

Calcium Can Prevent Toxic Effects of Na⁺ on Tomato Leaf Photosynthesis but Does Not Restore Growth

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ABSTRACT. The availability of good-quality irrigation water is decreasing worldwide, and salinity is an increasingly important agricultural problem. To determine whether detrimental effects of NaCl on plant growth and leaf physiology can be minimized by additional Ca²⁺ supply, tomato (*Solanum lycopersicum* L.) ‘Supersweet 100’ was grown hydroponically. The basic nutrient solution contained 11.1 mM NO₃⁻ and 2.8 mM Ca²⁺. Three levels of NaCl (14.1, 44.4, and 70.4 mM) were added to the basic solution to determine Na⁺ effects on leaf physiology and growth. To determine if Ca²⁺ could alleviate the toxic effects of Na⁺, treatments with 10 or 20 mM Ca²⁺ combined with 44.4 or 70 mM NaCl were included as well. To distinguish between osmotic and ion-specific effects, there were three treatments in which all nutrient concentrations were increased (without NaCl) to obtain electrical conductivity (EC) levels similar to those of the NaCl treatments. Nutrient solutions with 70.4 mM NaCl reduced leaf photosynthesis, chlorophyll content, gas-phase conductance, carboxylation efficiency, and dark-adapted quantum yield. Inclusion of 20 mM Ca²⁺ prevented these effects of NaCl. NaCl also decreased leaf length and elongation rate. This could not be prevented by adding extra Ca²⁺ to the solution; reductions in leaf elongation were due to osmotic effects rather than to Na⁺ specifically. Likewise, plant dry weight was negatively correlated with solution EC, suggesting an osmotic effect. Leaf area development apparently was more important for dry matter accumulation than leaf photosynthesis. Adding 20 mM Ca²⁺ to the 70 mM NaCl solution reduced the Na⁺ concentration in the leaf from 79 to 24 mg·g⁻¹.

Salinity represents an increasing threat to agriculture around the world, while at the same time agricultural food production will need to increase to meet the needs of the increasing world population. This increases the likelihood that agricultural production will expand into marginal areas, which may be salt-affected (Flowers, 2006). Salt-affected soils occur in more than 100 countries around the world (Rengasamy, 2006), and according to a report published in 2000 by the Food and Agriculture Organization of the United Nations (FAO), the total global area of salt-affected soils, including saline and sodic soils, was 831 million ha (Martinez-Beltran and Manzur, 2005). Besides the occurrence of naturally saline soils, agriculture has contributed to salinization through use of poor-quality irrigation water, high fertilization rates, and forest clearance. Although NaCl is the dominant salt in many saline soils, calcium, magnesium, potassium, iron, boron, sulfate, carbonate, and bicarbonate can also accumulate (Szabolcs, 1989).

Greenhouse cultivation is one agrosystem that may be highly sensitive to salt stress because of the areas in which it usually occurs, the climatic conditions under which it is carried out, and the cultural techniques adopted (Leonardi and Martorana, 2005). Tomato is one of the most important crops grown under protected conditions. Tomato production is concentrated in arid or semi-arid areas, where it is often cultivated in saline soils or with poor-quality irrigation water. According to production

data from 2005, ≈30% of world production comes from countries around the Mediterranean basin and ≈10% from the United States (FAO, 2006), primarily California and Florida. These regions are characterized by intensive agriculture along coastal areas, where use of saline water and consequent soil salinization are common.

Many detrimental effects of salt stress have been reported on tomato, mainly concerning reductions in yield (Cuartero and Fernández-Muñoz, 1999). Although saline conditions are linked to reductions in growth and yield, in some cases moderate salt stress can improve fruit quality (Cornish, 1992; Plaut, 1997; Sakamoto et al., 1999; Serio et al., 2004). In soilless cultivation, growers may increase the electrical conductivity (EC) of the nutrient solution by adding NaCl or by increasing the overall nutrient concentration to apply a controlled salt stress to the plant and increase fruit quality.

Saline conditions usually refer to the presence of high NaCl concentrations in the irrigation water or in the soil, but few studies looked at Na-specific vs. nonspecific effects on tomato growth and physiology. The presence of salt in the nutrient solution decreases the osmotic potential of the root environment, but not all plant parameters are influenced the same way by different salinity sources (Adams, 1991).

Altering fertilization practices has been suggested as one possible way to increase the salt tolerance of tomato. In particular, providing additional Ca has been shown to reduce some of the detrimental effects of Na on tomato and other crops (Cabañero et al., 2004; Carvajal et al., 2000; Lopez and Satti, 1996; Navarro et al., 2000, 2005). On the basis of these findings,

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the objectives of our study were to quantify detrimental effects of NaCl on growth, leaf photosynthetic parameters, and nutrient uptake of hydroponically grown tomatoes; to determine which of these effects are ion-specific and which ones are not; and to determine if additional Ca can minimize or prevent detrimental ion-specific effects.

Materials and Methods

PLANT MATERIAL. The experiment was carried out in a glasshouse at the University of Georgia, Athens, Ga., during October and November of 2005. Seeds of tomato ‘Supersweet 100’ were germinated in sand-filled trays. At the first true-leaf stage (26 Sept.), the seedlings were transplanted into 4-L containers (one plant per container) after the sand was rinsed from the root system. For the first 2 weeks, all containers were filled with a nutrient solution with an EC of 1.6 dS·m⁻¹ and N concentration of 11.1 mM (see below) to allow the plants to become established in the hydroponic system. The solutions were continuously aerated with the use of a small compressor and aquarium aeration stones.

TREATMENTS. Treatments were started instantaneously on 10 Oct. by replacing the solution used during the first 2 weeks with the respective treatment solutions. A nutrient solution with an EC of 1.6 dS·m⁻¹, containing 2.8 mM Ca(NO₃)₂, 5.5 mM KNO₃, 0.87 mM KH₂PO₄, 1.71 mM MgSO₄·7H₂O, 7.6 mg·L⁻¹ of S.T.E.M. (Soluble Trace Element Mix; Scotts, Marysville, Ohio), and 1.5 mg·L⁻¹ of Sequestrene 330 Fe (0.15 mg·L⁻¹ Fe; Becker Underwood, Ames, Iowa), was used as the control treatment (referred to as 11.1 mM N). Three higher nutrient levels were obtained by increasing all nutrients in the 11.1 mM N treatment 2, 4, or 6 times (referred to 22.2, 44.4, and 66.6 mM N, respectively), resulting in ECs of 2.9, 5.7, and 8.2 dS·m⁻¹. Three levels of salt treatments were established by adding 14.1, 44.4, and 70.4 mM of NaCl to the 11.1 mM N nutrient solution, resulting in ECs of 2.8, 5.9, and 8.4 dS·m⁻¹. These NaCl concentrations were chosen because they resulted in EC levels similar to those of the 22.2, 44.4, and 66.6 mM N treatments. All solutions were made with deionized water.

