

Short communication

Physiological and growth responses of sour cherry (*Prunus cerasus* L.) plants subjected to short-term salinity stress

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Abstract – The gradual response of CAB-6P sour cherry (*Prunus cerasus* L.) plants to NaCl-induced salinity stress (60 mM NaCl) was investigated in a short-term hydroponic experiment, based on parameters relating to the growth, water relations, chlorophyll and mineral nutrition. The results showed that CAB-6P plants are very sensitive to salinity stress because their growth and leaf chlorophyll concentration were both affected negatively from the 3rd and 5th day, respectively, after incurring salinity stress. Since root growth was suppressed more severely than shoot growth, the shoot to root ratio was significantly increased under saline conditions. The concentrations of Na in leaves and stem of NaCl-treated plants were much lower than those measured in roots, suggesting Na exclusion mechanism from the shoot. The opposite trend was observed for Cl, indicating Cl inclusion mechanism to leaves. As regards the concentrations of Ca, Mg, P, K, Na, Fe, Zn and Mn, they were not changed in higher salinity conditions, apart from K, concentrations of which in leaves and roots were significantly increased and decreased, respectively (K translocation to leaves). Salinity further reduced K/Na ratio in root and stem as well as leaf water and osmotic potentials, whereas leaves of control and NaCl-treated plants presented similar turgor potential and K/Na ratio. These data add very important information to our knowledge about the physiological events occurring in sour cherry plants after even short-term exposure to salinity.

Keywords: chlorophyll, CAB-6P rootstock, Cl inclusion, ion concentrations, Na exclusion, osmotic adjustment, water relations

Introduction

An excess of soil salinity affects the performance of hundreds of crop species worldwide and may be particularly deleterious to most fruit tree crops, which are usually salt-sensitive (Ziska et al. 1990). It is also well documented that various *Prunus* species are included in the group of salt sensitive species (Andreu et al. 2011). Although, there are many published papers dealing with the effects of salinity on growth, antioxidants, photosynthesis, nutritional status and several other parameters of *Prunus* species (Ziska et al. 1990, Papadakis et al. 2007, Chatzissavvidis et al. 2008, Andreu et al. 2011), there are no detailed studies regarding the changes and/or possible adaptations occurring just after exposure to saline environments. Such data would add valuable informa-

tion to our knowledge about the initial events that take place during the exposure of fruit trees to salinity. In fact, salinity is not a real problem in the main world's regions where sweet and sour cherries are traditionally cultivated, since these areas have usually high levels of rainfall and, therefore, high availability of good quality irrigation water. Nowadays, however, there are some new low-chill cherry varieties, and, of course, more will be produced in the future. These new varieties can be cultivated in warmer/more arid areas of the world, including lowlands and coastal areas, where, at least during the summer months, irrigation water of poor quality containing high levels of salts is commonly used. According to Tattini et al. (1995), the maintenance of plant growth and

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productivity is very important for perennial crops that are subjected to transitional periods of saline conditions. On the other hand, rootstocks possess a critical role for the sustainable cultivation of plants, and are a tool with which farmers can alleviate salinity and/or other environmental constraints in their orchards (Papadakis 2016). Therefore, the current study aimed to investigate the growth and physiological responses (plant growth, chlorophyll concentration, water relations and nutrient status) of the well-known sour cherry genotype CAB-6P, which is widely used as a rootstock for both sour and sweet cherry cultivars, under salt stress.

Materials and methods

One-year-old, uniform, self-rooted plants of the clone CAB-6P (*Prunus cerasus* L.) were transplanted in black plastic bags filled with 3 L of a sand/perlite mixture (50/50, v/v). In fact, the use of small-asexually propagated plants of about 25 cm in height was aimed at determining even the initial, tiny changes caused by the salinity stress. The plants were irrigated with good quality tap water for seven weeks before NaCl treatment was imposed. Afterwards, the plants were irrigated, thrice a week, with 300 mL of the well-known Hoagland nutrient solution No 2 (Hoagland and Arnon 1950) containing either 0 or 60 mM NaCl. The concentration of 60 mM NaCl is within the range of NaCl concentrations usually observed in irrigation waters of the Mediterranean basin (Flowers 1999). The experiment was carried out in a glasshouse, with temperatures ranging between 18 and 30 °C, and lasted 13 days. Three plants per treatment were randomly selected, every two days after salinity imposition, for mea-

suring water (Ψ_w), osmotic (Ψ_π) and turgor (Ψ_p) potentials as well as chlorophyll concentration in fully expanded apical leaves. Finally, the plants were separated into root, stem and leaves. After estimating the fresh and dry weights of all these plant portions, they were further analyzed for Na, Cl, K, N, P, Ca, Mg, Fe, Mn and Zn. All the methods, procedures and equipments used for the aforementioned measurements and determinations have already been described in our previously published paper (Papadakis et al. 2007).

