# Effect of short-term salinity on lipid metabolism and ion accumulation in tomato roots

G. RACAGNI[1]*, H. PEDRANZANI*, S. ALEMANO*, E. TALEISNIK**, G. ABDALA* and E. MACHADO-DOMENECH*

*Facultad de Ciencias Exactas, U.N.R.C, 5800-Río Cuarto, Argentina**
*Instituto de Fitopatología y Fisiología Vegetal, INTA, 5119-Córdoba, Argentina***

## Abstract

To examine the ion accumulation and membrane lipid metabolism in response to salinity we compared two tomato cvs. Pera and Hellfrucht Fruhstamm (HF), considered to be salt-tolerant and sensitive respectively. $Na^+$ and $K^+$ accumulation was significantly higher in roots of cv. Pera after 24 h and 72 h of 100 mM NaCl. While in cv. HF, a temporary increase in $K^+$ accumulation at 24 h was accompanied by a sustained increase in $Na^+$ content. Both cultivars enhanced incorporation of [$^{32}$P]orthophosphate into phosphatidylinositol 4,5-bisphosphate at 24 h and 72 h of NaCl. In parallel to the increase of phosphatidylinositol 4,5-bisphosphate a decrease in phosphorylation of phosphatidic acid and phosphatidylcholine were observed in the sensitive cv. HF. Structural and signal lipid changes in response to salinity were more evident in the sensitive cv. HF. Salt tolerant cv. Pera accumulated $Na^+$ ions in the roots without considerable modifications in lipid metabolism.

*Additional key words*: Lycopersicon esculentum, salt stress, phospholipids, sodium, potassium.

## Introduction

Tomato is a widely distributed and consumed annual vegetable. It is adapted to a wide variety of climates, but production is concentrated in warm and rather dry areas, where soil salinity often represents a serious constraint for productivity (Cuartero and Fernández-Muñoz 1999). Much effort has been invested in selecting and improving plants with increased salt tolerance in this species. Understanding the physiological basis of salt tolerance can point to metabolic sequences that have relevant roles in salt tolerance.

Ion regulation is important in determining salt sensitivity (Munns 1993). Excess $Na^+$ and $Cl^-$, the predominant ions in saline soils, create ionic imbalances that may impair selectivity of root membranes (Bohra and Dörffling 1993). Potassium is an important nutrient in many physiological processes, and the maintenance of high $K^+$ cytosolic levels is essential for plant survival in saline habitats (Chow et al. 1990). Saline conditions can reduce $K^+$ uptake by plants (Chow et al. 1990, Romero et al. 1994, Botella et al. 1997).

Membranes are important for regulating cell ion content; changes in plasma membrane composition can affect membrane permeability, fluidity and also the activity of transporters. Membrane lipids are precursors for the synthesis of compounds that have intracellular or long-range signaling activities. The metabolic pathways of glycerophospholipid biosynthesis in plants have been studied (Munnik et al. 1998a).

Phosphatidylinositols, frequently referred to as phophoinositides, have important functions in signal transduction pathways and could serve as second messengers to extracellular signals. It has been shown

that increased synthesis of phosphatidylinositol 4,5 bisphosphate (PIP$_2$) and diacylglycerol pyrophosphate (DGPP), as well as increased turnover of phosphatidylcholine (PC), may be involved in the response to high salinity in plant cells (Pical *et al.* 1999). However, the effect of hyperosmotic stress on phosphoinositide turnover varies significantly among plant species. In *Arabidopsis thaliana*, the expression of one phosphatidylinositol 4P kinase (PIP-k) gene (Mikami *et al.* 1998) and one phospholipase C (PI-PLC) gene

(Hirayama *et al.* 1995) increased by salt stress, suggesting that phosphoinosites may participate in the response to high salinity.

