RESEARCH PAPERS

Differential Response of Two Almond Rootstocks to Chloride Salt Mixtures in the Growing Medium¹

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Abstract—It was examined how essential cations, Ca^{2+} and K^{+} , can mitigate the toxic effects of NaCl on two different almond species (*Prunus amygdalus* Batsch) rootstocks, Garnem (GN15) and Bitter Almond. The tree growth parameters (water potential (Ψ_w), gas exchange, nutrient uptake) and leaf chlorophyll (Chl) content were measured in control and NaCl-treated plants with or without KCl or CaCl_2 supplements. The addition of CaCl_2 and KCl to Bitter Almond trees reduced their dry weight, shoot growth and leaf number although net photosynthetic assimilation rate (A) was not affected. These results indicated that changing of photo-assimilates flux to proline and/or soluble sugars synthesis may help to increase leaf Ψ_w . The Garnem trees also did not respond to the CaCl_2 and KCl addition indicating that the plants are already getting enough of these two cations (Ca^{2+} and K^{+}). In both rootstocks, NaCl in the medium reduced growth attributes, Ψ_w , A , stomatal conductance (g_s), and leaf Chl content. When CaCl_2 and KCl fertilizers were added together with NaCl to Bitter Almond trees, leaf K^{+} and Ca^{2+} contents increased while Na^{+} and Cl^{-} decreased leading to higher Ca/Na and K/Na ratios, but shoot growth was not improved and even declined compared to NaCl-treated trees. It appears that the addition of salts further aggravated osmotic stress as indicated by the accumulation of proline and soluble sugars in leaf tissues. The addition of KCl or CaCl_2 to NaCl-treated GN15 trees did not increase A , leaf Ψ_w , and shoot growth but improved ionic balances as indicated by higher Ca/Na and K/Na ratios. The reduction in A was mainly due to non-stomatal limitations in GN15, possibly due to the degradation of Chl a , unlike Bitter Almond, for which the reduction of A was due to stomata closure. The improvement in ionic balances and water status of Bitter Almond trees in response to addition of KCl or CaCl_2 was apparently offset by a high sensitivity to Cl^{-} ; therefore, no-chloride salts should be the preferred forms of fertilizers for this rootstock. Both rootstocks were sensitive to soil salinity and cation supplements were of limited value in mitigating the effect of excessive salt concentrations.

Keywords: *Prunus amygdalus*, Garnem, Bitter Almond, rootstocks, proline, soluble sugars, Ca/Na, K/Na, cation nutrition, salinity

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INTRODUCTION

The problem of salinity is especially serious in arid areas due to excessive evaporation and the scarcity of good quality water for irrigation. The term salinity refers to the total concentration of the major dissolved inorganic ions, Na^{+} , Ca^{2+} , Mg^{2+} , K^{+} , HCO_3^{-} , SO_4^{2-} and Cl^{-} in the soil solution [1]. High salt levels lead to

osmotic stress coupled with ionic imbalances caused by the increased uptake of toxic ions such as Na^{+} and Cl^{-} [1]. The salt stress has also an adverse effect on mineral Ca^{2+} and K^{+} homeostasis [1, 2]. An excess or deficiency of the major elements in plant's tissues may cause disorders with respect to nutrient availability, uptake, transport or partitioning within the plant. Thus, there may be a need to use fertilizers to alleviate the harmful effects of excessive soil salinity. Indeed, Ca^{2+} is known to displace Na^{+} from soil exchange colloids and render it easier to be leached down the soil profile with drainage. Furthermore, maintaining enough supply of Ca^{2+} and K^{+} in saline soil solution is

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Abbreviations: A —net photosynthetic assimilation rate, Chl—chlorophyll, EC—electrical conductivity, E —transpiration rate, g_s —stomatal conductance, iWUE—instantaneous water use efficiency, TSS—total soluble sugars, Ψ_w —leaf water potential.

