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Full Length Research Paper

Enhanced accumulation of root hydrogen peroxide is associated with reduced antioxidant enzymes under isoosmotic NaCl and Na_2SO_4 salinities

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The inhibitory effect of salt stress on lettuce is one of the main reasons for the reduction of plant growth and crop productivity. In the present study, the response of two lettuce varieties Verte and Romaine to isoosmotic NaCl and Na_2SO_4 treatments were examined. Both varieties were grown in pots containing nutrient Hoagland solution with or without 100 mM NaCl or 77 mM Na_2SO_4. Relative growth rate (RGR), hydraulic parameters, root ion content, proline and several antioxidant activities in roots were measured after 12 days of treatment. After prolonged exposure to salt stress, relative growth rate and water content of lettuce significantly decreased. Roots accumulated high level of Na^+ under both salts, whereas the accumulation of K^+ and Ca^{2+} decreased. High level of Na^+ inside the cells inhibited the K^+ uptake and resulted in increased K^+/Na^+ ratio. In addition, salt stress also caused an increase in the accumulation of proline. This result suggests that proline may play a crucial role in protecting lettuce under salt stress especially in response to Na_2SO_4 treatment. Membrane damage estimated by electrolyte leakage (EL) increased especially in response to Na_2SO_4 treatment in both varieties, but Verte had significantly lower EL relative to Romaine under 100 mM NaCl. A reduction in the activities of CAT in both varieties under 100 mM, and GPX activity in Verte under Na_2SO_4 treatment coincided with an increase in H_2O_2 level, indicative of cellular damage and a general depression of the antioxidant enzymatic system in lettuce roots.

Key words: Lettuce, NaCl, Na_2SO_4, RGR, mineral nutrition, antioxidant activities, proline.

INTRODUCTION

Salinity affects plant growth by the osmotic stress induced by salt around the roots as well as the toxicity effects caused by excessive accumulation of salt in leaves. Growth response to salinity has two phases. The first phase is characterized by a large decrease in growth rate caused by the salt outside the roots, that is, an 'osmotic' response, whereas the second phase typically involves an additional decline in growth caused by salt having built up to toxic levels within plants, that is a 'salt-specific' response (Munns, 2005). As a consequence of these primary effects, secondary stresses such as oxidative damage and nutritional imbalance often occur (Zhu, 2001), also affecting plant growth. It has been

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shown that reduction of relative growth rate (RGR) is greater during the time immediately after salt treatment, and there is a partial difference in RGR between control and salt treatments during the long term (Cramer et al., 1994).

Membrane damage, as measured by electrolyte leakage (EL) has been documented for salt-treated plants and can be alleviated by adding Ca (Leopold and Willing, 1984), although the exact mechanism of membrane injury has not been determined. Excess salt concentrations cause enhanced generation of reactive oxygen species (ROS) in plants (Muscolo et al., 2003; Kim et al., 2005). These ROS can cause serious oxidative damages by disrupting lipids, proteins and nucleic acids (Ghorbanli et al., 2004).

Plants have developed various protective mechanisms to eliminate or reduce ROS, which are effective at different levels of stress-induced deterioration (Beak and Skinner, 2003). The enzymatic antioxidant system is one of these protective mechanisms, and includes superoxide dismutase (SOD: EC 1.15.1.1), which can be found in various cell compartments and catalyses the dispro-portion of two $O_2^{\cdot-}$ radicals to H_2O_2 and O_2 (Scandalios, 1993). H_2O_2 is eliminated by various antioxidant enzymes such as catalases (CAT: EC 1.11.1.6) (Scandalios, 1993) and peroxidases (POX: EC 1.11.1.7) (Gara et al., 2003) which convert H_2O_2 to water. Moreover, increased POX enzyme activity in responses to salinity was reported by Neto et al. (2006).

Several reports have suggested that the extent of oxidative cellular damage in plants exposed to abiotic stress is controlled by the capacity of their anti-oxidant systems (Silvana et al., 2003). We previously analyzed seedlings of two lettuce varieties (Verte and Romaine) for their physiological and biochemical responses to equivalent osmotic levels of two types of salinity (NaCl and Na_2SO_4) (Mahmoudi et al., 2010). In the present study, we aimed to study the implications of Na^+ accumulation in the roots, and the limitations of essential nutrients acquisition. We also evaluated the effects of NaCl and Na_2SO_4 salts on antioxidant enzymes.

MATERIALS AND METHODS

Plant material and culture condition

The experiments were carried out in a greenhouse under the following conditions: 16 h light/8 h dark photoperiod and day/night temperature cycle of 20°C/17°C). Seeds were germinated in Petri dishes at room temperature in the dark. Seven-day old seedlings were irrigated with distilled water during the first week. The tested plants (Verte and Romaine) were grown in pots containing Hoagland nutrient solution (Hoagland and Arnon, 1950) diluted 8-fold as the control. After 7 days of acclimation, salt treatment was initiated by adding increments of 20 mM Na_2SO_4 and 25 mM NaCl daily until the desired concentrations of 77 mM Na_2SO_4 and 100 mM NaCl in order to avoid osmotic shock. Individual plants were grown in the control (without salt) , control plus 100 mM NaCl, or control plus 77 mM Na_2SO_4 for 12 days prior to harvest. Fresh weight (FW), dry weigh (DW) of roots and rosette leaves of six plants from each treatment were separately recorded. Tissue water content was expressed as a percentage of (fresh weight-dry weight)/fresh weight. Tissues were harvested and stored at -80°C until biochemical analysis.

