added to NaCl (Fig. 3).

Effects of ionic interactions on the total antioxidant capacity: The addition of $CaCl_2$, KCl and NaCl all decreased total antioxidant capacity (TAC) in Bitter Almond significantly, but had no effect on 'Garnem' (Fig. 4). However, addition of KCl decreased further the antioxidant capacity in Bitter Almond, compared to control and in Garnem, compared to all treatments.

Effects of ionic interactions on polyamine: In 'Bitter Almond', neither fertilizer alone nor with NaCl affected the contents of polyamines (Fig. 5). Compared to control treatment, the addition of CaCl and 75 mM NaCl alone significantly increased spermidine in 'Garnem' in 62% and 71%, respectively compared to control. Similarly, putrescine did not change with salinity in Bitter Almond, but increased significantly (73%) with 75 mM NaCl alone in 'Garnem' (Fig. 5).

Correlations between salinity and target parameters: The relationships of relative changes in anthocyanins, polyphenols and polyamines with osmotic potential and Total Antioxidant Capacity (TAC) were determined for two almond rootstocks separately (Table 2). In Garnem, Ψπ was positively correlated with total anthocyanins and more specifically cyanidine contents in the leaves, but negatively correlated with the polyamine contents, while TAC was positively correlated with polyphenols in both almond rootstock, and with putrescine and spermidine in Garnem.

Discussion

Some secondary metabolites such as chlorophylls, carotenoids, polyphenols, flavonoids, and anthocyanins are involved in stress tolerance by scavenging free radicals generated by stress (Chalker-Scott, 1999). Those metabolites are synthesized from intermediates of primary carbon metabolism via phenylpropanoid, shikimate, and mevalonate pathways. Their synthesis is generally stimulated under abiotic stress (Parida & Das, 2006). Also, the stimulation of the synthesis of such component depends on the type of salts. In the present investigation, the modulations of the levels of anthocyanin and carotenoids in leaves of both almond rootstocks seemed to be related to the type of chlorides salts and to the interactions between ions. The modulations in the leaf levels of anthocyanins and carotenoids are important in the prevention of stress-induced oxidative damage (Manetas, 2006). Our results indicate that neither the presence of Ca^{+2} or K^{+} enhanced the carotenoid concentration in either almond rootstock. Red anthocyanins, found in both young and senescent leaves, have been speculated to be important key regulators of stress responses (Steyn *et al*., 2002) and, more recently, as protectants against photo-oxidative damage to chloroplasts (Zhang *et al*., 2016). Anthocyanins accumulate in the vacuoles of mesophyll cells and have been suggested to work as osmoregulators (Manetas, 2006). However, this role may be more prominent in red-leaf plants, as noticed in

'Garnem'. As such, increased salinity sharply decreased anthocyanins in 'Garnem', independently of the type of salt present in the irrigation water. Such decrease may be due to the toxicity of Cl^-, suggesting that anthocyanins protect chlorophylls against reactive oxygen species and played a role in photoprotection (Zrig *et al*., 2011). Furthermore, it was reported that leaves rich in anthocyanidin had a higher antioxidant capacity compared to green ones; anthocyanins contribute to antioxidant capacity more than other low molecular weight compounds (Zrig *et al*., 2011). Interestingly, in this investigation, it appeared that the red anthocyanin was involved in the osmotic adjustment since the positive correlation between the Ψπ and total anthocyanin was found mainly in 'GN15' (or 'Garnem'). The ability of 'GN15' to maintain leaf turgor and protect the photosynthetic machinery from the effects of stress helped this rootstock to survive an ECw = 12.45 dS m^{-1} (C +75 mM NaCl) as it showed a lower reduction in growth than BA, as reported in previous work (Zrig *et al*., 2011). Maintenance of leaf turgor pressure and photosynthetic capacity made 'GN15' more efficient than 'BA' in taking up water and nutrients from the soil, despite high salinity (Zrig *et al*., 2011). However, a previous study (Zrig *et al*., 2016) reported that 'BA' plants were capable of osmotically adjust to high salt environments by the addition of $CaCl_2$, which was then followed by a large accumulation of proline and soluble sugars in leaves. One the other hand, this large increase in total anthocyanins and, to a lesser extent in petunidin-3-glucoside, was only seen when $CaCl_2$ was added with NaCl in 'BA' rootstock. Zrig *et al*., (2016) found that the same treatment ($CaCl_2$ + NaCl) decreased the assimilation rate and the total chlorophyll content. These results agree with the ones reported by others who worked with tomato and red cabbage seedlings under salinity (Eryılmaz, 2006), but we cannot explain why other salt treatments did not produce similar effects on anthocyanins. Nevertheless, the responses of 'Garnem' rootstock to salinity with a general decrease in total anthocyanins, petunidin, and cyaniding all match the results obtained previously for the same rootstock when just NaCl was added to salinity treatments (Zrig *et al*., 2011). Previous results on both almond rootstocks reported that high salinity caused the degradation of total leaf chlorophyll (Zrig *et al*., 2016). These results, and those reported by Zrig *et al*., (2016), indicate a relationship between chlorophyll degradation and anthocyanin production. Our results show that anthocyanin do not change ('Bitter Almond') or decrease ('Garnem') with salinity, except when $CaCl_2$ was added to NaCl. Based on previous work (Zrig *et al*., 2016), a direct association between anthocyanin production and the period of increased vulnerability to photoinhibition during senescence can be seen, providing further evidence that anthocyanins may play a photoprotective role in leaves, and that they may also play a protective role against salinity stress. Indeed, anthocyanins were found to increase in an inverse proportion to chlorophyll in response to salinity in both tomato and red cabbage (Eryılmaz, 2006).

