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# Germination, Mineral Composition, and Ion Uptake in Walnut Under Salinity Conditions

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***Abstract.*** **Walnut (*J. regia* L.) is one of the most sensitive plants to abiotic stresses for which finding salt-tolerant genetic resources is very important. Effects of salt stress on seed germination of seven walnut cultivars (Serr, Lara, Pedro, Chandler, Hartely, Vina, and Roun de Montignac) were studied. Salt stress treatments were applied using NaCl solutions ranging from 50 to 250 mM. The increase in salinity levels resulted in a substantial decrease in root relative water content. The percent of germination was also affected by salinity level and cultivar and their interactions. The mean germination time differed among treatment media and cultivars and a significant interaction was observed between these two factors. According to the cluster analysis, 'Chandler' was classified as the most tolerant cultivar. 'Hartley', 'Pedro', and 'Round de Montignac' ('RDM') were also classified in another group (semisensitive), whereas 'Vina', 'Serr', and 'Lara' were classified as sensitive cultivars. With the increase in salt stress, resistant cultivars accumulated more potassium (K) and calcium, especially in their shoots. However, in the semitolerant cultivars, accumulation of K in the root was more than in the shoot. Differences in magnesium accumulation in roots and shoots of all samples were significant at all stress levels and were dependent on genotypes. Sodium levels in roots were higher than in the shoots in most cultivars, especially in the semisensitive and tolerant ones. There was a low correlation ($r^2 \leq 0.15$) between walnut seed traits (seed weight and kernel weight) and growth indices with respect to salinity tolerance.**

Land affected by salinization in arid and semiarid regions of South Asia is ≈42 million ha (FAO, 1994). Approximately 33 million ha are in I.R. in Iran, known as the major nut crop producer in the world such as walnut. Salinity can be harmful for plant growth in nearly every irrigated area of Iran. Therefore, to extend walnut plantation to marginal lands, it is important to know the genetic recourses and tolerance mechanisms of walnut cultivars to salinity.

Living systems are supported and sustained by the genome through the action of the transcriptome, proteome, metabolome, and ionome, the four basic biochemical pillars of functional genomics (Salt, 2004). These pillars represent the sum of all the expressed genes, proteins, metabolites, and elements within an organism. Studies on the functional relations between the genome and the transcriptome (Becher et al., 2004; Leonhardt et al., 2004; Martzivanou and Hampp, 2003), proteome (Koller et al., 2002), and metabolome (Fiehn et al., 2000) are well underway. However, the study of the ionome, in contrast, is still in its infancy (Lahner et al., 2003; for review, see Hirschi, 2003; Rea, 2003). The majority of genes and gene networks involved in such regulations are still unknown. Moreover, because the ionome is involved in such a broad range of important biological phenomena, including electrophysiology, signaling, enzymology, osmoregulation, and transport, understanding of the ionome and how it interacts with other cellular systems such as the genome, proteome, metabolome and environment is the prerequisite for the full understanding of how plants integrate their organic and inorganic metabolisms.

The stresses induced by salinity in plants are caused by two factors: hyperosmotic stress, resulting from a more negative soil water potential, and ionic imbalance, leading to the accumulation of toxic ions (sodium, chloride, boron). Morphological and physiological disturbances produced by salinity are often associated with $Cl^-$ and $Na^+$ accumulation. In some species like citrus, $Cl^-$ is more damaging and is more rapidly accumulated than $Na^+$ (Romero-Aranda et al., 1998), but walnut trees seem to accumulate high amounts of $Na^+$ in contrast to $Cl^-$ in their shoots, fine roots, and in the root framework as a whole (Girona et al., 1993). The toxic effect of sodium plays a central role when salinity is caused by the excess NaCl. Under high salt concentration, sodium can enter the cytoplasm by nonselective potassium channels and reach concentrations up to a toxic level. The ability of plants to grow under salinity stress is associated with the capacity of plant cells to maintain low cytosolic sodium concentrations and high $K^+/Na^+$ ratio. The main strategies for the maintenance of a high $K^+/Na^+$ ratio are sodium extrusion and/or intracellular sodium compartmentation in the vacuole. The excess sodium in the cytoplasm is reduced by an active transport operated mainly by $Na^+/H^+$ antiporters. These transporters exchange one proton with one sodium ion across the membranes. The energy for this active transport of sodium is provided by plasma membrane $H^+$-ATPases and tonoplast $H^+$-ATPases, which maintain the proton gradient by consumption of ATP energy (Beritognolo et al., 2007; Blumwald, 2000; Blumwald et al., 2000). The active response to maintain ion homeostasis under salt stress relies on signaling cascades that have been deeply investigated in *Arabidopsis thaliana* by molecular analysis of salt overly sensitive (SOS) mutants. The SOS pathway is triggered by a transient increase of cytosolic $Ca^{2+}$ as a first effect of salt stress. The increase in $Ca^{2+}$ concentration is sensed by a calcium binding protein (SOS3), which forms a complex with a serine/threonine proteine kinase (SOS2). The SOS3–SOS2 complex then phosphorylates the plasma membrane $Na^+/H^+$ antiporter (SOS1) and other ion transporters (Chinnusamy et al., 2004; Zhu, 2003).