Table 1. Nutrient solution composition and electrical conductivity

Treatment	Irrigation solution composition	EC, dS/m
Control	Basal nutriment solution (C)	2.97
CaCl ₂	C + 8 mM CaCl ₂	4.77
KCl	C + 10 mM KCl	4.17
NaCl	C + 75 mM NaCl	9.95
NaCl + CaCl ₂	C + 75 mM NaCl + 8 mM CaCl ₂	11.77
NaCl + KCl	C + 75 mM NaCl + 10 mM KCl	11.17

a key factor in controlling specific ion toxicities, especially in crops prone to Na⁺ and Cl⁻ toxicity [1]. Potassium and Ca²⁺ are essential for plants; Ca²⁺ plays integrity role in cell membrane integrity, signal transduction and the control of enzyme activity [3], while K⁺ is involved in a number of metabolic processes such as photosynthesis, enzyme activation, protein synthesis, osmotic adjustment, and as a counter ion to both inorganic anions and organic biopolymers [3]. K⁺ uptake and transport in plants are strongly depressed in the presence of Na⁺ and enhanced by Ca²⁺ supplements.

Most fruit trees are considered sensitive to soluble salts especially Cl⁻. Irrigation with saline water may greatly reduce their yield [4]. The earliest symptoms of ion toxicity are typically caused by Cl⁻ excessive contents in the leaves while Na⁺ tends to be retained in the roots, trunk, and branches, so its content in the leaves remains relatively low for several years [4]. Therefore, the role of the rootstock is crucial in determining the tree performance under saline conditions [5].

In the Mediterranean region, Garnem (Garfi Almond × Nemared) peach hybrid with reddish leaves, has gained popularity among nurserymen for its tolerance of drought and calcareous soils [6]. 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 [7].

The present study was set up to evaluate how increased essential cations supply may mitigate the harmful effects of NaCl salinity on two distinct widely-planted almond rootstocks.

MATERIALS AND METHODS

Plant material and culture. One-year-old rooted cuttings of Garnem (GN15) and seedlings of Bitter Almond (*Prunus amygdalus* Batsch) were grown in 4-L plastic pots containing desert dune sand under a non-heated lath house (average PPFD of 1100 μmol/(m² s)). The plants were obtained from a commercial nursery then cultivated during two months (June and July) with daily maximum air temperatures ranging between 27°C and 31°C, while relative air humidity ranged between 49 and 60%. Dur-

ing the first 3 weeks of cultivation, the plants received a complete nutrient solution prepared with deionized water to which salts were added so to obtain an electrical conductivity (EC) of 2.77 dS/m and an initial total ion concentration of 4.5 mM consisting of 1.8 mM KNO₃, 0.64 mM KH₂PO₄, 1.0 mM Ca(NO₃)₂, 0.35 mM MgSO₄, 0.03 mM Fe-EDTA, 0.4 mM ZnSO₄, 0.5 mM MnSO₄, 0.1 mM CuSO₄ and 0.023 mM H₃BO₃.

After this initial acclimation phase, 24 plants of each rootstock were selected for uniformity in size and growth. Each set of four trees of each stock was watered with one of the following six solutions (treatments): i—control (basic nutrient solution), ii—control + 10 mM KCl, iii—control + 8 mM CaCl₂, iv—salt stress (basic nutrient solution + 75 mM NaCl), v—salt stress + 10 mM KCl, and vi—salt stress + 10 mM CaCl₂. The electrical conductivity (EC) of each solution is given in Table 1. The surface of pots was covered with black plastic to minimize evaporation and to prevent algal growth. The experimental design was a completely randomized block with four replicates (each pot being a replicate).

The plants were harvested after 4 weeks of imposing the treatments. The leaves of each plant were collected and divided into two batches, one was immediately frozen in liquid nitrogen then stored at -80°C for further biochemical analyses, the other was briefly washed free of dust in de-ionized water, dried at 80°C in a forced-air oven for 48 h and ground into a fine powder to pass through a 40-mesh screen for mineral analyses.

