

## Mitigating effect of salicylic acid and nitrate on water relations and osmotic adjustment in maize, cv. Lluteño exposed to salinity

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<sup>2</sup>Laboratorio de Suelo, Agua y Planta, Facultad de Ciencias Agronómicas, Universidad de Chile, Santa Rosa 11315, La Pintana, Santiago, Chile.

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### Abstract

**H. Escobar, R. Bustos, F. Fernández, H. Cárcamo, H. Silva, N. Frank, and L. Cardemil. 2010. Mitigating effect of salicylic acid and nitrate on water relations and osmotic adjustment in maize, cv. Lluteño exposed to salinity. Cien. Inv. Agr. 37(3): 71-81.** We analyzed the mitigating effect of  $\text{NO}_3^-$  and salicylic acid (SA) on the detrimental effects of salt stress by studying the water status of plants of maize grown in Hoagland's medium with NaCl 100 mM as the saline component, to which SA and  $\text{NO}_3^-$  were added in different concentrations as mitigating agents. We evaluated water potential ( $\Psi_w$ ), osmotic potential ( $\Psi_s$ ), relative water content (RWC), turgor potential ( $\Psi_p$ ), and the osmotic adjustment (OA) of leaves and roots. SA 0.5 mM mitigated the effects of salinity by increasing the  $\Psi_w$  of the leaf, the  $\Psi_s$  of the root, the  $\Psi_p$  of the leaf, RWC and OA of the leaf; while  $\text{NO}_3^-$  was only effective in combination with SA, mitigating the effects of salinity by increasing RWC and OA. However, the interaction SA- $\text{NO}_3^-$  reduced leaf  $\Psi_w$  and  $\Psi_s$  of leaves and roots. Mitigation of salt stress was also detected by a positive effect on plant growth. The greatest effect on growth was produced by the  $\text{NO}_3^-$  treatments and SA 0.5 mM combined with  $\text{NO}_3^-$ .

**Key words:** nitrate, osmotic potential, osmotic adjustment, salinity mitigation, salicylic acid, water potential, water relative content.

### Introduction

Salinity may cause water stress in plants, which is first manifested as an osmotic stress and then as ionic toxicity, due mainly to an excess of  $\text{Na}^+$  and  $\text{Cl}^-$  in the tissues. Plants may also have a

nutritional deficiency due to the competition of  $\text{Na}^+$  y  $\text{Cl}^-$  for the ionic nutrient transporters in the external zone of the roots.

Maize, cv. Lluteño, is the main cultivated species in the Lluta Valley, and the most widely cultivated crop in terms of area in the desert of northern Chile. It is especially interesting due to its high tolerance to extreme conditions of salt stress and the excess of boron in the irrigation water. The

main drawback with this cultivar is its low yield, which fluctuates between 12.000 and 20.000 ears/ha with a planting density of 30.000-40.000 plants/ha, what means less than one ear per plant. This low yield may be due to an excessive absorption of toxic ions such as boron, and to high concentrations of sodium and chlorine in the irrigation water (Bastías, 2005). In the Lluta valley the water has concentrations of  $\text{Na}^+$  from 194 to 480 ppm,  $\text{Cl}^-$  from 397 to 900 ppm and B from 11.7 to 28.7 ppm (Sotomayor *et al.*, 1995). However, the concentration of these ions should not be higher than 186, 200 and 0.75 ppm, respectively, to avoid toxic effects on crops, as has been reported by the Chilean Instituto Nacional de Normalización (1987). The toxicity induced by NaCl may be exacerbated by a deficient water absorption generated by the saline stress of the environment. This salinity can decrease the relative water content (RWC) and cause cell dehydration (Hasegawa *et al.*, 2000; Ortiz *et al.*, 2003; Chartzoulakis, 2005). Water stress may activate molecular signals to counteract the physiological damage of stress, such as the synthesis of abscisic acid (ABA) causing closure of the stomata to avoid water loss. The closure of stomata, however, decreases  $\text{CO}_2$  assimilation by plants; this might be a cause of the low yield of maize cv. Luteño (Sharp *et al.*, 1993; Wahbi *et al.*, 2005; Centritto *et al.*, 2005).

