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Contrasting physiological responses to high salinity between two varieties of corn 'Lluteño' (salt tolerant) and 'Jubilee' (salt sensitive)

Libertad Carrasco-Ríos[1*], Carla Rojas[1], and Manuel Pinto[2]

'Lluteño' is the only one corn capable to prosper in the Valley of Lluta under saline conditions (EC = 9.1 dS m^{-1}). This cultivar has been little studied and there is no current information about its growth and about the possible mechanisms involved in its tolerance to salts. The aim of this research was to compare the growth of young plants of corn (*Zea mays* L.) 'Lluteño' to that of the bred 'Jubilee', both grown under different salt concentrations, to characterize the absorption and distribution of Na^+ and other nutrients in the plant and to evaluate the effect of the saline conditions in the osmotic adjustment in both cultivars. The plants of 21 d old were subjected for 15 d to two saline treatments: 50 and 100 mM NaCl. The accumulation of DM was reduced from 5.12 to 1.80 g $plant^{-1}$ in 'Jubilee' and 5.53 to 4.12 g $plant^{-1}$ in 'Lluteño' ($P \leq 0.05$). 'Lluteño' showed to be more tolerant to salt stress that 'Jubilee' by greatest accumulation of biomass under saline conditions, it was associated with a lower accumulation of Na^+, steadiness of K^+ and Ca^{2+} content and accumulation of osmolytes in leaves. The latter affecting positively the maintenance of relative water content and the osmotic adjustment of this cultivar in the leaves.

Key words: Ion content, osmotic adjustment, salt stress, *Zea mays*.

INTRODUCTION

When plants are exposed to salinity, growth is initially inhibited by a cellular response to the osmotic effect. New leaves emerge more slowly, and there is little branching or formation of lateral sprouts (Niu et al., 1995; Munns and Tester, 2008). Leaves are more sensitive to salinity than roots, a phenomenon which is not yet completely understood (Munns and Tester, 2008). One teleological explanation is that the relative reduction in the development of leaf area and of root growth is produced in order to decrease the use of water and thus to prevent the gradual increase of salts in the plant (Munns and Tester, 2008). For many researchers, however, the control of growth under stress conditions is produced by a restricted coordination between cell division and elongation, in which abscisic acid (ABA) participates by inhibiting DNA synthesis and thus cell division (Wang et al., 1998).

Plant growth in saline conditions may also be inhibited by the toxic effect of the salts, since excessive accumulation within the plant generates various nutritional disorders, interfering with the absorption of nutrients such as K^+, Ca^{2+}, and NO_3^- (Niu et al., 1995). When salts are accumulated in toxic concentrations, which occur mainly in old leaves, they accelerate leaf senescence and finally produce mortality. If mortality surpasses the rate of production of new leaves, the photosynthetic capacity of the plant becomes insufficient to satisfy the carbohydrate requirements of the new leaves, which also leads to a reduction in growth rate (Munns and Tester, 2008).

Since NaCl is the most soluble salt, plants species have developed several different mechanisms to regulate its accumulation. Some species avoid the toxic effect of Na^+ actively transporting Na^+ through tonoplast and accumulating it in the vacuole against a high electrochemical gradient of Na^+ (Yokoi et al., 2002). The difference in the accumulation of Na^+ in different organs of the plant and/or its transport to the aerial part may partly explain the salt tolerance which some genotypes of corn (*Zea mays* L.) possess (Gorham et al., 1990). Corn 'Pioneer 3906' grown in greenhouse conditions with 100 mM NaCl, demonstrated a great capacity to exclude Na^+ (Mass et al., 1983; Schubert and Läuchli, 1986), with a significant reduction in the concentration of Na^+ in leaves. The compartmenting of Na^+ also appears to diminish the osmotic potential and adjust the cell water potential to permit the absorption of water during saline stress (Glenn et al., 1999).

Another strategy that plants have to tolerate salinity is the synthesis of organic solutes known as osmolytes. Compounds such as sugars, proline and glycine betaine do not interfere with the cell metabolism at high

[1]Universidad de Tarapacá, Facultad de Ciencias, Velásquez 1775, Arica, Chile. [*]Corresponding author (lcarrasc@uta.cl).
[2]Instituto de Investigaciones Agropecuarias INIA, Santa Rosa 11610, La Pintana, Santiago, Chile.
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concentrations but they participate in retaining water, which allows the plant to maintain its physiological functions (Hasegawa et al., 2000).