To investigate the role of Ca²⁺ in relieving NaCl stress, additional treatments increased the Ca²⁺ concentration of the 11.1 mM N, 44.4 mM NaCl, and 70.4 mM NaCl solutions from the control level of 2.8 mM to 9.6 and 19.6 mM by adding CaCl₂.

These treatments are referred to as 11.1 mM N + 10 mM Ca²⁺, 11.1 mM N + 20 mM Ca²⁺, 44.4 mM NaCl + 10 mM Ca²⁺, 44.4 mM NaCl + 20 mM Ca²⁺, 70.4 mM NaCl + 10 mM Ca²⁺, and 70.4 mM NaCl + 20 mM Ca²⁺. This resulted in a total of 13 treatments: four N treatments (including the control), two 11.1 mM N + Ca²⁺ treatments, three NaCl treatments, and four NaCl + Ca²⁺ treatments. Composition and EC of the solutions are summarized in Table 1.

Every other day, the level of the nutrient solution in the containers was checked to estimate the water consumption of the plants. All containers were refilled with fresh nutrient solution as needed, without discarding the solution still present in the container. Containers were refilled with their respective treatment solutions, and the EC was not adjusted to the EC of the original solution. There were no large changes in the EC of the nutrient solutions over time because the solutions were refilled only when the solution level in the containers was low. This ensured that the effect of the remaining solution on the nutrient solution composition after refill was minimal. The pH was checked and adjusted to 5.5 using H₂SO₄ at every refill.

MEASUREMENTS. Six days after the start of the treatments, one young leaf was selected from each plant, and its leaf length was recorded about every other day for 31 d, until the end of leaf elongation. Once per week, water, osmotic, and turgor potential were measured using individually calibrated thermocouple psychrometers (model 76-2VC; JRD Merrill Specialty Equipment, Logan, Utah) on a 5.5-mm-diameter leaf disc from the leaf below the leaf used to control leaf elongation. Psychrometers were equilibrated in a water bath for 4 h at 25 °C before measurement of water potential. Subsequently, the samples were frozen overnight to disrupt cell membranes, and osmotic potential was measured after the samples were again equilibrated for at least 4 h at 25 °C. Turgor potential was calculated as the difference between osmotic and water potential.

Leaf net CO₂ assimilation rate (*A_n*) and *g_s* were measured 26 d after the start of the treatments. Leaves were exposed to a photosynthetic photon flux (*PPF*) of 1000 μmol·m⁻²·s⁻¹ and a CO₂ concentration of 400 μmol·mol⁻¹ for at least 20 min, using a portable photosynthesis system (CIRAS-1; PP Systems, Haverhill, Mass.). The measurements were taken on the same leaf that was used to measure elongation.

Table 1. Composition and EC of the nutrient solutions (in mM) used to determine the effects of salinity on tomato growth and physiology (only macronutrients, Na⁺, and Cl⁻ are shown).

Treatment	NO ₃ ⁻	H ₂ PO ₄ ⁻	K ⁺	Ca ²⁺	Mg ²⁺	SO ₄ ²⁻	Na ⁺	Cl ⁻	EC (dS·m ⁻¹)
11.1 mM N	11.1	0.87	6.37	2.8	1.71	1.71	0	0	1.6
22.2 mM N	22.2	1.74	12.74	5.6	3.42	3.42	0	0	2.9
44.4 mM N	44.4	3.48	25.48	11.2	6.84	6.84	0	0	5.7
66.6 mM N	66.6	5.22	38.22	16.8	10.26	10.26	0	0	8.2
14.1 mM NaCl	11.1	0.87	6.37	2.8	1.71	1.71	14.1	14.1	2.8
44.4 mM NaCl	11.1	0.87	6.37	2.8	1.71	1.71	44.4	44.4	5.9
70.4 mM NaCl	11.1	0.87	6.37	2.8	1.71	1.71	70.4	70.4	8.4
11.1 mM N + 10 mM Ca ²⁺	11.1	0.87	6.37	9.6	1.71	1.71	0	13.6	2.9
11.1 mM N + 20 mM Ca ²⁺	11.1	0.87	6.37	19.6	1.71	1.71	0	33.6	4.6
44.4 mM NaCl + 10 mM Ca ²⁺	11.1	0.87	6.37	9.6	1.71	1.71	44.4	58.0	6.9
44.4 mM NaCl + 20 mM Ca ²⁺	11.1	0.87	6.37	19.6	1.71	1.71	44.4	78.0	8.4
70.4 mM NaCl + 10 mM Ca ²⁺	11.1	0.87	6.37	9.6	1.71	1.71	70.4	84.0	9.4
70.4 mM NaCl + 20 mM Ca ²⁺	11.1	0.87	6.37	19.6	1.71	1.71	70.4	104.0	11.1

At 27 and 36 d after the start of the treatments, chlorophyll content was measured non-destructively (SPAD-502; Minolta, Ramsey, N.J.) in relative units, here referred to as SPAD units. Although SPAD readings do not give an absolute measure of chlorophyll content, they do provide a useful relative index, which is closely related to leaf chlorophyll content (Markwell et al., 1995). Measurements were taken on leaves from the top, middle (the leaf used to control elongation rate), and bottom layers of the canopy. Five measurements were taken in each layer, and the average was recorded.

The response of A_n to the internal CO_2 concentration (C_i) of the leaf (hereafter, $A-C_i$ curve) was measured 31 d after the start of the treatments in the 11.1 mM N, 70.4 mM NaCl, and 70.4 mM NaCl + 20 mM Ca^{2+} treatments. Because these measurements are time-consuming, not all treatments could be measured; these treatments were chosen on the basis of results of earlier A_n measurements. The $A-C_i$ responses were measured after initial exposure of individual leaves to a PPF of 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and a CO_2 concentration of 600 $\mu\text{mol}\cdot\text{mol}^{-1}$ for a period of at least 40 min and subsequently after exposing the leaf to decreasing CO_2 concentrations in decrements of ≈ 50 $\mu\text{mol}\cdot\text{mol}^{-1}$ for a period of 5 min each. The PPF was maintained at 1000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ throughout these measurements. At each CO_2 concentration, measurements were taken after the photosynthetic rate had stabilized.