Plant growth, water potential, ion content, and gas exchange measurements. The total leaf number, tree dry weight, and shoot length were used to estimate plant growth. The predawn water potential (Ψ_w) of shoots was measured with a pressure chamber (Soil Moisture Equipment Co., United States). The net photosynthetic assimilation rate (*A*), transpiration rate (*E*), and stomatal conductance (*g_s*) were determined using an LCpro+ portable photosynthesis system (ADC, Bio-Scientific, Ltd, United Kingdom). The instantaneous water use efficiency (iWUE) was calculated as the ratio between *A* and *E* [8].

For determination of mineral contents, they were extracted from dried leaves (1 g) with 20 mL of 0.1 M

HNO₃. Then after filtration, the K⁺, Na⁺, and Ca²⁺ contents were determined with an atomic absorption spectrometer (Avanta, GBC, Australia). The Cl⁻ content was determined with chloride analyzer (Corning M926 chloride analyzer, Sherwood Scientific, United Kingdom).

Determination of proline, soluble sugars, and chlorophyll contents. The frozen leaves (0.2 g) were ground to a fine powder in a pre-cooled mortar with liquid nitrogen, homogenized with 5 mL of 3% aqueous sulfosalicylic acid and centrifuged at 8000 g for 15 min. Then 2 mL 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 for 1 h, after which the reaction was stopped by placing the test tube in an ice bath. Then 4 mL of toluene were added to each test tube and vortexed for 20 s. The absorbance at 520 nm of the organic toluene phase containing the chromophore was used to quantify the amount of proline as described by Bates et al. [9].

The total soluble sugars in leaf tissue were determined according to the method of Robyt and White [10]. The leaves powder (100 mg) was extracted by 80% (v/v) methanol heated to 70°C in a water bath. The extract was then centrifuged at 5000 g for 10 min. The supernatant was used for the estimation of soluble sugars contents. The reaction mixture consisted of 1 mL 5% phenol and 5 mL 98% sulphuric acid. After the extract had been cooled, its absorbance was determined at 490 nm using D-glucose as standard [10].

The leaf chlorophylls (Chl) were extracted with acetone (80%, v/v) as described by Arnon [11]. The absorbance of the extract was read at 647 and 664 nm with a spectrophotometer.

The Chl contents were calculated as follows:

$$\text{Chl } a \text{ (mg/g fr wt)} = [12.7 \times (A_{664}) - 2.69 \times (A_{647})],$$

$$\text{Chl } b \text{ (mg/g fr wt)} = [22.9 \times (A_{647}) - 4.69 \times (A_{664})].$$

Statistical analyses. The variance of data was analyzed with the GLM procedure of SAS software (SAS Institute, United States) [12] for a Randomized Complete Block design with four replicates. Where applicable, means were separated by Duncan's test ($P \leq 0.05$).

RESULTS

Effects of NaCl and Ionic Interactions on Plant Growth and Cation Contents

The addition of CaCl₂ and KCl to control plants (plants which did not receive additional NaCl) of Bitter Almond reduced their growth attributes (Fig. 1). Similarly, the addition of NaCl alone to the medium reduced tree dry weight, leaf number, and shoot length. For Bitter Almond trees fed additional NaCl, the addition of CaCl₂ or KCl further reduced leaf number by 51 and 36%, respectively, compared to the trees which received NaCl alone. Tree dry weight and shoot extension were further reduced (compared to

Fig. 1. Total leaf number, shoot length and dry weight of two almond rootstocks grown in a medium without NaCl (control), with NaCl, or containing KCl or CaCl₂ alone or together with NaCl. 1—control; 2—8 mM CaCl₂; 3—10 mM KCl; 4—75 mM NaCl + 8 mM CaCl₂; 5—75 mM NaCl + 10 mM KCl; 6—75 mM NaCl. Each point represents the mean ($\pm$ SE) of four replicates, values marked by different letters are significantly different at $P < 0.05$.

those of the trees which received NaCl alone) by the addition of CaCl₂ but not KCl. Some leaves became scorched on the margins then fell off.