Some plants confront salinity by osmotic adjustments to absorb and retain water while maintaining cell turgor (Serraj and Sinclair, 2002; Silva *et al.*, 2007) by means of the accumulation of compatible solutes and osmoregulators (Hasegawa *et al.*, 2000; Chinnusamy *et al.*, 2005; Munns y Tester, 2008).

Due to its biological and physiological actions, SA has been considered as a plant hormone (Canet *et al.*, 2010). As in the case of other plant hormones, SA may act as a plant regulator and signal messenger in plants under stress conditions (Harfouchea, 2008). SA activates defense mechanisms in pathogenicity and tolerance mechanisms to counteract different environmental stress conditions, such as ozone increase, low and high temperatures, salinity, anaerobiosis, etc. (Cakmak, 2003; Sawada *et al.*, 2006; Shi y Zhu, 2008).

The application of SA to cereal plants appears to decrease the concentrations  $\text{Na}^+$ ,  $\text{Cl}^-$  and B in plant tissues and significantly improves the nitrogen absorption of these plants when there is high salinity associated with boron (Shakirova *et al.*, 2003; Gunes *et al.*, 2005). However, the signals induced by SA to counteract saline stress of plants are unknown (Gunes *et al.*, 2005; Gunes *et al.*, 2007).

In glycophytic plants the lack of nitrogen produces severe consequences in the synthesis of proteins, nucleic acids, lipids and amino acids. Nitrogen deficiency also induces the synthesis of compatible solutes in plants to perform osmotic adjustments (Huber and Kaiser, 1996; Viégas and Gomes da Silveira, 2002). The decrease in  $\text{NO}_3^-$  is correlated with a high absorption of  $\text{Cl}^-$ . However, the application of  $\text{NO}_3^-$  in the soil compensates the decrease of N in leaves caused by an excess  $\text{Cl}^-$  (Tabatabaei, 2006). Salinity may affect nitrogen uptake by a direct competition between  $\text{Cl}^-$  and  $\text{NO}_3^-$  ions of the  $\text{NO}_3^-$  transport system (Pessarakli *et al.*, 1989; Campbell y Kinghorn, 1990) and/or by alteration of the plasmalemma by affecting the integrity of the proteins of this membrane (Cramer *et al.*, 1985).

Since SA seems to improve nitrogen absorption and nitrogen stimulates plant growth by synthesis of the fundamental biomolecules and reduces water stress by stimulating the synthesis of compatible solutes, it is necessary to test the combined effects of SA and  $\text{NO}_3^-$  in the induced salinity tolerance of maize, cv. Luteño. The objective of this study was to evaluate the combined mitigating effect of SA and  $\text{NO}_3^-$  on the detrimental effects cause by salinity on the maize plants. If there is an alleviating effect on salinity stress induced by SA different from that induced by  $\text{NO}_3^-$  the combined presence of SA with  $\text{NO}_3^-$  will increase the mitigation induced by SA or by  $\text{NO}_3^-$  separately, suggesting two interacting routes of transduction signals.

To evaluate this hypothesis, the water status of the plants was determined (water and osmotic potentials, relative water content (RWC), pressure potential (turgor potential), and the osmotic adjustment (OA). For this, experiments

were performed with 28 days old maize plants, grown in pots and irrigated with Hoagland's medium to which 100 mM NaCl was added. For mitigation of the stress effects caused by salinity, SA,  $\text{NO}_3^-$  and combinations of SA and  $\text{NO}_3^-$  were added to the Hoagland's medium supplemented with 100 mM NaCl (Acevedo *et al.*, 1998, Munns and Tester, 2008).

## Materials and methods

### *Growth conditions and experimental design*

The experiment was performed with plants of *Zea mays* L., cv. Lluteño, in a greenhouse with natural light, mean maximum temperature 27.3° C, mean minimum 11.4° C, PAR 359.8  $\mu\text{mol}/\text{m}^2 \text{ s}^{-1}$  and relative humidity 50%-80% (day-night). Plants were established in 15 L pots with a Perlite substrate. Three seeds were planted in each pot. After 10 days, one of the three seedlings of each pot was selected to obtain plants with a uniform size for all the experimental groups; the other