In the present study we analyzed Na$^+$ and K$^+$ accumulation and membrane lipid phosphorylation in response to salinity in two tomato cultivars differing in their salt sensitivity. We also studied the phosphorylation of minor lipid-precursors in order to assess the rate of lipid signal molecules production.

## Materials and methods

Tomato (*Lycopersicon esculentum* Mill.) cultivars Pera (salt tolerant) and Hellfrucht Fruhstamm (HF, salt sensitive) were grown hydroponically in half-strength Hoagland solution. Plants with 5 - 6 leaves were exposed to salt stress by addition of 100 mM NaCl to the culture medium. The control plants were not exposed to NaCl treatment. At 6, 24, and 72 h after continuous salinization, control and treated roots of both cultivars were harvested and processed for ion accumulation and phospholipid analysis. Na$^+$ and K$^+$ ions were measured in triplicate by atomic emission spectrometry using a *Shimadzu ICPS 1000* spectrometer (Tokyo, Japan). Statistical analysis of data was performed using General Linear Model of *SAS* (Statistical Analysis System) and Duncan's multiple range test.

Lipid analysis was performed in three independent experiments; each sample consisted of roots excised from four plants. Roots were incubated at 25 °C with [$^{32}$P]orthophosphate [(1.1 × 10$^4$ Bq mg$^{-1}$(tissue)] in 50 mM sodium cacodylate (pH 6.5) for 18 h. The tissue

was then filtered, washed and processed. Total lipids were extracted according to Bligh and Dyer (1959). The extract was washed with neutral upper-phase chloroform : methanol : 0.1 M KCl (3:48:47, v/v), dried under a stream of nitrogen and redisolved in chloroform : methanol (9:1, v/v) (Racagni *et al.* 1992). Phospholipids were separated by thin layer chromatography (TLC). The samples were spotted on silica gel plates impregnated with potassium oxalate solution and heated at 110 °C for 60 min just before use. The chromatoplate was developed with either chloroform : methanol : acetone : acetic acid : water (40:14:15:12:7, v/v) or chloroform : methanol : 25 % amonium hydroxide (90:70:20, v/v). Chromatograms were exposed to iodine vapour and inorganic phosphorous content was quantified as described Racagni *et al.* (1992) after mineralization of the sample with perchloric acid at 180 °C. The position of radiolabeled lipids was determined by autoradiography on a 3M ray film. Spots were scraped off the plates, and fractions were counted in a liquid scintillation counter.

## Results

The effect of NaCl on Na$^+$ and K$^+$ accumulation in both cultivars showed different patterns (Table 1). In cv. Pera a significant Na$^+$ and K$^+$ accumulation after 24 h and 72 h of salinization was observed. The most significant difference in the cv. HF was the transient K$^+$ concentration increase at 6 h and 24 h. The increment of Na$^+$ showed a similar pattern as that found for cv. Pera, but after 72 h accumulation was lower.

Under control conditions, total lipid phosphorus was 80 ± 8.4 µg(Pi) g$^{-1}$(f.m.) and 110 ± 15.5 µg(Pi) g$^{-1}$(f.m.), in cv. Pera and HF, respectively. After 24 h the value decreased to 80 ± 11.0 µg(Pi) g$^{-1}$(f.m.) tissue in cv. HF, while in cv. Pera it remained unchanged.

Radioactivity analysis of each phospholipid (in steady state) showed that [$^{32}$P]Pi was mainly incorporated into phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and phosphatidic acid (PA) (Fig. 1). PE appeared as a major band comprising approximately 63 - 65 % of the

total labeled phospholipids. The phosphoinositides phosphatidylinositol 4,5-bisphosphate (PIP$_2$), phosphatidylinositol 4-monophosphate (PIP) and phosphatidylinositol (PI) were minor constituents, as in other

Table 1. Na$^+$ and K$^+$ accumulation [mmol g$^{-1}$(d.m.)] in roots of tomato cultivars at different duration of NaCl treatment (* - differences significant at $P \leq 0.05$; Duncan's multiple range test).