The Garnem trees did not respond to the addition of CaCl₂ and KCl (Fig. 1). The leaf number and shoot

Fig. 2. Contents of K^+ , Ca^{2+} , Na^+ , and Cl^- in the leaves of two almond rootstocks grown in a medium without NaCl (control), with NaCl, or containing KCl or $CaCl_2$ alone or together with NaCl. 1—control; 2—8 mM $CaCl_2$; 3—10 mM KCl; 4—75 mM NaCl + 8 mM $CaCl_2$; 5—75 mM NaCl + 10 mM KCl; 6—75 mM NaCl. Each point represents the mean ($\pm$ SE) of four replicates, values marked by different letters are significantly different at $P < 0.05$.

length did not improve after fertilization with $CaCl_2$ and KCl; to the contrary, tree dry weight was even slightly lower. Furthermore, when NaCl was added alone, these parameters were 50% reduced. The addition of $CaCl_2$ or KCl did not reverse this reduction in shoot growth and leaf number but increased tree dry weight.

The addition of $CaCl_2$ to Bitter Almond trees increased leaf Ca^{2+} content by 62% compared to control plants but had no effect on K^+ content (Fig. 2). On the contrary, the addition of KCl to these plants decreased leaf Ca^{2+} content by 38% but had no significant effect on K^+ content. The addition of NaCl alone to the base solution increased considerably leaf Na^+ and Cl^- content and suppressed K^+ and Ca^{2+} contents leading to large reductions in Ca/Na and K/Na ratios (Table 2). When NaCl-treated Bitter Almond trees were fertilized with $CaCl_2$, leaf Na^+ and Cl^- contents significantly decreased while leaf K^+ and Ca^{2+} contents and Ca/Na and K/Na ratios increased.

The addition of $CaCl_2$ to GN15 trees had no effect on leaf Ca^{2+} and K^+ contents but increased the content of Cl^- and reduced Ca/Na and K/Na ratios compared to control plants (Fig. 2; Table 2). When KCl was added alone, it increased leaf K^+ content by 33% but reduced Ca^{2+} content; Ca/Na and K/Na ratios were also lower. The addition of NaCl alone to the base solution increased leaf Na^+ and Cl^- content by 75 and 82%, respectively, while it reduced the contents of Ca^{2+} and K^+ by 28 and 37%, respectively, leading to large reductions in Ca/Na and K/Na ratios. Furthermore, increasing the supply of calcium to NaCl-treated trees slightly increased leaf Ca^{2+} and K^+ contents and suppressed Na^+ and Cl^- contents. Likewise, the addition of KCl to such trees led to large increase in K^+ content and decrease in Na^+ and Cl^- contents compared to NaCl-fed plants. Fertilization significantly increased Ca/Na and K/Na ratios (Table 2). Regardless of treatments, Garnem leaves always had more Cl^- than Na^+ while Bitter Almond leaves had more Na^+ than Cl^- .

Table 2. K/Na and Ca/Na ratios in the leaves of Bitter Almond and GN15 rootstocks exposed to different combinations of chloride salts

Variety	Control (C)	C + CaCl ₂	C + KCl	NaCl + CaCl ₂	NaCl + KCl	NaCl
K/Na						
Bitter Almond	1.2 ^a	1.4 ^a	1.2 ^a	0.7 ^b	0.6 ^b	0.3 ^c
GN15	8.8 ^a	5.7 ^{bc}	8.0 ^a	4.8 ^c	6.7 ^b	3.2 ^d
Ca/Na						
Bitter Almond	0.9 ^b	1.9 ^a	0.5 ^c	0.4 ^c	0.3 ^{cd}	0.2 ^d
GN15	2.7 ^a	1.9 ^b	1.6 ^{bc}	1.5 ^{bc}	1.4 ^c	1.1 ^d

Each point represents the mean of four replicates; mean separation within rows by Duncan's Multiple Range Test $P < 0.05$. Different letters means significance at $P < 0.05$.

Effects of NaCl and Ionic Interactions on Gas Exchange and Water Status

Increasing Ca²⁺ supply to control trees of BA did not improve their *A* but increased their *g_s* and *E* considerably (Fig. 3). Similarly, adding KCl to the growing medium had no effect on *A* and *E* but reduced *g_s*. The addition of NaCl reduced all three parameters by more than 50% compared to control values. Similarly, adding of CaCl₂ to NaCl-treated trees further reduced their *A*, *g_s*, and *E*, whereas KCl did not affect *A* and *E* but reduced *g_s*. The *A/g_s* ratio increased by the addition of salts to the nutrient solution.