two were removed from the pot. During the first 28 days plants were irrigated with 100% Hoagland's solution, pH 6-7 (Hoagland and Arnon, 1950). The plants were watered every two days with one liter of Hoagland's solution per pot when the substrate reached a humidity of 30% of the field capacity (FC) (Fuentes, 2003). To avoid the accumulation of nutrients and salts in the substrate, every third irrigation the substrate was washed with distilled water until the electrical conductivity of the substrate was less than that of the Hoagland's solution. After 28 days the experimental treatments with NaCl,  $\text{NO}_3^-$  and SA began. All these chemical compounds were added to the Hoagland's medium (Gunes *et al.*, 2007). Treatments are indicated in Table 1; there were 9 treatments with 5 repetitions using 5 plants per treatment. Treatments were continued for 58 days; measurements started after 30 days of treatment. The parameters determined included water potential ( $\Psi_w$ ), osmotic potential ( $\Psi_s$ ), relative water content (RWC), turgor potential and osmotic adjustment (OA).

**Table 1.** Experimental Treatments. In the experiments there were 5 plants for treatment. Plants were grown in individual pots and irrigated with Hoagland's medium for 28 days. After this time the experimental treatments began.

Treatment group	Treatments
T1	Control (Hoagland's solution only)
T2	Hoagland's solution + 100 mM NaCl (HS100)
T3	HS100 + 6 mM $\text{NO}_3^-$
T4	HS100 + 0.1 mM SA
T5	HS100 + 0.5 mM SA
T6	HS100 + 1.0 mM SA
T7	HS100 + 0.1 mM SA + 6 mM $\text{NO}_3^-$
T8	HS100 + 0.5 mM SA + 6 mM $\text{NO}_3^-$
T9	HS100 + 1.0 mM SA + 6 mM $\text{NO}_3^-$

### *Measurement of water relations*

The water potential ( $\Psi_w$ ), the osmotic potential ( $\Psi_s$ ) and the relative water content (RWC) were measured at 9:00 in the sixth complete-

ly expanded leaf. At the same time, the root osmotic potential ( $\Psi_s$ ) was measured. The reported results are the mean of two values measured two days apart, each measurement performed 16 hours after watering.

Leaf  $\Psi_w$  was measured with a pressure bomb (PMS Model 600, USA) according to Scholander *et al.* (1965). The osmotic potential of leaves and roots was measured in tissue sections which were frozen at  $-20^\circ\text{C}$  for 2 hrs and then macerated and centrifuged at 13,200 g for 5 min to extract the cell sap. Osmolality was measured in an osmometer (Roebbling Messtechnik D-14129) using 100  $\mu\text{L}$  of sap in an Eppendorf tube calibrated with distilled water. Van't Hoff's equation was used to calculate the osmotic potential ( $\Psi_s$ ) of the solution (Nobel, 1991):

$$\Psi_s = -C R T \quad [1]$$

C = Concentration of the solution, expressed as molality.

R = Universal gas constant, 0.083 kg bar mol<sup>-1</sup> K<sup>-1</sup>.

T = Absolute temperature in degrees Kelvin (298 °K).

RWC is expressed as:

$$\text{RWC} = 100 \times (\text{fresh weight} - \text{dry weight}) / (\text{turgid weight} - \text{dry weight}) \quad [2]$$

The turgor potential of leaves ( $\Psi_p$ ) was estimated as the difference between the water potential ( $\Psi_w$ ) and osmotic potential ( $\Psi_s$ ):

$$\Psi_p = \Psi_w - \Psi_s \quad [3]$$

The leaf osmotic adjustment (OA) was obtained using the value of  $\Psi_s$  at maximum turgidity ( $\Psi_s^{100}$ ), which was estimated as the product of the values of  $\Psi_s$  and RWC (Irigoyen *et al.*, 1996):

$$\Psi_s^{100} = (\Psi_s \times \text{RWC}) / 100 \quad [4]$$

OA was then calculated as the difference between the values of the osmotic potential at maximum turgidity of the plants treated with salts ( $\Psi_s^{100s}$ ) and the control plants ( $\Psi_s^{100c}$ ). The water condition of the substrate must be optimum for this measurement, to eliminate the possibility of plant dehydration due to a deficiency of irrigation that could mask the effect of the treatment.

