

# Influence of salinity on transport of Nitrates and Potassium by means of the xylem sap content between roots and shoots in young tomato plants

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## Abstract

Salinity is well known to reduce plant growth and yield by reducing water availability; it does so by interfering with both nutrient uptake and translocation. The objective was to determine the nitrate and potassium contents in xylem sap and the root-shoot transportation of both as a function of the salinity of the nutritional solution provided. We compared NO<sub>3</sub><sup>-</sup> and K<sup>+</sup> contents and flux in xylem sap collected from cut stems of tomato seedlings, based on electric conductivity (EC) tests among five nutrition solutions for soilless crops ranging from medium to high salinity. The EC was 2.2, 3.5, 4.5, 6 and 12 dS m<sup>-1</sup>. The concentration of nitrates and potassium in the xylem sap remained constant, while the external concentration in the rhizosphere varied greatly. Notwithstanding, the xylematic flux was strongly affected by the salinity of the nutritional solution: at maximum salinity, EC reached 3.5 dS m<sup>-1</sup>; at minimum, EC was 12 dS m<sup>-1</sup>. For similar reasons, the longest NO<sub>3</sub><sup>-</sup> and K<sup>+</sup> transportation distance between root and shoot was achieved when the EC read 3.5 dS m<sup>-1</sup>, but was reduced by up to 80% when EC was 12 dS m<sup>-1</sup>.

**Keywords:** Exudate, root, salinity, sap, Xylem sap flow

## 1. Introduction

From very old to the present, the salinity is one of the most serious environmental stresses limiting growth and yield of horticultural plants (Rameeh, 2012; Mastrogiannidou *et al.*, 2016); it does so by interfering with both nutrient uptake and translocation to the shoot (Perez-Alfocea *et al.*, 2000).

Xylem sap content analysis through stem exudate has been widely known for some time (Vaadia, 1960), used for decades (e.g. Ferrario *et al.*, 1992;

Urrestarazu *et al.*, 1996b; Kato *et al.*, 2001; Masuda *et al.*, 2001; Rep *et al.*, 2003; Yamasaki, 2003) and still today (Ariga *et al.*, 2014) for herbaceous plants. For these plants, their daily variations have been studied (e.g. Wallace *et al.*, 1966; Schurr and Schulze, 1995; Urrestarazu *et al.*, 1996a, 1996b), as well as the effect of salinity on their ionic composition (Perez-Alfocea *et al.*, 2000).

Root-to-shoot signaling via xylem sap has been reported as an important mechanism by which plants respond to stress (Fernández-García *et al.*, 2011). Therefore, the flow rate and chemical composition of xylem sap have been reported as indicators of root activity or root health under field conditions (e.g. Engels *et al.*, 2000; Morita *et al.*, 2000; Yamasaki, 2003).

Among the anions present in the xylematic flow, nitrate is of those in highest concentrations. Additionally, nitrates are usually the most representative of nitrogen compounds circulating through the xylem sap from root to shoot (Goto *et al.*, 1987). In terms of majority cations in xylem sap, K<sup>+</sup> is present; however, there is not much information on its concentration or variation in different environmental conditions.

There is little information on the effect of salinity on nitrate and potassium contents in xylem sap.

The objective was to determine the nitrate and potassium contents in xylem sap and the root-shoot transportation of both as a function of the salinity of the nutritional solution provided.

## 2. Materials and Methods

### 2.1. Plant growth and treatments

Tomato seeds (cv. Gold Era) were sown January 1st, 2015, with seedling trays filled with Pelemix® coconut fiber as described by Morales and Urrestarazu (2013) with an alveolar volume of 50 cm<sup>3</sup>, and were given a solution similar to that described by Sonneveld and Straver (1994) in order to establish an EC of 2.2 dS m<sup>-1</sup> as the control group.

Once the seedlings had reached a state with 4 true leaves, the treatments were applied. These treatments consisted of fertigation of nutritional solutions for which their electrical conductivities were measured at 2.2, 3.5, 4.5, 6 and 12 dS m<sup>-1</sup>. These EC increments in

the solution were achieved by means of a mother solution with macronutrient concentrates, while the proportions of micronutrients remained constant through further mixing. The pH of the nutritional solutions also remained at a level of 5.8 with the addition of diluted nitric acid.