Walnut (*Juglans* spp.) has been described as a very sensitive crop to specific ion toxicity (Catlin and Schreader, 1985) and because of that, finding mechanisms involved in resistance to abiotic stresses, especially from the ionomics point of view, are important in this crop. Current studies are focused on developing hypothesis that variation in accumulation of mineral elements is closely related to the evolution of different walnut cultivars with different nutrient requirements. It was our goal to understand differences among walnut cultivars in accumulating essential elements that are involved in the cell wall firmness, ionic pumps, and in ion transfer through plasma and other cell membranes as well as having a positive effect on resistance to salinity stress. We were also interested to know how and which elements will differ in their effects on improving the genetic capacity

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and the salt tolerance of tolerant and sensitive walnut cultivars.

## Materials and Methods

*Plant material.* The half-sib seeds from seven open-pollinated walnut (*J. regia* L.) cultivars [Hartley, Pedro, Vina, Lara, Serr, Roun de Montignac (RDM), and Chandler] were supplied by the walnut collection of Seed and Plant Improvement Institute South of Karaj City, Iran. Meteorologically, Karaj Ccity is characterized by 242 mm of average annual rainfall, a relatively short (≈5 to 6 months) drought period in spring and summer, cold winters (minimum temperature –1 °C) and 5 months of frost period (December to April).

*Salt stress treatment.* The salt stress experiments were carried in 2007 from 15 Mar. to 8 Apr., under greenhouse conditions. Salt stress was induced by NaCl treatment at five stress levels (50, 100, 150, 200, and 250 mM) corresponding to electrical conductance of 0 (control), 4.91, 9.31, 13.10, 17.30, and 21.78 $mS \cdot cm^{-1}$, respectively. The seeds were soaked in water for 10 d and then were pretreated with Captan fungicide before chilling treatment (Vahdati and Hoseini, 2006). Seeds were then placed in a refrigerator for 2 to 4 weeks at 4 to 6 °C to overcome their chilling requirements. Seeds were planted in small-sized polyethylene pots (250 mL v) filled with pure medium-sized perlite granules. Fifty milliliters of salt solution with known osmotic potential were added to each pot. Pots were weighed soon after adding the NaCl and their soil surfaces were covered with plastic films to prevent evaporation and were then placed in a growth chamber at 25 ± 1 °C and 46% relative humidity. Pots were weighed every evening and distilled water was added to make up for the amount lost by evaporation. No nutrient solution was added to the pots in the germination test. Seeds were allowed to germinate at 25 ± 1 °C in light (12-h photoperiod at a light intensity of 8 μmol/E). Germination percentages were recorded every 24 h during the experiment and the final percentages of germination were determined for all cultivars.

*Growth index measurements.* When the primary root lengths of control seedlings (0 water potential) were 4 cm long, the following growth indices were recorded: root and shoot length, root and shoot fresh and dry weight, root and shoot thickness, shoot to root length ratio, shoots to roots fresh and dry weight ratio, and shoots to roots thickness ratio. Length and thickness of roots and shoots were measured by Vernier calipers (LG Co., Korea) by 125 × 0.02 mm or by 5 × 1.1000 in AACO accuracy. Furthermore, to study the correlation between seed characteristics and tolerant to salinity, seed size and kernel weight of all the studied cultivars were measured using a Vernier calipers by 20 replicates for each traits.

Also, the final germination percentage (FGP), mean germination time, relative water content (RWC), and tissue water content were calculated. The mean germination time (MGT) was estimated as $MGT = \sum_i^j ni.ti/N$; where $n_i$ is the number of seeds that germinated within consecutive intervals of time, $t_i$ is the time between the beginning of the test and the end of a particular interval, and N is the total number of germinated seeds (Hartmann et al., 2001). The values of final germination simply represent the number of seeds that germinated during the 28 d in the growth media. These values were expressed as percentages of the initial number of seeds placed on the trays. Values of total water content (TWC) were determined as Eq. [1].

$$TWC = 100\ (FM - DM)/FM \qquad [1]$$

where FM is tissue fresh weight and DM is tissue dry mass. Dry mass was determined after drying the tissues samples at 80 °C for 24 h. Values of TWC were expressed as RWC by determining FM, DM, and saturated mass (SM) as Eq. [2].

$$RWC = (FM - DM)/(SM - DM) \qquad [2]$$

For SM determination, tissues were rehydrated by immersing in distilled water in a large beaker sealed with parafilm. Full rehydration was achieved within 24 to 48 h in complete darkness at 2 to 4 °C.