Adding of CaCl₂ to GN15 control trees increased their *A* and *g_s* but did not affect *E* (Fig. 3). On the contrary, adding of KCl to the growing medium had no effect on *A* and *g_s* but reduced *E*. The addition of NaCl alone reduced all three parameters. The CaCl₂ or KCl adding to NaCl-treated trees did not affect their *A* but increased *g_s* and reduced *E*. The *A/g_s* ratio was reduced by the addition of salts to the nutrient solution.

Bitter Almond leaf Ψ_w was increased by the CaCl₂ addition but was unchanged by KCl (Fig. 3). On the contrary, it was reduced by more than 80% by NaCl. The CaCl₂ or KCl adding to NaCl-treated trees increased their leaf Ψ_w . All combinations of supplemental chloride salts except KCl improved water use efficiency (iWUE) of Bitter Almond plants compared to the control. All combinations of supplemental chloride salts reduced leaf Ψ_w of GN15 plants. Leaf Ψ_w of NaCl-treated GN15 trees was further reduced by the addition of CaCl₂ or KCl. Adding CaCl₂ or KCl to control and NaCl-treated trees slightly improved their iWUE.

Effects of NaCl and Ionic Interactions on Chlorophyll Content

The addition of CaCl₂, KCl or NaCl to Bitter Almond trees decreased leaf Chl (*a + b*) and Chl *a* contents but increased the content of Chl *b* (Fig. 4). However, when CaCl₂ was added together with NaCl, Chl (*a + b*) content increased considerably mainly as a result of more Chl *b* content. Content of Chl *a* was suppressed with Chl *a/b* ratio. In contrast, when KCl was added to NaCl-treated Bitter Almond trees, con-

tents of Chl (*a + b*), Chl *a*, Chl *b* decreased. The ratio Chl *a/b* was not affected.

The addition of CaCl₂ or KCl alone to the growing medium of GN15 trees increased leaf Chl (*a + b*) content by 23 and 22%, respectively, as compared to control plants (Fig. 4). Similarly, fertilization increased Chl *a* but not Chl *b* contents. CaCl₂ reduced Chl *a/b* ratio while KCl had no effect. The NaCl in the culture medium did not affect total Chl content but increased Chl *a* and reduced Chl *b* content resulting in a high increase in the ratio Chl *a/b*. Likewise, the additional supply of CaCl₂ to NaCl-treated trees did not affect total Chl and Chl *a* contents but increased Chl *b* content resulting in a lower Chl *a/b* ratio compared to NaCl-treated trees. The addition of KCl to NaCl-treated trees largely reduced total Chl and Chl *a* contents but increased Chl *b* content resulting in a dramatically lower Chl *a/b* ratio compared to NaCl-treated trees.

Effects of NaCl and Ionic Interactions on Proline and Total Soluble Sugar Contents

The proline content in Bitter Almond leaves increased more than two folds when CaCl₂ was added to the growing medium of control and NaCl-treated plants (Fig. 5). On the contrary, it decreased when KCl was added instead of CaCl₂. The addition of NaCl or (NaCl + KCl) did not change leaf proline content. In the GN15 trees, the addition of CaCl₂ or KCl alone had no significant effect on proline content. The addition of NaCl increased proline content more than 2 fold. The addition of CaCl₂ or KCl reduced proline content back to control level.

The addition of CaCl₂ increased the content of total soluble sugars (TSS) in the leaves of Bitter Almond rootstock whereas KCl and NaCl did not (Fig. 5). The addition of CaCl₂ or KCl to NaCl-treated plants largely increased TSS content. In the case of GN15, all combinations of supplemental chloride salts except (KCl + NaCl) did not change the content of leaf TSS. The latter treatment reduced TSS content by 84% compared to its level in NaCl-treated trees.