$$\text{OA} = (\Psi_s^{100c} - \Psi_s^{100s}) \quad [5]$$

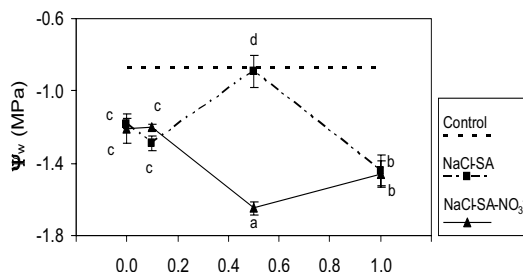
### Design and statistical analysis

A completely randomized experimental design was established with nine treatments and five replicates for the measurements of the plant water relations parameters. The results obtained were subject to an analysis of variance (ANOVA) and the means were compared according to Tukey's test ( $P \leq 0.05$ ).

### Results

#### Water Potential ( $\Psi_w$ )

Water potential decreased after treatment with NaCl. 0.5 mM SA increased the water potential to a similar value to that of the control without salinity, annulling the osmotic effect of NaCl. However, its interaction with  $\text{NO}_3^-$  decreased the water potential significantly, as concentrations of SA- $\text{NO}_3^-$  increased. Concentrations inferior or superior to 0.5 mM were not effective in reverting  $\Psi_w$  (Figure 1).

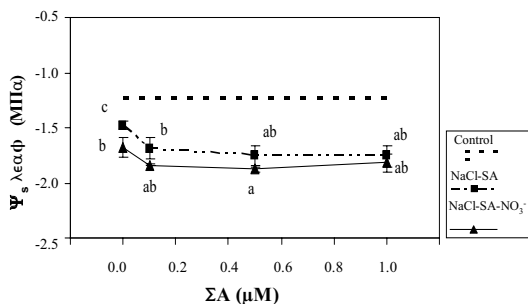


**Figure 1.** Mitigating effects of SA and SA with 6 mM  $\text{NO}_3^-$  on the leaf  $\Psi_w$  of plants of maize, cv. Llutefño. The determinations were performed 30 days after treatment. Each dot corresponds to five independent determinations with their SD (vertical bars). Different letters represent significant differences among treatments (Tukey test,  $P \leq 0.05$ ).

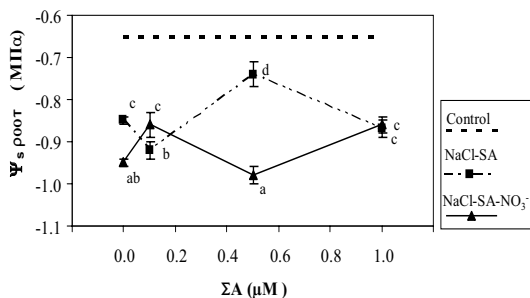
#### Osmotic Potential ( $\Psi_s$ ) of leaves and roots

Treatment with 100 mM NaCl caused a decrease in  $\Psi_s$  in both leaves and roots; the decrease was

greater in the leaves (Figures 2 and 3). In leaves, the  $\Psi_s$  of the treatments with SA and SA- $\text{NO}_3^-$  decreased more than the NaCl treatment. In roots, the treatment with 0.5 mM SA produced a  $\Psi_s$  greater than that of the NaCl treatment and close to the value of the control. The responses of osmotic potential to the treatments were similar to those of the water potential.



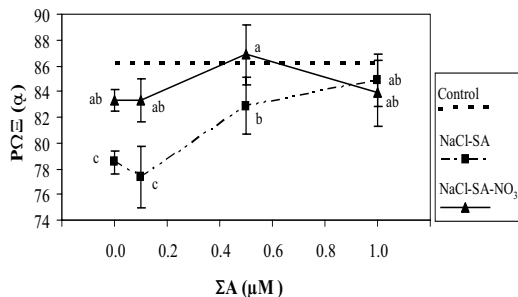
**Figure 2.** Mitigating effects of SA and SA with 6 mM  $\text{NO}_3^-$  on the leaf  $\Psi_s$  of plants of maize, cv. Llueteño. The determinations were performed 30 days after treatment. Each dot corresponds to five independent determinations with their SD (vertical bars). Different letters represent significant differences among treatments (Tukey test,  $P \leq 0.05$ ).