The component limitations to photosynthesis were quantified using the differential approach described by Jones (1985). Briefly, quadratic curves were fitted to the relationship between the CO_2 concentration of the air in the leaf chamber and C_i and between C_i and A_n . These regressions were used to first estimate C_i at an external CO_2 concentration of 370 $\mu\text{mol}\cdot\text{mol}^{-1}$ and then to estimate the net assimilation rate at this C_i . The gas-phase resistance for CO_2 assimilation, consisting of the boundary layer and stomatal resistance, was calculated as $(\text{CO}_{2,\text{air}} - C_i)/A_n$. Mesophyll resistance was determined as the inverse of the slope of the $A-C_i$ curve at the operating point, which is the C_i at an external CO_2 concentration of 370 $\mu\text{mol}\cdot\text{mol}^{-1}$ (as per Jones, 1985). Mesophyll resistance includes such factors as diffusion of CO_2 inside the leaf, conversion of gaseous CO_2 to dissolved HCO_3^- , light and dark reactions of photosynthesis, and potential feedback inhibition of photosynthesis. Carboxylation efficiency, an indicator of Rubisco activity, was determined as the slope of the $A-C_i$ curve when C_i is equal to the CO_2 compensation point. The CO_2 compensation point was calculated as the internal CO_2 concentration at which A_n was zero. Leaf photosynthesis, chlorophyll, and related parameters were determined on a leaf-area basis.

At 35 d after the start of the treatments, the maximum quantum yield of photosystem II (F_v/F_m) was measured in dark-adapted leaves, using a portable chlorophyll fluorometer (mini-PAM; Heinz Walz GmbH, Effeltrich, Germany). Five measurements were taken on each plant.

At the end of the experiment, 37 d after the start of the treatments, total dry weight was measured for each plant. Water use of the individual plants was recorded throughout the course of the study and was used to calculate total water use during the experiment. Water-use efficiency was then calculated as plant dry weight divided by water use.

A sample of the middle layer of the leaves was used for tissue nutrient analysis. Total C, N, and S were determined with a CNS 2000 analyzer (LECO Corp., St. Joseph, Mich.), while P, K, Ca, Mg, and micronutrients were determined by dry

ashing and inductively coupled plasma spectrophotometry. All nutrient concentrations are expressed on a dry-weight basis.

The total daily PPF during the experiment was 12.1 ± 4.3 $\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ (mean \pm SD), relative humidity averaged 62% \pm 14%, and the daily maximum and minimum temperatures were 25.3 ± 1.6 $^\circ\text{C}$ and 18.4 ± 1.4 $^\circ\text{C}$, respectively.

EXPERIMENTAL DESIGN AND DATA ANALYSIS. The experiment was designed as a randomized complete block with 13 treatments and two replications. The experimental unit was a single plant in a hydroponics container. Leaf elongation rates were analyzed by fitting a sigmoidal curve to the data:

$$\text{Leaf length} = a/[1 + e^{-(t-t_0)/b}],$$

where a = length of fully elongated leaf (mm), t = time (days), t_0 = time to reach half of the final length, and $a/4b$ = maximum elongation rate (slope of the regression curve at the inflection point, in mm per day).

Effects of different concentrations of the basic fertilizer solution, different NaCl concentrations, and nutrient solution EC were tested using linear and quadratic regressions, using proc GLM of SAS (SAS Institute, Cary, N.C.), with solution N concentration, Na^+ concentration, or EC as the treatment variable. The 11.1 mM N treatment (control) was included in the regression analyses for the Na^+ treatments as the 0 mM Na^+ treatment. To determine whether treatment effects were due to ion-specific or nonspecific effects, regression analyses were done separately for the Na^+ and N treatments, using the EC of the fertilizer solution as the independent variable. The regression lines from the Na^+ and N treatments were then compared, and, in the case of significant differences between these regressions, the effect was considered to be ion-specific. If there was no difference between the regressions, the effect was considered to be nonspecific and likely due to osmotic effects. In that case, the data were re-analyzed by combining the data from all treatments and performing a regression analysis with the EC of the fertilizer solution as the independent variable. To determine whether calcium additions affected the plant responses, analysis of variance was used followed by mean separation using Fisher's protected least-significant difference at $P = 0.05$. To determine whether there were differences in leaf chlorophyll content among the different canopy layers, the data were analyzed as a split-plot design, with the nutrient solution composition as the main variable and canopy layer as the split.

Results and Discussion

LEAF ELONGATION. Leaf elongation followed a sigmoidal pattern ($R^2 = 0.99$ for all leaves) and was greatly affected by the EC of the nutrient solution. Both final leaf length and maximum elongation rate decreased linearly with increasing EC of the nutrient solution (Fig. 1). There were no treatment effects on the duration of leaf elongation. On average, it took 7.0 d for leaves to reach one-half of their final size. The effects of the fertilizer solutions on leaf elongation were similar regardless of the type of salts in the nutrient solution (i.e., NaCl vs. high fertilizer concentrations). The addition of extra Ca^{2+} to the nutrient solution did not diminish the inhibitory effect of high EC on leaf elongation. These results are consistent with the finding that leaf elongation of corn (*Zea mays* L.) is affected similarly by PEG-8000, mannitol, and NaCl, suggesting that the effect of these solutions was osmotic in nature rather than being specific toxic effects of these solutes (Chazen and Neumann, 1994).

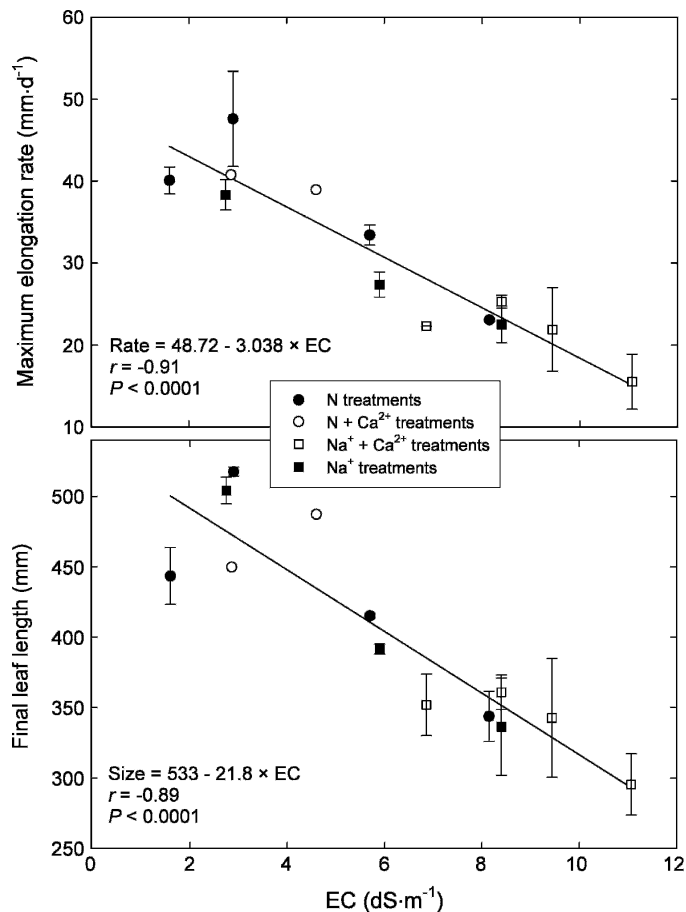


Fig. 1. Effect of the EC of the nutrient solution on the maximum leaf elongation rate and final leaf size of tomatoes (mean \pm SD) grown in nutrient solutions with different amounts of fertilizer, NaCl, and Ca²⁺.