The experimental design was completely randomized. In all, 36 plants were used: 18 were controls (0 mM NaCl) and 18 were NaCl-treated (60 mM NaCl). From the third day (3rd day) after salinity imposition until the last day of experimentation (13th day), 3 plants from each treatment were harvested every two days, i.e., 2 treatments (control, salinity) \times 3 plants per sampling day and treatment \times 6 sampling days (3rd, 5th, 7th, 9th, 11th and 13th). For each parameter and sampling day, means of control and NaCl-treated plants were compared with each other using the Student's t-test ($P \leq 0.05$).

Results and discussion

Salinity reduced the weights of leaves (Fig. 1A), stems and roots (Fig. 1B), and therefore the total weight of CAB-6P plants (Fig. 1C), even from the 3rd day of the experiment. Furthermore, NaCl-treated plants presented significantly higher values of shoot to root ratio (on a dry weight basis) than the control plants, from the 7th day of the experiment and thereafter (Fig. 1D). A decline in dry weight of leaves and stems was also observed in two cherry cultivars

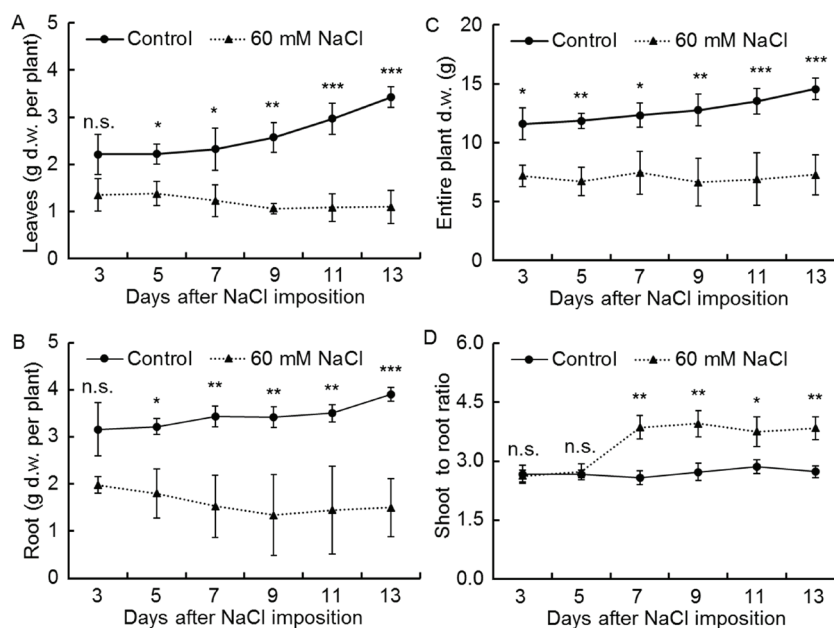


Fig. 1. Dry weight (d.w.) of leaves (A), root (B), and entire plant (C); shoot to root ratio (D) of control (0 mM NaCl) and salinity-treated (60 mM NaCl) *Prunus cerasus* plants at different days (3, 5, 7, 9, 11 or 13) after NaCl imposition. Results are expressed as mean values \pm SE. For each parameter and day after salt stress imposition, asterisks indicate significant differences between control and NaCl-treated plants, at $P \leq 0.05$ (*), $P \leq 0.010$ (**) or $P \leq 0.001$ (***) ; n.s. indicates non-significant differences.