| NaCl | cv. Pera Na$^+$ | K$^+$ | cv. HF Na$^+$ | K$^+$ |
|---|---|---|---|---|
| Control | 1.4 ± 0.26 | 10.0 ± 0.14 | 1.2 ± 0.04 | 10.3 ± 1.10 |
| 6 h | 3.6 ± 0.63 | 9.4 ± 0.90 | 4.9 ± 0.73* | 13.7 ± 1.15* |
| 24 h | 6.5 ± 0.29* | 14.0 ± 2.11* | 6.5 ± 1.95* | 28.5 ± 1.15* |
| 72 h | 24.7 ± 1.14* | 15.7 ± 1.60* | 9.1 ± 0.65* | 10.5 ± 0.52 |

eukaryotic cells (Irvine *et al.* 1989). PI did not separate from lyso-phosphatidylethanolamine (LPE) with the developing solvent used in this case, and comprised approximately 4 - 6 % of the total labeled phospholipids in all the samples analyzed. Salinized plants of cv. HF showed a significant, yet temporary increase in [$^{32}$P] PI/LPE after 24 h of salt treatment (data not shown).

The 6 h salt treatment did not produce modifications

## Discussion

In this species, salinity increased Na$^+$ concentration in shoots as well as in roots (Cuartero and Fernández-Muñoz 1999); thus, in the present study the characteristic accumulation of this ion in response to salinity was observed for both cultivars. However, sodium accumulation rate in the tolerant cv. Pera roots from 24 h was higher than in the sensitive cv. HF; this fact is consistent with reports that cv. Pera is considered to be an ion accumulator (Pérez-Alfocea *et al.* 1993) and this response is similar to that found for wild tomato cultivars.
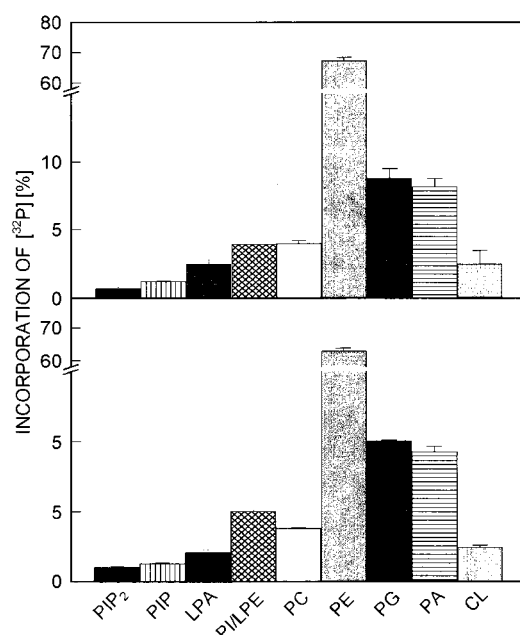


Fig. 1. Root phospholipid pattern of *Lycopersicon esculentum*, cv. Pera (*above*) and cv. HF (*below*) control plants. Mean ± SE from 3 independent experiments. Roots were incubated with [$^{32}$P]Pi for 18 h at 25 °C. Phospholipids were resolved by TLC and the radioactivity was determined (see Materials and methods).

Unlike the report of Cuartero and Fernández-Muñoz (1999), who sustain that K$^+$ concentration in roots of salinized-tomato plants showed less variations than those cultivated under salinity, our results indicate that K$^+$ was mainly accumulated in the roots of cv. HF during the first hours of salinization, although at 72 h the difference between both cultivars was highly significant for

in the phospholipid pattern of either cultivar. A decrease in PA content was significant only for the cv. HF at 72 h of the salt treatment. PIP$_2$ showed a significant increase at 24 h and 72 h representing a 70 % increase for the cv. Pera and 39 % for cv. HF. Another modification observed at 24 h and 72 h was a decrease in the label of PC in cv. HF (Fig. 2). Nevertheless, [$^{32}$P] incorporation into lipids showed earlier and more significant changes in cv. HF.