According to what was mentioned above, the maintenance of water and ionic homeostasis in saline conditions is therefore achieved by various processes at the cell level, among which are the mechanisms destined to exclude salts from the cytoplasm, the cellular compartmenting of salts in the vacuole and the synthesis of compatible solutes to accomplish the osmotic adjustment (Munns and Tester, 2008).

Between the bred and commercial varieties of corn, difference with respect to salt tolerance is scarce. This is because breeding has generally been performed under non-saline conditions; thus the genetic variation which could produce salt tolerance tends to disappear. However, corn genotypes that have been cropped for long time by local communities under high salinity, they are acclimated to those conditions and can be used as source of tolerance to salt in breeding programs. This is the case of 'Lluteño' corn, which is a land race from the Lluta Valley situated in the extreme north of Chile and where soils and water have a conductivity of 9.1 and 2.85 dS m^{-1}, respectively. Under these conditions, it has been proved by local farmers that commercial varieties of corn are not definitely able to prosper, and that the only corn possible to crop in the valley is 'Lluteño', which can produce total biomass of 22 Mg ha^{-1}. This cultivar has been little studied and there is no current information about its growth and about the possible mechanisms involved in its tolerance to salts. Therefore the aim of this study was to compare growth of 'Lluteño' to that of 'Jubilee', both grown under different salt concentrations. A characterization of the absorption and the distribution of Na was also done and the synthesis of osmolytes was determined to evaluate whether osmotic adjustment is a mechanism used by 'Lluteño' to tolerate salt stress. Comparisons were done during the first month of growth.

MATERIALS AND METHODS

Plant material and experimental conditions

The study was performed at the Experimental Station of the Tarapacá University (18º35' S, 60º30' W) using young plants of 'Lluteño' and 'Jubilee' corn, this last is a corn variety bred (USA) commercialized in Chile by Bioamerica S.A. and normally cultivated in the North of Chile in non-saline soils. Seeds were sown in plastic pots containing perlite-vermiculite 1:1 (v/v) as substrate. To keep the humidity, pots were covered with a plastic wrap until the epicotyls appeared which occurred 4 to 5 d after sowing. Then plants were cultivated for 2 wk under greenhouse conditions where the mean maximum temperature was 35 ºC and the mean relative humidity was 34%. Along this time, plants were cultivated in hydroponic conditions using a non-saline Hoagland solution composed of 2.08 mM $Ca(NO_3)_2 \cdot 4H_2O$, 1.99 mM $MgSO_4 \cdot 7H_2O$, 2.00 mM $NH_4H_2PO_4$, 10.09 mM KNO_3, 46.26 nM H_3BO_3, 0.45 nM $Na_2MoO_4 \cdot 2H_2O$, 0.32 nM $CuSO_4 \cdot 5H_2O$, 9.19 nM $MnCl_2 \cdot 4H_2O$, 0.76 nM $ZnSO_4 \cdot 7H_2O$, 19.75 nM $FeSO_4 \cdot H_2O$ (Arnon and Hoagland, 1940).

Salt treatment and growth conditions

Twenty one days after sowing, plants were randomly placed outdoors and subjected to the treatments for 15 d as listed in Table 1. To obtain different salt treatments the Hoagland solution was supplemented with 50 mM NaCl and 100 mM NaCl. The control was the basic nutrient solution without NaCl. Conductivity and pH of the solution were monitored periodically and ion concentrations were maintained at the corresponding treatment value. During the experiment, midday PAR (photosynthetically active radiation) intensity was 2200 μmol m^{-2} s^{-1}, the mean maximum temperature 27.6 ºC and the mean minimum 16.9 ºC. The relative humidity averaged 50% in the day and 80% in the night.

Leaf area and DM accumulation measurements

After 15 d of saline treatments, five plants per treatment were harvested and divided into leaves, stems, and roots, for their respective fresh weight (FW) and dry weight (DW) determination. Total leaf area was estimated according to Francis et al. (1969). The increment in leaf area was expressed as the difference between leaf area at the end of treatments and leaf area recorded at the beginning of treatments. Dry weight was obtained after drying each part of the plant in an oven at 80 ºC for 48 h. Accumulation of DM was expressed as the plant weight at the end of the treatments minus the weight of the plants recorded at the beginning of the treatments.

Analysis of mineral contents

For Ca^{2+}, Na^+, K^+, and P analysis, samples of dried leaves and roots were ashed in a furnace at 500 ºC for 6 h. Then ashes were dissolved in 2 M HCl, and filtered through Whatman filter paper. Ca^{2+} was measured with atomic absorption spectrophotometer (AA240, Varian, Mulgrave, Victoria, Australia). Na^+ and K^+ contents were determined with flame emission photometer (PFP7, Jenway, Stone, Staffordshire, UK). Phosphorus was measured by the molybdenum blue method described by Murphy and Riley (1962).