subjected to salinity conditions (Papadakis et al. 2007). The osmotic stress leads to an instant decline in cell expansion of young roots and leaves, causing stomatal closure as well. Enhanced resistance to osmotic stress would be beneficial for leaf growth and stomatal conductance, but the resulting increased leaf area would be productive only if plants had adequate soil water (Munns and Tester 2008), such as under the current experimental conditions. The latter consideration could possibly explain the fact that the root growth of CAB-6P plants was inhibited by salinity to a greater extent than shoot growth (increased values of shoot/root ratio). In other words, the root of CAB-6P plants is more sensitive to salinity than the shoot, at least in the first two weeks after the imposition of NaCl stress. This finding is not in accordance with the results of other researchers working with many different crops, like tomato (Albacete et al. 2008). More specifically, the shoot to root ratio in salt-treated tomato plants was decreased (an important adaptive response) due to the rapid inhibition of shoot growth (which limits plant productivity) while root growth was maintained (Albacete et al. 2008). The reduction of shoot to root ratio is one of the most usual symptoms of plants subjected to salinity stress resulting in improvement of water use efficiency within the plant. It was suggested that the plants subjected to high salinity save water by minimizing their shoot growth and by keeping a normal water status for growth (Sohan et al. 1999). In contrast to these reports, our results are in agreement with those stated by Bernstein and coworkers (2004), who found that the shoot growth of avocado plants was less sensitive to salinity than root growth. The strong inhibition of root growth is expected to affect the entire plant and thus the root growth could be considered an important criterion for rootstocks' tolerance to NaCl (Bernstein et al. 2004). Overall, since the root growth of CAB-6P plants was dramatically affected by salinity, even from the first days of the current short-term experiment, the use of CAB-6P as rootstock has to be avoided when sweet or sour cherry varieties are going to be cultivated in areas having low quality irrigation water.

Genotypes with high capabilities to control Cl and Na absorption by roots and/or Cl and Na movement from roots to stems and especially to leaves, present high tolerance to salinity (Storey and Walker 1999). Ion exclusion and compartmentation at the root level regulates ion concentration in the xylem sap, preventing thus the accumulation of potentially toxic ions in the aboveground plant parts. This mechanism is effective at levels of salinity up to 50 mM NaCl, but it considerably impedes plant growth. The accumulation of Na in roots provides a mechanism for many crops, such as olive, to cope with salinity in the root zone and may indicate the existence of an inhibitory mechanism of Na transport to leaves (Chartzoulakis 2005). In the current study, the concentrations of Na in the shoots (leaves and stems) of NaCl-treated plants (Fig. 2A-B) were much lower than those in the roots (Fig. 2C). Moreover, lower Na concentrations were found in leaves (Fig. 2A) than in stems (Fig. 2B). Therefore, CAB-6P sour cherry plants must have had the capability to control the absorption of Na and/or its transport

to stems and leaves. In general, Na exclusion from the shoot has often been reported as a crucial mechanism for plants to tolerate high salinity (Jha et al. 2010). On the other hand, the increase of Cl concentration in the NaCl-treated plants was simultaneous in all plant parts (leaves, stems and roots), from the 9th day of NaCl imposition and thereafter (Figs. 2D-F). Furthermore, higher Cl concentrations were determined in leaves of NaCl-treated plants (Fig. 2D) than in the stems (Fig. 2E) and roots (Fig. 2F), especially after the 11th day of the experiment, which indicates that a Cl inclusion mechanism (export to leaves) was implicated. Therefore, it could be concluded that CAB-6P sour cherry plants are very sensitive to salinity, since the mechanism controlling (restricting) Cl absorption by roots collapsed quickly (from the 9th day of plants' exposure to salinity and thereafter), although the concentration of NaCl (60 mM) in the nutrient solution was relatively low. In other words, a high susceptibility of CAB-6P plants to Cl absorption when they were subjected to saline conditions was revealed. Leaf Cl concentration has been commonly used as a reliable criterion for rating plant species according to their efficiency under saline conditions (Antcliff et al. 1983, Storey and Walker 1999). In short, the sensitivity of sour cherry plants to salinity is closely correlated with Cl concentration in various plant parts (mainly in the leaves). This criterion could be applied for future screening studies of other sour cherry genotypes for their relative sensitivity to saline conditions.