*Analysis of mineral elements.* Total magnesium, calcium, potassium, and sodium were analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES) after digestion in a 4:10 mixture of sulfuric acid and hydrogen peroxide. Acid digestion of samples for measuring minerals by ICP-AES was done using the method explained by Bottin et al. (1999). For mineral analysis, samples were taken from 28-d-old seedlings after germination. The root and shoot samples were dried at 70 °C for 72 h. The dried samples were then separately ground to fine powder. Two hundred fifty milligrams of each sample was used to analyze for mineral composition.

*Statistical analyses.* The experimental design was factorial arranged as a completely randomized design with four replications and 12 seeds per replicate. The first factor was the six salinity levels (50, 100, 150, 200, 250 mM NaCl) and the second factor included the seven cultivars (Hartley, Pedro, Vina, Lara, Serr, Roun de Montignac, and Chandler). Normality of experimental errors was determined by Proc Univariate and Proc Capability (SAS Institute Inc., Cary, NC). Analysis of variance and comparison of means were performed using SAS software (SAS Institute Inc.). Significant differences among the mean values were compared by Duncan's multiple range test ($P \leq 0.05$). To screen tolerant cultivars and study their mineral compositions, the cultivars were classified by cluster analysis using SPSS (SPSS Inc., Chicago, IL). Genetic distances were calculated based on the mean of all measured traits mentioned in M&M that were made at 200 mM NaCl using square Euclidean distance and groupings were performed by the unweighted pair group method with averages method. A resultant dendrogram was cut by comparing variations in square Euclidean distances of each step (Jobson, 1992).

## Results

*Germination indices.* The walnut cultivars showed differential responses to salt stress under greenhouse conditions. There was a negative correlation between salinity level and most of the vegetative growth parameters. Measuring growth indices indicated that by increasing salinity levels, root and shoot length, their thicknesses, and also their fresh and dry masses, especially those of shoots, were decreased. 'Lara' and 'Chandler' seedlings were most and least affected by salt stress, respectively. The increase in salinity levels was accompanied by a substantial decrease in root RWC (Table 1). Differences among cultivars at different salinity levels were highly significant. In general, seed germination rates were more rapidly in control (no salt stress) than in salt-containing media. Consequently, the time it took for the complete seed germination in control media was shorter than for those in salt-treated ones. The final seed germination percentages were affected by salinity levels, cultivars, and also by their interactions (Fig. 1). The FGP values were significantly lower at higher salinity levels and there were also differences in FGP among species (Fig. 1).

The mean germination time differed both among different treatments and cultivars and also a significant interaction was found between these two factors (Fig. 2). For the seven walnut cultivars studied, the mean germination time was shorter in the control than in the other treatments (Fig. 2). There were also differences in the mean germination time among cultivars. At high salinity levels (200 and 250 mM), the average mean germination time for 'Chandler' was 5.2 to 2.3 d shorter, respectively, than those observed in the other cultivars.

*Cluster analysis.* Cluster analysis resulted in a dendrogram with three different groups of tolerance to salinity stress (Fig. 3). There were highly significant differences among three salt-tolerant classes (data not shown). According to the cluster analysis, 'Chandler' was classified in an independent group as the most tolerant cultivar. 'Hartley', 'Pedro', and 'Roun de Montignac' ('RDM') were also classified in the other (semisensitive) group, whereas 'Vina', 'Serr', and 'Lara' were classified as sensitive cultivars (Fig. 3).

*Mineral elements.* Differences in the range of sodium accumulation were minimal as compared with other minerals at different salt stress levels. In control plants, the average sodium content ranged from 0.34 to 1.82 $mg \cdot g^{-1}$ dry weight (DW), whereas the shoots of the sensitive cultivars (Lara, Vina, and Serr) had significantly higher sodium contents than other cultivars (Table 2).

Table 1. Relative water content in roots of control and salt-stressed walnut seeds 28 d after the beginning of the experiment.[z]

| Cultivars | Salinity levels (mM NaCl) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 50 | 100 | 150 | 200 | 250 |
| Lara | 81.45 ± 0.08 | 77.35 ± 0.09 | 74.26 ± 0.05 | 72.89 ± 0.09 | 64.12 ± 0.07 | 61.15 ± 0.03 |
| Pedro | 82.89 ± 0.12 | 80.24 ± 0.08 | 78.36 ± 0.06 | 74.91 ± 0.04 | 72.37 ± 0.06 | 67.05 ± 0.08 |
| RDM | 83.60 ± 0.04 | 81.94 ± 0.07 | 79.56 ± 0.10 | 76.98 ± 0.03 | 74.77 ± 0.05 | 71.48 ± 0.09 |
| Hartley | 87.69 ± 0.03 | 86.59 ± 0.03 | 85.64 ± 0.07 | 83.14 ± 0.05 | 81.97 ± 0.08 | 80.45 ± 0.03 |
| Chandler | 91.57 ± 0.06 | 91.34 ± 0.06 | 89.45 ± 0.08 | 88.46 ± 0.06 | 87.72 ± 0.14 | 85.38 ± 0.04 |
| Vina | 84.43 ± 0.09 | 82.97 ± 0.03 | 80.53 ± 0.05 | 77.68 ± 0.07 | 74.19 ± 0.05 | 71.92 ± 0.06 |
| Serr | 89.13 ± 0.08 | 86.45 ± 0.07 | 84.23 ± 0.04 | 81.98 ± 0.09 | 76.46 ± 0.09 | 72.83 ± 0.16 |