These results indicate that leaf elongation depends on the total amount of salts in the nutrient solution, suggesting that high EC inhibits leaf growth mainly through the osmotic effect of the nutrient solution. A strong inhibition of leaf area expansion under saline conditions is well documented (An et al., 2005; Elia et al., 2001; Gao et al., 1998; Li and Stanghellini 2001; van Ieperen, 1996). For example, when corn is exposed to saline conditions, leaf elongation stops immediately, and it subsequently returns to a lower, steady-state growth rate (Cramer, 1992). Root elongation of corn also is reduced by NaCl, but contrary to our findings with tomato leaves, the root elongation of NaCl-stressed corn can be partly restored by the addition of additional Ca to the growth medium (Cramer et al., 1988; Zidan et al., 1990).

WATER RELATIONS. Both salinity sources (NaCl and increased nutrient solution concentrations) linearly decreased water and osmotic poten-

tial with increasing concentrations, but the magnitude of this decrease depended on the source of the salinity (Fig. 2). There was a slightly larger decrease in water potential as the result of NaCl (-1.19 MPa at 70.4 mM NaCl) than with high fertilizer concentrations (-1.10 MPa at 66.6 mM N compared with -0.7 MPa in the control), but this difference was not significant. Leaf osmotic potential was -0.8 MPa in the control treatment and was decreased much more by high NaCl than by high fertilizer concentrations (-1.55 MPa at 70.4 mM NaCl vs. -1.28 MPa at 66.6 mM N, $P = 0.015$).

The decrease in osmotic potential with increasing NaCl concentrations was larger than the decrease in water potential. This resulted in an increase in leaf turgor potential from 0.10 MPa in the absence of NaCl to ≈ 0.35 MPa in the presence of NaCl, without much difference among the 14.1, 44.4, and 70.4 mM NaCl treatments (Fig. 2, $P = 0.014$). Conversely, increasing the fertilizer concentration of the nutrient solution did not have a significant effect on leaf turgor. Romero-Aranda et al. (2001) found similar effects of NaCl on leaf water, osmotic, and turgor potentials of tomato. The different effects of NaCl and high fertilizer concentrations on turgor potential were surprising, because both sources of salinity affected leaf elongation similarly. We did not find an effect of additional calcium in the nutrient solution on leaf water, osmotic, or turgor potentials (results not shown).

LEAF CHLOROPHYLL CONTENT. There was no interaction between the composition of the nutrient solution and the canopy layer on leaf chlorophyll content; in all treatments, chlorophyll decreased from the top to the bottom of the canopy (Fig. 3). Chlorophyll content was affected differently by the two salinity sources. NaCl decreased leaf chlorophyll content when applied at 70.4 mM but not when applied at lower concentrations. Addition of either 10 or 20 mM Ca²⁺ to the solution containing 70.4 mM NaCl prevented this decrease in chlorophyll. However, increasing the overall nutrient concentration increased chlorophyll content linearly (Fig. 3), likely because of the increasing N availability for chlorophyll synthesis. This suggests that the effect of salinity on leaf chlorophyll content is ion-specific and not due to a decrease in the osmotic potential of the nutrient solution. However, it is important to keep in mind that increasing the overall concentration of the nutrient solution

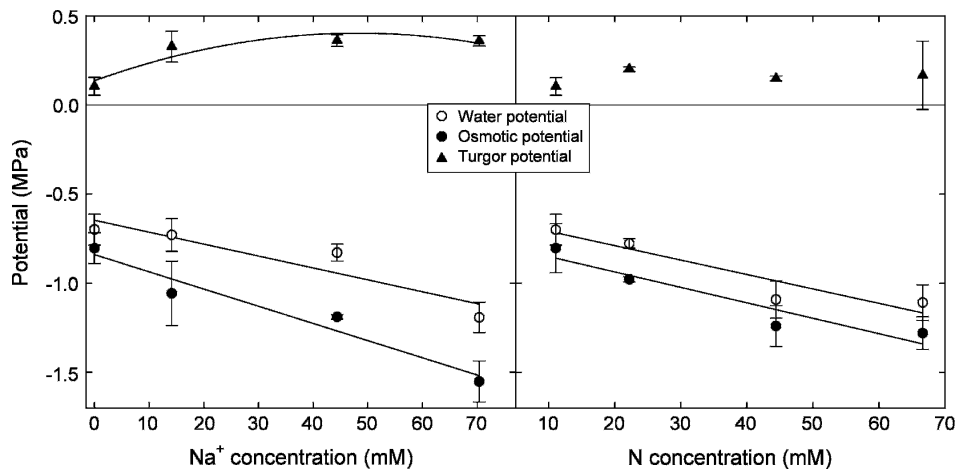


Fig. 2. Leaf water, osmotic, and turgor potentials of tomato leaves (mean \pm SD) as affected by different NaCl or fertilizer concentrations of the nutrient solution. Lines indicate significant linear or quadratic effects ($P < 0.05$). Because there were no significant interactions involving sampling date, data are averaged over 4 different sampling times.