In a previous study where the effects of NaCl in the mineral nutrition of two sweet cherry varieties were investigated, it was found that the concentrations of all nutrients, except for N and Mg, in leaves, stems and roots of both cultivars were altered under the treatment of 50 mM NaCl. In addition, the root was the main plant organ whose nutrient content was mostly affected by NaCl, probably because it is the plant part that salts enter first (Papadakis et al. 2007). Based on the concentrations of various mineral elements (N, P, Mg, Ca, Mn, Zn and Fe) determined in roots, stems and leaves of CAB-6P plants, no one significant difference was found between control and NaCl-treated plants (data not shown). This finding could be mainly ascribed to the short duration of the salinity stress imposition which was not enough to affect the overall nutritional status of sour cherry plants. The only exception was potassium (K) which was strongly affected by NaCl (Fig. 2G-I). Liu and van Staden (2001) suggested that a constant or increased level of internal K is of major importance for a better response of entire plants and/or plant tissues to salinity stress. In the current study, although control and NaCl-treated CAB-6P plants had similar concentrations of K in their stems (Fig. 2H), salinity decreased or increased K concentrations in roots (at 11th and 13th day) (Fig. 2I) and leaves (at 13th day) (Fig. 2G), respectively. A possible explanation could be the existence of a mechanism that removes K from roots to aerial plant parts, especially to leaves (Romero and Marañón 1994). Furthermore, the relatively high salt tolerance is usually correlated with relatively high values of K/Na ratio in plant tissues (Liu and van Staden 2001). Giri et al. (2007) experimenting with the mycorrhizal