Relative growth rate (RGR) was calculated from the increase in the dry weight of plant at initial and final sampling. Plant harvests were taken of six replicate plants for each genotype and each treatment at two sampling times 0, and 12 days after final salt concentrations (100 mM NaCl and 77 mM Na_2SO_4). Plant DW was determined after drying fresh tissues at 60°C for 48 h. Relative growth rate was calculated for each period as per the equation:

$$RGR = (\ln W_2 - \ln W_1) / (t_2 - t_1) \quad (1)$$

Where, W is the plant dry weight and t is time in days at the start and finish of each period.

Nutrition parameters

Ions were extracted with 0.5% HNO_3; K^+, Na^+ and Ca^{2+} concentrations were assayed by flame photometry (Eppendorf apparatus) and Cl^- by coulometry (Butcher Cotlove apparatus). The ability of the plants to maintain tissue K^+ or Ca^{2+} concentration in saline conditions is indicated as K^+ selectivity. It is defined as the ratio of $K^+/(K^+ + Na^+)$ in the tissue divided by the ratio of $K^+/(K^+ + Na^+)$ in the external medium (Ashraf and McNeilly, 1990).

Poline content

Proline in root and leaf tissues was extracted and analysed according to Bates et al. (1973). Ten milligram (10 mg) of powder of dry plant material was mixed with 1.5 ml aqueous sulfosalicylic acid (3%, w/v). The homogenate was centrifuged at 14,000 x g for 10 min, and the supernatant was transferred to a fresh 1.5 ml tube. The extracted solution was reacted with an equal volume of glacial acetic acid and ninhydrin reagent (1.25 g ninhydrin in 30 ml glacial acetic and 20 ml 6 M H_3PO_4) and incubated at 100°C for 1 h. The reaction was terminated by placing the tube in an ice bath. The reaction mixture was vigorously mixed with 2 ml of toluene. After warming at 25°C, the chromophore was measured at 520 nm. L-proline was used as a standard.

Electrolyte leakage (Relative ion leakage ratio)

EL was measured to assess membrane permeability according to the procedure of Lutts et al. (1996) using an Electrical Conductivity Meter (EC). Leaf samples were taken and cut into 1 cm sections. The samples were then placed in individual stoppered vials containing 10 ml of distilled water after three washes with distilled water to remove surface contamination and incubated at room temperature (Ca 25°C) on a shaker (100 rpm) for 24 h. Electrical conductivity (EC) of bathing solution (EC_1) was recorded after incubation. The same samples were then placed in an autoclave at 120°C for 20 min and a second reading (EC_2) was taken after cooling the solution to room temperature. The electrolyte leakage was calculated as EC_1/EC_2 and expressed as a percentage.

Hydrogen peroxide assay

The hydrogen peroxide (H_2O_2) content was determined according to Kotchoni et al. (2006) and as described by Mahmoudi et al. (2011). Briefly, fresh tissue (100 mg) was homogenized with 1 ml of 25 mM H_2SO_4 and then centrifuged at 14000 rpm for 10 min at 4°C. Twenty

microliter (20 µl) of the supernatant was added to 200 µl of working reagent (WR) using PeroXoquant Quantitative Peroxide Assay Kit (Cat no. 23285, Thermo Fisher Scientific, Ottawa, ON, Canada). The absorbance of the supernatant was measured at 560 nm in a spectrophotometer. H_2O_2 was used as a standard calibration curve.

Antioxidant activities

Fresh root samples were submersed for 5 min in liquid nitrogen. The frozen roots were kept at –80°C for further analyses. Enzymes were extracted from 0.5 g leaf tissue using a mortar and pestle with 5 ml extraction buffer containing 50 mM potassium phosphate buffer, pH 7.6 and 0.1 mM Na-EDTA. The homogenate was centrifuged at 15,000 g for 15 min and the supernatant fraction was used to assay for the various enzymes. All steps in the preparation of enzyme extracts were performed at 4°C Gaiacol peroxidase activity (GPX) according to Srinivas et al. (1999) was assayed using guaiacol as an electron donor with a reaction mixture containing 20 mM phosphate buffer (pH 7.0), and 30 mM H_2O_2. The increase of absorbance due to tetraguaiacol formation was recorded at 470 nm. One unit of peroxidase activity catalyzes the oxidation of 1 µmol of guaiacol.APX activity was determined by measuring the consumption of ascorbate following the absorbance at 290 nm. One unit of APX activity was defined as the amount of enzyme required to consume 1 µmole ascorbate min^{-1} (Cakmak and Marschner, 1992).SOD activity was estimated by recording the decrease in absorbance of superoxidenitro blue tetrazolium complex by the enzyme (Sairam and Srivastava, 2002). About 3 ml of reaction mixture, containing 0.1 ml of 200 mM methionine, 0.1 ml of 2.25 mM nitro-blue tetrazolium (NBT), 0.1 ml of 3 mM EDTA, 1.5 ml of 100 mM potassium phosphate buffer, 1ml distilled water and 0.05 ml of enzyme extraction were taken in test tubes in duplicates from each enzyme sample. Two tubes without enzyme extract were taken as the control. The reaction was started by adding 0.1 ml riboflavin (60 µM) and placing the tubes below a light source of two 15 W florescent lamps for 15 min. Reaction was stopped by switching off the light and covering the tubes with a black cloth. Tubes without enzyme developed maximal colour. A non-irradiated complete reaction mixture which did not develop colour served as blank. Absorbance was recorded at 560 nm and one unit of enzyme activity was taken as the quantity of enzyme which reduced the absorbance reading of samples to 50% in comparison with tubes lacking enzymes.CAT activity was measured according to Aebi (1984). About 3 ml reaction mixture containing 1.5 ml of 100 mM potassium phosphate buffer (pH=7), 0.5 ml of 75 mM H_2O_2, 0.05 ml enzyme extraction and distilled water to make up the volume to 3 ml. Reaction was started by adding H_2O_2 and the decrease in absorbance was recorded at 240 nm for 1 min. Enzyme activity was computed by calculating the amount of decomposed H_2O_2.