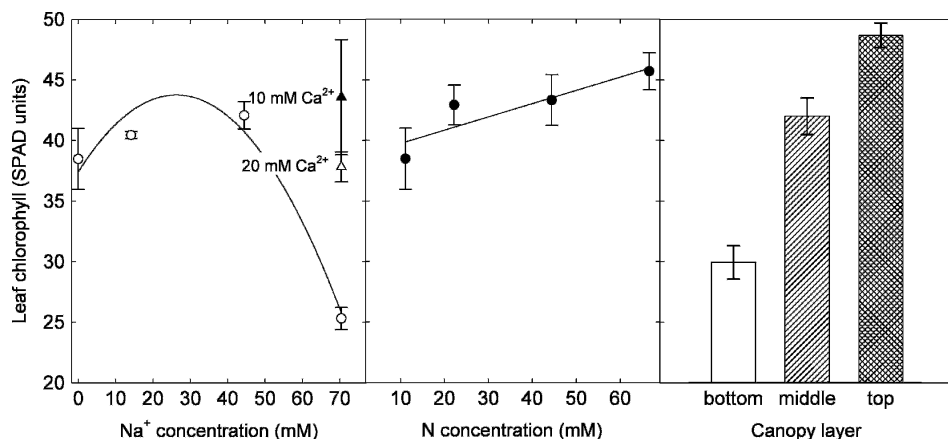


Fig. 3. Leaf chlorophyll content of tomato (mean \pm SD) as affected by the solution Na^+ concentration (left), fertilizer concentration (middle), and canopy layer. Lines indicate significant linear or quadratic effects ($P < 0.05$). Inclusion of 10 or 20 mM Ca^{2+} in the 70.4 mM Na^+ solution significantly increased leaf chlorophyll content as compared with the 70.4 mM Na^+ treatment ($P < 0.001$), but there was no difference between the addition of 10 or 20 mM Ca^{2+} . Chlorophyll levels were significantly different among top, middle, and bottom parts of the canopy ($P < 0.0001$).

also increases Ca^{2+} , from 2.8 mM in the control to 16.8 mM in the 66.6 mM N solution. Thus, we cannot rule out that increasing the solution EC would decrease leaf chlorophyll if Ca^{2+} concentrations would be kept constant, as in the Na^+ treatments.

Al-aghabary et al. (2004) also found that NaCl (97.5 mM) decreased leaf chlorophyll of tomato, while Romero-Aranda et al. (2001) found an increase in leaf chlorophyll content under saline conditions (35–70 mM). The leaf chlorophyll content of soil-grown cucumber (*Cucumis sativus* L.) decreased with increasing NaCl, and supplementing the soil with $\text{Ca}(\text{NO}_3)_2$ diminished this decrease in leaf chlorophyll (Kaya and Higgs, 2002).

PHOTOSYNTHESIS. Leaf photosynthesis decreased with increasing EC of the nutrient solution [$A_n = 17.0 - (0.60 \times \text{EC})$, $P = 0.046$, $r = -0.41$, data not shown] and was positively correlated with leaf chlorophyll content [$A_n = 0.603 + (0.301 \times \text{SPAD})$ reading, $P = 0.047$, $r = 0.41$, data not shown], which was due to the low chlorophyll (26 SPAD units) and low A_n ($6 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) in the 70.4 mM NaCl treatment. Photosynthetic rates in all other treatments were similar and averaged $14.1 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, with an average chlorophyll content of 44 SPAD units. To examine NaCl effects on photosynthesis in more detail, $A-C_i$ curves were determined in the control (11.1 mM N), 70.4 mM NaCl, and 70.4 mM NaCl + 20 mM Ca^{2+} treatments (Fig. 4). $A-C_i$ curves for the control and the 70.4 mM NaCl + 20 mM Ca^{2+} treatments were similar, with no statistically significant differences. However, the response of A_n to C_i was dramatically affected by NaCl (Fig. 4).

On the basis of the $A-C_i$ curves, A_n was much lower in the 70.4 mM NaCl treatment than in the control treatment when compared at an atmospheric CO_2 concentration of $370 \mu\text{mol}\cdot\text{mol}^{-1}$ (Table 2). Inclusion of 20 mM Ca^{2+} with the 70.4 mM NaCl restored A_n close to the level of the control treatment. Further analysis of the $A-C_i$ curves revealed that NaCl increased the gas-phase resistance for CO_2 diffusion and reduced the carboxylation efficiency. Addition of 20 mM Ca^{2+} to the 70.4 mM NaCl solution resulted in a similar gas-phase resistance and carboxylation efficiency as the control treatment (Table 2). The $A-C_i$ curves did not reveal significant differences in mesophyll resistance among the treatments, which may have been due to the large coefficient of variation (cv; 45%) for this parameter. Leaf photosynthetic rates at an atmospheric CO_2

concentration of $370 \mu\text{mol}\cdot\text{mol}^{-1}$ were closely correlated with both the gas-phase resistance ($r = -0.98$, $P = 0.0006$) and the carboxylation efficiency ($r = 0.93$, $P = 0.009$) (results not shown).

QUANTUM YIELD. The dark-adapted quantum yield of photosystem II, which is the maximum efficiency with which photosystem II can use absorbed photons to drive photochemistry (i.e., electron transport) and an indicator of damage to photosystem II, was decreased in the 70.4 mM NaCl treatment but similar to the control level when 10 or 20 mM Ca^{2+} was added (Fig. 5). Lower concentrations of NaCl did not affect the quantum yield. Although the effect of NaCl on the quantum yield was significant, the

decrease in quantum yield in the 70.4 mM NaCl treatment was small (0.820 vs. 0.837 in the control) and unlikely to have an important effect on photosynthesis. Clearly the small decrease in quantum yield in the 70.4 mM NaCl treatment (2% compared with the control) was not enough to explain the large decrease in A_n (60% compared with the control treatment). Thus, the decrease in A_n by 70.4 mM NaCl instead was due likely to a decrease in the amount of light absorbed by the leaves, which is consistent with the reduction in leaf chlorophyll (Fig. 3), an increase in the gas-phase resistance for CO_2 , or a reduction in the carboxylation efficiency (Table 2). Al-aghabary et al. (2004) also found a small, but at times significant, decrease in the dark-adapted quantum yield of photosystem II of NaCl-stressed tomato.

PLANT GROWTH. Total dry-matter production by the plants decreased with increasing EC, irrespective of the salinity source (Fig. 6). Growth reductions induced by NaCl were not prevented by additional Ca^{2+} in the nutrient solution. Likewise,

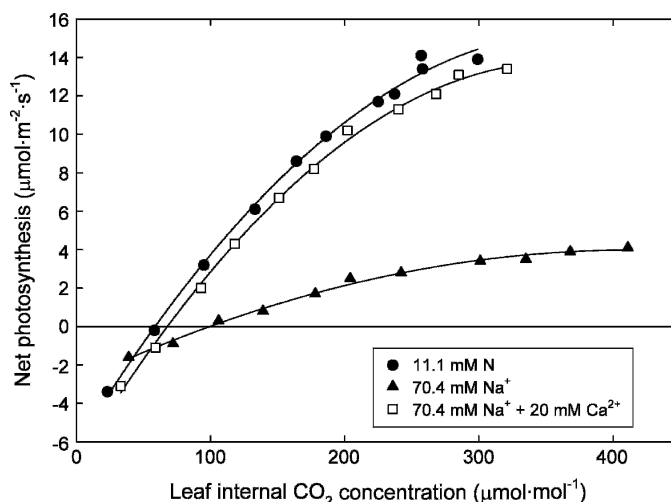


Fig. 4. Relationship between the internal CO_2 concentration and leaf photosynthesis of tomato ($A-C_i$ curves) in the control (11.1 mM N), 70.4 mM Na^+ , and 70.4 mM Na^+ + 20 mM Ca^{2+} treatments. Data for only one replication are shown. The lines show quadratic regression curves ($R^2 > 0.99$).

