[1]Hungarian Department of Biology and Ecology, Babes-Bolyai University, Cluj-Napoca, Romania
[2]Plant Nutrition Department, CEBAS-CSIC, Espinardo, Murcia, Spain
[3]Molecular Biology Centre, Institute for Interdisciplinary Experimental Research, Babes-Bolyai University, Cluj-Napoca, Romania

Sodium accumulation contributes to salt stress tolerance in lettuce cultivars

Csaba Bartha[1*], Laszlo Fodorpataki[1], Maria del Carmen Martinez-Ballesta[2], Octavian Popescu[3], Micaela Carvajal[2]

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Summary

Increasing soil salinity of irrigated agricultural areas represents a major environmental stress factor that impairs the production of many salt-sensitive crop plants. Different lettuce cultivars were studied for identification of more tolerant ones, based on physiological properties. Five selected lettuce cultivars were grown hydroponically, salt stress was induced by 50 mM and 100 mM of NaCl. The cultivars exhibited differential reduction in shoot fresh weight. The highest sodium and free proline accumulation in the shoot of the most tolerant cultivar, associated with a moderate decrease of the root hydraulic conductance and of the leaf stomatal conductance, can be related to a better defense mechanism against osmotic stress. Salt exposure increased the potassium and calcium ion content of the xylem sap, which may be important for an efficient osmotic adjustment needed to support leaf expansion. The fact that the highest amount of Na^+ was found in the shoot of the most tolerant cultivar, and the lowest in the most sensitive one, reflects that in lettuce Na^+ exclusion is not a main strategy for salt tolerance. Lettuce is a good example for the case in which salinity tolerance is related not to exclusion, but to inclusion of sodium ions in the shoot system. For salt tolerant varieties the marketable yield has a higher dry biomass percentage, leaves of plants grown under high salinity are crispier, darker green and have a slight salty taste.

Introduction

High salinity is a major stress factor that restricts crop productivity. Worldwide more than 800 million hectares of land are salt-affected, most of these are located in arid and semiarid regions (FAO, 2008). Because the irrigated area has at least twice the productivity of rainfed land, more than one third of all crop production is coming from these salt affected regions (MUNNS and TESTER, 2008). Among other crop plants, the majority of the lettuce world production is increasingly being affected by salinity (FAO, 2009).

High concentrations of salt increase the osmotic potential, making it harder for roots to extract water (osmotic effect), and result in toxicity symptoms (ionic effect), leading to metabolic imbalance and premature senescence of leaves (MUNNS, 2002; TESTER and DAVENPORT, 2003). The loss of water and the invading ions activate a concerted acclimation process that may lead to salt tolerance associated with a new steady state of growth. This acclimation includes three basic processes: restoration of turgor, regulation of ion transport across membranes, and induction of the accumulation of osmoprotectants and stress proteins. Besides these processes, several secondary responses are needed to ensure salt tolerance, e. g. the scavenging of overproduced reactive oxygen species, an increase in energy-supplying reactions, and the adjustment of the whole metabolism to the new situation. This is why multiple molecular and physiological changes may be observed in plants exposed to salt stress: drop of stomatal and root hydraulic conductance, osmotic adjustment, reduced growth rate, changes of the root to shoot ratio, nutritional disorders (MUNNS and TESTER, 2008; RAJENDRAN et al., 2009).

A rapid whole-plant response to salt stress is a decrease in stomatal conductance, caused by the osmotic effect of high NaCl concentration. The effect is very fast, but a new steady-state rate of transpiration becomes stabilized after hours, depending on the species and cultivars. In the first phase of stress reactions, the osmotic effect of the salt stress is responsible for the reduction of shoot growth (CRAMER, 2002), usually showing a good correlation between stomatal conductance and growth rate (JAMES et al., 2008).

To maintain turgor, plant cells have to increase the osmotic potential. Energetically, the most effective way to do this in salt-rich environments is accumulating Na^+ and Cl^- ions, but the chemical toxicity will appeared (MUNNS and TESTER, 2008). Another strategy is to synthesize osmotically active organic compounds (compatible solutes) which accumulate as a result of the reprogrammed metabolism (SOUDRY et al., 2005). One of the most widespread osmoprotectants is proline, which may play multiple roles in stress tolerance. Besides keeping the osmotic balance stable, it may protect enzymes and membrane lipids from degradation (ASHRAF and FOOLAD, 2007), it may be a radical scavenger in the process of antioxidative defense (HONG et al., 2000), and it can constitute an energy source for after-stress recovery mechanisms (HARE et al., 1999). Therefore, in many plants, proline accumulation can be considered an indicator of stress tolerance (HAJLAOUI et al., 2010; ZHU et al., 2008). However, the achievement of the proper balance to maintain turgor, depends on the species, on cultivars or ecotypes, and on the environmental conditions (GREENWAY and MUNNS, 1980).