Fig. 3. Net photosynthesis (A), stomatal conductance (g_s), transpiration rate (E), leaf water potential (Ψ_w), A/g_s , and instantaneous water use efficiencies ($iWUE = A/E$) in the leaves of two almond rootstocks grown in a medium without NaCl (control), with NaCl, or containing KCl or $CaCl_2$ alone or together with NaCl. 1—control; 2—8 mM $CaCl_2$; 3—10 mM KCl; 4—75 mM NaCl + 8 mM $CaCl_2$; 5—75 mM NaCl + 10 mM KCl; 6—75 mM NaCl. Each point represents the mean ($\pm$ SE) of four replicates, values marked by different letters are significantly different at $P < 0.05$.

DISCUSSION

The present study tested the effect of increased Ca^{2+} and K^+ concentrations in the culture medium on the response of two almond rootstocks to NaCl stress.

The logic behind the experiment is the common assumption that increasing soil Ca^{2+} and K^+ supply would shift root uptake in favor of Ca^{2+} and K^+ at the expense of Na^+ thus protecting plant tissues from the adverse effects of high NaCl salinity.

Fig. 4. Total chlorophyll (Chl (a + b)), chlorophyll a (Chl a), chlorophyll b (Chl b), as well as Chl a/b ratio in the leaves of two almond rootstocks grown in a medium without NaCl (control), with NaCl, or containing KCl or CaCl₂ alone or together with NaCl. 1—control; 2—8 mM CaCl₂; 3—10 mM KCl; 4—75 mM NaCl + 8 mM CaCl₂; 5—75 mM NaCl + 10 mM KCl; 6—75 mM NaCl. Each point represents the mean ($\pm$ SE) of four replicates, values marked by different letters are significantly different at $P < 0.05$.

It was no surprise that all three measured growth parameters were reduced by the addition of 75 mM NaCl to the growing medium for both rootstocks confirming previous reports indicating that almond tree is sensitive to salinity [13]. The electrical conductivity threshold in vegetative growth of almond trees was only 1.5 dS/m, i.e., 15 mM total salinity [13]. Hence, almond is considered a salt-sensitive glycophyte [7]. However, certain woody fruit crops differ in their ability to absorb and transport Na⁺ and Cl⁻ and have, therefore, different reactions to salt [14]. This appears to be the case of these two rootstocks. It was noticeable that Bitter Almond rootstock accumulated more Na⁺ in its leaves while Garnem accumulated more Cl⁻. The addition of NaCl reduced Ψ_w , g_s , and E in both almond rootstocks (Fig. 3) as occurred in other species [2]. This is an expected response to the osmotic stress brought about by the medium high salt concentration.

The addition of CaCl₂ or KCl to BA plants reduced their shoot growth, dry weight and leaf number. The basic nutrient solution was not concentrated enough to cause a significant reduction in vegetative growth. We think that Bitter Almond tree is sensitive to Cl⁻ in the medium which concentration was further increased after addition of CaCl₂ (8 mM) or KCl (10 mM). Since Cl⁻ level of 10 mM in a soil extract is considered toxic to sensitive rootstocks, the reduction of growth was expected [14]. The growth reduction happened even though A was not affected indicating a possible diversion of photo-assimilates from growth processes to proline and/or soluble sugars synthesis which helped, along with the reduction in water loss by transpiration, increased leaf Ψ_w . The role of these osmolytes may have been the osmo-protection of proteins against Cl⁻ toxicity rather than osmo-regulation [15]; in fact, leaf Ψ_w only slightly increased by the addition of CaCl₂ and remained unchanged by KCl.

Fig. 5. Proline and total sugar content in the leaves of two almond rootstocks grown in a medium without NaCl (control), with NaCl, or containing KCl or CaCl₂ alone or together with NaCl. 1—control; 2—8 mM CaCl₂; 3—10 mM KCl; 4—75 mM NaCl + 8 mM CaCl₂; 5—75 mM NaCl + 10 mM KCl; 6—75 mM NaCl. Each point represents the mean ($\pm$ SE) of three replicates, values marked by different letters are significantly different at $P < 0.05$.