cv. Pera. The augmented level of K$^+$ in the roots of both cultivars might be associated with a mechanism to counter the toxic effect of the salt (Cuartero *et al.* 1992). In general, K$^+$ slightly diminished by the salt effect although not all the genotypes responded in the same way. For example in the cv. Edkawy the K$^+$ concentration remained unchange (Cruz *et al.* 1990) and in *L. pennellii* it slightly increased (Bolarín *et al.* 1995).
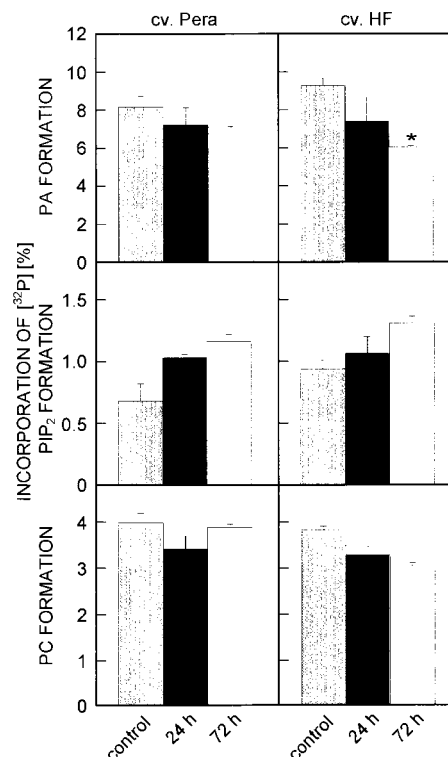


Fig. 2. [$^{32}$P]PA, [$^{32}$P]PIP$_2$ and [$^{32}$P]PC formation in *Lycopersicon esculentum* roots, cv. Pera and cv. HF in the presence of NaCl at 24 h and 72 h of salinization. Others specifications as in figure legend 1. * - the difference statistically significant at $P < 0.05$; Duncan's multiple range test.

Oscillations in lipid phosphorous were observed in cv. HF: at 24 h a decrease of total lipid phosphorous was observed which was counter-balanced at 72 h. These changes suggest an increased metabolism of the membrane compounds. This effect may reflect changes in

phospholipid synthesis as a response to salt stress in the sensitive cv. HF.

Our results from control and treated roots of both cultivars showed a high labeling of PE, which may indicate that this lipid was formed from rapidly labeled precursors, suggesting entry of the phosphate first into PA by phosphorylation of diacylglycerol (DAG). Phosphatidylserine (PS), another PE precursor, was not identified in our system suggesting that the decarboxylase that forms PS might not be present in the tomato roots. Hence, the heavily labeled lipids, PE, PG and PA, may form one metabolic sequence.

Likewise, DAG may also be a precursor to other lipids such as phosphatidycholine (PC), which is synthesized from cytidyl-diphospho-choline (CDP-choline). Since PC decreased in treated roots only in cv. HF, the data suggest that enzymatic activity in the biosynthesis pathway may have decreased in response to salt treatment. Our results are in agreement with Wu *et al.* (1998) who, working with *Spartina patens*, a well-known halophyte, reported that the content of PC and PE showed a decrease in salt-treated plants, but the PC/PE ratios were not affected. Another possible explanation for the change in PC could be an increased phospholipase D (PLD) activity. If this were the case, the adaptation process could also involve a change in the signaling pathway. PLD has been identified as an important signaling enzyme that produces both PA and choline (Munnik *et al.* 1998a,b). This activity was considered to play a role in mediating the salt stress process (Munnik *et al.* 2000).

The observed increase in PIP$_2$ and decrease in PA could be related to the activation of phospholipases such as PLC and PLD, or, alternatively, changes in phosphatidylinositol 4-monophosphate kinase and phosphatidate phosphatase.