Although the ability to exclude Na^+ or Cl^- from the shoot is often a primary determinant of variability in salinity tolerance within a species, there is not necessarily an inverse relationship between shoot Na^+ or Cl^- concentration and salinity tolerance. There is rather a difference in the ability of ion homeostasis maintenance in the root, in the xylem sap and in the leaves (MUNNS and TESTER, 2008; RAJENDRAN et al., 2009; SHABALA et al., 2010).

Lettuce (*Lactuca sativa* L.) has many cultivars, which are grown on extended areas and have become a much appreciated healthy food source, rich in mineral nutrients and vitamins. As most commercially grown cultivars are growing under non-saline conditions, very limited information is available about salt stress response of the different lettuce cultivars. Also, the physiological and biochemical parameters that may be useful for the screening of salt tolerant varieties are not well stated, and influence of salinity on marketable yield quality of lettuce is poorly documented.

The aim of this study is to compare salt stress tolerance of five, largely commercialized lettuce cultivars, and to identify more tolerant cultivars that may be suited for large-scale cultivation in areas with increasing soil salinity.

Materials and methods

Plant material and growth conditions

Five different cultivars of lettuce (*Lactuca sativa* L.) were used in the experiments. These were selected from among sixteen cultivars examined for salt stress sensitivity, largely cultivated in Europe.

* Corresponding author

Seeds of all cultivars were provided by B&T World Seeds (Paguignan, France). The cultivars were chosen from all of the four main lettuce convarieties (butterhead, roman, looseleaf and steam lettuce). The Valdor and Parella Green cultivars belong to the butterhead type (*Lactuca sativa var. capitata*), Paris Island is part of the roman (Cos) type (*Lactuca sativa var. romana*), Salad Bowl Red is included in the looseleaf type (*Lactuca sativa var. crispa*), and Asparagina is a cultivar belonging to the steam lettuce type (*Lactuca sativa var. asparagina*).

The lettuce seeds were pre-hydrated with distilled water and continuously aerated for 24 h. After this, the seeds were germinated in vermiculite substrate at 20 °C and kept in dark for 3 days. On the 4th day germinated seeds were transferred under controlled conditions using a daily photoperiod of 16 h light and 8 h darkness. The relative humidity was set to 75 % during daytime and to 60 % for the night. The temperature was 23 °C during daytime and 20 °C during the dark period. A photosynthetically active radiation of 400 μmol m^{-2} s^{-1} was provided by a combination of fluorescent tubes (Philips TLD 36 W/83 and Sylvania F36 W/GRO) and metal halide lamps (Osram HQI, T 400 W). On the 7th day after germination the seedlings were placed in 15 L containers with a continuous circulation of full-strength Hoagland's nutrient solution (HOAGLAND et al., 1950). The solution was replaced every four days. Salinization treatment was initiated on 21 d old seedlings, after two weeks of growth in Hoagland's solution. Salt stress was induced by 50 mM and 100 mM of NaCl (p. a.). The 100 mM of NaCl was added in two steps, in two consecutive days. Plantlets were harvested after ten days of exposure (31 days old). Fresh weight (FW) of roots and shoots (stem with leaves) was recorded separately, for five plants from each cultivar and salinity combination. For DW determination, the plant material was kept at 65 °C for 5 days. The youngest fully-expanded leaves were also collected from five plants per cultivar and salinity treatment, and kept frozen at -80 °C for proline content measurements.

Determination of mineral elements

The concentrations of Na^+, Ca^{2+} and K^+ were determined in plant material (roots and shoots) ground finely in a mill grinder, after drying at 65 ºC for 5 days. The samples were digested in a microwave oven (CEM Mars Xpress, North Carolina, USA), reaching 200 ºC in 20 min, and held at this temperature for 2 h. Digestion of 0.1 g DW plant material was performed with 5 mL 65 % HNO_3, 17 mL H_2O dist. and 3 mL 30 % (v/v) H_2O_2. For determining the sodium, potassium and calcium ion content of the xylem sap, 0.1 ml of xylem sap was diluted with distilled water to a final volume of 10 mL. The concentrations of Na^+, Ca^{2+} and K^+ were determined by inductively coupled plasma spectrometry (Iris Intrepid II, Thermo Electron Corporation, Franklin, USA).

Leaf gas exchange parameters

Stomatal conductance (Gs) was measured using a portable photosynthesis system (model LCA-4, ADC Bioscientific Ltd., Hoddesdon, UK). The abaxial stomatal conductance (on the lower epidermis) was measured on the youngest fully-expanded leaves, in the middle of the daily photoperiod, under constant photon flux density, air humidity and temperature (AHMED et al., 2006).