When CaCl₂ and KCl fertilizers were added along with NaCl to Bitter Almond trees, leaf Ca²⁺ and K⁺ contents increased while Na⁺ and Cl[−] contents decreased leading to higher Ca/Na and K/Na ratios but shoot growth did not improve and even further declined compared to NaCl-treated trees. It appears that the addition of salts further increased osmotic stress and more importantly aggravated Cl[−] toxicity as indicated by the accumulation of proline and soluble sugars in leaf tissues (osmotic adjustment and osmoprotection). Plant growth has suffered as a result of fertilization despite the improvement in ionic balances because photosynthesis was suppressed possibly due to the reduction in leaf area (there was some leaf abscission), content of Chl *a*, stomatal conductance and *A*.

The addition of CaCl₂ to Bitter Almond plants improved their iWUE and *A* relative to *g_s* (higher *A/g_s*) possibly because of a more efficient carboxylation and reduced photorespiration due to partial closure of stomata [15].

The addition of KCl or CaCl₂ to NaCl-treated GN15 plants did not increase *A* or shoot growth but it improved ionic balances as indicated by higher Ca/Na and K/Na ratios. Apart from the expected Cl[−] toxicity, the addition of these fertilizers caused additional water stress as indicated by the lower Ψ_w . The leaf transpiration rate decreased due to lower *g_s*. Our results showed that when KCl or CaCl₂ were added alone (without NaCl) they increased *g_s* and, to a lesser extent, *A* and decreased *A/g_s* ratio. Therefore, the depressing effects of chloride salts on photosynthetic activity were predominantly due to non-stomatal limitations related to the toxic effect of Na⁺ and Cl[−]. The Garnem plants showed a slight increase in water use efficiency (iWUE) as response to fertilization.

It is worth mentioning that Bitter Almond leaves did not display a significant degradation of total Chl content under CaCl₂ treatments, but for the most part a clear conversion of Chl *a* to the Chl *b* (Fig. 4). It appeared, that Ca²⁺ boosted such conversion or, at least, stimulated de novo Chl *b* biosynthesis. Similar trend was seen in GN15 plants too. The conversion of Chl *a* to Chl *b* appears now to be a part of general Chl *a/b* inter-conversion cycle which is thought to play a vital role in the formation and reorganization of the photosynthetic apparatus and which enables plants to adapt to various environmental conditions [1, 16].

Despite the improvement in nutritional balances after Ca²⁺ and K⁺ fertilization, plant growth did not improve and even declined in the case of Bitter Almond because of the excessive concentration of NaCl in the growing medium for a salinity-sensitive species. Our results confirmed the sensitivity of these two rootstocks to both soil salinity and Cl[−] anions [13, 14].

The Bitter Almond, and to less extent GN15, are capable of osmotically adjust to high-salt environments. Indeed, the addition of CaCl₂ to Bitter Almond plants was followed by a large accumulation of proline and soluble sugars in the leaves. It is likely, that the increase in cytosolic free Ca²⁺ in leaf cells acted at the transcriptional level to induce the expression of genes encoding for enzymes involved in proline biosynthesis [17]. Similarly, the higher Ca²⁺ content may stimulate amylase activity which degrades starch into sugars [17], and the increase in soluble sugars content can stimulate proline biosynthesis [17, 18].

In conclusion, almond tree is a salt-sensitive glycophyte. However, two rootstocks considered in this study differed in their response to the addition of chloride salts to the growing medium. The Garnem plants

appeared to be slightly less sensitive to soil salinity than Bitter Almond plants, because they have the capacity to limit Na^+ uptake by roots and its translocation to the leaves [19]. The reduction in *A* was mainly due to non-stomatal limitations in GN15, possibly due to the degradation of *Chl a*, unlike Bitter Almond plants for which the reduction was due to stomata closure. The improvement in ionic balances and water status of Bitter Almond trees in response to the addition of KCl or CaCl_2 was apparently offset by a higher sensitivity to Cl^- which caused some leaf abscission; therefore, non-chloride salts should be the preferred form of fertilizers for this rootstock. Both rootstocks were sensitive to soil salinity and cation supplements were of limited value in mitigating the effect of salt at high concentrations.

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