Data analysis

The values of the parameters were subjected to two-way analysis of variance (ANOVA) and the mean differences were compared by Duncan Test. Each data point was the mean of indicated replicates and comparisons with P-values <0.05 were considered significantly different.

RESULTS

Effect of salts on growth

Some growth related characteristics such as plant biomass, relative growth rate (RGR), rosette diameter, leaf number, leaf area and root length of both lettuce varieties were studied to distinguish the effect of sodium sulfate on lettuce plants. In the control condition, relative growth rate was higher in Verte than in Romaine. Salinity significantly affected RGR in both lettuce varieties (Figure 1). However, a significant difference in RGR between genotypes and treatments was found but results showed that RGR reduced severely with Na_2SO_4. Verte showed higher relative growth rate at 100 mM NaCl than Romaine. Thus, Verte was more tolerant to NaCl than Romaine. Furthermore, RGR reduced more by Na_2SO_4 than by NaCl in both varieties suggesting that the toxic effect is more important with Na_2SO_4 than with NaCl.

Dry weights (DW) of roots and leaves were reduced by both sodic salts (Mahmoudi et al., 2010). This reduction was due to a decrease in root length, in leaf number, in rosette diameter and in total surface area. DW reduction was more significant with Na_2SO_4 than with NaCl in both varieties (Table 1). However, total surface area decreased by 36 and 86% in comparison with the control plants in Verte, and by 48 and 84% in Romaine under NaCl and Na_2SO_4, respectively. Leaf number (Table 1) did not change in Verte treated with NaCl, but decreased significantly under Na_2SO_4 in both varieties.

Growth of roots and leaves was similarly affected so that the roots/leaves ratio remained unchanged (Table 1)

Sodium uptake and distribution

Independently of the treatments, Na^+ was accumulated in both leaves and roots of Verte and Romaine (Figure 2) suggesting a possible saturation of the root tissues at approximately 1 $mmol.g^{-1}$ root DW. The accompanying anion did not exert a significant effect on Na^+ uptake in Romaine. However, sodium concentration reached high values [about 3 mmol g^{-1} dry weight (DW)] in Romaine-treated plants. In Verte, the increase in Na^+ concentration was more pronounced in response to Na_2SO_4 (5 $mmol.g^{-1}DW$) than to NaCl (3 $mmol.g^{-1}DW$). Thus, Verte seemed inefficient or unable to limit the uptake or translocation of Na^+ under Na_2SO_4.

Effect of salts on Na^+, K^+ and Ca^{2+} accumulation

Under control conditions, roots of both varieties had similar contents of Na^+ (Table 2). Under NaCl treatment, both lettuce varieties showed an increase in Na^+ accumulation at equivalent concentrations of salt, but Verte roots showed a stronger capacity than Romaine roots to accumulate Na^+ under Na_2SO_4 treatment (Table 2).

Under control conditions, roots of both varieties had similar contents of Ca^{2+} and K^+ (Table 2). Both salts decreased the K^+ and Ca^{2+} levels in roots of both varieties especially under Na_2SO_4 treatment. However,

Table 1. growth characteristics of Verte and Romaine plants grown in the presence of 0, 100 mM NaCl or 77 mM Na_2SO_4 salts for 12 days.

Parameter	Genotype	Growth parameter		
		No treatment	100 mM NaCl	77 mM Na_2SO_4
Leaf number	Verte	$8.50^{a} \pm 0.57$	$7.5^{a} \pm 0.57$	$6.17^{c} \pm 0.43$
	Romaine	$9.00^{a} \pm 0.00$	$8.00^{b} \pm 0.00$	$6.17^{c} \pm 0.43$
Leaf area, cm^2	Verte	$31.75^{a} \pm 2.73$	$20.40^{b} \pm 2.45$	$4.50^{c} \pm 0.54$
	Romaine	$30.86^{a} \pm 2.43$	$16.00^{b} \pm 1.01$	$5.07^{c} \pm 0.52$
Rosette diameter, cm	Verte	$15.79^{a} \pm 1.17$	$8.50^{b} \pm 0.88$	$5.08^{c} \pm 1.86$
	Romaine	$15.50^{a} \pm 0.88$	$8.63^{b} \pm 0.93$	$4.21^{c} \pm 1.47$
Root length, cm	Verte	$22.75^{a} \pm 2.43$	$18.83^{a} \pm 4.33$	$14,17^{b} \pm 1,84$
	Romaine	$20.33^{a} \pm 1.39$	$18.08^{a} \pm 2.58$	$15.00^{b} \pm 1.62$
R/L ratio	Verte	$0.29^{a} \pm 0.05$	$0.35^{a} \pm 0.08$	$0.21^{a} \pm 0.08$
	Romaine	$0.26^{a} \pm 0.05$	$0.36^{a} \pm 0.08$	$0.21^{a} \pm 0.02$

Means followed by different letters are significantly different at *P* inferior or equal to 0.05 as determined by two ways analysis (ANOVA). (mean ± S.E., n=6).