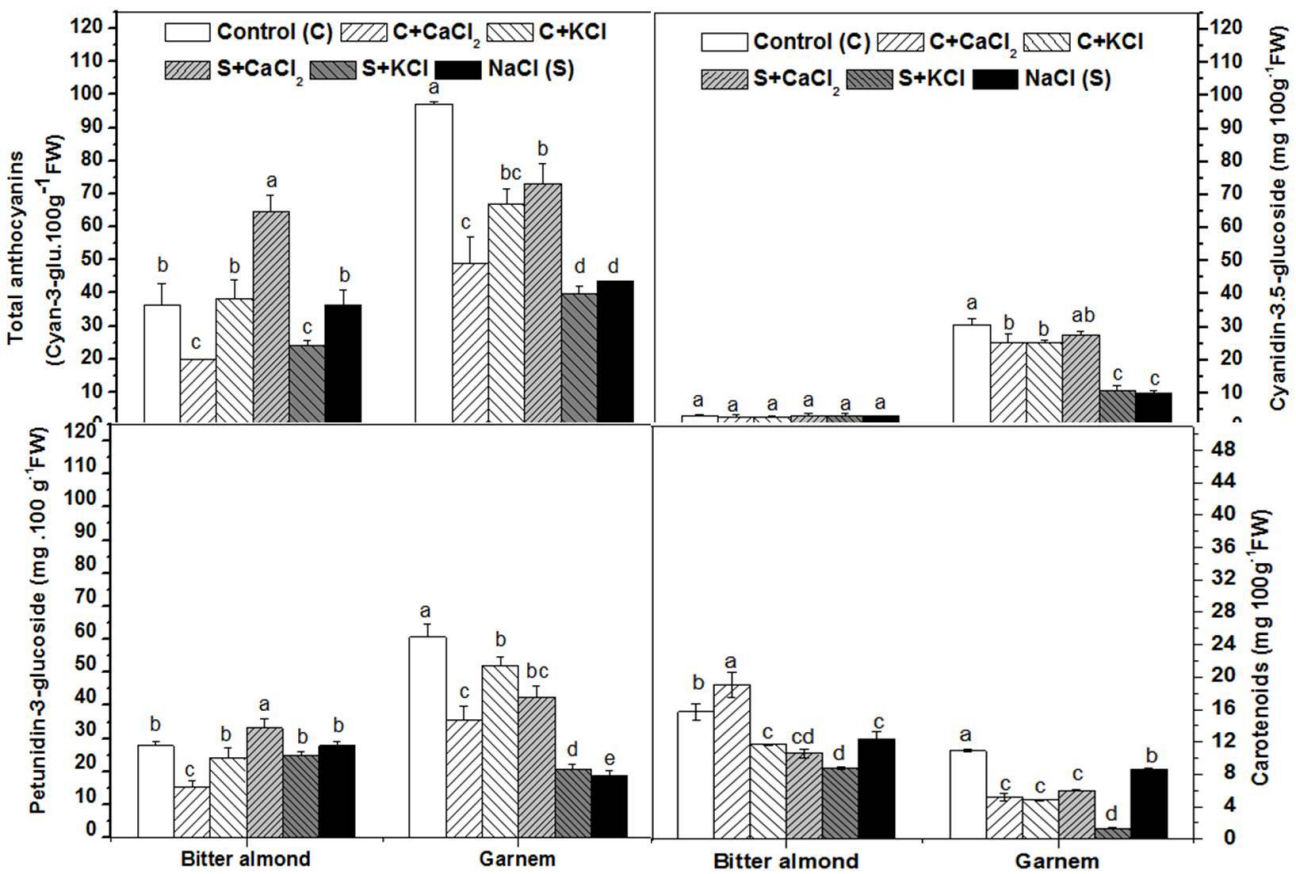

Fig. 2. Concentrations of leaf pigments of two almond rootstocks irrigated with low-salinity water (control), and with waters containing 75 mM NaCl (NaCl, S), 10 mM KCl or 10 mM $CaCl_2$ alone or combined with 75 mM NaCl. Each point represents the mean (± SE) of three replicates. Values marked by different small letters are significantly different at p≤5%.

Table 2. Correlation coefficients (r) between leaf osmotic potentical (Ψπ) and total antioxidant capacity (TAC) vs. anthocyanin, cyanidin, petunidin, polyphenols, putrescine, and spermidine leaf contents.

Leaf metabolites	'Bitter Almond'		'Garnem'	
	Ψπ	TAC	Ψπ	TAC
Anthocyanin	-0.0363^{ns}	0.0224^{ns}	0.3540 *	0.1507 ^{ns}
Cyanidin	-0.10.34^{ns}	-0.1241^{ns}	0.3540**	0.1507^{ns}