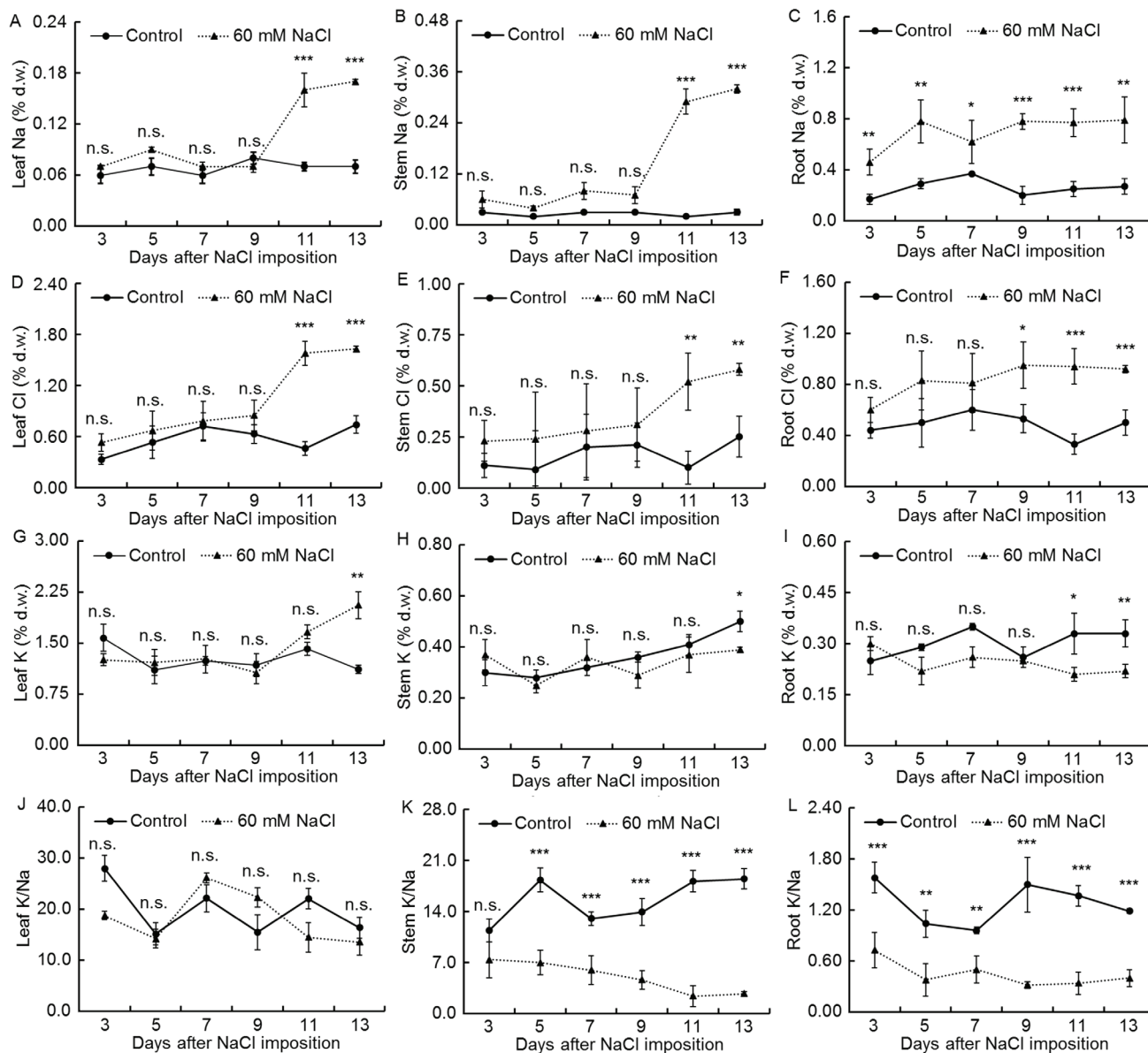


Fig. 2. Concentrations of Na (A, B, C), Cl (D, E, F) and K (G, H, I) in leaves (A, D, G), stem (B, E, H) and root (C, F, I); K/Na ratio in leaves (J), stem (K) and root (L) determined in control (0 mM NaCl) and salinity-treated (60 mM NaCl) *Prunus cerasus* plants at different days (3, 5, 7, 9, 11 or 13) after NaCl imposition. Results are expressed as mean values \pm SE. For each parameter and day after salt stress imposition, asterisks indicate significant differences between control and NaCl-treated plants, at $P \leq 0.05$ (*), $P \leq 0.010$ (**) or $P \leq 0.001$ (***); n.s. indicates non-significant differences.

Acacia nilotica under saline conditions found that elevated K/Na ratios in root and shoot may prevent K-mediated enzymatic processes from disruption. In contrast, our data have shown that stem and root K/Na ratios in NaCl-treated plants were much lower than those of control plants, from the 3rd (root) or 5th (stem) day after salinity stress imposition (Figs. 2K-L). Regarding the leaf K/Na ratio (Fig. 2J), no significant difference between control and salinity stressed plants during the experiment was found. Moreover, the K/Na ratio in leaves of CAB-6P plants (Fig. 2J) was much higher than in stems (Fig. 2K) and roots (Fig. 2L), irrespective of the NaCl treatment and/or sampling day. This latter finding was due to the relatively high concentrations of K and low

concentrations of Na found in CAB-6P leaves, a fact that was governed by the parallel existence of an Na-exclusion mechanism from the shoot and a K-inclusion (transport) mechanism to the leaves. In other words, salt-treated CAB-6P plants were capable of maintaining an appropriate (similar to that of control plants) leaf K-to-Na ratio, probably for protecting the most actively growing portion of plants (leaves). In accordance with the results of other studies (Chartzoulakis 2005, and references therein), the diminution of K concentrations in roots of NaCl-treated plants, which resulted in too low K/Na ratios, may also provide a mechanism by which CAB-6P plants achieved ionic balance following the high Na absorption by their roots.

In a previous *in-vitro* experiment with CAB-6P explants treated with 60 mM NaCl, one of the main effects of the high leaf Cl concentration (4.2-5.6 % d.w.) was the decline in chlorophyll concentration of leaves (Chatzissavvidis et al. 2008). Similarly, in another study with *Prunus salicina*, leaf chlorophyll concentration decreased when leaf chloride exceeded 0.25 mol kg⁻¹ d.w. (about 0.9 % d.w.) (Ziska et al. 1990). In the current short-term study, the total leaf chlorophyll concentration was also reduced by the salinity, from the 5th day of the experiment and thereafter (Fig. 3A). However, this decrease in leaf chlorophyll could not mainly be ascribed to either the toxic effects of accumulated Na (ranged between 0.07 % d.w. -5th day- and 0.19 % d.w. -13th day-) and Cl (ranged between 0.67 % d.w. -5th day- and 1.63 % d.w. -13th day-) ions in leaves or to deficiencies of other mineral nutrients (similar concentrations were found in salt-treated and control plants). Alternately, it might rather have been due to NaCl-induced changes in the overall physiological status of plants (low water potential, possible closure of stomata, a decrease of photosynthetic performance, oxidative damages, etc.). The lower values of water (Fig. 3B) and osmotic (Fig. 3C) potentials found in the leaves of NaCl-treated sour cherry plants, compared with those of control plants, resulted in turgor potential values (Fig. 3D) similar to those of control plants. This latter observation in combination with the increased concentrations of Na, K and Cl found in leaves of NaCl-treated plants leads to the conclusion that CAB-6P plants are osmoregulated by the accumulation of inorganic ions. In any case, however, the possible contribution of some compatible solutes in the osmotic adjustment of sour

