Influence of NaCl Salinity on Vegetative Growth, Nutrient Uptake and Proline Content in Two Tomato Cultivars Grown Under Greenhouse Conditions

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ABSTRACT

Effect of four NaCl salinity levels; 2.0 (control), 6.0, 8.3 and 15.0 dS m^{-1} on the performance of two tomato commercial F1 tomato hybrids, "Maha" (normal fruited) and Pepe (Cherry type), were investigated in pot experiments under greenhouse conditions to evaluate the relative degree of salt tolerance at the seedling stage. In general, application of 8.3 and 15.0 dS m^{-1} at the cotyledon stage, significantly reduced seedling shoot and root fresh and dry weights, increased Na, Cl and proline accumulation and significantly reduced accumulation of K, Mg and Ca. However, under low salinity levels (2.0 to 6.0 dS m^{-1}) both cultivars were able to stand certain amounts of Na and Cl in their shoots without significant reductions in weight. Under high salinity levels (8.3 and 15.0 dS m^{-1}), reductions in shoot fresh weights and leaf area in response to salinity were higher than the other vegetative growth parameters in both cultivars and to a greater extent in "Maha". "Maha" accumulated less Na and Cl in shoots, while "Pepe", accumulated higher Na and Cl. The reduction in vegetative growth parameters was associated with the increase in Na and Cl accumulation. Linear regression between ion regression index and relative shoot and root dry weights showed that the inhibitory effect of Cl was greater than Na on vegetative growth and to a higher extent in "Maha".

Abbreviations: Days After Salt Treatment: DAST, Ion Regression Index (IRI).

KEYWORDS: Lycopersicon esculentum L., salinity, salt stress.

1. INTRODUCTION

In general, salinity has been reported to slow tomato shoot growth (Cuartero and Fernandez-Munoz, 1999). The effect of salinity on shoot growth varied with salt concentration (Alarcon *et al*., 1994b; Bolarin *et al*., 1991; Mohammad, *et al*., 1998; Smith, *et al*. 1992), cultivars (Alian *et al*., 2000; Cruz *et al*., 1990; Shannon *et al*., 1987), plant age and time of salt application (Bolarin *et al*., 1993), and duration of salt treatment (Shannon *et al*., 1987; Smith *et al*., 1992).

Tomato shoot fresh weight decreased with increasing salt concentration from 1 to 14.2 and to 20.7 dS m^{-1} in the nutrient solutions (Shannon *et al*., 1987). Significant reduction in shoot fresh weight was found by increasing salinity of the solution from 2.4 to 11.9 dS m^{-1} (Mohammad *et al*., 1998). About 30 and 77% of the control (EC$_e$ 3.2 dS m^{-1}) reduction in shoot fresh weight was obtained by moderate (7.3 dS m^{-1}) and high (12.2 dS m^{-1}) soil salinity levels, respectively (Perez-Alfocea *et al*., 1996).

Exposure of plants to salt stress usually begins with the exposure of the roots to that stress (Cuartero and Fernandez-Munoz, 1999). Tomato root fresh weight was decreased by 18% and 36% under moderate (EC$_e$ 7.3 dS m^{-1}) and high (12.2 dS m^{-1}) salinity levels, respectively as compared with 3.2 dS m^{-1} (Perez-Alfocea *et al*., 1996). However, Shannon *et al*. (1987) reported that root fresh weight of the cultivated tomato species was increased by increasing salinity of the irrigation solution to 7.9 dS m^{-1} and decreased by 14.2 and 20.7 dS m^{-1} salinity levels.

Genetic variability in the lowering of root fresh weight under salt stress within cultivated tomato has been

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reported (Alian *et al.*, 2000; Shannon *et al.*, 1987). Root fresh weight of "Patio" was decreased significantly by 135 mM NaCl salinity while that of "Fireball" was not significantly affected even though both are considered fresh market tomato cultivars (Alian *et al.*, 2000).

The mechanisms determining plant responses to salinity involve complex interactions. Plants grown under saline conditions are subjected to different types of stress including water stress caused by osmoticum (Schwarz and Kuchenbuch, 1998) and mineral toxicity (Shannon, 1985) and disturbance in the mineral nutrition of the plant (Perez-Alfocea *et al.,* 1996; Franco *et al.,* 1999). Many studies on physiological responses of plants to salinity were based on the assumption that plants can survive under salty conditions expressing their pre-existent genetic information for tolerance. However, there is evidence that plants do show adaptive responses to salinity (Cuartero and Fernandez-Munoz, 1999). Moreover, the physiological mechanisms that prevent damage at moderate salinity may differ from those required for survival at high salinity (Shannon, 1985; Shannon *et al.*, 1987).

Although there was strong evidence that proline accumulation under salt stress is a symptom of stress injury rather than an indicator of stress tolerance in some plant species (Liu and Zhu, 1997), this does not negate the adaptation role for proline (Delauney and Verma, 1993). Proline accumulation in tomato leaves and roots is considered as a salt sensitive trait that may be used to select plants with different degrees of tolerance (Bolarin *et al.*, 1995).