Table 1. Treatments given during 15 days.

Treatments	NaCl concentration in the Hoagland solution	EC
	mM	dS m^{-1}
Low salinity	0	2.99
Intermediate	50	8.75
High salinity	100	13.01

EC: electrical conductivity.

Water relations measurements

Relative water content (RWC), leaf water potential (Ψ_w) and osmotic potential (Ψ_s) were measured in the fifth leaf of each corn plant. Relative water content was expressed as 100 × (fresh weight - dry weight)/(turgid weight - dry weight). Leaf water potential was measured with a pressure chamber (PMS Model 600, USA) according to Scholander et al. (1965). Osmotic potential of leaves was measured in tissue sections which were frozen at -20 ºC for 2 h and then macerated and centrifuged at 13 636 × g for 5 min to extract the cell sap. Osmolality was measured with the help of an osmometer (D-14129, Roebling Messtechnik, Berlin, Germany), using 100 μL sap in 1.5 mL centrifuge vials calibrated with distilled water. Van't Hoff's equation was used to calculate Ψ_s of the solution (Nobel, 1991). The turgor potential of leaves (Ψ_p) was estimated as the difference between Ψ_w and Ψ_s. The leaf osmotic adjustment (OA) was obtained using the value of Ψ_s at maximum turgor (Ψ_s^{100}), which was estimated as the product of the values of Ψ_s and RWC (Irigoyen et al., 1996):

$$\Psi_s^{100} = (\Psi_s \times RWC)/100.$$

The OA was then calculated as the difference between the values of the Ψ_s at maximum turgor of the plants treated with salts (Ψ_s^{100s}) and the control plants (Ψ_s^{100c}).

Determination of osmolytes active and/or protector compounds

Soluble sugars (glucose, fructose, and saccharose) were determined in samples of leaf tissue macerated with 80% ethanol. The alcoholic extracts were transferred to 15 mL tubes and warmed in an oven at 30 ºC for 30 min, then centrifuged at 1957 × g for 10 min. The pellet was extracted a second time using the same procedure. The two alcoholic extracts were then combined and treated with an anthrone solution.

Soluble sugars were then quantified using a molecular absorption spectrophotometer (Hewitt, 1958).

Proline was determined in samples of leaf tissue macerated with 10 mL of 3% sulfosalicylic acid. Two milliliters of the extract were reacted with 2 mL glacial acetic acid and 2 mL acid ninhydrine for 1 h at 100 ºC. After cooling the sample on ice, 4 mL of toluene were added to extract the proline to the organic phase. Proline was quantified at 520 nm according to Bates et al. (1973).

Experimental design and statistical analysis

A factorial design in randomized complete block was used, with two factors: cultivars ('Lluteño' and 'Jubilee') and salinity (0, 50, and 100 mM NaCl) and five replicates for the measurements. All data were analyzed statistically by ANOVA using the Statgraphics Plus program 5.1 version. The Tukey's test was used for multiple comparisons, with a 95% level of significance.

RESULTS

Effect of salinity on DM accumulation and the leaf area

Saline treatments produced a significant reduction in the total DM accumulation in both cultivars (Table 2); however this reduction was more important in 'Jubilee'. In this cultivar after 15 d growing in 50 mM NaCl, DM accumulation was reduced from 5.12 to 2.44 g plant^{-1}. This means a reduction of 52%. This reduction was only 20% in 'Lluteño' and surprisingly non additional DM reduction was observed when the NaCl concentration was doubled to 100 mM. Under such conditions 'Jubilee' reduced its DM production from 5.12 to 1.80 g plant^{-1}. This means a reduction of 65%, revealing the sensitivity of 'Jubilee' to the saline conditions. 'Lluteño' is also shown by the non-significant reduction in leaf area observed at any of the given treatments (Table 2).