In *Arabidopsis thaliana* suspension-cultured cells, NaCl treatments induced dramatic increases in the levels of PIP$_2$, DGPP and also affected PC turnover. PIP$_2$ increase was also observed during non-ionic hyperosmotic shock produced by sorbitol (Pical *et al.* 1999). Nevertheless, the change in PIP$_2$ pool in tomato roots may indicate that PIP kinase is relatively more active under salt stress than PLC.

Considering the complexity of signal transduction mechanisms, several speculations may be formulated to explain the response found in tomato roots.

External signal activation may imply modifications of PIP$_2$ levels, regardless of a stimulated PLC activity. PIP$_2$-specific PLC has been established to play a signal transduction role in osmoregulation in some plant cell types (Einspahr and Thompson 1990, Coté *et al.* 1996). Generally, an increase of PA turnover parallels this stimulation. Under our assay conditions, the salt treatment induced an increase in the $^{32}$P labeled of PIP$_2$ but it was accompained by a decrease of [$^{32}$P]PA. Possibly, it was the salt stress that induced a more significant response in tomato roots of the sensitive cv. HF with biphasic changes in phospholipase activation. In such a case, NaCl may have induced an activation of PLC parallel to an elevated PA turnover before 24 h. Afterwards, PA was rapidly metabolized and its levels decreased. Thus, in the second phase, PIP$_2$ may be involved in other processes. In this respect, it has been reported that osmotic stress induced long term changes in polyphosphoinositide metabolism (Chapman 1998). In addition, it is involved in the recovery of plant cells from hyperosmotic stress (Cho *et al.* 1993).

Changes in the pattern of [$^{32}$Pi] incorporation and Na$^+$ accumulation in response to salinity were more pronounced in the salt-tolerant Pera than in the sensitive HF. Thus, salt tolerance was associated with the capacity to accumulate Na$^+$ ions in the roots of cv. Pera without considerable changes in lipid metabolism.

## References

Bligh, E.G., Dyer, W.J.: A rapid method of total lipid extraction and purification. - Can. J. Biochem. Physiol. **37**: 911-918, 1959.

Bohra, J.S., Dörffling, K.: Potassium nutrition of rice (*Oryza sativa* L.) varieties under NaCl salinity. - Plant Soil **152**: 299-303, 1993.

Bolarín, M., San Cruz, A., Cayuela, E., Perez-Alfocea, F.: Short term solute changes in leaves and roots of cultivated and wild tomato seedling under salinity. - Plant Physiol. **147**: 463-468, 1995.

Botella, M.A., Martínez, V., Pardines, J., Cerda, A.: Salinity induced potassium deficiency in maize plants. - J. Plant Physiol. **150**: 200-205, 1997.

Chapman, K.D.: Phospholipase activity during plant growth and development and in response to environmental stress. - Trends Plant Sci. **3**: 419-426, 1998.

Cho, M.H., Shears, S.B., Boss, W.F.: Changes in phosphatydilinositol metabolism in response to hyper-

osmotic stress in *Daucus carota* L. cells grown in suspension culture. - Plant Physiol. **103**: 637-647, 1993.

Chow, W.S., Ball, M.C., Anderson, J.M.: Growth and photosynthetic responses of spinach to salinity: Implications of K$^+$ nutrition for salt tolerance. - Aust. J. Plant Physiol. **17**: 563-578, 1990.

Coté, G.G., Yueh, Y.G., Crain, R.C.: Phosphoinositide turnover and its role in plant signal transduction. - In: Biswas, B.B., Biswas, S. (ed.): Subcellular Biochemistry. Volume 26. Myo-Inositol Phosphate, Phosphoinositides and Signal Transduction. Pp. 317-343. Plenum Press, New York 1996.