Root hydraulic conductance (L_0)

The root hydraulic conductance of lettuce plants exposed to salt stress treatments was measured by natural exudation of detached roots. The measurements were made in the middle of the photoperiod, 10 days after the beginning of salt treatment. The aerial parts of the plants were removed and the stems were put in silicon tubes.

The roots were kept in the same nutrient solution which was used for their growth. The sap that accumulated during a certain time, according to the treatment, was collected in Eppendorf tubes. The roots and the tubes were weighed with a precision balance. Sap flow (J_v) was expressed in mg g^{-1} (root fresh weight) h^{-1}. The osmotic potentials of the sap samples and of the nutrient solutions were measured using an osmometer (Digital osmometer, Roebling, Berlin, Germany). The osmotic potential difference between the xylem sap and the external solution, $\Delta\Psi\pi$, was calculated from their osmolarity values. The hydraulic conductance, L_0, expressed in mg (g root FW)$^{-1}$ h^{-1} MPa^{-1}, was calculated according to the relation $L_0 = J_v / \Delta\Psi\pi$, where J_v = mg xylem sap h^{-1} g^{-1} root, and $\Delta\Psi\pi = \Psi_0$ xylem sap – Ψ_0 solution (CABANERO and CARVAJAL, 2007).

Free proline content

Free proline content in the plant material was determined by generation of a colored product with ninhidrine (BATES, 1973). 0.5 g of fresh plant material was homogenized in a pre-chilled mortar with 3 ml of 3 % sulfosalicilic acid cooled on ice. The extract was centrifuged for 10 minutes at 20 000 g, then 600 μl of 96 % acetic acid and 600 μl ninhidrine solution (containing 2.5 % w/v ninhidrine, 60 % v/v 96 % acetic acid and 40 % v/v of 6 M ortophosphoric acid) was added to 600 μl of supernatant. The samples were incubated in test tubes for 1 hour at 100 °C, and after cooling, 3 ml of toluene was added to extract the reaction product. The mixtures were stirred, and when two layers were separated, 2 ml of the upper layer was transferred in a cuvette. The concentration of the red product was determined on the base of its absorbance at 520 nm using toluene as reference. L-proline (Sigma) was used for the preparation of standard curve.

Statistical analysis

The data were analyzed statistically, using the SPSS 18.0 software package, by analysis of variance (ANOVA) and by Tukey's test. Significant differences were determined at $P < 0.05$.

Results

Upon exposure to salt stress, physiological and biochemical changes, related to disturbance in growth, water relations and mineral nutrition, occurred in the different lettuce cultivars. It is worth mentioning that under the given growth conditions, 30 days old lettuce plants may be considered well developed, having at least 12 fully extended leaves and being suitable for consumption.

The high amount of NaCl in the nutrient solution reduced significantly the growth rate of shoot fresh weight in all five lettuce cultivars (Fig. 1, A). The most pronounced reduction, related to the control, was observed in the Asparagina cultivar, at both salt concentrations (31 % decrease in the presence of 50 mM NaCl and 51 % reduction with 100 mM NaCl). The mildest shoot growth inhibition was registered for the Paris Island cultivar (24 % reduction at 50 mM NaCl and 41 % reduction at 100 mM NaCl). Root growth was not inhibited, but it was stimulated by salt stress, except for the Asparagina cultivar. In the case of Salad Bowl Red and Parella Green cultivars, exposure to 50 mM sodium chloride resulted in a statistically significant increase of root fresh weight during the treatment (Fig. 1, B).