[z]Each value is the mean ± SE of three measurements each with four seeds per salinity treatment.

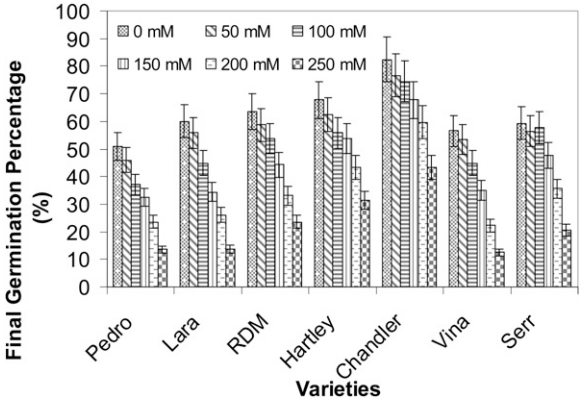


Fig. 1. Effect of different salinity level on the final germination percentage. Each bar represents the mean (±SE) of 10 trays. Seventy-two seeds were used for each trial.

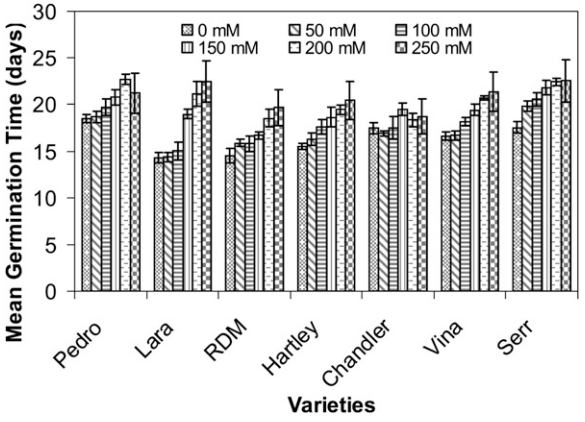


Fig. 2. Effect of different salinity levels on the mean germination time. Each bar represents the mean (±SE) of 10 trays. Seventy-two seeds were used for each tray.

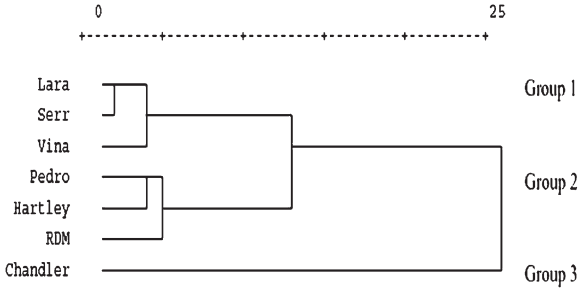


Fig. 3. Cluster analysis of different cultivars based on the all measured traits at 200 mM NaCl salinity level. Unweighted pair group method with averages (UPGMA) dendrogram of the cultivar was drawn using square Euclidean distance based on rescaled Jaccard similarities and the UPGMA clustering algorithm.

In salt-treated plants, the average sodium content was higher than in control plants (nearly twice) and ranged from 0.52 to 7.92 $mg \cdot g^{-1}$ DW (Table 2), and the Chandler cultivar had significantly less sodium content than the others. Sodium levels in roots were higher than in the shoots in almost all cultivars, especially in the tolerant and semitolerant groups (Table 2). In contrast, the increase in sodium content was more evident in shoots of sensitive and semisensitive groups.

Results of mineral composition analysis showed that the calcium and potassium accumulations were increased by the increase in salt stress levels, especially in shoots of semisensitive and tolerant cultivars (Tables 3 and 4; Fig. 4). Also, variations of magnesium accumulation in root and shoot samples were significant at all stress levels and were dependent on cultivar (data not shown).

*Regression analysis.* Weak correlations were observed between the seed characteristics (seeds size, seed weight, and kernel weight) and growth indices under salinity ($r^2 \leq 0.15$). Similarly, regression analysis failed to show a relationship between percent germination and seed weight ($r^2 = 0.0541$; $P < 0.7800$) and kernel weight ($r^2 = 0.0438$; $P < 0.2364$). As a result, analysis of covariance was not used as a statistical procedure for species-level and family-level analyses.