### 2.2. Collection of xylem sap

The xylem sap was obtained after cutting incisions in the leaves of the cotyledons that had been eliminated. The stalks were incised following a procedure similar to the one described in Masuda *et al.* (2001) and in Gil de Carrasco *et al.* (1994). The incision on the stalk was performed at noon, according to the criteria set by Urrestarazu *et al.* (1996a, 1996b).

A silicone tube was fixed over the stump of mesocotyl, and the exudate was removed by means of a microaspirator. The volume was measured with a calibrated micro-syringe. pH and EC were measured using a pH-meter and an EC-meter with microelectrode, respectively. The xylem exudate was centrifuged for 10 min at 3000 g, and the floating solution was recovered and stored in 1.5 mL Eppendorf tubes and frozen until analysis.

### 2.3. Ion analysis

For ion analysis, NO<sub>3</sub><sup>-</sup> and K<sup>+</sup> were diluted and measured by ionic chromatography, and injected into a Dionex-2000i/sp with AS12 anionic and CS12 cationic columns, respectively, as described by Gil de Carrasco *et al.* (1994).

### 2.4. Statistical analysis

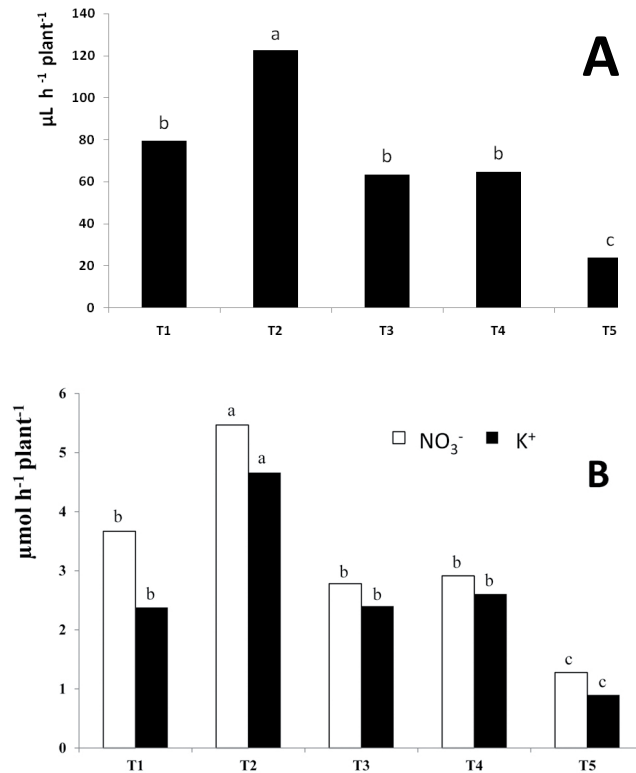
The experimental design was performed by means of a random system of complete plots, with four plots per condition. Each block contained 15 plants.

Statistical analyses were conducted with Statistical Products and Service Solutions Software for Windows, and the software packages used were Statgraphics Centurion® 16.06.15 and Microsoft Office 2010. Data were analyzed with analysis of variance (ANOVA), and differences between the means were tested using Tukey's test ( $P \leq 0.05$ ).

### 3. Results and Discussion

#### 3.1. Xylematic Flow, Xylem sap EC and pH

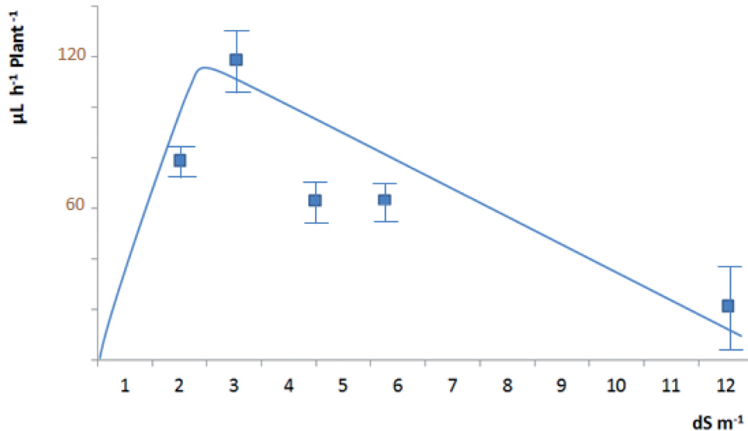
A highly significant effect on xylematic flow was detected due to the increment of salinity in the nutritional solution (Figure 1A).