Table 2. Root ions concentrations and K/Na ratio of Verte and Romaine plants grown in the presence of 0, 100 mM NaCl or 77 mM Na_2SO_4 salts for 12 days.

Parameter	Genotype	Ions concentrations, $mmol.g^{-1}DW$		
		No Treatment	100 mM NaCl	77 mM Na_2SO_4
Root Na^+	Verte	$0.38^{c} \pm 0.21$	$1.52^{b} \pm 0.12$	$2.36^{a} \pm 0.03$
	Romaine	$0.38^{c} \pm 0.02$	$1.16^{b} \pm 0.24$	$1.67^{a} \pm 0.08$
Root K^+	Verte	$1.77^{a} \pm 0.51$	$0.92^{b} \pm 0.11$	$0.07^{c} \pm 0.01$
	Romaine	$1.78^{a} \pm 0.22$	$1.13^{b} \pm 0.28$	$0.35^{c} \pm 0.02$
Root Ca^{2+}	Verte	$0.05^{a} \pm 0.02$	$0.02^{b} \pm 0.01$	$0.02^{b} \pm 0.01$
	Romaine	$0.05^{a} \pm 0.02$	$0.02^{b} \pm 0.01$	$0.02^{b} \pm 0.01$
Root $Na^+ + K^+ + Ca^{2+}$	Verte	$2.19^{a} \pm 0.45$	$2.47^{a} \pm 0.13$	$2.45^{a} \pm 0.03$
	Romaine	$2.21^{a} \pm 0.22$	$2.31^{a} \pm 0.47$	$2.04^{a} \pm 0.10$
Root K/Na ratio	Verte	$0.82^{c} \pm 0.10$	$50.69^{a} \pm 5.23$	$5.77^{b} \pm 0.55$
	Romaine	$0,82^{c} \pm 0,02$	$66.33^{a} \pm 5.43$	$36.08^{b} \pm 1.20$

(Mean ± SE., n=6). Means followed by different letters are significantly different at *P* inferior or equal to 0.05 as determined by two ways analysis (ANOVA).

accumulation of K^+ reduced by 48 and 96% in Verte roots, compared to 47 and 80% reduction in Romaine roots under NaCl and Na_2SO_4 treatments, respectively. The decrease of Ca^{2+} was similar under both treatments. A significant increase in root K/Na selectivity (Table 2) was found in Verte and Romaine under both salt treatments indicative of differential selectivity of favourable nutrient ions over toxic Na^+ between Verte and Romaine.

Water relationships

The maintenance of cell turgor by osmotic adjustment is

an important physiological adaptation to minimize the detrimental effects of salt stress (Munns et al, 1983). K^+, Na^+ and Cl^-, as main soluble inorganic intracellular ions participate in the osmotic adjustment in glycophytes. In varieties, the leaf (Figure 3A, B) and root (Figure 3C, D) water content decreased by Na^+ salt treatments. Thus, large Na^+ accumulation was probably associated with the absence of ion compartmentalization into the leaf and root cells, permitting its accumulation in the cytosol.

Effect of NaCl and Na_2SO_4 on proline accumulation

Proline content was measured in both varieties under control and saline conditions. In control conditions, both varieties contained equivalent amounts of Proline in leaves but roots of Verte variety contained higher levels of Proline as compared to those of Romaine (Figure 4). In control conditions, both varieties contained equivalent amounts of Proline in leaves (Figure 4). Under salt stress, leaf Proline content increased significantly at 100 mM NaCl in Verte but remained unchanged in Romaine. Moreover, this increase was more pronounced in the presence of Na_2SO_4 than of NaCl. However, leaf Proline content increased by 2.71- and 4.99-fold in Verte in the presence of 100 mM NaCl and 77 mM Na_2SO_4 respectively. In Romaine, only an increase by 4.64-fold was observed in Proline content under Na_2SO_4.

In Verte roots (Figure 4B), Proline contents increased by 2.2-fold at 100 mM NaCl and remained unchanged under Na_2SO_4 treatment , while in Romaine it increased by 2.8- and 2.2- fold in the presence of 100 mM and 77 mM Na_2SO_4, respectively. These results suggest that the relative sensitivity of proline accumulation to 100 mM NaCl in Verte compared to Romaine was related at least partially to transient leaf Proline accumulation.

Salts effect on relative ion leakage ratio

Electrolyte leakage (EL) was used to assess membrane permeability. The EL index of leaves and roots of the both varieties progressively increased in response to salt treatments (Figure 5A, 5B). Relative to the control, salt treatments increased EL by 1.6- and 3.2-fold in Verte leaves and by 3.1- and 3.5-fold in Romaine leaves under 100 mM NaCl and 77 mM Na_2SO_4, respectively. In roots (Figure 5B), EL increased by 4.5 and 13.5 in Verte under 100 mM NaCl and 77 mM Na_2SO_4 respectively. While in Romaine EL was increased by 6.7- and 11.4-fold compared to the control under both salt treatments.