**Figure 3.** Mitigating effects of SA and SA with 6 mM  $\text{NO}_3^-$  on the root  $\Psi_s$  of plants of maize, cv. Llueteño. The determinations were performed 30 days after treatment. Each dot corresponds to five independent determinations with their SD (vertical bars). Different letters represent significant differences among treatments (Tukey test,  $P \leq 0.05$ ).

*Relative water content (RWC)*

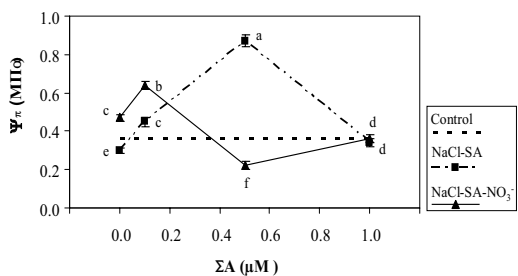
The application of NaCl caused a significant decrease in RWC. The treatments with  $\text{NO}_3^-$ , 0.5 mM SA- $\text{NO}_3^-$  and 1.0 mM SA counteracted the effect of NaCl, returning the RWC to the value of the control plants without salinity (Figure 4). Although 0.5 mM SA mitigated the effect of 100 mM NaCl, it did not return RWC to the level of the control.



**Figure 4.** Mitigating effects of SA and SA with 6 mM  $\text{NO}_3^-$  on the leaf RWC of plants of maize, cv. The determinations were performed 30 days after treatment. Each dot corresponds to five independent determinations with their SD (vertical bars). Different letters represent significant differences among treatments (Tukey test,  $P \leq 0.05$ ).

*Turgor potential ( $\Psi_p$ )*

Turgor potential was significantly affected by the treatment with 100 mM NaCl. Four of the treatments mitigated the effect of salinity:  $\text{NO}_3^-$ , 0.1 mM SA, 0.1 mM SA- $\text{NO}_3^-$  and 0.5 mM SA; these all produced a turgor potential greater than that of the control without salt (Figure 5). The greatest positive effect was produced by 0.5 mM SA, however, when combined with  $\text{NO}_3^-$  it produced a greater decrease in turgor than that produced by NaCl. The  $\Psi_p$  of the treatment with 0.5 mM SA- $\text{NO}_3^-$  was significantly different from the control without salt; however, the differences between turgor values are small. The three treatments with greatest growth (Table 2) (control,  $\text{NO}_3^-$  and 0.5 mM SA- $\text{NO}_3^-$ ) had very similar turgor values.



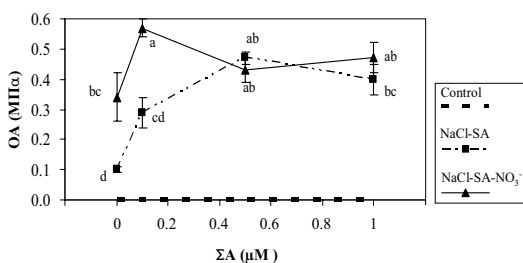
**Figure 5.** Mitigating effects of SA and SA with 6 mM  $\text{NO}_3^-$  on the leaf  $\Psi_p$  of plants of maize, cv. Llueteño. The determinations were performed 30 days after treatment. Each dot corresponds to five independent determinations with their SD (vertical bars). Different letters represent significant differences among treatments (Tukey test,  $P \leq 0.05$ ).

**Table 2.** Mitigating effects of SA and  $\text{NO}_3^-$  on plant growth. The table shows the plant height, total leaf area, foliage fresh weight and root fresh weight as % of control plants (plants grown in Hoagland solution). The figures correspond to five different determinations with their SD. Different letters represent significant differences among treatments (Tukey test,  $P \leq 0.05$ ).