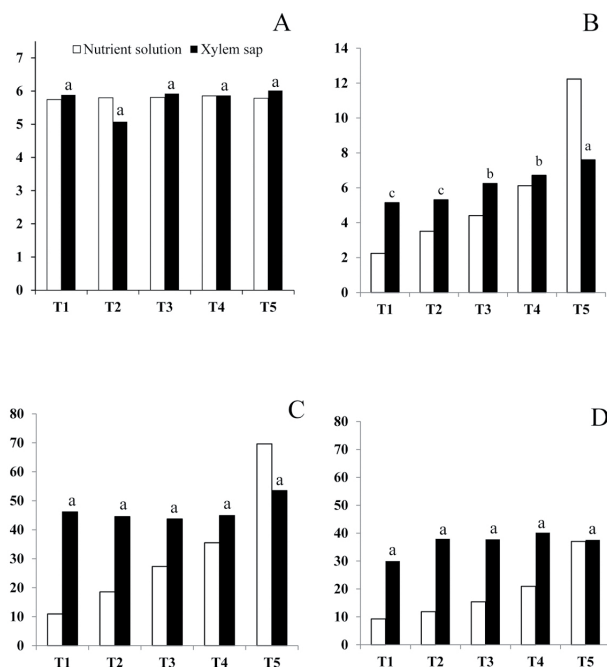
**Figure 1.** Xylem sap volume (A), nitrate and potassium flux (B) as a function of the salinity of the nutritional solution supply. T1, T2, T3, T4, and T5 are treatments of EC for 2.2, 3.5, 4.5, 6.0 and 12.0  $\text{dS m}^{-1}$ , respectively. Different letters indicate significant differences at  $P \leq 0.05$  (Tukey test).

The maximal flow was  $123 \mu\text{L h}^{-1} \text{plant}^{-1}$  for T2, which is 80% more volume over the plot with the highest EC (T5), and 50% more than the rest of the conditions (T1, T3, and T4). The xylematic flow data coincide with those reported by White *et al.* (1981) and Urrestarazu *et al.* (1996a, 1996b). The behavior of an optimal flow for a determined EC range, with a minimum threshold and maxima above and below, coincides with the model proposed by Sonneveld and Vooght (2009), which was in turn derived from the classical Maas and Hoffman (1977) model for crop yield in relation to the EC of the nutritional solution or rhizosphere (Figure 2). The xylem sap pH was shown to have no significant differences among the groups, rather hovering between 5.08 and 6.02 (Figure 3A). Similar exudate pH values from young tomato plants have been reported from between 5.56 and 6.13 (e.g. Gil de Carrasco *et al.*, 1994; Else *et al.*,

1995; Urrestarazu *et al.*, 1996a), although higher values, from 6.45 to 6.50, have been reported for nighttime extracts (White *et al.*, 1981), we found similar values in all cases for the different saline treatments tested, probably to maintain the appropriate values for xylem sap, as it is well known—for example—that metal solubility, dissociation constants, hydrolysis reactions and metal binding in general are all pH-dependent (White *et al.*, 1981). The EC of the xylem saps varied from 5.2 to  $7.6 \text{ dS m}^{-1}$  (Figure 3B); the highest values were obtained from group T5, and the minimum, from T1 and T2. Although the EC of the nutritional solution increased by  $9.8 \text{ dS m}^{-1}$ , the xylem sap itself only increased by  $3.6 \text{ dS m}^{-1}$ . As such, the EC in the plants remained much more stable than that of the existing rhizosphere variation, which implies a significant level of metabolic regulation for EC in xylem sap, described from very old since the decade of the 80s (White *et al.*, 1981).