This study was initiated to evaluate the relative degree of salt tolerance of two tomato cultivars, normal fruited "Maha" and cherry fruit type "Pepe" in terms of vegetative growth, nutrient uptake and proline accumulation in response to salt stress NaCl at cotyledon stage.

2. MATERIALS AND METHODS

Plant Material

Seeds of two commercial indeterminate tomato "Maha" from Bruinsma Co. (Holland) and "Pepe" from Takji (Japan) were used. Seeds were sown directly on April 1st 1999 in polystyrene trays (67x40x6 cm) in washed silica sand. Sown seeds were irrigated every two days with half-strength Hoagland solution (Taleisnik-Gertel *et al.*, 1983) for the first 10 days. Thereafter,

seedlings were transplanted into 0.5 L plastic pots filled with washed silica sand and irrigated with full-strength Hoagland solution throughout the experiment.

Salt Treatment

Four salinity treatments (EC$_{is}$: 2.0, 6.0, 8.3 and 15.0 dS m^{-1}) were applied at cotyledon stage by dissolving 0, 2.05, 4.10 and 8.19 g NaCl per L of nutrient solution, respectively. Extra nutrient solutions were added to avoid salt accumulation in the root zone.

The experiment was carried out under greenhouse conditions (Fig.1) using split plot arranged in RCBD with 4 replicates. Main plots were assigned for salt treatments and sub-plots for the cultivars.

Vegetative Growth Measurements

Seedlings were harvested at 2 different times during the experiment: 30 and 45 days after salt treatment. Time was replicated 4X with 10-20 seedlings per replicate. Seedlings were washed, air dried on filter paper, separated into shoots and roots, weighed, dried to constant weight at 60°C and weighed.

Chemical Analysis

For Na, Cl, K, Mg and Ca analysis, dry shoots and roots were grinded. Samples were ashed at 450 -500°C for 4 hrs using Thermolyne Muffle Furnace (6000 Furnace). Wet ashing was done by adding 10 ml of 0.1N HNO$_3$ to 100 mg ashed plant materials and heating for 4 min. The extract was filtered into 50 ml volumetric flasks, 10 ml of HNO$_3$ were added and de-ionized water was added to bring the volume to 50 ml. Chloride was determined by adding 5 drops of potassium chromate to 5 ml of the solution. The solution was then titrated to an end point of light brown color using 0.05N AgNO$_3$. Sodium, K, Mg and Ca contents were determined using Varian Spectra AA 200 Atomic Absorption.

Free proline was extracted and measured colorimetrically following the procedure described by Bates *et al*. (1973). To measure absorbence, a Milton Roy Spectrophotometer (1001 plus) at 520 nm. was used.

3. RESULTS

Vegetative Growth

Irrespective of salinity, shoot fresh and dry weights of both cultivars increased with age but to a higher extent in "Maha" (Table 1). In general, increasing salinity to 8.3

and 15.0 dS m^{-1} significantly reduced shoot fresh and dry weights of both cultivars, while 6.0 dS m^{-1} did not induce significant reductions in shoot fresh and dry weights. Rate of reduction in shoot fresh and dry weights by increasing salinity, represented by slope values, was more pronounced with increasing time of salt treatment (Table 2); fresh weight reduction slopes were 49.9 and 144.2 for "Maha" and 15.4 and 61.7 for "Pepe" 30 and 45 DAST, respectively. On the other hand, dry weight reduction slopes were 3.0 and 6.4 for "Maha" and 0.8 and 2.6 for "Pepe" 30 and 45 DAST, respectively.

Average root fresh and dry weights of tomato seedlings were affected by salinity and cultivar interaction (Table 1). Although root fresh and dry weights of "Maha" were significantly higher than that of "Pepe" regardless of sampling date, a general significant reduction, due to increasing salinity to 8.3 and 15.0 dS m^{-1} which occurred in both cultivars. Reduction (% of the control) by increasing salinity to 8.3 and 15.0 dS m^{-1} was generally greater in "Maha" than in "Pepe" except 30 DAST. In contrast, the reduction in root dry weight with increasing salinity to 8.3 and 15.0 dS m^{-1} was significant except for 8.3 dS m^{-1} in "Pepe" 30 and 45 DAST as compared with 6.0 dS m^{-1} (Table 2). The reduction slope due to increasing salinity was more pronounced with increased period of salinity treatment (Table 3), "Maha"

reduction slopes for fresh weight were 9.7 and 26.1 and were 4.0 and 10.0 in "Pepe" 30 and 45 DAST, respectively. However, reduction slopes for dry weight were 0.4 and 1.1 in "Maha" and were 0.1 and 0.5 in "Pepe" 30 and 45 DAST, respectively.