Root H_2O_2 accumulation under salt treatments

Under control condition, root H_2O_2 content was 2-fold higher in Verte than in Romaine (Figure 6). Root H_2O_2 content increased significantly in plants treated with NaCl (3.1- fold and 9.0-fold in Verte and Romaine respectively). This increase was more pronounced in response to Na_2SO_4; it reached the values of 3.6- and 10.1-fold in Verte and Romaine, respectively.

Effect of isoosmotic salts on root antioxidative enzyme activities

GPX activity (Figure 7A) increased by 193% in Verte roots treated with 100 mM NaCl, but decreased by 52% in roots treated with 77 mM Na_2SO_4. In Romaine variety, GPX activity did not change irrespective of salt treatment. CAT activity (Figure 7B) decreased by 75 and 66% in comparison with the control under 100 mM NaCl treatment, in Verte and Romaine, respectively. Treatment with 77 mM Na_2SO_4, did not cause any change in CAT activity in the roots of either variety.

APX activity (Figure 7C) remained unchanged in comparison with the control when plants were treated with 100 mM NaCl. However, increases of 14- and 13-fold in Verte and Romaine, respectively were observed when plants were treated with 77 mM Na_2SO_4.

SOD activity represents the most activity as compared with the other three enzymes under control and treatment condition (Figure 7D). Under control condition, both varieties have the same SOD activity. Both NaCl and Na_2SO_4 salts increased significantly SOD activity by 2.18- and 1.87-fold respectively in Verte. In Romaine, SOD activity increased by 2.71- and 3.45- fold under the same conditions.

DISCUSSION

Lettuce showed a moderate tolerance to NaCl constraint than to Na_2SO_4, as shown by the reduction of RGR and a number of morphological traits (leaf area, rosette diameter, leaf number, and root length) (Table 1, Figure 1)). Green leaves and dry matter production per plant were reported to be reduced with the increase in soil salinity (Bal and Dutt, 1984). Inhibition of the formation of leaf primodia under salinity stress could be the probable reason for low leaf number. Moreover, RGR provides a relative basis to compare plant growth rates of plants because it takes into account both the initial and the final plant weights over a specified time. Our results show that plant biomass (RGR), leaf number and leaf area (Table 1) were particularly affected by both salts. This finding suggests that salinity modified growth through its effects on leaf expansion and on the initiation of new leaves. Indeed, salt-induced growth reduction might be related to salt osmotic effects, which affect cell turgor and expansion (Rozema, 1991). Our growth parameters data indicate that the inhibitory effect of Na_2SO_4 was stronger than that of NaCl. In contrast, in Sorghum bicolour (Khan et al., 1995), the inhibitory effect of NaCl on growth was more pronounced than that of Na_2SO_4. Sodium sulphate

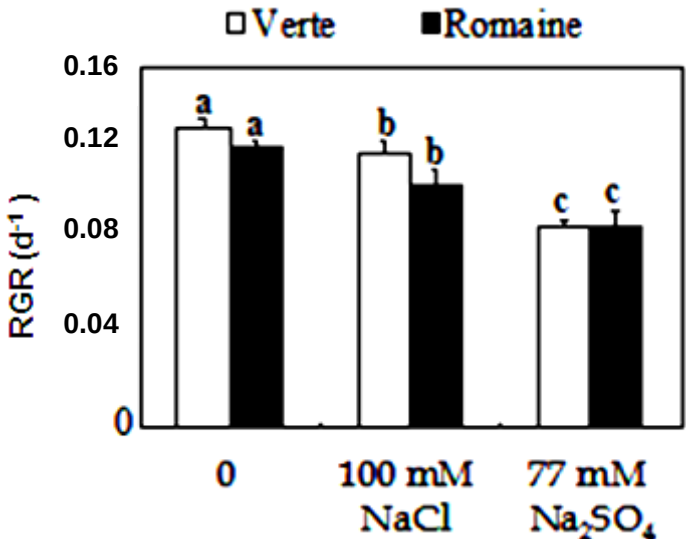

Figure 1. Relative growth rate (RGR, d^{-1}) of Verte and Romaine. Plants were grown for 12 days in the presence of the indicated salts medium,100 mM NaCl or 77 mM Na_2SO_4. Means of six replicates ± SE. R, roots; L leaves.

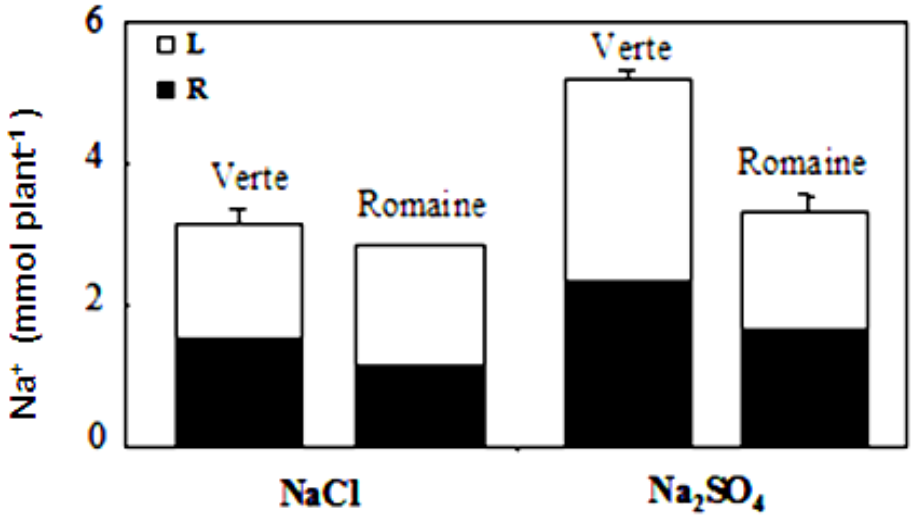

Figure 2. Na^+ content of the different plant organs of Verte and Romaine. Plants were grown for 12 days in the presence of the indicated salts medium,100 mM NaCl or 77 mM Na_2SO_4. Means of six replicates ± SE. R, roots; L leaves.