**Figure 2.** Xylematic flux for tomato seedlings, adjusted to the model from Sonneveld and Voogt (2009) for production as a function of electrical conductivity of the nutritional solution.



**Figure 3.** Values for pH (A), EC (dS m<sup>-1</sup>) (B), and NO<sub>3</sub><sup>-</sup> (C) and K<sup>+</sup> (D) content (mmol L<sup>-1</sup>) in xylem sap flux as a function of the salinity of the nutritional solution supplied. T1, T2, T3, T4, and T5 are the treatment of EC for 2.2, 3.5, 4.5, 6.0 and 12.0 dS m<sup>-1</sup>, respectively. Different letters indicate significant differences at  $P \leq 0.05$  (Tukey test).

### 3.2. Xylem sap NO<sub>3</sub><sup>-</sup> and K<sup>+</sup>

Although the NO<sub>3</sub><sup>-</sup> concentration was increased progressively to almost seven times the original value in the nutritional solution (Figure 3C), the NO<sub>3</sub><sup>-</sup> concentration in the xylem sap remained constant at around 50 mM; indeed, there were no significant differences between any of the groups. Masuda (1989) and Masuda and Shimada (1993) had already reported similar concentrations for nitrates found in sap although the external concentration was much higher, and Goto *et al.* (1987) also concluded that, with and without additional external fertilization for groundnut plant, the

nitrate concentration in xylem sap remained the same. On the other hand, Bialczyk *et al.* (2004) found that the concentration of nitrates and ammonium is dependent on the proportion of these in the cultivation media.

The K<sup>+</sup> concentration in the xylem sap was between 30 and 40 mM (Figure 3D), again without statistically significant differences. Masuda (1989) and Perez-Alfocea *et al.* (2000) reported a somewhat lesser potassium level (20 mM). The tendency for potassium was similar to that of NO<sub>3</sub><sup>-</sup>, and remained constant independently of increases in the rhizosphere; reflecting this, similar reports from the works of Perez-Alfocea *et al.* (2000) found K<sup>+</sup> concentration stability by

increased exposure time to greater salinity. As for our case, the  $K^+$  concentration stability in the xylem sap was achieved by the incrementally increased saline concentration with all the macronutrients.

### 3.3. Long way transport

Considering the average concentration of  $K^+$  and  $NO_3^-$  nutrients, and the rate of xylem sap flow, Figure 1B shows the transport between root and shoot. There is a similar tendency between both ions being transported. Although the concentration of both ions in the xylem sap is constant, flow is the predominant factor; and since flow is highly variable as a function of the EC of the nutrition solution, the final result is highly significant, wide variation in the root-shoot ascendant transport. Treatment T2 significantly transported 56% more  $NO_3^-$  and  $K^+$  did treatments T1, T3, or T4. The most saline treatment, T5, reduced the transportation of these ions by up to 50% with respects to T1, T3, and T4. This implies that the reduction of transport of these elements as much as  $NO_3^-$  a long distance from the root to shoot is mainly due to a decrease in volume of xylem flow. This work is studied the major cation and anion macronutrient (Potassium and Nitrate), but probably this general roles can be extrapolate to other macronutrient under its ionic nutrient fertilizer like Calcium, Phosphate or Sulfate inside xylem transport. This behavior is showing the same as that modeled by Sonneveld and Voogt (2009) for the correlation between EC and yield.

## 4. Conclusions

The concentration of nitrates and potassium in xylem sap remained constant, while the external rhizosphere concentration varied greatly in terms of EC, between 2.2 and 12.0 dS  $m^{-1}$ . Nevertheless, since the xylematic flow was strongly affected by this incremental EC

in the nutritional solution, long distance root-shoot transportation of these ions increased significantly, with a gap of up to 80% of both  $NO_3^-$  and  $K^+$  for EC from 3.5 to 12 dS  $m^{-1}$ .

## Acknowledgements

The authors gratefully acknowledge the support of Mexican National Council for Science and Technology (CONACYT) for its financial support for this work.

The authors gratefully acknowledge the support of CONICYT through Project FONDECYT INITIATION INTO RESEARCH 2014 n° 11140154.

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