Effect of salinity on mineral contents

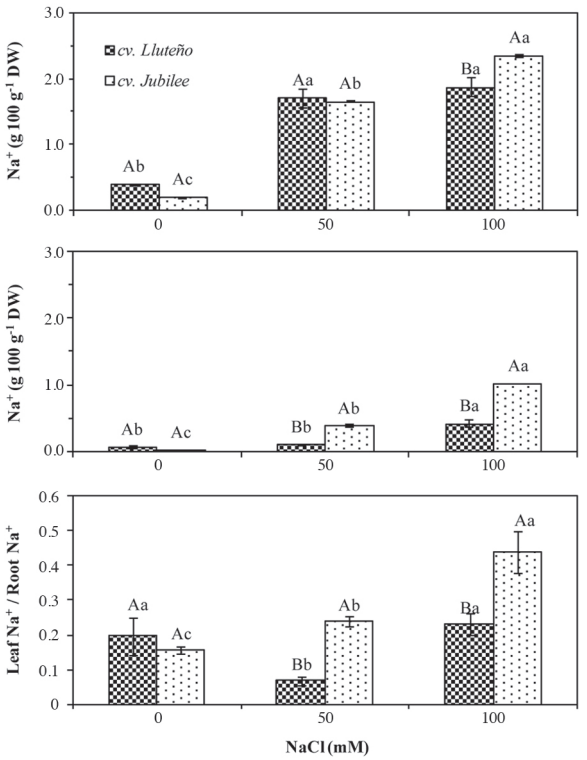
The levels of Na^+ were almost three times higher in roots than in leaves (Figure 1). The rising of NaCl in the solution allowed the Na content to increase in roots and shoots of both corn cultivars. When 'Lluteño' plants were subjected to 50 mM NaCl solution, only 0.11 g 100 g^{-1} DW reached the leaves. This amount increased to 0.43 g 100 g^{-1} DW when plants were in the 100 mM NaCl solution. In 'Jubilee' 0.39 g 100 g^{-1} DW reached the leaves when plants were in the 50 mM NaCl solution. This means that 'Jubilee' accumulated around four times the amount of Na^+ in leaves more than 'Lluteño' under similar conditions. To accumulate a similar amount of Na^+ in leaves, 'Lluteño' needed to be in a 100 mM NaCl solution for 15 d. 'Jubilee' accumulated 1.03 g 100 g^{-1} DW when plants were in 100 mM NaCl, which is almost the double of what 'Lluteño' accumulated in the same solution.

The Na^+ exclusion from leaves or the translocation of Na^+ from root to leaf (ratio of total leaf Na^+ content to the total root Na^+ content), was less in 'Lluteño' at both salinities (Figure 1).

Table 2. Dry matter accumulation in 'Lluteño' and 'Jubilee' corns after growing 15 d in different concentrations of NaCl.

NaCl concentration	Leaves	Stems	Roots	Total DM	Ratio roots:leaves	Total leaf area
mM	g plant^{-1}					cm^2 plant^{-1}
	'Lluteño'					
0	2.72Aa	1.81Aa	0.99Aa	5.53Aa	0.22Aa	68.75Aa
50	2.14Ab	1.28Aa	0.99Aa	4.40Ab	0.30Aa	58.12Aa
100	2.08Ab	1.23Aa	0.81Aa	4.12Ab	0.26Aa	47.66Aa
	'Jubilee'					
0	3.00Aa	1.23Ba	0.88Aa	5.12Aa	0.20Aa	62.67Aa
50	1.39Bb	0.64Bb	0.41Bb	2.44Bb	0.20Ba	46.84Ab
100	1.05Bb	0.46Bb	0.28Bb	1.80Bb	0.17Ba	30.79Ab

Values correspond to the average of five replicates ± standard error. Capital letters indicate comparisons between cultivars for the same treatment; lower case letters indicate comparisons between treatments of the same cultivar. Different letters denote significant differences among treatments ($P \leq 0.05$).



Values are the average of five replicates ± standard error. Capital letters indicate comparisons between cultivars for the same treatment; lower case letters indicate comparisons between treatments of the same cultivar. Different letters over the bars indicate differences according to Tukey test ($P \leq 0.05$).

Figure 1. Effects of different NaCl concentrations on the content of Na^+ in roots, leaf and on the leaf Na^+/root Na^+ ratio of corn 'Lluteño' and 'Jubilee' after 15 d of saline treatment.

The K^+ content in leaves and roots of 'Jubilee' decreased significantly as the concentration in NaCl increased (Figure 2). 'Lluteño' only showed a decrease of K^+ in roots. Both cultivars translocated K^+ mainly to the leaf.

Under saline conditions, the Ca^{2+} content in roots was similar to that of the non-saline conditions in both varieties (Figure 2). In leaves of 'Lluteño' the Ca^{2+} content tended to increase by 30% over the control value. By contrast, in 'Jubilee' no changes were observed in Ca^{2+} content in leaves due to salinity (Figure 2). Figure 2 shows that the salinity generated a decrease in P content in leaves and roots of both corn cultivars. Nevertheless, 'Jubilee' accumulated more P in leaf tissues than 'Lluteño' (Figure 2).