4. MINERAL COMPOSITION

Sodium

Shoot Na increased with salinity and age with significant differences between the two cultivars at 8.3 and 15.0 dS m^{-1} (Table 3). Throughout the two sampling dates, Na in "Pepe" shoots was significantly higher at 15.0 dS m^{-1} compared with those of the other cultivar, while similar levels of Na were observed in shoots of both cultivars at 2.0 dS m^{-1}. Shoot Na of "Pepe" was significantly higher at 6.0 dS m^{-1} 30 DAST only, and was consistently and significantly higher than that of "Maha" shoots at 8.3 dS m^{-1} for the sampling dates.

Root Na increased with increasing salinity and age (Table 3). At the two sampling dates, the highest and significant root Na was found in "Maha" at 15.0 dS m^{-1} followed by "Pepe" at the same salinity level. At 8.3 dS m^{-1}, "Pepe" root Na was significantly higher than that of "Maha" except for 30 DAST, while at 6.0 dS m^{-1}, root Na was significantly higher in "Pepe" than in "Maha".

Table 1: Interactive effects of cultivar and salinity [1]
on fresh and dry weights (mg seedling^{-1}) of tomato shoots and roots.

Cultivar	Salinity (dS m^{-1})	Days after salt treatment							
		30				45			
		Shoot		Root		Shoot		Root	
		Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
Maha	2.0	1121 a	82.2 a	284 a	15.6 a	2992 a	210.4 a	664 a	38.4 a
	6.0	1116 a	82.4 a	296 a	16.1 a	2784 b	208.5 a	631 a	36.1 a
	8.3	655 b	53.9 b	201 b	12.2 b	1649 c	159.0 b	423 b	30.0 b
	15.0	532 c	47.2 c	172 c	10.9 c	1240 d	134.2 c	348 c	24.8 c
Pepe	2.0	377 d	29.0 d	123 d	6.4 d	1322 d	90.1 d	285 d	16.7 d
	6.0	360 d	26.8 d	113 d	6.0 de	1296 d	91.1 d	261 de	15.9 d
	8.3	240 e	21.3 e	86 e	5.3 de	709 e	64.3 e	204 ef	13.9 de
	15.0	192 f	18.4 e	74 f	5.0 e	591 e	60.2 e	160 f	10.9 e

(1) Salinity treatment started at cotyledon stage.

Means within columns having different letters are significantly different according to DMRT(p<0.05).

**Table 2: Linear regression equations for shoot and root fresh and
dry weights of two tomato cultivars as affected by salt treatment at two different dates after salt treatment.**

Parameter	cultivar	30 DAST		45 DAST	
		Equation	$R^{2(1)}$	Equation	R^2
Shoot fresh weight	Maha	$Y = -49.87x +1246$	0.78	$Y = -144.2x +3294$	0.85
	Pepe	$Y = -15.3x +411.8$	0.85	$Y = -61.7x +1462.2$	0.77
Shoot dry weight	Maha	$Y = -2.97x +89.67$	0.76	$Y = -6.4x +108.3$	0.85
	Pepe	$Y = -0.85x +30.50$	0.90	$Y = -2.57x +96.51$	0.72
Root fresh weight	Maha	$Y = -9.73x +314.5$	0.75	$Y = -26.12x +720.9$	0.84
	Pepe	$Y = -3.96x +103.15$	0.88	$Y = -10.02x +305.7$	0.94
Root dry weight	Maha	$Y = -0.41x +16.85$	0.76	$Y = -1.09x +40.89$	0.94
	Pepe	$Y = -0.11x +6.55$	0.91	$Y = -0.47x +18.02$	0.97

(1) Coefficient of determination.

**Table 3: Interactive effects of cultivar and salinity[1]
level on Na, Cl and K content in shoots and roots of tomato seedlings.**

Cultivar	Salinity (dS m^{-1})	(mg g^{-1} DW)					
		Na		Cl		K	
		Shoot	Root	Shoot	Root	Shoot	Root
		30 DAST					
Maha	2.0	2.4 f[2]	1.5 f	15.3 de	10.3 e	47.2 ab	25.6 c
	6.0	3.9 e	2.1 ef	18.3 d	14.4 d	50.5 a	20.8 f
	8.3	11.2 d	5.9 d	37.4 c	30.2 c	42.1 cd	34.3 b
	15.0	18.6 b	12.7 a	52.6 b	46.7 a	38.9 d	37.5 a
Pepe	2.0	2.5 f	1.7 f	11.6 e	9.7 e	51.5 a	24.0 cd
	6.0	4.7 e	2.6 e	17.9 d	10.8 e	50.4 a	21.2 ef
	8.3	13.9 c	8.1 c	54.6 b	31.3 c	45.4 bc	22.8 de
	15.0	25.4 a	9.6 b	79.9 a	38.4 b	42.4 cd	24.8 c
		45 DAST					
Maha	2.0	4.5 g	3.7 f	17.3 fg	11.2 f	55.4 b	29.6 cd
	6.0	7.1 f	6.1 e	23.8 e	17.3 d	55.5 b	28.0 de
	8.3	20.8 d	16.2 c	52.3 d	34.2 c	47.3 cd	35.2 b
	15.0	37.0 b	34.6 a	85.9 b	53.9 a	44.6 d	40.4 a
Pepe	2.0	4.1 g	4.3 f	13.3 g	9.8 f	58.4 a	19.6 f
	6.0	11.8 e	11.2 d	19.8 ef	14.2 e	59.7 a	21.1 f
	8.3	23.3 c	15.8 c	66.0 c	35.2 c	54.9 b	26.6 e
	15.0	46.9 a	25.5 b	105.9 a	42.1 b	49.8 c	30.6 c

(1) Salinity treatment started at cotyledon stage.