## Discussion

Generally, the effects of salinity levels on germination were similar for the seven cultivars studied. High salinity delayed and reduced germination in all cultivars. However, these cultivars differed in several characteristics. The lengths of dry seeds were 3.88 (± 0.78) cm for 'Serr', 3.65 (± 0.78) cm for 'Hartley', 3.37 (± 1.15) cm for 'Pedro', 3.06 (± 0.32) cm for 'Chandler', 2.97 (± 0.28) cm for 'Round de Montignac' ('RDM'), 2.36 (± 0.63) cm for 'Vina', and 2.07 (± 0.47) cm for 'Lara'. Seed size could be a factor affecting germination in stressed media (Sedia and Ehrenfeld, 2003; Zamfir, 2000). Many studies have shown that various seed sizes and weights may behave differently in terms of germination under saline conditions (Khan and Ungar, 1984a, 1984b; Philipupillai and Ungar, 1984; Schat, 1981; Ungar, 1977, 1995). It is generally believed that large seed sizes have a higher propensity for germination in saline media. However, Mohammed and Sen (1988), found a negative relationship between seed size and germination capacity in *Trianthema triquetra* L. Moreover, the smallest seeds were collected from the site with the greatest salinity. The hypothesis tested in this study showed that walnut seed size does not have any effect on germination capacity in saline media. Within the range of seed sizes studied, we did not observe any significant differences in the germination response. Also, regression analysis failed to show a relationship between percent germination and seed weight ($r^2 = 0.0541$; $P < 0.7800$) and kernel weight ($r^2 = 0.0438$; $P < 0.2364$).

Table 2. Concentrations of sodium (mg·g$^{-1}$ dry weight) in root and shoot of different salt-tolerant groups of the studied walnut cultivars.[z]

| Cultivars | Tissue | Salinity levels (mM NaCl) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 50 | 100 | 150 | 200 | 250 |
| Lara | Root | 1.64 ± 0.02 c[y] | 2.52 ± 0.04 a | 3.67 ± 0.08 a | 4.21 ± 0.08 c | 5.67 ± 0.05 c | 6.98 ± 0.02 c |
| | Shoot | 1.67 ± 0.03 c | 2.45 ± 0.04 b | 3.54 ± 0.06 b | 3.78 ± 0.06 e | 5.53 ± 0.04 d | 7.68 ± 0.05 b |
| Pedro | Root | 0.87 ± 0.03 i | 0.94 ± 0.07 h | 1.54 ± 0.04 g | 1.97 ± 0.05 h | 2.32 ± 0.04 h | 2.79 ± 0.06 i |
| | Shoot | 1.12 ± 0.07 g | 1.46 ± 0.06 g | 2.21 ± 0.03 f | 2.87 ± 0.05 g | 3.56 ± 0.02 g | 4.34 ± 0.04 g |
| RDM | Root | 0.86 ± 0.05 h | 0.97 ± 0.03 h | 1.32 ± 0.05 h | 1.78 ± 0.04 i | 2.24 ± 0.03 i | 2.67 ± 0.08 j |
| | Shoot | 1.24 ± 0.05 f | 1.67 ± 0.03 f | 2.18 ± 0.05 f | 3.07 ± 0.03 f | 3.95 ± 0.06 f | 4.21 ± 0.03 h |
| Hartley | Root | 0.89 ± 0.05 h | 0.95 ± 0.06 h | 1.35 ± 0.02 h | 1.76 ± 0.04 i | 2.08 ± 0.07 j | 2.86 ± 0.05 i |
| | Shoot | 1.37 ± 0.05 e | 1.87 ± 0.03 e | 2.17 ± 0.04 f | 3.07 ± 0.05 f | 3.95 ± 0.04 f | 4.68 ± 0.01 f |
| Chandler | Root | 0.34 ± 0.06 k | 0.52 ± 0.06 j | 1.17 ± 0.09 g | 1.42 ± 0.07 j | 1.45 ± 0.10 k | 1.64 ± 0.05 k |
| | Shoot | 0.57 ± 0.05 j | 0.63 ± 0.08 i | 0.59 ± 0.05 j | 0.64 ± 0.08 k | 0.76 ± 0.06 l | 0.89 ± 0.03 l |
| Vina | Root | 1.54 ± 0.03 d | 1.95 ± 0.04 d | 2.75 ± 0.06 d | 3.89 ± 0.04 d | 4.73 ± 0.02 e | 5.03 ± 0.08 e |
| | Shoot | 1.76 ± 0.04 b | 1.86 ± 0.07 e | 2.56 ± 0.03 e | 4.67 ± 0.04 a | 5.94 ± 0.08 b | 7.92 ± 0.05 a |
| Serr | Root | 1.64 ± 0.03 c | 2.32 ± 0.05 c | 3.32 ± 0.04 c | 3.80 ± 0.03 e | 4.78 ± 0.10 e | 5.78 ± 0.03 d |
| | Shoot | 1.82 ± 0.04 a | 2.46 ± 0.06 b | 3.57 ± 0.04 b | 4.59 ± 0.01 b | 6.89 ± 0.08 a | 7.85 ± 0.03 a |