Effects of salinity on water condition of the plants

The salinity produced a significant decrease in Ψ_w in both cultivars. This decrease was also in both cases paralleled by the reduction in Ψ_s (Table 3). It must be emphasized that the irrigation system employed did not limit the entrance of water, thus the changes produced in the water condition of the plants are in this case exclusively due to the effect of the increased salinity. Salinity did not produce changes in the water content (WRC) in both corn cultivars. The leaf turgor pressure (Ψ_p) of 'Lluteño' did not show significant variation due to the salinity treatments; average values were around 0.75 MPa. Conversely, saline stress produced a significant reduction of Ψ_p of 'Jubilee' from -0.71 to -0.53 MPa. This means a reduction of 25.4% at 50 mM NaCl compared to the control and at 100 mM NaCl treatment, the reduction in Ψp from -0.71 to -0.46 MPa, was of 35.2% compared to the non-saline condition (Table 3).

The increase in salinity imposed in this experiment in both corn cultivars made OA; however, 'Lluteño' presented more significant adjustment.

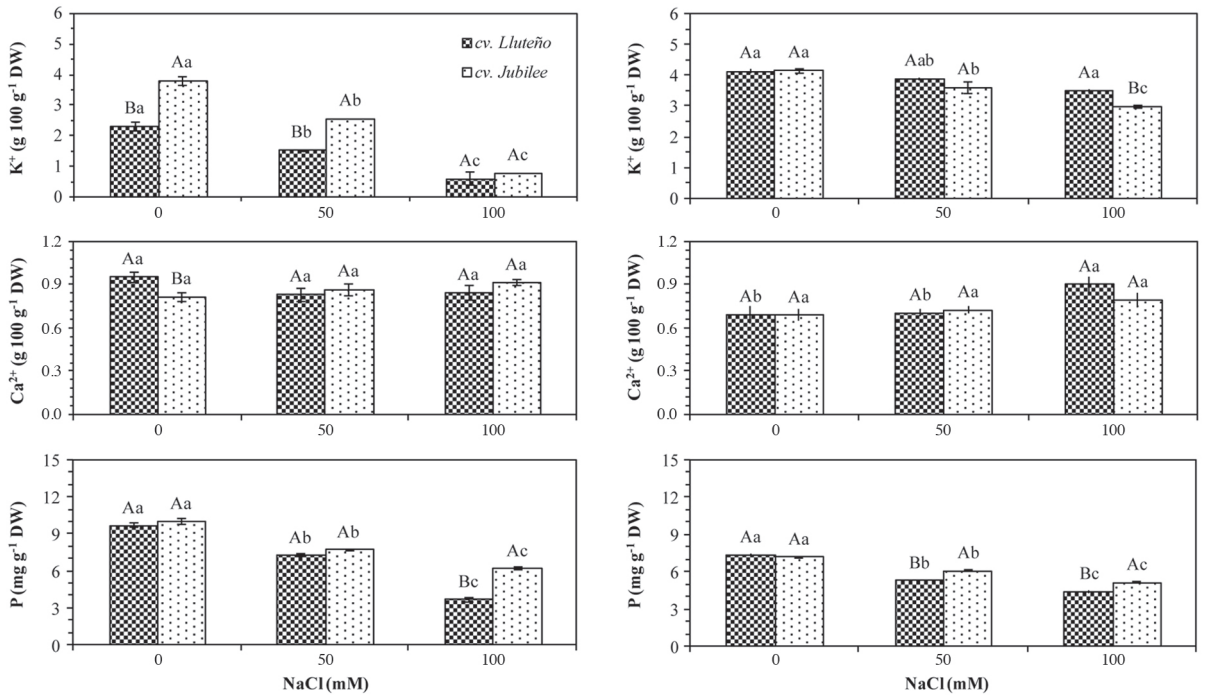
Effects on salinity on soluble sugar and proline content

In 'Jubilee' an increase in NaCl concentration did not induce any increase in the synthesis of the soluble sugar (Figure 3). But in 'Lluteño' the soluble sugar content increased from 72.40 mg g^{-1} DW in control to 99.48 mg g^{-1} DW when the plants were cultivated during 15 d in 50 mM NaCl. This means an increase of 37.4% with respect to the sugar content in non-saline conditions. However, when plants of 'Lluteño' were in 100 mM NaCl concentration for 15 d no changes in sugar content were detected. On the other hand, salinity increased significantly proline accumulation in 'Jubilee', whereas in 'Lluteño' the accumulation of this compound did not show any variation in all the treatments (Figure 3).