(2) For each age category, means within columns having different letters are significantly different according to DMRT($p<0.05$).

Chloride

Shoot Cl increased with salinity and age (Table 3). At 2.0 and 6.0 dS m^{-1} no significant differences between the two cultivars were detected at any of the two sampling dates. Highest and significant shoot Cl was observed at 15.0 dS m^{-1} in "Pepe" followed by "Maha" at the same salinity level. "Pepe"shoot Cl was significantly higher than that of "Maha" at 8.3 and 15.0 dS m^{-1} but not at 6.0 dS m^{-1}.

Root Cl increased as salinity and age increased with significant differences between cultivars at 6.0 and 15.0 dS m^{-1} (Table 3). The highest root Cl was found at 15.0 dS m^{-1} 30 and 45 DAST in "Maha" seedlings. There were no significant differences between the two cultivars at 8.3 dS m^{-1}, while at 6.0 dS m^{-1}, "Maha" root Cl was significantly higher than that of "Pepe" in the two sampling dates.

Potassium

Shoot K increased with sampling date (Table 3); however, shoot K varied in response to increased salinity with significant differences between the two cultivars at the 4 salinity levels 45 DAST. "Pepe" shoot K was significantly higher 45 DAST than in "Maha" seedlings at 2.0, 6.0 and 8.3 dS m^{-1} but no significant differences were observed in shoot K 30 DAST. Shoot K at 15.0 dS m^{-1} was similar in both cultivars 30 DAST but was significantly higher and lower for "Maha" compared to "Pepe" 45 DAST, respectively. Root K was generally increased with sampling date and salinity, but to variable extents, in both cultivars (Table 3). Significantly higher root K was found in "Maha" over "Pepe" at 6.0 and 8.3 dS m^{-1} at the two sampling dates.

Magnesium

Accumulation of shoot Mg increased with time and varied between the two cultivars (Table 4). Significantly the highest Mg accumulation was observed in both cultivars at 15.0 dS m^{-1} and in "Maha" at 8.3 dS m^{-1} 30 DAST. At the same sampling date, "Maha" was significantly superior to "Pepe"with respect to shoot Mg at 2.0 and 6.0 dS m^{-1}.

Root Mg was not strongly affected by salinity or sampling date. Highest root Mg was found in "Maha" at 8.3 dS m^{-1} regardless of sampling date but differences were insignificant from those accumulated in roots 45 DAST at 6.0 and 15.0 dS m^{-1}.

Calcium

Shoot Ca increased with sampling date and changed slightly due to salinity. However, sporadic significant differences were observed between the two cultivars (Table 4). Highest Ca values were recorded at 15.0 dS m^{-1} in "Pepe" shoots regardless of sampling date and at 8.3 dS m^{-1} 45 DAST in "Maha" seedlings; however, the differences in shoot Ca between the two cultivars were narrower and almost diminished 45 DAST.

Root Ca changed with cultivar, sampling date and salinity (Table 4). Significantly higher Ca accumulation was observed at 15.0 dS m^{-1} in "Pepe" roots regardless of sampling date and at 8.3 dS m^{-1} in roots of the same cultivar 30 DAST. In general, roots of "Pepe" accumulated significantly higher amounts of Ca than those of "Maha" with each increase in salinity and at the two sampling dates except for 2.0 and 8.3 dS m^{-1} 45 DAST where roots of both cultivars accumulated similar amounts of Ca at each of the two levels of salinity.

Proline Content

Shoot and root proline varied with salinity, sampling date and cultivar (Table 4). Increasing salinity to 8.3 and 15.0 dS m^{-1} was associated with significant proline increases in shoots of both cultivars at the two sampling dates. These increases, however, became more pronounced when sampling date was delayed. At the highest two levels of salinity (8.3 and 15.0 dS m^{-1}), shoot proline was significantly higher in "Maha" than in "Pepe" regardless of sampling date. Roots of "Maha" showed significantly higher amounts of proline at 8.3 and 15.0 dS m^{-1} regardless of sampling dates. At 6.0 dS m^{-1}, and 30 and 45 DAST, roots of "Pepe" accumulated significantly higher amounts of proline over those of "Maha".