Petunidin	-0.3574^{ns}	-0.1011^{ns}	0.1487^{ns}	0.1428^{ns}
Polyphenols	0.1731^{ns}	0.3204*	0.2582^{ns}	0.4722**
Putrescine	-0.1781^{ns}	0.2309^{ns}	-0.3792**	0.4726**
Spermidine	0.0261^{ns}	-0.0995^{ns}	-0.3353*	0.4810**

Asterisks indicate statistically significant correlations, considered at probability levels of ≤0.1 (*) and p≤0.05 (**), while ^{ns} mean not significant

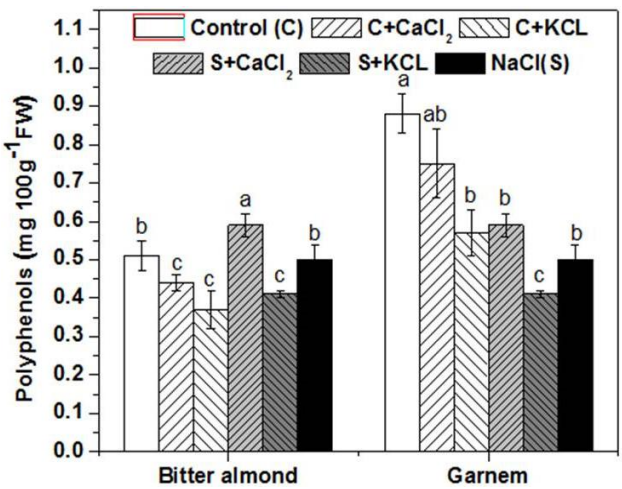

Fig. 3. Concentrations of leaf polyphenols of two almond rootstocks irrigated with low-salinity water (control), and with waters containing 75 mM NaCl (NaCl, S), 10 mM KCl or 10 mM $CaCl_2$ alone or combined with 75 mM NaCl. Each point represents the mean (± SE) of three replicates. Values marked by different small letters are significantly different at p≤5%.