Treatment	Plant growth (% control)			
	Plant height	Total leaf area	Foliage fresh weight	Root fresh weight
1 Control	100.0 ± 5.8 a	100.0 ± 13.2 a	100.0 ± 4.0 a	100.0 ± 9.6 b
2 NaCl	46.9 ± 3.1 c	36.9 ± 5.9 d	36.8 ± 1.2 d	71.1 ± 11.4 cd
3 NaCl- $\text{NO}_3^-$	98.3 ± 5.8 a	67.5 ± 4.4 b	97.0 ± 6.3 a	147.1 ± 13.9 a
4 NaCl-0.1 SA	25.4 ± 0.4 d	19.4 ± 3.7 e	15.7 ± 0.7 e	10.4 ± 1.0 e
5 NaCl-0.5 SA	52.8 ± 5.6 c	47.8 ± 7.0 cd	44.5 ± 0.7 c	80.2 ± 5.2 c
6 NaCl-1.0 SA	18.2 ± 0.3 d	16.9 ± 3.4 e	14.1 ± 0.8 e	15.0 ± 3.1 e
7 NaCl-0.1 SA- $\text{NO}_3^-$	47.0 ± 3.5 c	35.7 ± 4.1 d	39.7 ± 2.8 cd	59.5 ± 4.0 d
8 NaCl-0.5 SA- $\text{NO}_3^-$	59.9 ± 2.5 b	57.6 ± 7.7 bc	65.2 ± 6.0 b	96.3 ± 5.8 b
9 NaCl-1.0 SA- $\text{NO}_3^-$	24.4 ± 0.9 d	15.9 ± 2.5 e	13.9 ± 1.2 e	15.1 ± 1.7 e

### Osmotic adjustment (OA)

OA was lower in the treatment with NaCl 100 mM. All the treatments with SA and  $\text{NO}_3^-$  increased the osmotic adjustment significantly above the level of the NaCl treatment. The most efficient conditions of mitigation and increase of OA were found in the treatments with all the combinations SA- $\text{NO}_3^-$  and with 0.5 mM SA (Figure 6).



**Figure 6.** Mitigating effects of SA and SA with 6 mM  $\text{NO}_3^-$  on the leaf OA of plants of maize, cv. Llueteño. The determinations were performed 30 days after treatment. Each dot corresponds to five independent determinations with their SD (vertical bars). Different letters represent significant differences among treatments (Tukey test,  $P \leq 0.05$ ).

### Discussion

NaCl 100 mM caused a significant decrease in the water relation parameters RWC,  $\Psi_w$ , leaf  $\Psi_s$ , root  $\Psi_s$ ,  $\Psi_p$  and OA in maize cv. Llueteño. Our results demonstrate that this decrease may be reverted with an appropriate concentration of SA interacting with 6 mM  $\text{NO}_3^-$  applied in the irrigation solution. The mitigating effect of 0.5 mM SA on the effects of salinity was shown by increases in leaf RWC, leaf  $\Psi_w$ , root  $\Psi_s$ , leaf  $\Psi_p$  and leaf OA, compared to the treatment with NaCl. The addition of both compounds might favor water absorption and plant growth, and therefore also have a mitigating effect. Thus growth, measured by plant height, leaf area and fresh weight of greenery and of roots was greater in the treatment with 0.5 mM SA- $\text{NO}_3^-$ , in spite of the decrease in the values of  $\Psi_w$  in leaves and  $\Psi_s$  in leaves and roots.



### *Water potential ( $\Psi_w$ )*

It is known that SA with  $\text{NO}_3^-$  reduces  $\Psi_w$  (Song *et al.*, 2006, Szepesi *et al.*, 2009). The magnitude of this reduction will depend on how they are applied, their concentrations, and the plant species (Hayat *et al.*, 2008). In the case of maize cv. Llueteño, concentrations lower than 0.5 mM SA were inefficient, while greater concentrations were supraoptimal. This reinforces the idea that SA acts as a hormonal factor with a specific optimum concentration.

In contrast to the action of 0.5 mM SA, its combination with 6 mM  $\text{NO}_3^-$  caused a decrease in leaf  $\Psi_w$  and root  $\Psi_s$ . A number of authors (Wahbi *et al.*, 2005; Centritto *et al.*, 2005) have suggested that this decrease favors the absorption of water under saline conditions, and thus this treatment is positive in terms of producing greater growth. Nevertheless, the significant differences between the  $\Psi_w$  of the leaves and the  $\Psi_s$  of the roots favored growth in plants with 0.5 mM SA, with or without  $\text{NO}_3^-$ .