5. DISCUSSION

Although seedling shoot growth of both cultivars, represented as shoot weight (Table 1), increased with age, significant reductions in fresh and dry weights were observed with salinity > 8.3 dS m^{-1}, and became more pronounced as seedlings grew older. Such reductions are common responses to salt stresses in tomato (Alarcon *et al.,* 1994a; Alarcon *et al.*, 1994b; Bolarin *et al.*, 1991; Perez-Alfocea *et al.*, 1996; Smith, *et al.*, 1992).

Salinity effect on shoot growth reduction was more pronounced in "Maha" than in "Pepe" (Table 2). According to Caro *et al.* (1991) and Cruz *et al.* (1990),

vegetative growth is the most widely used index in studies of plant salt tolerance. Considering absolute growth under stress conditions, however, as a combined measure of plant vigor and tolerance (Shannon *et al*., 1987), "Pepe" seems to be more salt tolerant than "Maha" at the seedling stage. To differentiate vigor and tolerance one can view growth under stress as either absolute or relative (Fooland, 1996). Nevertheless, the use of absolute values for vegetative growth or yield parameters in response to salinity to differentiate between cultivars may lead to differences in plant salinity tolerance classification (Caro *et al*., 1991). However, when shoot growth and yield were considered, shoot growth was less dependable as an index of tolerance to salinity (Caro *et al*., 1991).

Bolarin *et al*. (1991) and Shannon (1985) considered, on the other hand, relative growth (Tolerance index) as a measure of plant stress tolerance. When Fooland (1996) studied the behavior of several generations of salt tolerant and salt sensitive tomatoes under 20 dS m^{-1} salinity, shoot absolute growth and shoot relative growth (Tolerance index) were significantly correlated (r=0.86). However, this is not always the case, as plants with higher shoot growth under salt stress were not always the most tolerant ones (Shannon *et al*., 1987). Threshold values (highest salinity in dS m^{-1} without vegetative growth reduction) and slope of the curve (vegetative growth decrease per unit salinity increase) must be taken into account (Caro *et al*., 1991; Shannon *et al*., 1987). In the present study, we cannot differentiate between the two cultivars tested in their tolerance to salinity as the maximum salinity without vegetative growth reduction in our experiment was 6.0 dS m^{-1} for both cultivars; to better understand differences in salt tolerance of both cultivars on basis of salinity threshold, more levels of salinity above 6.0 dS m^{-1} and less than 8.3 dS m^{-1} need to be investigated. According to Caro *et al*. (1991), the use of threshold or slope values to determine plant salinity tolerance depends on salinity level; under low to medium salinity levels (< 7.0 dS m^{-1}) threshold could be used as a parameter for plant salt tolerance, while for medium to high salinity range (> 7.0 dS m^{-1}) slope will be the index parameter.

Under our growing conditions, the coefficient of determination (R^2) for shoot weight, fresh and dry, in both cultivars (table 2) was higher than 0.5 indicating a significant correlation between shoot growth and salinity. Threshold values 45 DAST are apparently less than 6.0

dS m^{-1} for "Maha" and below 8.3 dS m^{-1} for "Pepe" on fresh weight basis and for both cultivars at salinity less than 8.3 dS m^{-1} when dry weights are considered.

Slopes of relative shoot fresh and dry weights reduction (Tolerance index) were higher in "Maha" (Table 5) indicating that this cultivar is more sensitive to salinity. Irrespective of cultivar, slopes of dry weights were lower, indicating a reduced response to salinity. Growth reduction in salt stressed plants could be due to reduction in leaf turgor (Alarcon *et al*., 1993; Alarcon *et al*., 1994b), reduction in net assimilation rate due to reduced photosynthesis (Alarcon *et al*., 1994b), increase in energy cost resulting from osmotic adjustment (Alarcon *et al*., 1993), or due to toxic effects and imbalanced nutrition provoked by NaCl (Alarcon *et al*., 1993; Greenway and Manns, 1980).

Reduction in root growth is a common response to increased salinity (Rodriguez *et al*., 1997; Snapp and Shennon, 1994). Salinity above 6.0 dS m^{-1} had significant influence on seedling root growth (Tables 1-2). A negative correlation was also observed between root weight and salinity (R^2= 0.84 to 0.97). Reduction in the relative root fresh and dry weights 45 DAST due to salinity were not as much as those in the shoot (Table 5); relative root fresh weight reduction slopes were higher for "Maha" and almost similar for root dry weights in both cultivars. These results suggest that "Maha" root fresh weight is more sensitive to salinity and hence less salt tolerant than "Pepe". Similarly, genetic variability in the lowering of root growth under salt stress has been reported in tomato (Cuartero and Fernandez-Munoz,1999; Snapp and Shennon, 1994). Differences among L. *esculentum* cultivars were also described at 140 mM (Alian *et al*., 2000). That slope values of relative root dry weights were lower than relative root fresh weights, could also indicate that accumulation of dry matter in roots is less susceptible to salinity.