Regarding fresh biomass, dry matter and water content of leaves, our results showed that high salinity reduces water content, and in a smaller extent it decreases fresh shoot biomass. As a consequence, the dry weight percentage of lettuce leaves increases under elevated salinity (Fig. 2). In absolute values, leaf dry biomass is higher in plants exposed to 50 mM NaCl than in plants grown in the presence of 100 mM sodium chloride.
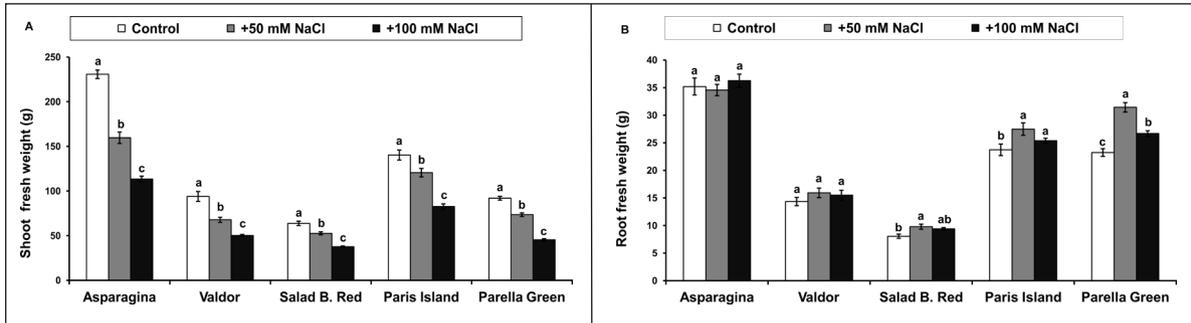
Fig. 1: Effect of 50 mM and 100 mM NaCl on the shoot and root fresh weight (FW) of five lettuce cultivars. Data are means of five plants ± SE. Different letters indicate significant differences among treatments of the same cultivar, at P < 0.05, according to the Tukey test.
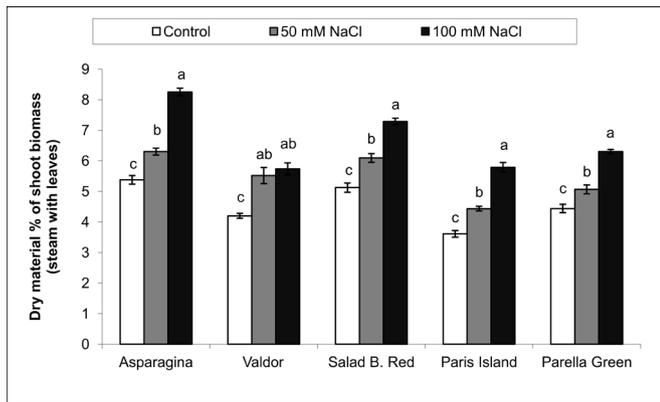

Fig. 2: Percentage of dry matter in the biomass of leaves of five lettuce cultivars developed under different salinity levels in the nutrient medium. Vertical bars represent ± SE from means (n = 5). Different letters indicate significant differences among treatments of the same cultivar, at P < 0.05.

The values of root hydraulic conductance (L_0) are shown in Fig. 3. There was a significant difference between the hydraulic conductance of the cultivars without salinity treatments. Salt stress caused a significant L_0 decrease in all cultivars. The most pronounced reduction was shown in the Asparagina cultivar, for both NaCl concentrations. In the case of Paris Island cultivar, both salt concentrations showed the same degree of reduction. In the case of Parella Green it was not possible to collect xylem sap from plants exposed to 100 mM NaCl.
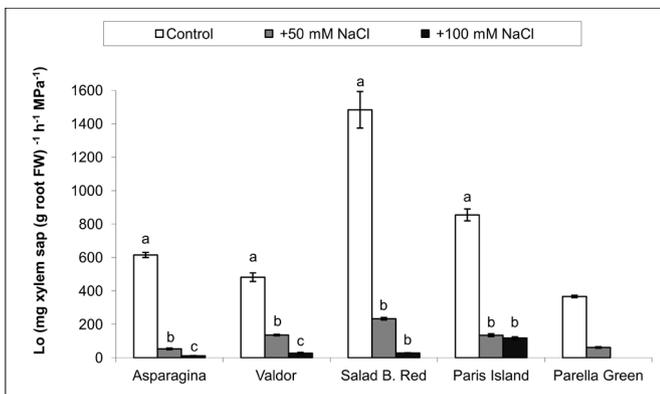
Fig. 3: Root hydraulic conductance (L_0) of five lettuce cultivars exposed to salt stress. FW – fresh weight. Each value is the mean of five samples ± SE. Different letters indicate significant differences among treatments of the same cultivar, at P < 0.05, according to the Tukey test.