### *Osmotic potential ( $\Psi_s$ ) of leaves and roots*

Osmotic potential decreased significantly in plants treated with NaCl, which has also been shown for many other species that grow in saline conditions (Çiçek and Çakırlar, 2002; Wahbi *et al.*, 2005, Carillo *et al.*, 2008). As in the case of  $\Psi_w$ ,  $\Psi_s$  decreased in plants treated with 0.5 mM SA- $\text{NO}_3^-$ , while 0.5 mM SA returned the  $\Psi_s$  of the roots to the values of control plants. However, in the leaf 0.5 mM SA did not have this effect. 0.5 mM SA alone and in combination with  $\text{NO}_3^-$  increased the concentrations of sugars in maize cv. Llueteño (unpublished results), which are osmolytes, favorable for the retention of water in the cell. This retention of water due to increase in sugars may explain the greater growth of plants subjected to these treatments.

### *Relative water content (RWC)*

The decrease in the  $\Psi_w$  of the plant, caused by salinity, produced a reduction in  $\Psi_s$ , which resulted in a reduction in RWC in leaves of the plants of

maize cv. Llueteño. These effects of salinity have been reported for other species (Çiçek and Çakırlar, 2002; Chartzoulakis, 2005). The high concentration of salt retains water in the substrate, which would imply less water absorption by the roots, aggravated by a loss of water through the roots (Burgess and Bleby, 2006). The consequence of this water loss is a lower RWC. SA and  $\text{NO}_3^-$  revert these adverse effects of salinity, possibly by means of an osmotic regulation at the level of the leaf and root (Song *et al.*, 2006). This reversion of the RWC appears to indicate that these mitigating agents favor the entrance of water in the roots and/or avoid water loss by the roots (Carvajal *et al.*, 1999; Hasegawa *et al.*, 2000; Zhu, 2001; Martinez-Ballesta *et al.*, 2006; Burgess and Bleby, 2006). The increase in RWC caused by SA was directly related to its concentration in the experimental range used, supporting the idea that SA may be considered as a hormone. However, its molecular role is unknown (Gunes *et al.*, 2005).  $\text{NO}_3^-$  has been considered an osmotic regulator due to its ability to replace other solutes, especially in halophytic plants (Veen and Kleinendorst, 1986; Song *et al.*, 2006). If  $\text{NO}_3^-$  is an osmotic regulator, it will diminish the negative effects caused by the entrance of NaCl and will facilitate water transport by the roots, increasing water absorption as well as providing a nutritional effect (McIntyre *et al.*, 1996; Song *et al.*, 2006). It is interesting to note that in halophytic plants a greater salt concentration induces the expression of aquaporin genes, allowing water to enter the plant (Qi *et al.*, 2009). This may also be the case for  $\text{NO}_3^-$ ; it might induce the expression of aquaporin genes of maize cv. Llueteño as salinity does for halophytic plants (Qi *et al.*, 2009).

Because the RWC increased significantly in the treatments with 0.5 mM SA,  $\text{NO}_3^-$  and with 0.5 mM SA- $\text{NO}_3^-$  compared to the NaCl treatment, the greater growth observed is due to the recovery of the RWC. The reversion of the RWC in plants by these treatments suggests that the mitigation is produced by root water absorption. It may be that these treatments (SA,  $\text{NO}_3^-$ , and SA 0.5 mM- $\text{NO}_3^-$ ) activate the expression of aquaporins in the plasmalemma of the root and leaves, as it occurs with salt in halophytic plants (Qi *et al.*, 2009).

### *Turgor potential ( $\Psi_p$ )*

According to Hasegawa *et al.* (2000), a plant cell exposed to a saline medium equilibrates its water potential by decreasing cell water, which causes a decrease in  $\Psi_p$ . We observed this effect in maize cv. Lluteño only in the treatments Table 1 with NaCl and 0.5 mM SA-NO<sub>3</sub><sup>-</sup>. The treatments with NO<sub>3</sub><sup>-</sup>, 0.1 mM SA-NO<sub>3</sub><sup>-</sup>, 0.1 mM SA and 0.5 mM SA caused an increase in turgor. In contrast, treatments with 1.0 mM SA with or without NO<sub>3</sub><sup>-</sup> did not cause variation from control values.