DISCUSSION

It is evident from the present study that 'Lluteño' appeared to be more tolerant than 'Jubilee'. In 'Lluteño', the lack of effect of the NaCl concentrations on the leaf area correlates well with the low reduction of the total DM accumulation and particularly with leaf DM production. This is not the case for 'Jubilee' which leaf area was reduced to a half after the plants were growing for 15 d in 100 mM NaCl. It is known that under saline conditions growth is initially inhibited by the osmotic effect of the salts, which limits the availability of water for the plant. Later on, necrotic symptoms were not generated by the toxic effects in the tissues due to the excessive accumulation of salts within the plant. Nevertheless, in high salinity we visually observed wilting and leaf rolling only in 'Jubilee' at the end of the treatment. Furthermore, it has been reported that most of the species reduce their DM accumulation by 50% when they are cultivated at a concentration of salts of 40 mM, which in turn generates an osmotic pressure of approximately 0.2 MPa (Munns and Tester, 2008).

The leaves and stems are the most sensitive plant organs to this osmotic effect, which has been observed in corn even in the absence of nutritional deficiencies and ionic toxicity (Hu et al., 2007). The growth of roots appears to be less affected by the salinity than the aerial tissues;



Values are the average of five replicates ± standard error. Capital letters indicate comparisons between cultivars for the same treatment; lower case letters indicate comparisons between treatments of the same cultivar. Different letters over the bars indicate differences according to Tukey test ($P \leq 0.05$).

Figure 2. Effects of different NaCl concentrations on the content of K^+, Ca^{2+} and P content in roots tissues (left) and leaf tissues (right) of corn plants 'Lluteño' and 'Jubilee' after 15 d of saline treatment.

this is probably due to changes which are generated in the properties of the cell wall of root cells. For example, in corn, root cells are capable of recovering their turgor after an osmotic shock of 0.7 MPa (150 mM NaCl); while leaves do not recover completely under such conditions (Frensh and Hsiao, 1994). However, the mechanisms involved in this response are still unknown (Munns and Tester, 2008). The growth inhibition produced by NaCl affected the growth of the entire plant of 'Jubilee' (Table 2), while in 'Lluteño' we only found a decrease in leaf growth; the DM accumulation in stems and roots did not vary significantly. One of the criteria that indicate tolerance to salinity is the maintenance of the development of the root system (Maggio and Joly, 1995). As the principal organ of absorption of water and ions, roots are very important in the short and long term responses to salt stress; both anatomical and morphological characteristics of the roots may affect the capacity of adaptation of plants to salinity (Maggio and Joly, 1995). We speculate that the reduction in root growth generated by the saline treatments would limit the capacity of 'Jubilee' plants to explore the soil and absorb water and nutrient. In contrast in 'Lluteño' no significant salinity effect in root growth was observed and was more dispersed and deeper. At 100 mM NaCl in 'Lluteño' the roots/aerial tissues ratio had greater values than in 'Jubilee' (Table 2).

Table 3. Effects of different NaCl concentrations on the leaf relative water content (RWC), water potential (Ψ_w), osmotic potential (Ψ_s), turgor potential (Ψ_p), osmotic potential at maximum turgor (Ψ_s^{100}) and the degree of osmotic adjustment ($OA = \Psi_s^{100s} - \Psi_s^{100c}$) of two corn cultivars: Lluteño and Jubilee.

NaCl concentration	WRC	Ψ_w	Ψ_s	Ψ_p	Ψ_s^{100}	OA
mM	%	MPa				
		'Lluteño'				
0	92.6Aa	-0.07Aa	-0.86Aa	-0.79Aa	-0.83Aa	-
50	91.9Aa	-0.31Ab	-1.08Ab	-0.77Aa	-1.03Bb	0.20Ab
100	91.6Aa	-0.52Ac	-1.22Ac	-0.70Aa	-1.17Bc	0.34Aa
		'Jubilee'				
0	91.6Aa	-0.20Ba	-0.91Aa	-0.71Aa	-0.87Aa	-
50	91.2Aa	-0.45Bb	-0.98Ab	-0.53Bb	-0.91Ab	0.04Bb
100	90.3Ba	-0.70Bc	-1.16Ab	-0.46Bb	-1.06Ab	0.19Aa

Values are the average of five replicates ± standard error. Capital letters indicate comparisons between cultivars for the same treatment; lower case letters indicate comparisons between treatments of the same cultivar. Different letters denote significant differences among treatments ($P \leq 0.05$).