Mineral Composition in Shoot

The absolute amounts of Na and Cl in tomato shoot increased with salinity in both cultivars but to higher levels in "Pepe" (Tables 3-4). This increase is associated with reduction in shoot dry weights (Table 6). Similarly, Bolarin *et al*. (1993) and Alian *et al*.(2000) found both elements to accumulate with increased salinity and their accumulation was associated with reduction in shoot fresh and dry weights. Those plants with higher Na (Bolarin *et al*.,1993; Alian *et al*., 2000) and Cl (Alian *et*

al. 2000) were the most tolerant ones (Bolarin *et al.*,1993; Alian *et al.*, 2000). It is therefore possible that "Pepe" is more tolerant than "Maha" as it accumulated more Na and Cl in shoots with less reduction in fresh and dry weights. In contrast, Sacher *et al.* (1983) reported that the relation between Cl accumulation and plant tolerance to salinity varied with tomato lines; some tolerant tomato lines accumulated higher or lower amounts of Cl, but others with low Cl accumulation were salt sensitive.

According to Sacher *et al.* (1983), the use of absolute levels of ions in the leaves of a particular tomato line may lead to its classification as a salt includer or excluder compared with other lines; none of the tested lines was able to prevent a substantial increase in either Na or Cl, nor did any line exhibited a salt uptake response proportional to salt application. Regressing the growth of mesophytic plants, including tomato, by the Ion Regression Index (IRI) (Foliar ion content under salt stress/ foliar ion content in the control) gave a much better fit to growth data than did regression on the amounts of each ion accumulated (Sacher *et al.*, 1983).

Forty five DAST, highly significant correlation (R^2 = 0.91-0.97) was established between either Na or Cl IRI and tomato relative shoot dry weight in both cultivars (Table 6); reduction slopes for Na were -2.99% and -2.57% and for Cl were about -9.68% and -5.36% in "Maha" and "Pepe", respectively. These results lead to the conclusion that at this growth stage, "Maha" was more sensitive to Na and Cl accumulation than "Pepe".

Under low salinity levels (2.0 to 6.0 dS m^{-1}) both cultivars were able to stand certain amounts of Na and Cl in their shoots (Table 3) without significant reduction in weight (energy saving) and therefore both cultivars might have used these ions for osmotic adjustment. Plants may adjust osmotically either by absorbing ions through roots and accumulating them in shoots or by synthesizing organic osmotica (Bolarin *et al.*, 1993). On the other hand, under high salinity levels (8.3 and 15.0 dS m^{-1}), "Maha" could be considered an excluder as it accumulated less Na and Cl and osmotic adjustment in this cultivar was probably achieved by accumulation of higher amounts of proline with expenditure of energy (shoot growth was significantly reduced).

Salinity > 8.3 dS m^{-1} reduced shoot K, Ca and Mg accumulation (Tables 3-4). Those plants which take up more K, Ca and Mg from the medium reduce Na/K, Na/Ca and Na/Mg ratios (Table 7) and thus tolerate salt stress more (Perez-Alfocea *et al.*,1996). However, neither "Maha" nor "Pepe" were able to maintain lower Na/K, Na/Ca and Na/Mg ratios at high salinity levels. It seems more plausible to consider the higher accumulation rate of Na and Cl, at this growth stage, a symptom of specific ion toxicity rather than adaptation to salinity as deduced from the fact that seedlings of both cultivars died 7-10 days after the last sampling date (45 DAST). Rush and Epestien (1981) reported that tolerant tomato freely accumulated Na in the shoot while the salt sensitive excluded it from the leaves where it has been shown to be toxic. In addition to osmotic shock, specific accumulation of Cl and Na ions in plants is often toxic and may be one of the main causes for growth inhibition under high salinity (Greenway and Munns, 1980). According to Caro *et al.* (1991) and Shannon *et al.* (1985), the fundamental mechanisms determining response of plants to salinity might be due to osmotic shock and toxic effect (Caro *et al.*, 1991; Shannon *et al.*, 1985).

Table 4: Interactive effects of cultivar and salinity(1) level on Mg, Ca and Proline content in shoots and roots of tomato seedlings.

Cultivar	Salinity (dS m^{-1})	(mg g^{-1} DW)				μmole.g g^{-1} FW	
		Mg		Ca		Proline	
		Shoot	Root	Shoot	Root	Shoot	Root
		30 DAST					
Maha	2.0	9.2 b[(2)]	4.7 bc	26.3 f	10.2 e	1.3 g	1.5 g
	6.0	8.9 b	4.7 bc	29.5 de	10.6 e	2.8 f	2.9 f
	8.3	11.2 a	6.1 a	32.0 c	12.4 d	24.0 b	12.0 b
	15.0	11.1 a	5.4 b	33.9 b	14.5 c	40.2 a	16.3 a
Pepe	2.0	7.7 c	4.8 bc	28.7 e	12.0 d	1.5 g	1.9 g
	6.0	7.8 c	4.6 c	28.3 e	16.5 b	3.8 e	3.8 e
	8.3	9.3 b	4.8 bc	30.7 cd	17.0 ab	13.2 d	6.6 d
	15.0	10.8 a	4.8 bc	35.7 a	18.3 a	15.6 c	10.7 c