Therefore, the mitigating action of 0.5 mM SA is not only due the increase of  $\Psi_w$  and  $\Psi_s$ , but also because it increases  $\Psi_p$ . The greater turgor induced by SA 0.5 mM was 241.7% of the control value, which may explain the reversion of growth to 50% of the control. The reversion of root growth was even more notable, reaching 80% of the control value. We may speculate that this greater root growth could be induced by an increase in ABA in the root, also induced by SA 0.5 mM (Sharp *et al.*, 1993; Szepesi *et al.*, 2009). The lowest turgor was observed in the treatment 0.5 mM SA-NO<sub>3</sub><sup>-</sup> (61.1 % of the control), which was even lower than the NaCl treatment (83% of control). However, greater growth was produced when 0.5 mM SA interacted with NO<sub>3</sub><sup>-</sup>.

### *Leaf osmotic adjustment (OA)*

100 mM NaCl decreased the leaf OA of maize, cv. Lluteño. All treatments which included SA and SA-NO<sub>3</sub><sup>-</sup> reverted the OA, possibly due to an increase in the osmolyte concentration in the vacuoles. If this is the case, the increase of osmolytes would cause the cell to increase the flow of water towards the vacuole, which would increase its volume without losing water. The result would be an increase in  $\Psi_p$ , and plant growth (Parida and Das, 2005). OA may also be

produced by the participation of other organic solutes as well as sugars, by which plants may also recover their  $\Psi_w$  and  $\Psi_p$  (Hasegawa *et al.*, 2000; De Costa *et al.*, 2007). However, in our experiments 0.1 mM SA and NO<sub>3</sub><sup>-</sup> increased OA less than other treatments did. In summary, the rest of the treatments produced a highly significant effect on osmotic regulation of maize, cv. Lluteño, and their mitigating effects led to a recovery of water in the cell.

In summary, our results of determinations of water relations in plants of maize cv. Lluteño treated with 100 mM NaCl lead us to conclude that: 1) SA is a good mitigator of the effects of salt stress at a concentration of 0.5 mM. 2) Treatment with 0.5 mM SA in combination with 6 mM NO<sub>3</sub><sup>-</sup> is a better treatment than with only one of them reverting the negative effect of NaCl on growth. 3) The reversion of the deteriorating effects of NaCl by these mitigants implies the reversion of  $\Psi_p$  due to the increase of OA, which induces the uptake of water and plant growth.

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### Resumen

**H. Escobar, R. Bustos, F. Fernández, H. Cárcamo, H. Silva, N. Frank y L. Cardemil. 2010. Efecto mitigante del ácido salicílico y nitrato en las relaciones hídricas y ajuste osmótico en maíz, cv. Lluteño expuesto a salinidad. Cien. Inv. Agr. 37(3): 71-81.** Se evaluó el efecto mitigante de  $\text{NO}_3^-$  y AS sobre el deterioro fisiológico inducido por salinidad en plantas de maíz crecidas en solución Hoagland con 100 mM de NaCl. La evaluación se realizó mediante determinaciones del potencial hídrico ( $\Psi_w$ ), potencial osmótico ( $\Psi_s$ ), contenido relativo de agua (RWC), potencial de turgor ( $\Psi_p$ ) y el ajuste osmótico (AO). A la solución de Hoagland con 100 mM de NaCl se adicionó AS,  $\text{NO}_3^-$  y combinaciones de diferentes concentraciones de ambos mitigadores. AS 0.5 mM puede mitigar el efecto de la salinidad incrementando el  $\Psi_w$  de la hoja,  $\Psi_s$  de la raíz, el  $\Psi_p$  de la hoja, el RWC y el AO de la hoja. El  $\text{NO}_3^-$  6 mM solo y la interacción AS- $\text{NO}_3^-$  6 mM puede mitigar el efecto salino incrementando el RWC y el AO. Sin embargo, la interacción AS- $\text{NO}_3^-$  6 mM disminuye el  $\Psi_w$  de la hoja y el  $\Psi_s$  de hojas y raíces. La mitigación del estrés salino puede ser detectada, también, por un efecto positivo en el crecimiento de la planta. El mayor efecto en el crecimiento fue obtenido cuando las plantas fueron tratadas con  $\text{NO}_3^-$  6 mM y con AS 0.5 mM combinado con  $\text{NO}_3^-$  6 mM.

**Palabras claves:** Ácido salicílico, mitigación de salinidad, nitrato, RWC, potencial hídrico, potencial osmótico, potencial de turgencia, ajuste osmótico.

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