If we consider the accumulation of Na^+ in leaves as an indicator of the transport rate of Na^+ in the plant, after this results is therefore possible to argue that under high saline conditions transport rate of Na^+ in 'Lluteño' is almost the half of that of 'Jubilee'. This certainly represents an enormous advantage for 'Lluteño' and can explain why it performs better under salty conditions. The capacity to transport Na^+ ions from the roots to the aerial tissues and the posterior inverse re-translocation is one of the characteristics found in species tolerant to saline stress (Zhu, 2002). It has been reported that corn cultivars with low concentrations of Na^+ in the leaf are more salt tolerant, suggesting that Na^+ exclusion may

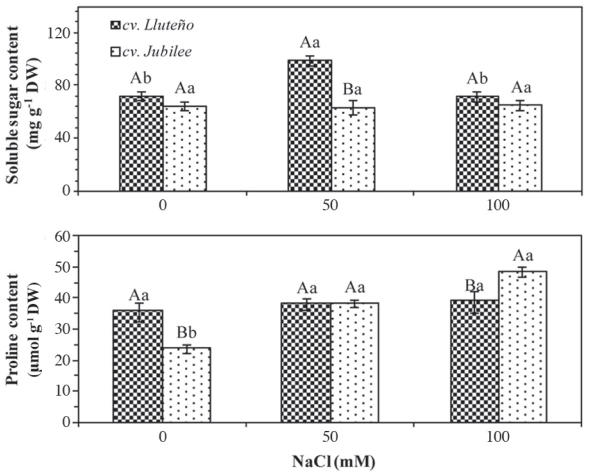


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Figure 3. Effects of different NaCl concentrations on the content of the soluble sugar content (left) and proline (right) of corn 'Lluteño' and 'Jubilee' after 15 d of treatment.

be positively correlated with salt tolerance (Fortmeier and Schubert, 1995). In this process of maintenance of ionic homeostasis, a number of protein transporters have been identified in loading and unloading of Na^+ ions in the xylem (Apse and Blumwald, 2002). One of these is the anti-transporter of Na^+/H^+ called SOS1 (*salt overly sensitive mutant 1*) first identified in *Arabidopsis*, which is preferentially expressed in vascular tissues, in the interphase between the xylem parenchyma and vessels and in the epidermis of little-differentiated tissues near the root meristem (Shi et al., 2002). SOS1 appears to function bi-directionally to control the Na^+ content in the xylem. In conditions of low salinity, SOS1 actively loads Na^+ to the xylem for its controlled translocation to the aerial tissues and accumulation in leaf mesophyll. When Na is in excess, SOS1 may expulse Na^+ to the medium from the root apex and re-absorb the Na^+ from the xylem of more differentiated tissues, thus delaying the transport of Na^+ to the aerial tissues through the transpiration current (Shi et al., 2002; Munns and Tester, 2008). Thus, the strong Na^+ exclusion from leaves of 'Lluteño' can be traced back to two different strategies: a low Na^+ uptake at the root surface and a low Na^+ translocation from root to leaves. Whereas the first trait could be inherited by 'Jubilee', both strategies are successfully combined in 'Lluteño' (Figure 1). The parameter that describes Na^+ exclusion from leaves or the translocation of Na^+ from root to leaf (ratio of total leaf Na^+ content to the total root Na^+ content), was less in 'Lluteño' at both salinities, which suggests that this cultivar is capable of decreasing the Na^+ content in the leaves and thus preventing the toxic effect of this ion (Figure 1).

The salinity diminished K^+ uptake by both cultivar of corn. This can be explained by the existing antagonism between Na^+ and K^+ (Azevedo and Tabosa, 2000). For example Cramer et al. (1985) showed that an excess of NaCl leads to the loss of K from roots due to the membrane depolarization caused by Na ions. The translocation of K^+ to the leaves in both cultivars would appear to be facilitated by the small ionic radius of this ion, which permits an easy translocation through membranes. Potassium is one of the cations with the greatest intercellular concentration and very active osmotically (Hu and Schmidhalter, 2005). In conditions of saline stress, an adequate level of K^+ in tissues will depend upon its selective entrance, distribution and compartmenting in leaves (Carden et al., 2003). Different K transporters perform these processes, such as the entrance channels of K^+ of low affinity (KORCs) and the voltage-independent cation canals (VICs), which play an important role in the cellular maintenance of K^+ (Amtmann and Sanders, 1998). These kinds of transporters may be involved in the maintenance of K^+ content observed in 'Lluteño', since the entrance of this nutrient was not inhibited by salinity. By contrast, the significant decrease in K observed in 'Jubilee' in saline conditions is probably due to an inhibition caused by Na^+ to the entrance of K^+ into roots and its posterior transport through the xylem.