The stomatal conductance (Gs) of leaves decreased in all cultivars when salinity treatments were applied. The most significant difference from control was observed, with both salt treatments, in the case of Aparagina and Salad Bowl Red cultivars. In the Paris Island cultivar stomatal conductance decreased significantly only upon exposure to 100 mM NaCl. From all cultivars, at the 50 mM salt regime, the smallest reduction was observed in Paris Island (Fig. 4).
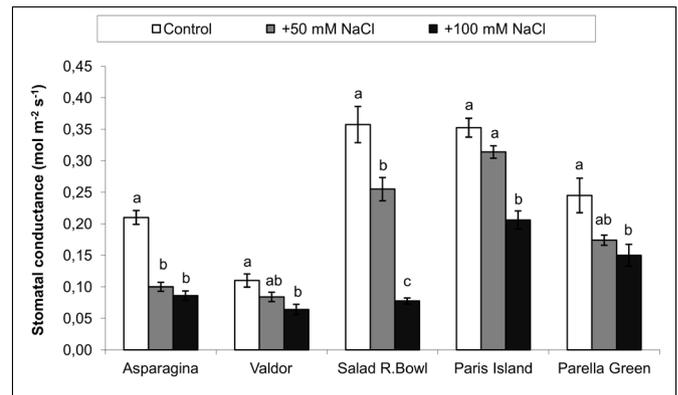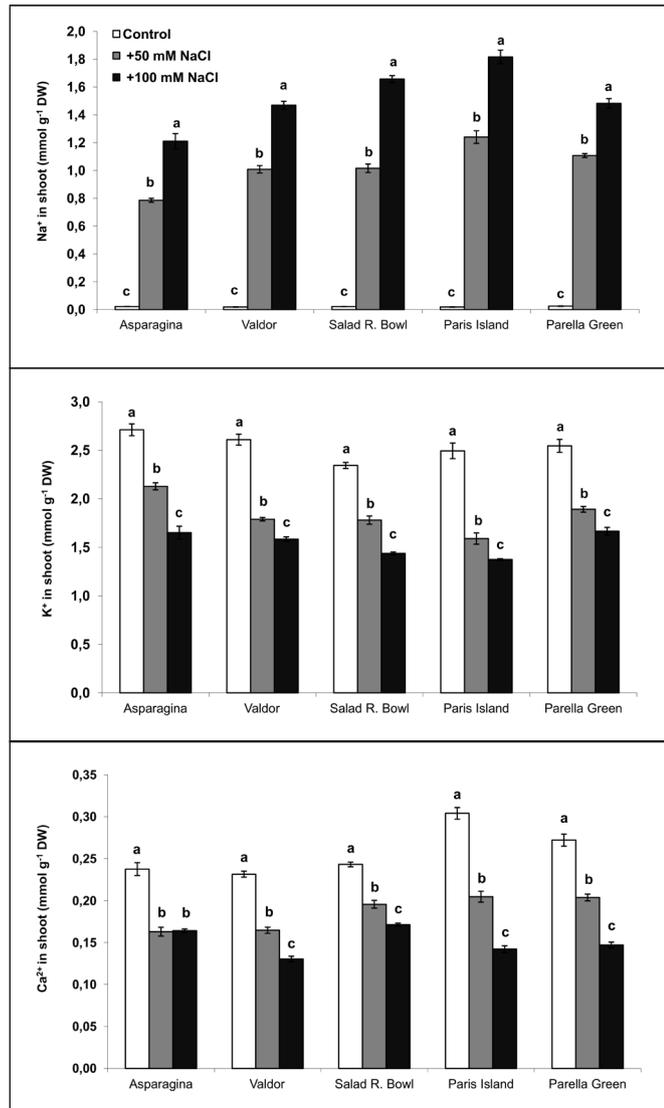

Fig. 4: Stomatal conductance (G_s) in leaves of five lettuce cultivars exposed to different degrees of saltstress. Bars represent means ± SE, n = 5. Different letters indicate significant differences among treatments of the same cultivar, at P < 0.05, according to the Tukey test.

Fig. 5 summarizes the Na^+, Ca^{2+} and K^+ content of the shoot of five lettuce cultivars. At both salinity levels, the highest Na^+ accumulation was found in Paris Island, while the lowest was measured in Asparagina. The roots exhibited an opposite trend of sodium content as compared to shoots, the highest accumulation being achieved by Asparagina (data not shown). Asparagina, and in a smaller extent Salad Bowl Red, accumulated higher amounts of Na^+ in the root than in the shoot, while in the case of Paris Island, Parella Green and Valdor the concentration of sodium ions was higher in shoots than in roots, at both salinity levels (data not shown). Calcium ion content was reduced in all cultivars as a result of salt stress. Salt stress also decreased K^+ concentration of all cultivars. In Paris Island, Parella Green and Valdor there was no obvious difference in the K^+ accumulation upon exposure to 50 mM and to 100 mM NaCl.

Free proline level of the leaves was increased by both salt regimes in all five cultivars. A significant difference between proline concentrations of different cultivars was observed only upon exposure to 100 mM of NaCl (Fig. 6). The highest proline content was determined in the Paris Island cultivar exposed to 100 mM NaCl, where free proline concentration was about twenty times higher than in control plants. In the other cultivars treated with 100 mM NaCl, this
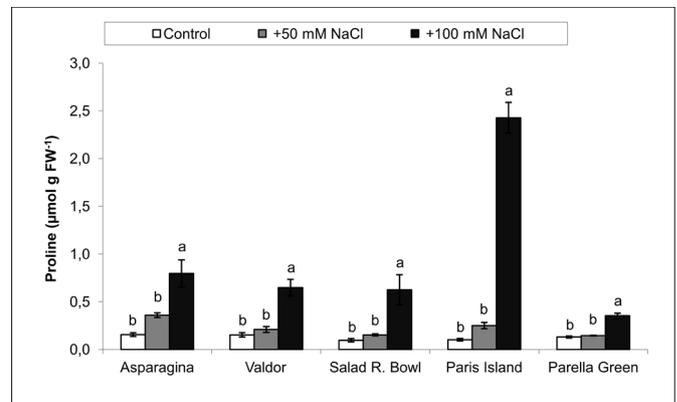
increase was smaller: threefold for **Parella Green, fourfold for As**paragina and Valdor, and sevenfold for the leaves of the Salad Bowl Red cultivar.