Table 2. Leaf photosynthetic parameters (mean \pm SD) of tomato in a standard hydroponics solution (11.1 mM N) and as affected by the addition of 70.4 mM NaCl and 70.4 mM NaCl + 20 mM Ca²⁺ to the nutrient solution.^z

Treatment	A_n (mmol·m ⁻² ·s ⁻¹)	Gas-phase resistance (s·m ² ·mmol ⁻¹)	Mesophyll resistance (s·m ² ·mmol ⁻¹)	Carboxylation efficiency (mmol·m ⁻² ·s ⁻¹)
11.1 mM N	11.6 \pm 0.0 a	12.2 \pm 1.3 b	21.2 \pm 0.2 a	89 \pm 11 a
70.4 mM NaCl	3.9 \pm 1.2 b	34.7 \pm 5.0 a	63.6 \pm 21.4 a	32 \pm 9 b
70.4 mM NaCl + 20 mM Ca ²⁺	10.3 \pm 0.0 a	17.9 \pm 4.0 b	16.0 \pm 7.4 a	102 \pm 12 a

^zParameters were determined from $A-C_i$ curves. A_n is the estimated net photosynthesis at an atmospheric CO₂ concentration of 370 μ mol·mol⁻¹. Means separated by Fisher's protected LSD_{0.05}.

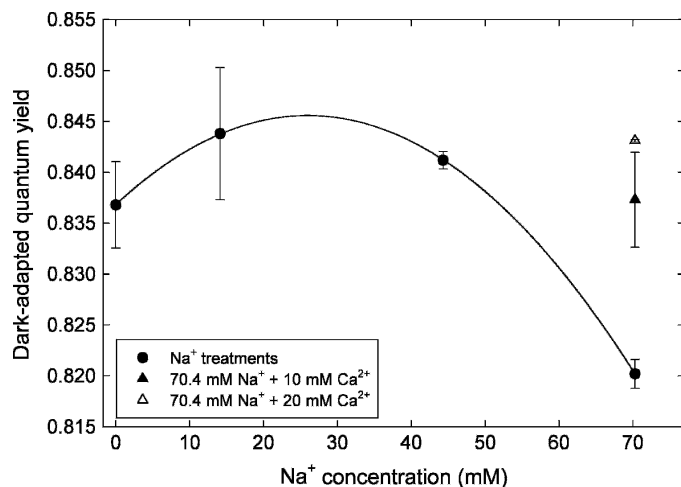


Fig. 5. Dark-adapted quantum yield of photosystem II of tomato (mean \pm SD) as affected by the Na⁺ concentration of the fertilizer solution. The line indicates a significant quadratic effect of Na⁺ on the quantum yield ($P = 0.0054$). Inclusion of 10 or 20 mM Ca²⁺ in the 70.4 mM Na⁺ solution significantly increased the quantum yield as compared with the 70.4 mM Na⁺ treatment ($P < 0.006$), but there was no difference between the addition of 10 or 20 mM Ca²⁺ ($P = 0.25$).

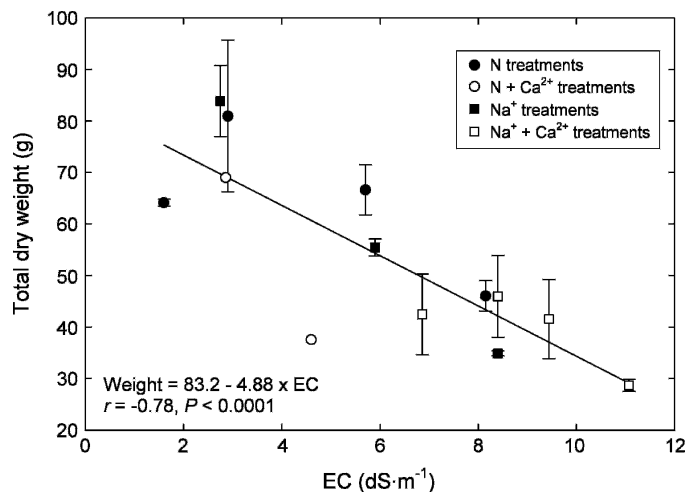


Fig. 6. Correlation between EC of the nutrient solution and dry weight of the tomato plants. Data points represent the mean \pm SD.

Navarro et al. (2005) found that the NaCl-induced reduction in total and marketable yield of tomato could not be prevented by additional Ca²⁺ in the hydroponics solution. In contrast, Navarro et al. (2000) found that additional calcium partially prevented the growth-inhibiting and physiological (root hydraulic conductance, g_s) effects of Na⁺ (60 mM) on tomato.

Lopez and Satti (1996) found that adding 20 mM Ca²⁺ to a saline nutrient solution (50 mM NaCl) increased root volume, fresh weight, and Ca²⁺ concentration as well as leaf fresh weight and fruit yield per tomato plant compared with the same solution without the additional Ca²⁺. Similarly, Kaya and Higgs (2002) found that Ca(NO₃)₂ supplementation increased yield and dry matter of cucumber in a soil high in NaCl.

There was no correlation between leaf photosynthesis and total plant dry weight ($r = 0.24$, $P = 0.26$), indicating that photosynthesis was not useful in the prediction of plant growth under saline conditions. This also is evident from the $A-C_i$ curves, which were similar for the 11.1 mM N and 70.4 mM NaCl + 20 mM Ca²⁺ treatments (Fig. 4), although growth in the 70.4 mM NaCl + 20 mM Ca²⁺ treatment was reduced by 55% as compared with the 11.1 mM N treatment. Other reports indicate that NaCl reduces growth more severely and at lower concentrations than photosynthesis (Alarcon et al., 1994; An et al., 2005; Seemann and Critchley, 1985; Yeo et al., 1991). Gao et al. (1988) found that moderate salinity (50 mM NaCl) reduced the photosynthesis rate per unit leaf area only slightly but caused considerable inhibition of leaf area expansion. To understand salinity effects on plant growth, reductions in leaf expansion incited by nutrient solutions with high EC need to be taken into account. We found a strong correlation between the maximum length of the measured leaf and total plant dry weight [dry weight = $-28.2 + (0.210 \times \text{final leaf length})$, $r = 0.84$, $P = 0.018$]. This suggests that salinity effects on the development of photosynthetically active leaf area were more important for dry matter accumulation than were differences in the photosynthetic rate per unit leaf area.