cherry plants could not be excluded since relevant measurements were not carried out. Therefore, since (a) it is nearly impossible for a cell to adjust osmotically using only inorganic ions, and (b) *Prunus* species produce and accumulate significant amounts of sorbitol and proline under dehydration stress (Jiménez et al. 2013, Zrig et al. 2016), which may work as a membrane and as metabolism stabilizers and antioxidants, the contribution of soluble carbohydrates and other compatible organic solutes in the osmoregulation of sour cherry plants subjected to salinity stress has to be further investigated.

Overall, CAB-6P sour cherry plants are very sensitive to salinity stress since their growth and leaf chlorophyll concentration were both affected negatively from the 3rd and 5th day, respectively, after the imposition of salinity. Moreover, the results of this short-term study revealed some interesting responses of sour cherry plants under saline conditions. Firstly, their root growth was affected more than their shoot growth (dry weight basis). Secondly, a Na-exclusion mechanism from the shoot (stems and leaves) was found to take place. Thirdly, the existence of Cl- and K-inclusion mechanisms to leaves were also a fact. Therefore, CAB-6P plants were able to control the uptake of Na and/or its transport from roots to leaves more appropriately than they could Cl. Fourthly, plants had the capability of osmoregulation, a fact that apparently limited the detrimental effects of salinity. Finally, from a pomological point of view, the use of the CAB-6P sour cherry clone as rootstock for sweet and sour cherry plantations has to be avoided in areas having low quality irrigation water, even if salinity is just a short-term-seasonal problem.

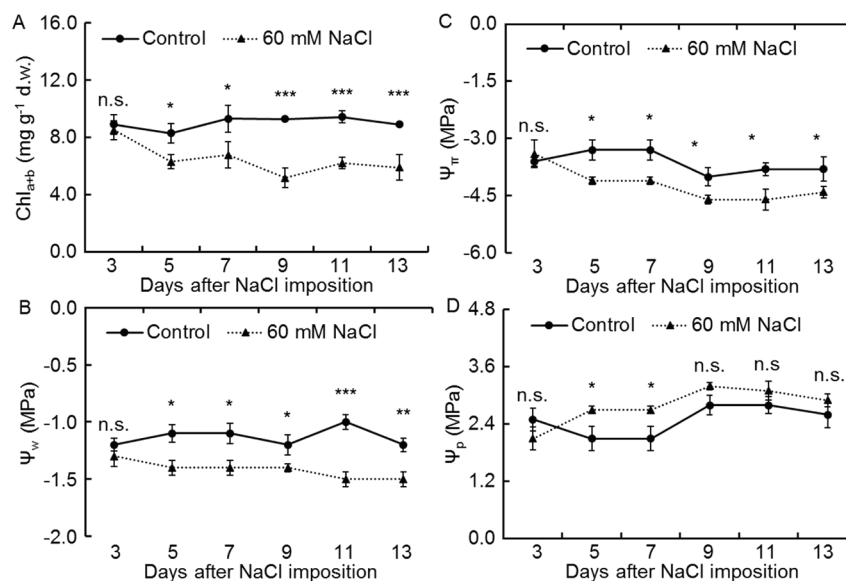


Fig. 3. Total leaf chlorophyll concentration (A); leaf water potential (Ψ_w) (B), osmotic potential (Ψ_π) (C) and turgor potential (Ψ_p) (D) determined in control (0 mM NaCl) and salinity-treated (60 mM NaCl) *Prunus cerasus* plants at different days (3, 5, 7, 9, 11 or 13) after NaCl imposition. Results are expressed as mean values \pm SE. For each parameter and day after salt stress imposition, asterisks indicate significant differences between control and NaCl-treated plants, at $P \leq 0.05$ (*), $P \leq 0.010$ (**) or $P \leq 0.001$ (***); n.s. indicates non-significant differences.

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