and sodium chloride have been found to have verydifferent effects on plant growth, pigment concentration, protein metabolism, cellular structure (Strogonov, 1973), nutrient interactions (Franklin et al., 2002b), and water relations (Renault et al., 2001; Redfield and Zwiazek 2002). Plants may avoid accumulation of potentially toxic ions in leaf tissues by restricting ion uptake or translocation, and our data suggest that lettuce has a limited ability to regulate both processes for both Na^+ and Cl^-. In our conditions, the capacity of the roots to sequester ions appears to be exceeded after 2 weeks of treatment (Figure 2), resulting in increases of Na^+ accumulation in roots and led to the dehydration of this organ (Figure 3, illustrated by high correlation R) especially in Verte under Na_2SO_4 despite the high proline accumulation (Figure 4). The important increase in the proline contents of leaves and roots of both varieties seemed to be related to a neo-synthesis and an inhibition of the catabolism of this compound (Abdul Jaleel et al., 2007). Our results showed that RGR decreased significantly with salt treatment. This result could indicate that proline accumulation would occur at the expense of

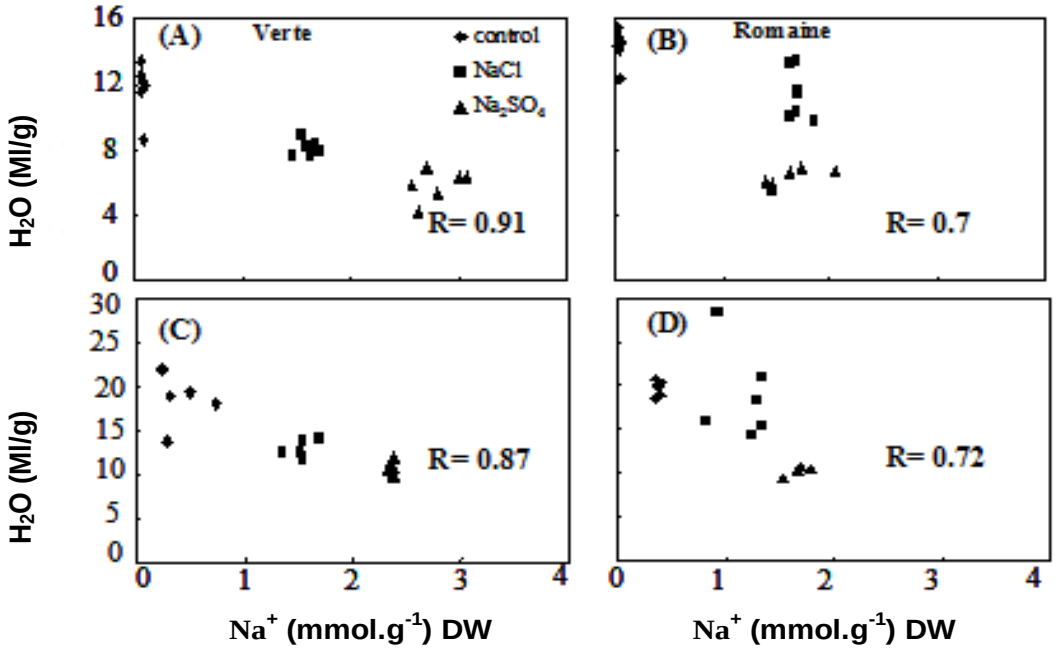

Figure 3. Relationship of leaf (Figure 3A, B) and root (Figure 3C,D) hydration (ml H_2O. g^{-1} DM) to Na^+ concentration (mmol. g^{-1} DM) in the two lettuce varieties cultivated on the absence (control) or on presence of 100 mM NaCl or 77 mM Na_2SO_4. Individual value of six replicates.