[z]Each value is the mean ± SE of three measurements each with four seeds per salinity treatment.
[y]Means in each column followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

Table 3. Concentrations of calcium (mg·g$^{-1}$ dry weight) in root and shoot of different salt-tolerant groups of the studied walnut cultivars.[z]

| Cultivars | Tissue | Salinity levels (mM NaCl) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 50 | 100 | 150 | 200 | 250 |
| Lara | Root | 2.47 ± 0.02 c[y] | 2.49 ± 0.02 c | 1.96 ± 0.05 c | 2.25 ± 0.07 e | 2.37 ± 0.10 d | 214 ± 0.06 e |
| | Shoot | 1.58 ± 0.03 i | 1.44 ± 0.05 j | 1.37 ± 0.02 h | 1.64 ± 0.07 i | 1.59 ± 0.08 i | 1.45 ± 0.01 hi |
| Pedro | Root | 1.94 ± 0.05 d | 1.98 ± 0.02 d | 1.89 ± 0.05 e | 1.99 ± 0.07 g | 2.06 ± 0.04 f | 2.13 ± 0.01 e |
| | Shoot | 0.60 ± 0.05 k | 0.82 ± 0.02 l | 0.93 ± 0.04 j | 1.13 ± 0.05 k | 1.42 ± 0.07 j | 1.53 ± 0.04 g |
| RDM | Root | 1.84 ± 0.03 e | 1.89 ± 0.05 e | 1.94 ± 0.02 d | 2.07 ± 0.05 f | 2.14 ± 0.03 e | 2.21 ± 0.07 d |
| | Shoot | 0.73 ± 0.04 j | 0.83 ± 0.05 l | 0.95 ± 0.07 j | 1.08 ± 0.03 l | 1.24 ± 0.08 l | 1.68 ± 0.04 f |
| Hartley | Root | 1.72 ± 0.03 fg | 1.78 ± 0.09 h | 1.84 ± 0.05 f | 1.98 ± 0.07 g | 1.97 ± 0.09 g | 2.18 ± 0.04 de |
| | Shoot | 0.54 ± 0.05 m | 0.87 ± 0.05 k | 1.07 ± 0.05 i | 1.28 ± 0.04 j | 1.35 ± 0.06 k | 1.49 ± 0.05 h |
| Chandler | Root | 1.68 ± 0.06 g | 1.81 ± 0.06 g | 1.85 ± 0.09 f | 3.68 ± 0.07 b | 2.52 ± 0.10 c | 2.13 ± 0.05 e |
| | Shoot | 2.79 ± 0.05 b | 2.88 ± 0.08 a | 6.79 ± 0.05 a | 6.92 ± 0.08 a | 7.61 ± 0.06 a | 7.94 ± 0.03 a |
| Vina | Root | 2.98 ± 0.02 a | 2.86 ± 0.05 a | 2.65 ± 0.02 b | 2.67 ± 0.04 c | 2.74 ± 0.05 b | 3.46 ± 0.08 b |
| | Shoot | 1.65 ± 0.02 h | 1.57 ± 0.03 i | 1.87 ± 0.07 e | 1.69 ± 0.08 i | 1.82 ± 0.01 h | 1.23 ± 0.05 j |
| Serr | Root | 2.58 ± 0.04 c | 2.78 ± 0.01 b | 2.64 ± 0.08 b | 2.41 ± 0.06 d | 2.53 ± 0.04 c | 2.72 ± 0.06 c |
| | Shoot | 1.76 ± 0.03 f | 1.83 ± 0.07 f | 1.69 ± 0.08 g | 1.85 ± 0.06 h | 1.57 ± 0.02 i | 1.89 ± 0.02 ef |

[z]Each value is the mean ± SE of three measurements each with four seeds per salinity treatment.
[y]Means in each column followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