		45 DAST					
Maha	2.0	10.4 e	5.2 bc	31.8 cd	13.9 d	1.9 f	1.8 f
	6.0	11.4 de	6.3 a	31.9 cd	14.9 d	3.5 ef	3.0 f
	8.3	14.5 b	6.5 a	35.5 b	18.4 bc	33.8 c	15.6 c
	15.0	14.7 b	5.8 ab	38.1 a	19.7 b	68.6 a	47.7 a
Pepe	2.0	12.3 cd	5.9 ab	29.9 e	13.8 d	2.2 f	1.1 f
	6.0	12.9 c	5.5 bc	30.6 de	17.3 c	4.1 e	6.3 e
	8.3	14.9 b	5.3 bc	32.8 c	18.5 bc	14.3 d	10.1 d
	15.0	17.1 a	4.9 c	38.6 a	21.8 a	56.9 b	38.1 b

(1) Salinity treatment started at cotyledon stage.

(2) For each age category, means within columns having different letters are significantly different according to DMRT($p<0.05$) .

Table 5: Linear regression slopes between salinity and relative shoot and root weights of tomato seedlings 45 DAST.

Parameter	Slope[1]		R^2 [2]	
	Maha	**Pepe**	**Maha**	**Pepe**
Relative shoot fresh weight	-4.82	-4.67	0.85	0.77
Relative shoot dry weight	-3.03	-2.85	0.86	0.72
Relative root fresh weight	-3.93	-3.52	0.84	0.94
Relative root dry weight	-2.85	-2.79	0.94	0.97

(1) Percent decrease per unit salinity increase.

(2) Coefficient of determination.

Table 6: Linear regression slopes between Na and Cl regression indices and relative shoot and root weights of tomato seedlings 45 DAST.

Parameter	Slope[1]		R^2 [2]	
	Maha	Pepe	Maha	Pepe
Na				
Relative shoot dry weight	-2.99	-2.57	0.97	0.93
Relative root dry weight	-2.80	-3.59	0.99	0.84
Cl				
Relative shoot dry weight	-9.68	-5.36	0.96	0.91
Relative root dry weight	-9.36	-9.15	0.99	0.90

(1) Percent decrease per unit Na or Cl increase.

(2) Coefficient of determination.

Mineral Composition in Roots

A marked increase in Na and Cl accumulation was observed in seedling roots of both cultivars as salinity increased but to a higher level in "Maha" (Table 3). The correlation between root dry weight and Na and Cl ion regulation indices (Table 6) showed high negative correlations; the root dry weight slopes were lower for Na (-2.80% and -3.59%) than for Cl (-9.39% to -9.15%). These results show that "Pepe" roots are more sensitive to Na accumulation but both cultivars are similar in response to Cl accumulation. Moreover, at this growth stage (45 DAST) seedling roots were more sensitive to Cl than to Na as deduced from the greater slope value reductions in root dry weights.

According to Greenway and Munns (1980), the regulation of ion transport in plant organs is an essential factor of the mechanism of salt tolerance. Although both cultivars, "Maha" and "Pepe", were unable to avoid excessive accumulation of Cl and Na in shoots and roots, "Maha" showed considerable avoidance to Na accumulation in shoots but accumulated more Na in the roots at high salinity levels (Table 3). Therefore, the salt tolerance of this cultivar seems to be related to the capacity of Na exclusion from the shoot to avoid toxic effects of Na. Specific ion accumulation of Cl and Na in plant tissue is toxic and may be one of the main causes for growth inhibition under high salinity (Greenway and Munns, 1980). "Pepe", however, was unable to maintain low Na concentration in shoots but reduction in relative shoot growth was lower than that of "Maha" (Table 6), suggesting that salt tolerance of this cultivar is possibly related to osmotic adjustment; the greater reduction in relative root dry weight as indicated by Na regulation indices, casts in the possibility of specific ion toxicity.

The inhibitory effect of Cl on shoot and root relative dry weights was greater than that of salinity and Na; therefore, by early application of salt treatment (20 days from seed sowing), the inhibitory effect of salinity on vegetative growth, as recognized by slope values, is due mainly to specific ion toxicity of Cl followed by the effect of salinity on shoot and root turgidity (water deficit). Furthermore, the higher inhibition of relative shoot and root dry weights by Cl, tempts us to conclude that the accumulation of this ion could be used to discriminate salt injury. As cited by Bolarin *et al*. (1993), Marschner (1986) reported that water deficit is the principal constraint in plants exposed to high salinity for short periods. However, in plants exposed for longer periods, in addition to water deficit, ion toxicity and imbalance limited plant growth.