Fig. 5: Na^+, Ca^{2+} and K^+ content of shoot in five lettuce cultivars exposed to 50 mM and 100 mM NaCl. Bars represent means ± SE, n = 5. Different letters indicate significant differences among treatments of the same cultivar, at P < 0.05, according to the Tukey test.

Fig. 6: Proline content of five lettuce cultivars exposed to salt stress. FW – fresh weight. Bars represent means ± SE, n = 5. Different letters indicate significant differences among treatments of the same cultivar, at P < 0.05, according to the Tukey test.

Discussion

Differences among cultivars in growth inhibition caused by salt stress were reported for several crop plants, e.g. for *Triticum monococcum* (RAJENDRAN et al., 2009), for maize (HAJLAOUI et al., 2010), for rice (QUINET et al., 2010). Relatively few studies have been undertaken to investigate the effects of NaCl on different lettuce cultivars. PASTERNAK et al. (1986) found that roman lettuce types are generally more salt tolerant than iceberg types, however, MAHMOUDI et al. (2010; 2011; 2013) reported that for four lettuce varieties (including Butterhead, Romain and Verte) roman type was the most sensitive to salinity. Another study compared several cultivars of lettuce, and demonstrated different salt sensitivity during the germination stage (COONS et al., 1990; NASRI et al., 2010). In our experiments, a significant reduction in shoot fresh weight of all five lettuce cultivars exposed to salt stress was found. This reflects that there is

no inherited salt stress resistance that could avoid growth inhibition caused by high salinity, regardless of the different cultivars. The cultivar Aspargina suffered the largest shoot growth reduction at both salinity levels. This indicates that from among the five cultivars that were investigated, this is the most sensitive to salt stress in terms of biomass production. By contrast, as Paris Island cultivar did not significantly altered the growth after 50 mM NaCl addition, may be considered more tolerant (Fig. 1).

Root growth was less affected by salinity than leaf growth, and root elongation rate recovered remarkably well after several days of exposure to NaCl. This can be related to the osmotic effect of salt stress, the impaired water supply stimulating root growth to increase water uptake surface (MUNNS, 2002). Even though the absolute chlorophyll content does not increase upon salt stress (data not shown), the lower water content of the fresh biomass confers a darker green colour to lettuce leaves. For the cv. Vera of lettuce, grown hydroponically for 32 days, ANDRIOLO et al. (2005) reported that leaf number was not affected by salinity treatments, why dry biomass of leaves increased under mild salinity (up to 2.81 dS m^{-1} electrical conductivity, approximately equivalent to 28 mM NaCl), and decreased above this salinity level. This reflects that cv. Vera is most probably a salt-sensitive lettuce cultivar. In an attempt to use diluted seawater for irrigation of lettuce, TURHAN et al. (2014) found that dry biomass, total fresh yield and marketable fresh yield of cv. Funly of lettuce, after 40 days of irrigation with 2.5 % and 5 % seawater were similar to control, but decreased in response to 10 % and 20 % seawater. They concluded that low amounts of salt (2.5 % - 5 % seawater) are necessary in irrigation water to reach the optimal yield of the studied lettuce cultivar. Our results showed that salt tolerant lettuce varieties may produce a slightly increased dry biomass percentage even under considerably higher salinity values. In another set of experiments, the Verte de cobhain variety of lettuce was found to be salt tolerant, and upon treatment for 12 days with NaCl concentrations increasing progressively up to 100 mM, displayed better growth and superior antioxidative capacity than the control grown hydroponically in Hoagland's nutrient solution (MAHMOUDI et al., 2010). These results, in agreement with our findings, underline the broad range of salt tolerance of different lettuce cultivars, and the fact that some degree of salinity may increase the marketable yield of this vegetable.