Table 4. Concentrations of potassium (mg·g$^{-1}$ dry weight) in root and shoot of different salt-tolerant groups of the studied walnut cultivars.[z]

| Cultivars | Tissue | Salinity levels (mM NaCl) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 50 | 100 | 150 | 200 | 250 |
| Lara | Root | 10.55 ± 0.04 g[y] | 10.67 ± 0.07 h | 10.34 ± 0.02 j | 10.64 ± 0.08 k | 10.73 ± 0.03 l | 10.23 ± 0.06 m |
| | Shoot | 11.24 ± 0.03 f | 11.14 ± 0.04 g | 11.29 ± 0.07 g | 11.73 ± 0.08 j | 11.56 ± 0.08 k | 11.12 ± 0.03 l |
| Pedro | Root | 15.79 ± 0.02 b | 16.74 ± 0.05 b | 17.54 ± 0.03 c | 19.01 ± 0.07 c | 21.03 ± 0.11 d | 22.67 ± 0.07 c |
| | Shoot | 8.74 ± 0.02 k | 9.64 ± 0.04 i | 10.79 ± 0.07 i | 13.67 ± 0.04 g | 14.93 ± 0.07 f | 16.34 ± 0.01 f |
| RDM | Root | 14.34 ± 0.04 c | 15.34 ± 0.05 d | 17.50 ± 0.06 c | 18.37 ± 0.04 d | 21.38 ± 0.03 c | 23.26 ± 0.06 b |
| | Shoot | 9.12 ± 0.03 i | 9.34 ± 0.02 k | 9.47 ± 0.06 l | 11.78 ± 0.07 j | 14.54 ± 0.04 g | 16.73 ± 0.04 e |
| Hartley | Root | 16.74 ± 0.03 a | 16.02 ± 0.04 c | 17.62 ± 0.03 b | 20.76 ± 0.05 b | 21.78 ± 0.09 b | 23.35 ± 0.06 b |
| | Shoot | 8.87 ± 0.03 j | 9.90 ± 0.02 i | 10.96 ± 0.05 h | 11.76 ± 0.04 j | 13.94 ± 0.08 h | 15.23 ± 0.02 g |
| Chandler | Root | 12.68 ± 0.03 e | 13.12 ± 0.02 e | 15.98 ± 0.04 d | 17.55 ± 0.05 e | 18.72 ± 0.08 e | 21.94 ± 0.12 d |
| | Shoot | 16.06 ± 0.04 a | 16.97 ± 0.05 a | 19.27 ± 0.06 a | 21.55 ± 0.07 a | 23.85 ± 0.07 a | 23.81 ± 0.09 a |
| Vina | Root | 9.94 ± 0.02 h | 9.47 ± 0.05 j | 9.99 ± 0.06 k | 10.46 ± 0.05 l | 11.82 ± 0.08 j | 11.56 ± 0.02 k |
| | Shoot | 11.25 ± 0.03 f | 11.05 ± 0.05 g | 11.64 ± 0.01 f | 12.05 ± 0.02 i | 11.50 ± 0.05 k | 11.65 ± 0.02 j |
| Serr | Root | 13.12 ± 0.03 d | 13.54 ± 0.05 e | 13.05 ± 0.07 e | 13.87 ± 0.06 f | 13.43 ± 0.08 i | 12.74 ± 0.12 h |
| | Shoot | 12.43 ± 0.04 e | 12.48 ± 0.03 f | 12.91 ± 0.07 e | 12.83 ± 0.07 h | 11.57 ± 0.04 k | 12.26 ± 0.13 i |

[z]Each value is the mean ± SE of three measurements each with four seeds per salinity treatment.
[y]Means in each column followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

We observed differential responses in the uptake of sodium and in the pattern of germination in walnut cultivars, which could account for the differences in response to salinity. The ability to maintain low sodium concentration in leaves and in growing shoots is crucial for plant growth in saline media. When sodium accumulates in the cytoplasm of shoot or leaf cells, it can lead to tissue necrosis and leaf abscission; thus, the photosynthetic apparatus is impaired and plant growth is hindered. The sodium content in salt-stressed shoots was generally higher than in shoots of control plants. The accumulation of sodium in shoots was significantly different in the three salt tolerance classes, but they presented distinct responses to the increasing concentration of NaCl. Similarly, Sixto et al. (2005) observed differences in leaf sodium content among *P. alba* cultivars from different Spanish origins subjected to salt stress. Possibly, cultivar Chandler, which accumulated significantly less sodium in shoots, has mechanisms for sodium exclusion at the root level, which reduces sodium uptake and its translocation to the shoot tissues. Mechanisms for sodium exclusion in roots are well studied in *P. euphratica* (Chang et al., 2006; Chen et al., 2001, 2002, 2003), which is the most salt-tolerant poplar species. In *P. alba*, the ability to maintain lower sodium content in leaves has also been associated with less severe symptoms of salinity stress (Sixto