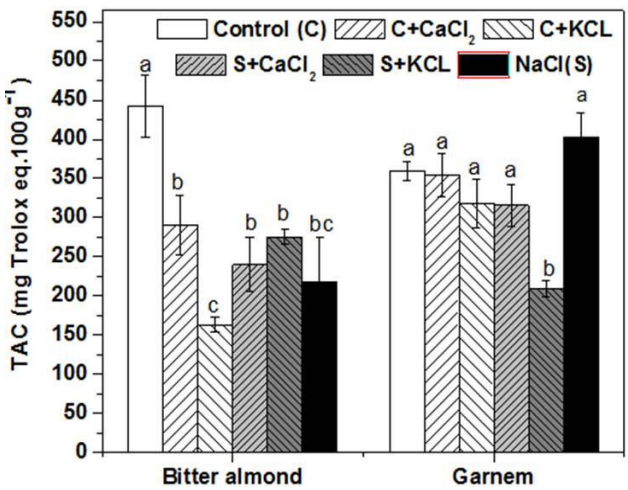

Fig. 4. Total antioxidant capacity (TAC) of leaves of two almond rootstocks irrigated with low-salinity water (control), and with waters containing 75 mM NaCl (NaCl, S), 10 mM KCl or 10 mM $CaCl_2$ alone or combined with 75 mM NaCl. Each point represents the mean (± SE) of three replicates. Values marked by different small letters are significantly different at p≤5%.

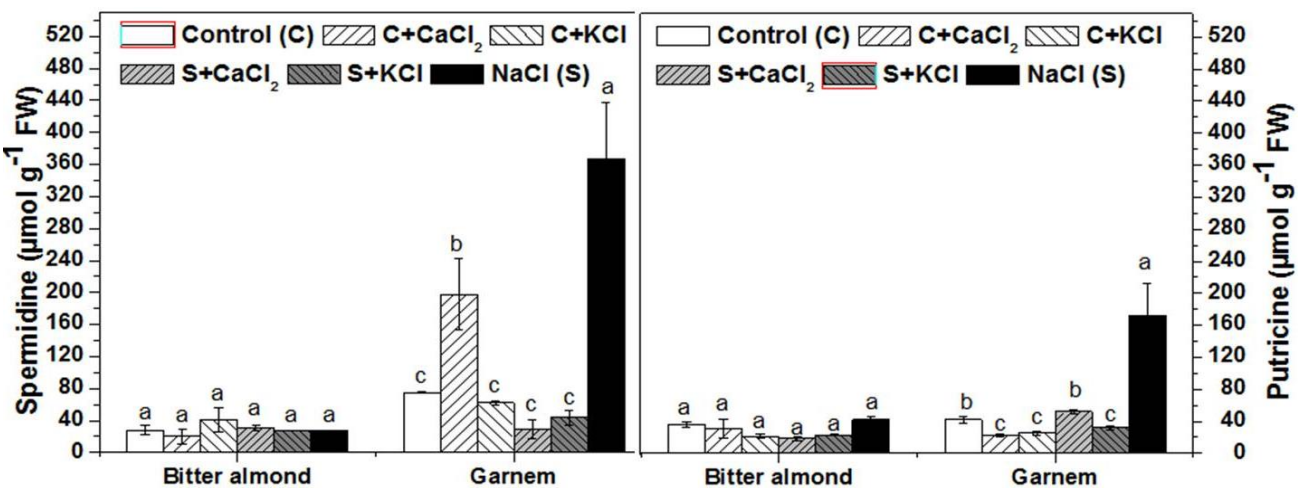

Fig. 5. Concentration of spermidine and putrescine in leaves of two almond rootstocks irrigated with low-salinity water (control), and with waters containing 75 mM NaCl (NaCl, S), 10 mM KCl or 10 mM $CaCl_2$ alone or combined with 75 mM NaCl. Each point represents the mean (± SE) of three replicates. Values marked by different small letters are significantly different at p<5%.

The positive correlations between the osmotic potential and both anthocyanin and cyanidin suggest an osmotic adjustment, regardless of the decrease of anthocyanin content in 'Garnem', or 'GN15', which can be accomplished through the synthesis of cyanidin. The high content of cyanidin in leaves of 'GN15' under $CaCl_2$ + NaCl compared to NaCl-treated plants suggests that cyanidin contribute most to osmoregulation. Indeed, anthocyanins are hypothesized to be involved in osmotic regulation (Chalker-Scott, 2002) and many studies have confirmed that plant tissues containing high levels of anthocyanins are usually tolerant to drought and salt (Chalker-Scott, 1999). According to our findings, previous reports have shown that, a purple-leaved pepper

tolerated salt stress better than a green-leaved cultivar (Bahler *et al*., 1991), whereas the negative correlation between the level of anthocyanin and TAC indicate that anthocyanin did not contribute to the antioxidant capacity of either almond rootstocks. This relationships between anthocyanin content and antioxidant capacity was observed in several plants such as tomato and red cabbage treated with NaCl (Eryılmaz, 2006).