When plants are subjected to salt stress, root hydraulic conductance (L_0) is usually reduced (CABANERO and CARVAJAL, 2007; MURIES et al., 2011). The main cause of this reduction could be the decrease in the activity or concentration of aquaporins in the root plasma

membrane (CARVAJAL et al., 2000), this effect being mainly due to the specific toxicity of Na^+ and Cl^- ions (MARTINEZ-BALLESTA et al., 2000). Secondly, reduced hydraulic conductance may be related to the hyperosmotic stress and ionic imbalance caused by the high apoplastic concentrations of Na^+ and Cl^- (MUNNS and PASSIOURA, 1984). Due to the morphology of the lettuce (rosette type plant) it is difficult to use the Scholander chamber for measuring hydraulic conductance without causing injuries to the very short stem (data not shown). The Scholander pressure chamber forces xylem sap out from decapitated plants showing higher L_0 than natural exudation method, since the water movement is forced to occur through the apoplast to a greater extent (FERNANDEZ-GARCÍA et al., 2002). However, the differences between salinity treatments were maintained (LOPEZ-PEREZ et al., 2007).

All five lettuce cultivars exposed to salt stress exhibited a certain reduction of root hydraulic conductance, but the magnitude of this decrease depends on the cultivar (Fig. 3). The reduction of the xylem transport from root to shoot can be beneficial for the plant, because it prevents the accumulation of toxic levels of Na^+ and Cl^- ions in the leaves, but causing decrease of water transport. This means that for finding a proper balance, the lettuce plants can exhibit a reduced hydraulic conductivity upon salt stress ensuring a better protection to the leaves against ion toxicity, being more salt tolerant. On the other hand, certain amount of shoot Na^+ content may be beneficial by helping the plant to maintain turgor. Again, a balance is needed to be established between the use of Na^+ and Cl^- by the plant to maintain turgor and the need to avoid their toxicity (MUNNS and TESTER, 2008). Taking all this parameter into account, Paris Island cultivar maintained a higher hydraulic conductance, which confers a more pronounced salt tolerance by enabling a better water supply of the leaves from the root.

As a reaction to the osmotic component of salt stress, stomata tend to close in order to reduce water loss by transpiration (MUNNS and TESTER, 2008). This impairs photosynthetic carbon uptake, but reduces moisture accumulation during storage of lettuce heads. Salt stress tends to reduce stomatal conductance (G_s) in a short period after exposure. The fact that stomatal conductance was less affected by salinity in the Paris Island cultivar (Fig. 4) may be related to the other parameters (particularly with shoot FW and root hydraulic conductance) in order to show its higher salt tolerance in comparison with the other cultivars. A higher conductance enables a better carbon dioxide supply for a sustained photosynthetic assimilation, resulting in a smaller reduction of biomass production. A direct correlation between stomatal conductance and salt stress tolerance was also observed in maize cultivars (AZEVEDO-NETO et al., 2004). In our experiments, the most pronounced reduction in stomatal conductance upon salt stress was recorded in the Salad Red Bowl lettuce cultivar, suggesting the sensitivity of the gas exchange regulation (MUNNS and TESTER, 2008).

All lettuce cultivars growth in saline conditions showed an increase in Na^+ concentration. The highest amount of Na^+ was found in the cultivar Paris Island, and the lowest Na^+ concentration was determined in Asparagina. Therefore, two main strategies of salt stress tolerance can be considered, i.e. salt exclusion and salt sequestration, the latter one is used by lettuce cultivars. This is why the marketable biomass gets a slight salty taste. In a recent study on different cultivars of barley, SHABALA et al. (2010) conclude that after one week of salt treatment (320 mM NaCl), shoot Na^+ content of the tolerant variety was about 20 % higher than in the sensitive genotype. In the first phase of the salt stress the rapidly accumulating Na+ is an osmolite with low energy cost in the leaf vacuoles for the adjustment of cell turgor, and ultimately of tissue growth under the hyperosmotic stress condition imposed by salinity (MUNNS and TESTER, 2008; SHABALA et al., 2010). Salt stress disturbs the uptake of essential mineral nutrients such as K^+ and Ca^{2+}, as Na^+ com-

petitively inhibits K^+ and Ca^{2+} transport through membranes (ZHAO et al., 2007). Fig. 5 shows the reduction of potassium content in shoots as a result of salt stress, and this reduction is most probably due to the competition of Na^+ for the same cation transporters (AZEVEDO-NETO and TABOSA, 2000). We have found no correlation between K^+ content and the salt tolerance of the examined lettuce cultivars. Similar results were published by NEOCLEOUS et al. (2014) for lettuce and AZEVEDO-NETO et al. (2004) for maize genotypes. Regulation of potassium to sodium ion ratio in plant cells subjected to salt stress needs to be elucidated by further investigations. Na^+ also reduces the influx of Ca^{2+} ions through the plasma membrane, and increases efflux of Ca^{2+} from plant cells (CRAMER et al., 1989) This is valid for the investigated lettuce cultivars, although the dynamics of cytosolic calcium content decrement varies among the cultivars. Under the influence of 100 mM NaCl Ca^{2+} content dropped to the approximately same value in all cultivars, except for Valdor. No correlation could be established between calcium ion content and salt stress tolerance of the different lettuce cultivars. For *Lactuca sativa* var. *Crispa*, identified as moderately sensitive to salinity, it was found that dry matter ratio increased with increasing salinity in the range of 0.75-7.0 dS m^{-1} (approx. 7.5-70 mM NaCl), calcium accumulation in leaves decreased, while the amount of potassium was unaffected (ÜNLÜKARA et al., 2008). They did not distinguish between the calcium and potassium content of the xylem sap in the veins and of the parenchyma tissue in the leaf blade. They also established that the taste of lettuce was not affected by salinity, even though salt accumulated in leaves.