Several studies have related the resistance to salinity of plant species to the accumulation of Ca^{2+} in the leaves (Unno et al., 2002). In saline conditions, the presence of Na^+ in the cell activates a complex system of signals which begins with the detection of this ion and increased in cytosolic free of Ca^{2+} (Hasegawa et al., 2000). This intracellular Ca^{2+} regulates the response of the plant to saline stress, participating in signaling mechanisms which include the expression of Ca^{2+}-dependent kinases and an essential role in osmoregulation (Urao et al., 1994; Nayyar, 2003). Thus the increase of Ca^{2+} observed in leaves of 'Lluteño' in 100 Mm NaCl may reduce the magnitude of the negative effect of salt stress on growth, facilitates the phosphorylation and thus the activation of the membrane bound Na^+/H^+ antiporter, SOS1 (Shi et al., 2002) it may also have a stabilizing effect on the membrane and maintenance of its selective capacity (Marschner, 1995). This is not the case for 'Jubilee' where the Ca^{2+} content did not change due to salinity.

The present study shows the reduction of P content in both cultivars of corn. The reduction of P content is often found in plant tissues under saline conditions. The P absorption from soil water is altered by the effect of the ionic force, which reduces the activity of P by the formation of Ca-P complexes which have a low solubility, impeding the entrance of P in the plant (Hu and Schmidhalter, 2005). Phosphorus is an essential component of molecules such as nucleic acids, phospholipids, phosphoproteins, and ATP. Thus a decrease in P observed in both cultivars of corn, may limit different metabolic processes such as

energy storage and transfer, photosynthesis, the regulation of some enzymes, carbohydrate transport and of course the maintenance of ionic homeostasis; processes which are dependent on ATP and ultimately on P.

The salinity caused a significant decrease in the water relation parameters Ψ_w, Ψ_s, in both cultivars. This trend was associated to a decrease of leaf water content and high accumulation of Na^+ and osmolytes in leaf tissues, suggesting the involvement of these solutes in osmotic adjustment. The synthesis of osmotically active compounds such as proline and soluble sugars is a strategy with high energy cost for plants, but it is necessary when the level of salinity increases; they may alter cellular metabolism (Serraj and Sinclair, 2002). In saline conditions, the organic compounds that are commonly accumulated in leaf tissues are proline and glycine betaine (Ashraf and Foolad, 2007). A wide range of other molecules such as saccharose, pinnitol, and mannitol may accumulate in leaf tissues to a lesser degree (Hasegawa et al., 2000). In this study, 'Lluteño' showed capacity for osmotic adjustment by synthesizing soluble sugars at intermediate salinity (50 mM NaCl). The increase of sugar observed in 'Lluteño' at 50 mM NaCl treatment can be explained due to the sucrose was initially metabolized to monosaccharides, which caused their content to rise. We speculated that, as respiratory substrates, the monosaccharides promote respiration and mitochondrial electron transport in this treatment. However, in the 100 mM treatment the sugar content was similar to the control conditions, probably due to the decreasing of the photosynthetic process or to accumulation of the fructan as reserve carbohydrates. On the other hand, salinity did not induce proline synthesis in this cultivar; its content in the control plants was almost 40% higher than in the control plants of 'Jubilee'. This means that proline is constitutively high in 'Lluteño' and further inductions of synthesis could not be necessary under salt stress. This could also mean an important economy of energy in this cultivar which in turn could favor its growth. In 'Jubilee' the situation seems to be different: in this cultivar salinity induced a high amount of proline that could represent a great energetic cost (Raven, 1985) and in turn a great effect in its vegetative growth. However, more exact calculations are necessary to link the proline synthesis to a lower accumulation of biomass in 'Jubilee'.

CONCLUSIONS

A notable distinction between the two tested cultivars was observed. 'Lluteño' showed to be more tolerant to salt stress that 'Jubilee' as indicated by increased growth and the maintaining of the leaf area. Moreover in saline conditions, 'Lluteño' is able to keep the contents of K^+, Ca^{2+} in leaves and accumulate osmolytes which contribute to maintain the relative water content in the leaves and enhanced the osmotic adjustment.

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