Schwarz et al. (2002) affirmed that single-leaf photosynthesis measurements are poor indicators of plant growth because such measurements do not take into account the total leaf area of plants. They found that high nutrient concentrations had no effect on photosynthesis per unit leaf area, but inhibited leaf elongation and thus whole-plant photosynthesis and dry-matter accumulation.

The apparent contradiction of a lack of correlation between leaf photosynthesis and dry-matter accumulation has long been recognized (Evans, 1975). Predicting plant growth on the basis of instantaneous measurements of leaf photosynthesis can result in misleading conclusions unless the effects of carbohydrate allocation and leaf-area development are taken into account. Lawlor (1995) reviewed the role of the leaf-area index and photosynthetic rate per unit leaf area and concluded that leaf-area index and leaf-area duration (the integral of leaf area index over time) are important factors in determining crop growth.

Inhibition of leaf elongation likely has a larger effect on growth of small plants, where light interception may be a more important growth-limiting factor than it is in closed canopies that intercept most of the available light and where light

interception may not be limiting. Thus, restoration of leaf photosynthesis to control levels by using additional Ca^{2+} may be more beneficial for closed canopies.

WATER USE. Water use per plant was linearly reduced by increasing EC, regardless of the salinity source (Fig. 7A). Additional Ca^{2+} did not restore the water uptake. At the highest EC level ($11.1 \text{ dS}\cdot\text{m}^{-1}$, $70.4 \text{ mM NaCl} + 20 \text{ mM Ca}^{2+}$), total water use was 30% of that under control conditions. This correlation between EC and water use was the result of two separate factors. Not surprisingly, water use was positively correlated with plant size at the end of the experiment ($r = 0.91$, results not shown). In addition, the water use efficiency of the plants increased with increasing EC (Fig. 7B). Thus, plants grown at high EC had lower dry weight and used less water per unit of dry weight produced, resulting in a large decrease in plant water use. Reina-Sanchez et al. (2005) also reported a strong correlation between vegetative dry weight and plant water use under saline conditions. Our results differ somewhat from those of Romero-Aranda et al. (2001), who found that NaCl reduced dry weight and water uptake of tomato but did not affect water use efficiency.

LEAF NUTRIENT CONCENTRATIONS. Increasing the fertilizer concentration of the nutrient solution increased leaf concentrations of N, K, B, and Fe, while decreasing the Na concentration in the leaves (results not shown). The decrease in Na had

little practical relevance, because the concentrations were low at all four fertility levels, decreasing from $0.68 \text{ mg}\cdot\text{g}^{-1}$ in the 11.1 mM N to $0.21 \text{ mg}\cdot\text{g}^{-1}$ in the 66.6 mM N treatment.

The Na^+ concentration in the leaf tissue increased with increasing NaCl concentration in the nutrient solution, from $0.68 \text{ mg}\cdot\text{g}^{-1}$ in the Na^+ -free nutrient solution to $79 \text{ mg}\cdot\text{g}^{-1}$ in the 70.4 mM NaCl treatment. Plants in this treatment were visibly chlorotic, but there were no signs of marginal leaf burn. Adding 20 mM Ca^{2+} to the 70.4 mM NaCl treatment reduced the Na concentration in the leaf tissue to $24 \text{ mg}\cdot\text{g}^{-1}$, or by 70% (Fig. 8), and prevented chlorosis (Fig. 3). This suggests that Ca^{2+} may prevent some of the toxic effects of Na on leaf photosynthesis by preventing the accumulation of Na^+ in leaves. Na^+ flow through nonselective channels is strongly impaired by application of external Ca^{2+} (Davenport and Tester, 2000; Roberts and Tester, 1997), and down-regulation of this channel type may be crucial for salt tolerance. For a recent review of the molecular genetics of the role of Ca^{2+} in salinity tolerance, see Maggio et al. (2006).

Ca^{2+} not only reduces the influx of Na^+ through nonselective cation channels in the plasma membrane, but also reduces Na^+ -induced efflux of K^+ (Shabala et al., 2006). Under saline conditions, Na^+ can displace Ca^{2+} from the plasma membrane, increasing membrane permeability and leakage of K^+ out of the cells (Cramer et al., 1985). This is consistent with our finding that the K^+ concentration in the leaf tissue was reduced from $57.8 \text{ mg}\cdot\text{g}^{-1}$ in the control plants to $22.4 \text{ mg}\cdot\text{g}^{-1}$ in presence of 70.4 mM NaCl (Fig. 8). The K^+ concentration in the leaf tissue was increased slightly by Ca^{2+} applied to the 70.4 mM NaCl treatment, but this effect was not statistically significant. Increased Ca^{2+} can inhibit the efflux of K^+ (Viets effect) from protoplasts (Bouteau et al., 1996), and Ca^{2+} may therefore be important in maintaining adequate K^+ concentrations in NaCl-stressed plants. Antagonism among cation uptake may also have contributed to the decrease in leaf K^+ concentration at 70.4 mM NaCl .

Leaf N concentrations decreased linearly with increasing NaCl (Fig. 8) and were unaffected by Ca^{2+} . This decrease in N concentration may result from an inhibition of NO_3^- uptake by $\text{NO}_3^-/\text{Cl}^-$ competition at the sites for anion transport and to membrane depolarization by Na^+ in tomato (Suhayada et al., 1990). If the decrease in leaf N concentrations was due to antagonism in uptake between NO_3^- and Cl^- , CaCl_2 should have decreased leaf N concentrations as well, but no such effect was found. Leaf Ca^{2+} concentrations were unaffected by NaCl (mean concentration = $21 \text{ mg}\cdot\text{g}^{-1}$), but addition of 10 or 20 mM Ca^{2+} increased the Ca^{2+} tissue concentration to $42 \text{ mg}\cdot\text{g}^{-1}$.

Conclusions

NaCl affected plant growth and leaf physiology of tomato through both osmotic and ion-specific, toxic effects. Plant growth, leaf elongation (maximum elongation rate and final leaf length), and water use decreased while water use efficiency increased with increasing EC of the nutrient solution, suggesting an osmotic effect. This decrease in leaf elongation was not incited by a decrease in turgor, which actually was increased by NaCl. Several physiological processes (leaf chlorophyll content, leaf photosynthesis, carboxylation efficiency, gas-phase resistance for CO_2 , dark-adapted quantum yield) were affected specifically by Na^+ . Ion-specific, toxic effects of Na^+ could be prevented by the addition of extra Ca^{2+} to the nutrient solution.