Cruz, V., Cuartero, J., Bolarín, M.C., Romero, M.: Evaluation of characters for ascertaining salt stress responses in *Lycopersicon* species. - J. amer. Soc. hort. Sci. **115**: 1000-1003, 1990.

Cuartero, J., Fernández-Muñoz, R.: Tomato and salinity. - Sci. Hort. **78**: 83-125, 1999.

Cuartero, J., Yeo, A.R., Flowers, T.J.: Selection of donors for salt-tolerance in tomato using physiological traits. - New Phytol. **121**: 63-69, 1992.

Einspahr, K.J., Thompson, G.A., Jr.: Transmembrane signaling via phosphastidylinositol 4,5-bisphosphate hydrolysis in plants. - Plant Physiol. **93**: 361-366, 1990.

Hirayama, T., Ohto, C., Mizoguchi, T., Shinozaki, K.: A gene encoding a phosphatidylinositol-specific phospholipase C is induced by dehydration and salt stress in *Arabidopsis thaliana*. - Proc. nat. Acad. Sci. USA **92**: 3903-3907, 1995.

Irvine, R.F., Letcher, A.J., Lander, D.J., Drøback, B.K., Dawson, A.P., Musgrave, A.: Phosphatidylinositol (4,5)bisphosphate and phosphatidylinositol (4)phosphate in plant tissues. - Plant Physiol. **89**: 888-892, 1989.

Mikami, K., Katagiri, T., Iuchi, S., Yamaguchi-Shinozaki, K., Shinozaki K.: A gene encoding phosphatidylinositol-4-phosphate 5-kinase is induced by water stress and abscisic acid in *Arabidopsis thaliana*. - Plant J. **15**: 563-568, 1998.

Munnik, T., Irvine, R.F., Musgrave, A.: Phospholipid signalling in plants. - Biochim. biophys. Acta **1389**: 222-272, 1998a.

Munnik, T., Meijer, H.J.G., Ter Riet, B., Frank, W., Bartels, D., Musgrave, A.: Hyperosmotic stress stimulates phospholipase D activity and elevates the levels of phosphatidic acid and diacylglycerol pyrophosphate. - Plant J. **22**: 1-8, 2000.

Munnik, T., Van Himbergen, J.A.J., Ter Riet, B., Braun, F., Irvine, R.F., Van den Ende, H., Musgrave, A.: Detailed analysis of the turnover of polyphosphoinositides and phosphatidic acid upon activation of phospholipases C and D in *Chlamydomonas* cells treated with non-permeabilizing concentrations of mastoparan. - Planta **207**: 133-145, 1998b.

Munns, R.: Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. - Plant Cell Environ. **16**: 15-24, 1993.

Pérez Alfocea, F., Estañ, M.T., Caro, M., Bolarín, M.C.: Response of tomato cultivars to salinity. - Plant Soil **150**: 203-211, 1993.

Pical, C., Westergren, T., Dove, S.K., Larsson, C., Sommarin, M.: Salinity and hyperosmotic stress induce rapid increases in phosphatidylinositol 4,5-biphosphate, diacylglycerol pyrophosphate, and phosphatidylcholine in *Arabidopsis thaliana* cells. - J. biol. Chem. **274**: 38232-38240, 1999.

Racagni, G., García de Lema, M., Domenech, C., Machado de Domenech, E.E.: Phospholipids in *Trypanosoma cruzi*: Phosphoinositide composition and turnover. - Lipids **27**: 275-278, 1992.

Romero, J.M., Maranon, T., Murillo, J.M.: Long-term responses of *Melilotus segetalis* to salinity. 2. Nutrient absorption and utilization. - Plant Cell Environ. **17**: 1249-1255, 1994.

Wu, J.L., Seliskar, D.M., Gallagher, J.L.: Stress tolerance in the marsh plane *Spartina patens*: impact of the NaCl on growth and root plasma membrane lipid composition. - Physiol. Plant. **102**: 307-317, 1998.