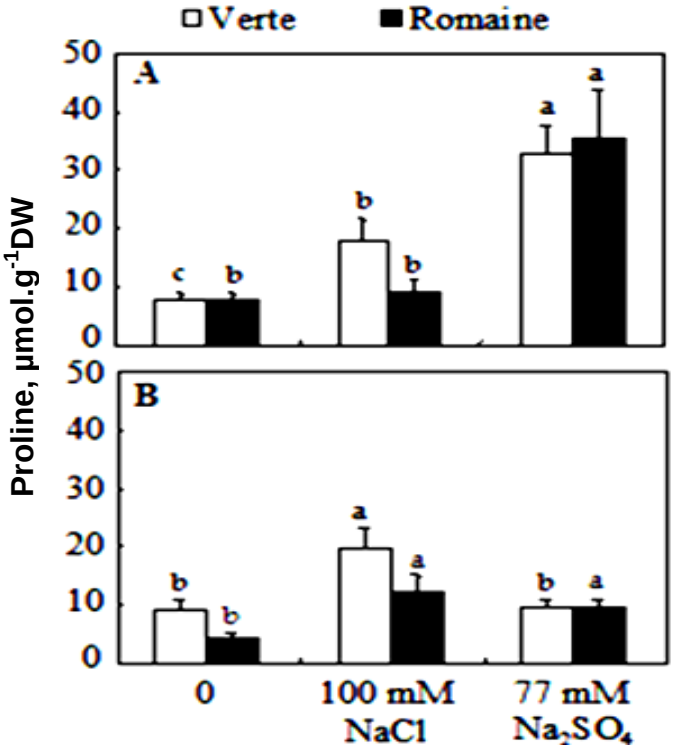

Figure 4. Effect of salt on leaf Pro (Figure 4A) and root pro (Figure 4B) contents. Seedlings of Verte (empty bar) and Romaine (filled bar) lettuce were grown in the presence of 0, 100 mM NaCl or 77 mM Na_2SO_4 for 12 days. Error bars represent standard error of the means of healthy leaves randomly selected from six plants for each genotype and treatment.

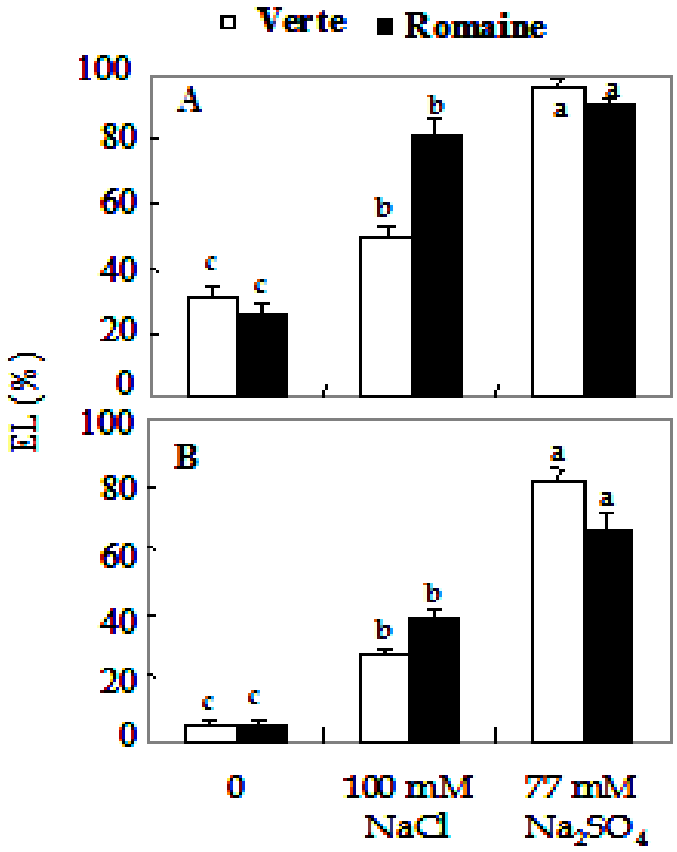

Figure 5. Effect of salt on electrolyte leakage and in leaves (A) and roots (B). Seedlings of Verte (empty bar) and Romaine (filled bar) lettuce were grown under 0, 100 mM NaCl or 77 mM Na_2SO_4 for 12 days. Error bars represent standard error of the means of 5 replicates for EL.

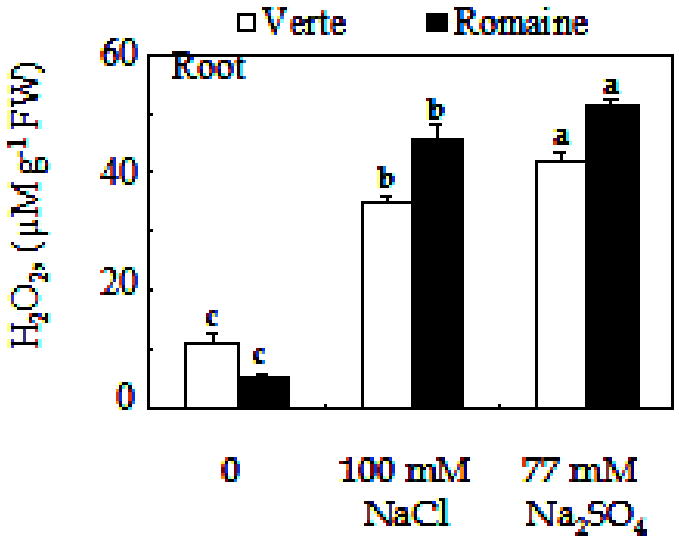

Figure 6. Root H_2O_2 content of Verte (empty bar) and Romaine (filled bar) lettuce grown under 0, 100 mM NaCl or 77 mM Na_2SO_4 for 12 d. Error bars represent standard error of the means of 4 independent replicates.