The reduction of K^+ and Ca^{2+} content in shoots, accompanied by an increased concentration of these ions in the xylem sap (data not shown), can be explained by the pronounced decrease of the hydraulic and stomatal conductances in lettuce plants exposed to salt stress, so although the xylem sap has higher amounts of Ca^{2+} and K^+, a smaller sap volume reaches the leaves of the salt-exposed plants.

The presence of 100 mM NaCl in the growth medium caused a significant increase in the free proline content in all of the five lettuce cultivars (Fig. 6). In the Paris Island cultivar the proline content increased twenty five times as compared to the control, which is a very pronounced metabolic reaction related to an effective osmoregulation with involvement of this compatible solute. Similar data were presented about the involvement of proline accumulation in salt stress tolerance of some other lettuce cultivars (YOUNIS et al., 2009), however, such an increase in prolin concentration like in case of Paris Island was not reported before. In terms of free proline content, we obtained significantly different results (considerably higher increases) from those reported by MAHMOUDI et al. (2011) for two other cultivars, even though the cultivation and treatment conditions were very similar. While their one month old plants, grown hydroponically and exposed to 100 mM NaCl, developed a proline content similar to control or at most two times higher, we have determined in our cultivars, under rather similar conditions, a three to twenty times increased proline concentration as a result of osmotic stress tolerance. These differences may be due to different developmental phases of the examined plants (their one month old plants had a generally lower biomass production than the ones obtained in our experiments). The higher proline accumulation of this cultivar could be a biochemical indicator of its better salt tolerance. Increment of free proline concentration in plant cells subjected to osmotic stress is well documented in the literature, consequently different levels of proline content may indicate the degree of environmental stress that affects water balance of plants. The higher proline content of leaves can be related to a higher capacity to accumulate Na^+ ions in the shoot, as osmotic adjustment is achieved by proline accumulation in the cytosol and by sodium sequestration in the vacuole (ASHRAF and FOOLAD, 2007; HASEGAWA et al., 2000).

In summary, we concluded that from among the investigated lettuce

cultivars, Paris Island exhibited the highest tolerance to salt stress exerted by 50 mM and 100 mM NaCl in hydroponic cultures, while Asparagina was the most sensitive to high salinity. The cultivars with the greater growth rate in saline solution have the higher concentrations of Na^+, thus suggesting that the involvement of Na^+ in osmotic adjustment is a contributor to the higher growth rate. Stomatal closure reduces moisture accumulation during storage of lettuce. Free proline content is a reliable, easy-to-determine and sensitive biochemical marker for selection of salt tolerant lettuce varieties even at an early developmental stage. Salt accumulation in leaves of more salt tolerant varieties (e.g. Paris Island, Valdor) confers a slight salty taste to the marketable yield of lettuce, while lower water content, resulting in a higher percentage of dry biomass, makes the leaves crispier and darker green, that may compensate for reduced leaf size of the plants exposed to high salinity.

Based on the above presented data, the Paris Island lettuce cultivar can be recommended for cultivation in areas affected by increased salinity. Even though a controlled relation between salt concentration and salinity stress reactions can be achieved only in laboratory conditions (because in the field salt concentration cannot be stabilized around the roots and many other variables appear), in the next step field experiments should complete those undertaken in the present study.

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Address of the authors:

Dr. Csaba Bartha and Assoc. Prof Dr. Laszlo Fodorpataki, Hungarian Department of Biology and Ecology, Babes-Bolyai University, RO-400084 Cluj-Napoca, Romania
E-mail: barthacsabi@gmail.com; lfodorp@gmail.com

Prof. Dr. Micaela Carvajal and Dr. Maria del Carmen Martinez-Ballesta, Plant Nutrition Department, CEBAS-CSIC, P.O. Box 164, Campus de Espinardo-Edificio 25, E-30100 Espinardo, Murcia, Spain
E-mail: mcarvaja@cebas.csic.es; mballesta@cebas.csic.es

Prof. Dr. Octavian Popescu, Molecular Biology Centre, Institute for Interdisciplinary Experimental Research, Babes-Bolyai University, RO-400084 Cluj-Napoca, Romania
E-mail: opopescu.ubbcluj@gmail.com