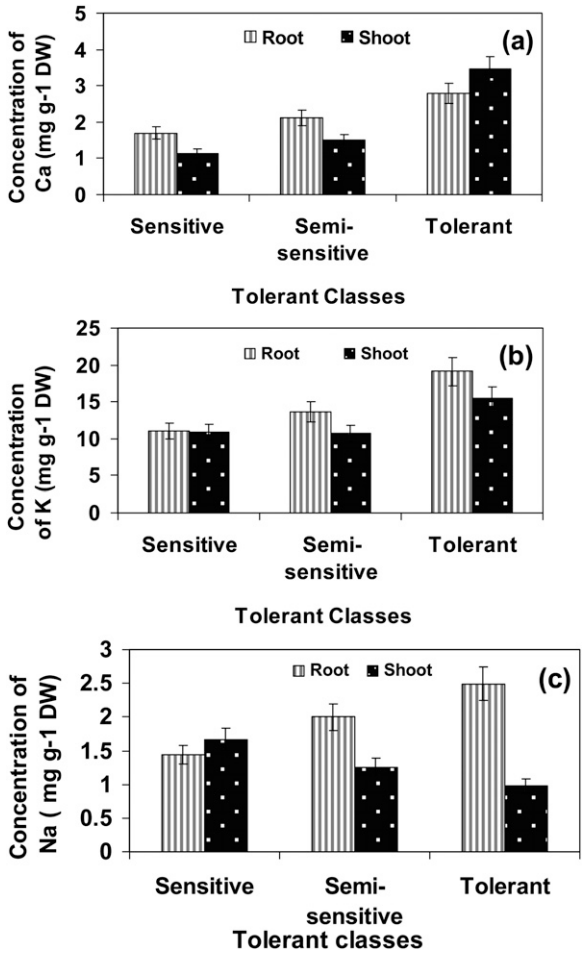


Fig. 4. Comparing amount of calcium (Ca) (**A**), potassium (K) (**B**), and sodium (Na) (**C**) (mg·g$^{-1}$ dry weight) in shoot and root of different tolerant classes of walnut. Each value is the mean±SE of three measurements each with four seeds per salinity treatment.

et al., 2005). Our results confirm a negative relationship between sodium accumulation in the shoots and its effects on shoot growth in 'Chandler'. Probably in 'Lara', the negative effect of long-term salt stress on shoot growth is more related to sodium toxicity than to its osmotic effect. The excess sodium could be both actively accumulated in the vacuole or be excreted into apoplast. Sodium compartmentation in the vacuole is an adaptation mechanism typical of halophytes (Blumwald, 2000; Greenway and Munns, 1980). Ottow et al. (2005) observed that *P. euphratica* could tolerate increasing sodium concentration by apoplastic accumulation of salt in the leaves' cell wall regions but not in the vacuole. A similar mechanism for apoplastic localization of sodium could operate in *P. alba* and accounts for the different behavior observed among the cultivars studied. These hypotheses need to be tested by further studies to determine the exact site of sodium localization at histological, cellular, and subcellular levels.

The results of the present study suggest that different walnut cultivars have different tolerance to salinity stress. This study demonstrated variability among walnut cultivars regarding their germination capacities in saline habitats, which implies that walnut trees may be able to regenerate as moderately salt-tolerant plants (Lotfi Tappeh et al., 2008a, 2008b). Salinity treatments caused a net $K^+$ uptake, which is likely to be the result of osmotic adjustment in tolerant cultivars. Net $Na^+$ uptake by sensitive cultivars was noticeably higher than tolerant cultivars. Interestingly, in control plants, the sodium content in shoots of cultivars that belong to the sensitive groups was significantly higher than in the shoots of the other cultivars. This suggests a constitutive ability of these cultivars to accumulate more sodium in the leaves. This feature could contribute to osmotic adjustment in response to salinity as has also been observed in *P. euphratica* plants exposed to salt stress, in which the osmotic adjustment was mainly resulted from sodium accumulation (Ottow et al., 2005).

In the tolerant and semitolerant groups, roots had higher potassium contents than shoots. This could reflect differences in the membrane transport properties of cells in different stress-tolerant groups (De Boer et al., 2004). The amount of calcium accumulation was increased by increase in salinity stress levels, especially in shoots of tolerant and semisensitive cultivars. Calcium is an essential plant nutrient that is required for its structural roles like in membrane integrity, as a counterion for inorganic and organic anions in the vacuole, as an intracellular messenger in the cytosol, and as an enzyme activator (Carvajal and Cabañero, 2004). In conclusion, different strategies for adaptation to salinity have been observed in walnut cultivars with different climatic origins when grown in a greenhouse trial. Thus, a different genetic basis underlies the different behaviors observed under salt stress. The degree of variation in salinity tolerance in these cultivars could be linked to their different abilities in sodium exclusion at the root level or to different regulation of ion transport across shoot cell membranes. Our results suggest that cultivar Chandler could also be a suitable model to be used for the study of physiology and genetics of salt tolerance in walnut.

In recent years, ionomics have been applied to bulk tissue samples. Such analysis only provides a very limited view of the tissue and cellular and subcellular complexities of ion homeostasis mechanisms (Pickering et al., 2000, 2003). To have the ability to profile the elemental content of different plant tissues such as meristemic and vascular tissues, one needs to know the natural elemental needs of different walnut tissues arising from their genetic ability for maximum absorption of essential elements involved in stress signaling and resistance to salinity.

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