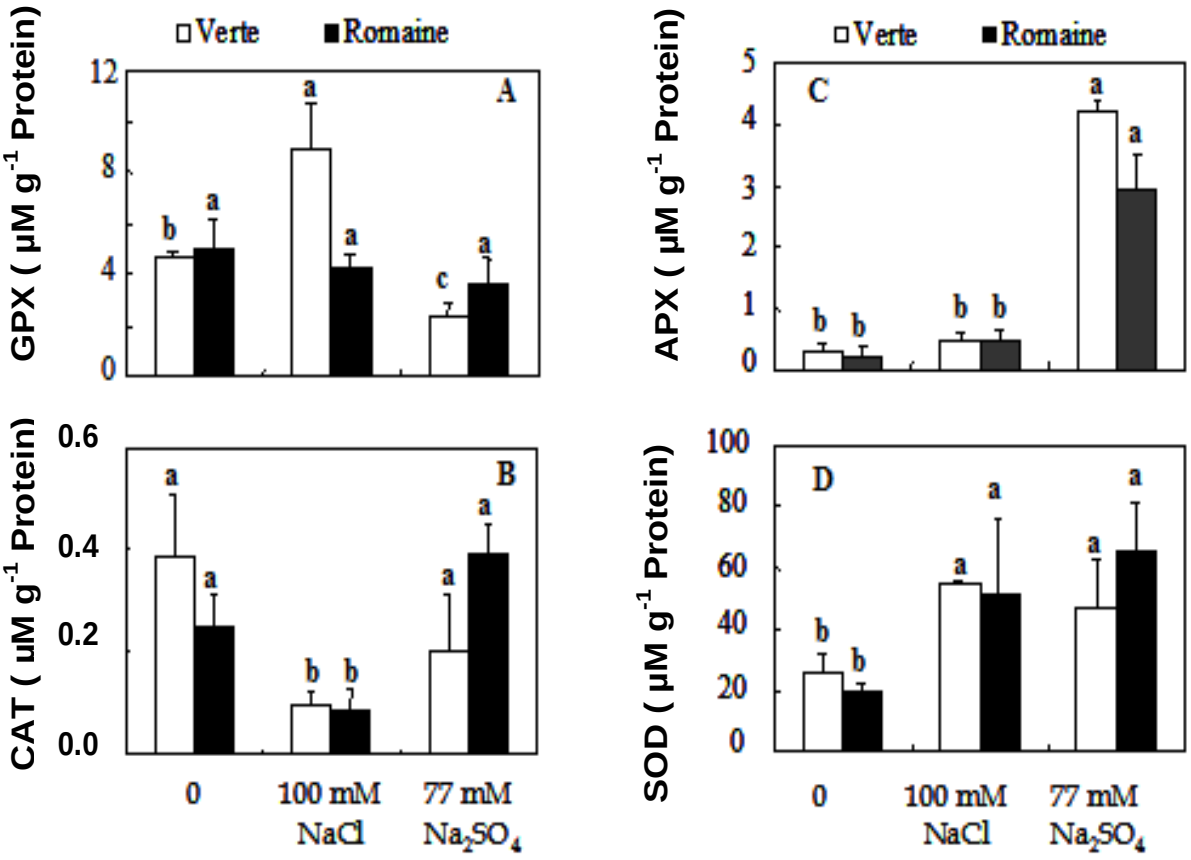

Figure 7. Root antioxidant activities of Verte (empty bar) and Romaine (filled bar) lettuce grown under 0, 100 mM NaCl or 77 mM Na_2SO_4 for 12 days. Error bars represent standard error of the means of 4 independent replicates.

growth activity and thus it could not be sufficient by itself to confer salt tolerance (Poustini et al., 2007).

Our previous data (Mahmoudi et al., 2010) showed a marked increase of leaf and root MDA content of both varieties subjected to 100 mM NaCl or 77 mM Na_2SO_4, which is indicative of severe oxidative injury to membrane lipids in these plants due to the inadequate response of the antioxidative systems. The apparent increase in membrane EL (Figure 5) seems to parallel the increase in salt content of the leaves in both NaCl and Na_2SO_4 treatments, and the movement of Na^+ is closely associated with the movement of the anion. The observed reduction in control over ion movement could result from high external levels of Na^+, since high Na^+ levels can result in membrane depolarization and K^+ efflux (Jacoby, 1994), but this does not explain the differences between NaCl and Na_2SO_4 treatments.

Salinity can affect growth and yield of plants and can also generate secondary oxidative stress that occurs when there is a serious imbalance between the production of reactive oxygen species (ROS) and antioxidant defence, leading to oxidative damage of macromolecules and, eventually, of cellular structure. In agreement with growth parameters, the production of H_2O_2 increased following the treatment (Figure 6), and was more significant in Na_2SO_4 than in NaCl treatments in both lettuce varieties, indicative of cellular damage. The increased production of H_2O_2 with both isoosmotic salts could be due to a significant decrease of GPX in Verte under Na_2SO_4 and CAT activities in Roots of Verte and Romaine under NaCl (Figure 7A, C). Although, APX activity was significantly increased in roots of both varieties in response to Na_2SO_4 associated with a significant increase in SOD activity under NaCl and Na_2SO_4 treatments associated to an increase of H_2O_2 level. These findings suggest a general depression of the antioxidant enzymatic system in lettuce roots.

In conclusion, our data indicate that both salts induced oxidative damage in *Lactuca sativa* L. The damage inflicted by salinity on plant physiology and development was shown to be caused by osmotic stress due to high concentrations of Na^+ ion in organs; leaves and roots associated with a nutritional disturbance (reduction of K^+ and Ca^{2+} accumulations) and the toxic effect promoted by Na^+ accumulation in plant tissues. However, despite concomitant increases in the activities of some antioxidant enzymes such as APX and SOD, an increase of H_2O_2 level was observed, indicative of cellular damage and a

general depression of the antioxidant enzymatic system in lettuce roots.

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Abbreviations: RGR, Relative growth rate; **GPX,** gaiacol peroxidase; **CAT,** catalase; **SOD,** superoxide dismutase; **EL,** electrolyte leakage.

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