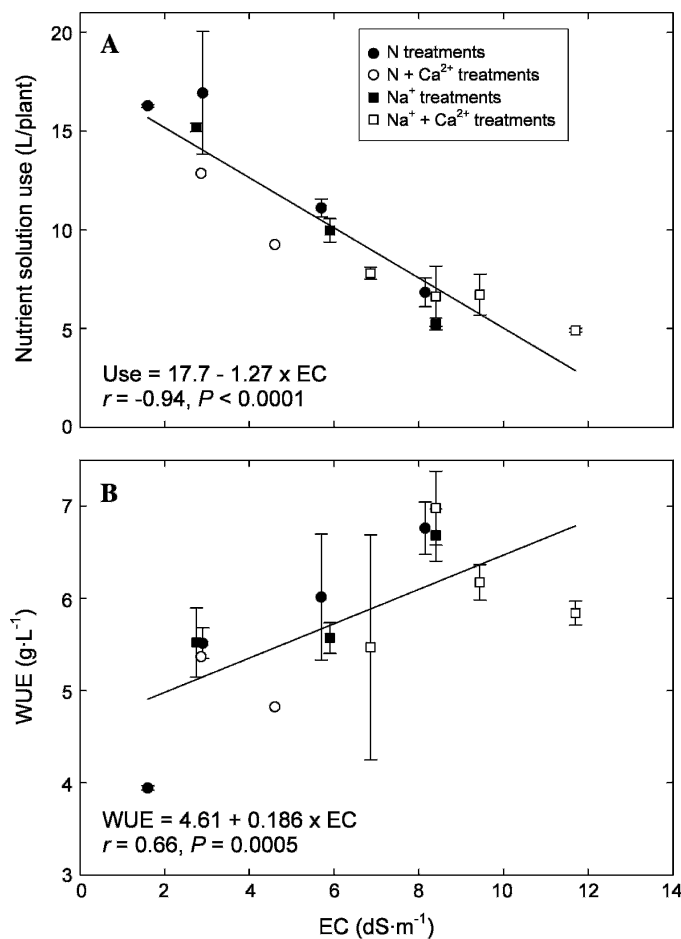


Fig. 7. Effect of EC of the fertilizer solution on the solution use per tomato plant (A) and the water use efficiency (WUE) (B). Data points represent the mean \pm SD.

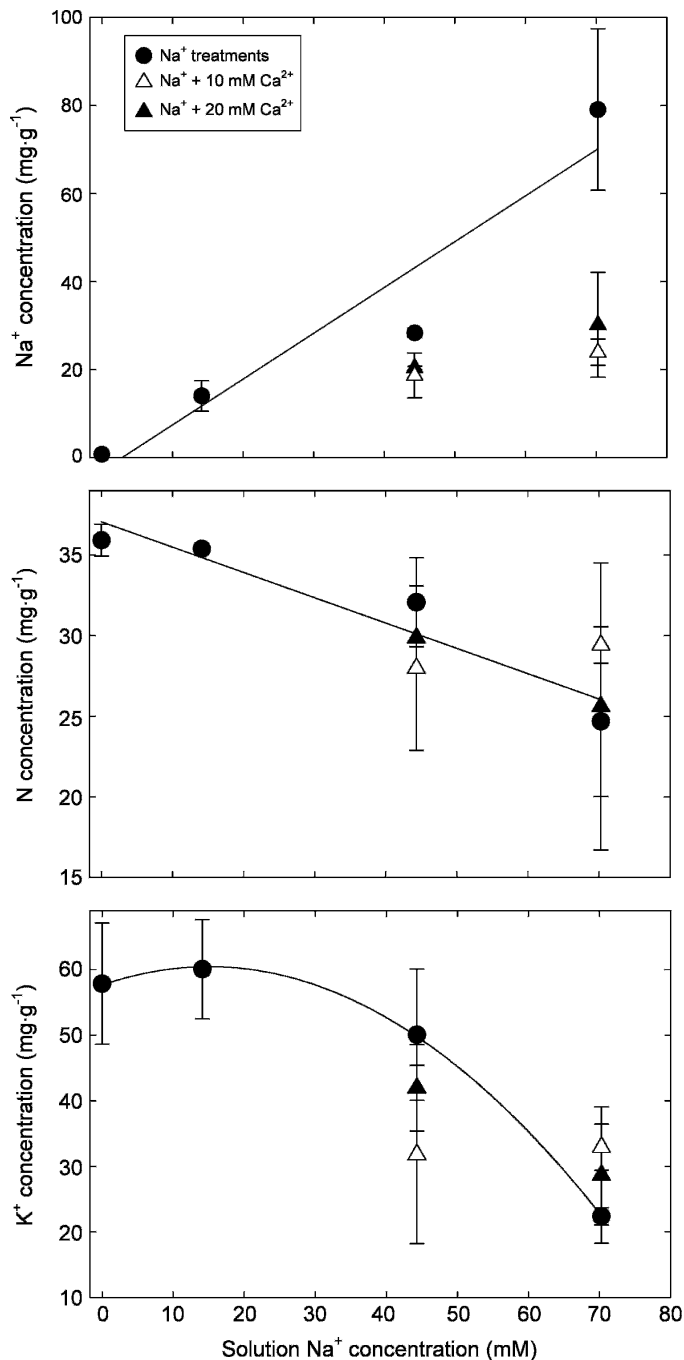


Fig. 8. Effect of the Na^+ concentration of the nutrient solution on the leaf Na^+ , N, and K^+ concentrations (mean \pm SD, on a dry weight basis) of tomato. Lines show significant linear or quadratic effects of the solution Na^+ concentration. Leaf Na^+ increased with increasing Na^+ ($P = 0.018$) in the solution, while leaf N ($P = 0.086$) and K^+ ($P = 0.015$) concentrations decreased. Addition of 10 or 20 mM Ca^{2+} to the 70.4 mM Na^+ solution decreased leaf Na^+ concentrations ($P = 0.026$), but there was no significant difference between the 10 and 20 mM Ca^{2+} treatments. Ca^{2+} additions did not have a significant effect on leaf N or K^+ concentrations.

This likely was due to decreased leaf Na^+ concentrations when 10 or 20 mM Ca^{2+} was added to the 70.4 mM NaCl solution. Although Ca^{2+} prevented many of the Na -specific toxic effects on leaf physiology, it did not increase the dry weight of the plants. Leaf area development appeared to be more important for plant growth than photosynthesis per unit leaf area. It is

important to note that the plants in these experiments were relatively small, so that light interception may have had limiting effects on growth. It is possible that leaf photosynthesis is a more important determinant of growth in closed canopies, where most of the available light is intercepted.

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