Salt stress and nutrient deficiencies induces higher levels of polyphenolic compounds in different tissues of several plant tissues (Hernández *et al*., 2008; Vitrac *et al*., 2000). However, according to our results, the effect of salinity on polyphenols depended on the rootstock (Fig. 4). However, polyphenol decreased in GN15 when salt was added to the culture medium. This reduction was probably due to the degradation of anthocyanins and matches the results obtained previously with the same rootstock and increasing salinity (Zrig *et al*., 2011). The positive correlation between TAC and the polyphenols in both rootstocks (Table 2) suggest a protective role of polyphenols against oxidative stress caused by salinity. The antioxidant capacity of phenolics act as reducing agents hydrogen donors, and singlet oxygen quenchers due to their redox properties, (Hernández *et al*., 2009).

Our results showed an accumulation of putrescine and spermidine in leaves of 'GN15' plants irrigated with 75 mM NaCl solution. A higher putrescine and spermidine concentration in plant tissues were reported to improve plant K^+/Na^+ homeostasis (Martin-Tanguy, 2001) and reduce salt-induced oxidative damage by activating enzymatic and non-enzymatic antioxidants (Martin-Tanguy, 2001). In particular, increased levels of cellular polyamines during salinity stress have shown dual effects. In fact, the accumulation of polyamines was correlated with higher plant tolerance to salt stress, partly due to their ability to inactivate oxidative radicals (Martin-Tanguy, 2001). Furthermore, our results indicated that spermidine increased considerably in 'GN15' rootstock when $CaCl_2$ was added to control plants, while putrescine decreased slightly. These changes may be attributed, in part, to the inhibiting effect of Ca^{2+} on ethylene biosynthesis. Such inhibition may result in the increased generation of spermidine due to the relative accumulation of S-adenosylmethionine (Zhang *et al*., 2000), a precursor of ethylene. However, when NaCl was added to $CaCl_2$, spermidine level decreased in the red leaves of 'GN15' indicating that some other mechanism, such as translocation, catabolism and/or further synthesis, effectively reduced polyamine content (Kovács *et al*., 2014). The role of the synthesized polyamines may be to maintain photosynthetic activity and prevent leaf senescence (Martin-Tanguy, 2001). The positive correlation between the TAC and polyamine suggested the antioxidant role of putrescine and spermidine to prevent the salt oxidative damage in red leaves of 'GN15'. Plants also exhibit increased polyamine degradation during salt stress, and polyamine turnover appears to be highly regulated. During salt stress, intracellular polyamines are exported from the cytosol to the apoplast, against the electrochemical gradient, and oxidized to generate hydrogen peroxide that is further converted to OH via the Fenton reaction (Pottosin *et al*., 2014). In addition, the lower level of polyamine indicated that the ion interactions enhanced the PA catabolism which produces hydrogen peroxide (H2O2), a signaling molecule that can modulate the stress signal transduction chain promoting the activation of the antioxidant defense response, but that can also act as a pro-oxidant agent mainly in red leaves of 'GN15' (Pottosin *et al*., 2014). The rootstock 'GN15' has been shown to accumulate less Na and Cl than other rootstocks, while increasing concentration of polyamines and decreasing anthocyanins in response to salinity imposed by NaCl (Zrig *et al*. 2011). These results indicate that polyamines may be involved in helping GN15 cope with salinity stress. The addition of $CaCl_2$ to NaCl, although increasing the salinity of NaCl treatments seemed to decrease the salinity effect on the reduction of anthocyanins, petunidin, and cyanidin, but had no advantage to either rootstock regarding to other metabolites.

Conclusion

In general, the almond rootstocks 'BA' and 'GN15' showed a differentiated response to salinity, but not necessarily to the type of salt added. This response seemed to be dictated by leaf biochemical composition, which varied between red-leaf ('GN15') and green-leaf ('BA') phenotypes. Indeed, rootstock response to salinity, assessed through leaf accumulation of secondary metabolites, is not straightforward. Still, polyamines and anthocyanins seemed to contribute differently to salt response in both rootstocks. Although both the decrease in anthocyanins and increase in polyamines in 'GN15' were in response to salinity, and their role in salinity tolerance has been speculated by others, further research is needed to establish if decreased anthocyanins and polyphenols, and increased polyamines in fact can be interpreted as mechanisms of salinity tolerance in 'GN15' rootstocks.

Acknowledgments

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