Proline Accumulation

That proline accumulation, in both cultivars, increased with salinity (Table 4) is in general agreement with several authors. Bolarin *et al*. (1995) recognized an increase in proline content in tomato seedlings at the 2-3 leaf stage, when 140 mM NaCl were applied. Such increase in proline at high salinity (12.2 dSm^{-1}) was reported in the cytoplasm only (Perez-Alfocea *et al*., 1996). Proline accumulation has been reported to increase in plants subjected to hyperosmotic stresses, primarily drought (Balibrea *et al*., 1997) and salt stress (Aziz *et al*., 1999; Perez-Alfocea *et al*., 1996; and Guerrier, 1998) and could be an adaptive response to salinity through decreasing the osmotic potential in the cytoplasm avoiding water loss [Perez-Alfocea and Larher (1995) as cited by Perez-Alfocea *et al*. (1996)].

In the present investigation (Table 4), proline accumulation was significantly higher in "Maha" than "Pepe" regardless of sampling date especially at 8.3 and 15.02 dSm^{-1}. However, the ability to decrease the internal osmotic potential was considered insufficient to attain salt tolerance (Rajasekaran *et al*., 1999). Bolarin *et al*. (1995) reported that proline accumulation increased only after sugar accumulation occurred in plants under salt stress, and because of its possible relation to inhibition of callus growth (Cano *et al*., 1996) these authors concluded that such accumulation of proline can be used as a salt sensitive trait.

Table 7: Na/K, Na/Mg and Na/Ca ratios in tomato shoots in response to salinity[1] 45 DAST.

Cultivar	Salinity (dS m^{-1})	45 DAST		
		Na/K	Na/Mg	Na/Ca
Maha	2.0	0.08	0.43	0.14
	6.0	0.28	1.35	0.48
	8.3	0.86	2.79	1.14
	15.0	1.42	4.31	1.66
Pepe	2.0	0.09	0.43	0.18
	6.0	0.30	1.38	0.58
	8.3	1.02	3.75	1.71
	15.0	1.68	4.89	2.17

(1) Salinity treatment started at cotyledon stage.
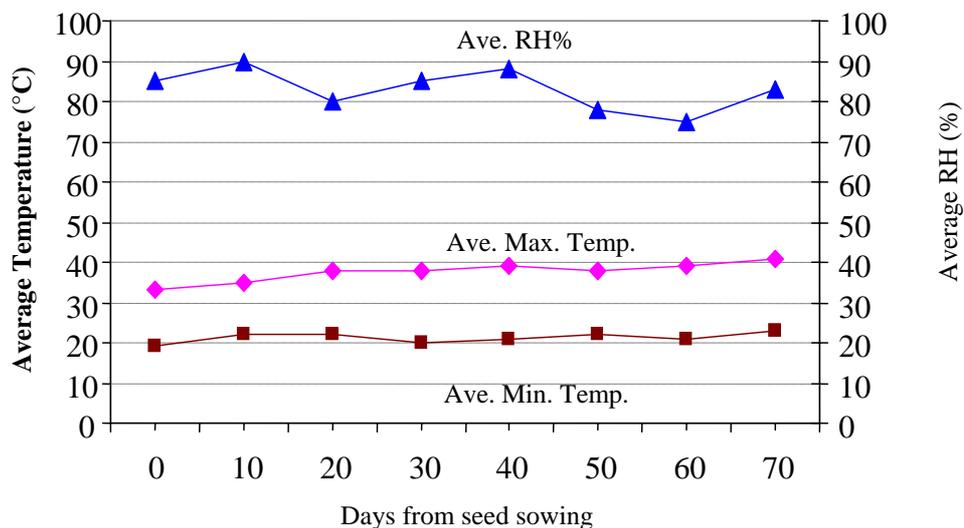
Fig 1: Average maximum and minimum temperature and RH inside the greenhouse during the growing period of tomato seedlings.

6. REFERENCES

Alarcon, J.J., Bolarin, M.C., Sanchez-Blanco, M.L. and Torrecillas, A. 1994a. Growth, Yield and Water Relations of Normal Fruited and Cherry Tomato Cultivars Irrigated with Saline Water. *J. Hort. Sci*., 69 283-288.

Alarcon, J. J., Sanchez-Blanco, M. J., Bolarin, M. C. and Torrecillas, A. 1993. Water Relations and Osmotic Adjustment in *Lycopersicon esculentum* and *L. pennellii* During Short-term Salt Exposure and Recovery. *Physiol. Plant.,* 89:441-447.

Alarcon, J.J., Sanchez-Blanco, M.J., Bolarin, M. C. and Torrecillas, A. 1994b. Growth and Osmotic Adjustment of Two Tomato Cultivars During and After Saline Stress. *Plant Soil*., 166 : 75-82.

Alian, A., Altman, A. and Heuer, B. 2000. Genotypic Difference in Salinity and Water Stress Tolerance of Fresh Market Tomato Cultivars. *Plant Sci*., 152:59-65.

Aziz, A., Martin-Tanguy, J. and Larher, F. 1999. Salt Stress-induced Proline Accumulation and Changes in Tyramine and Polyamine Levels are Linked to Ionic Adjustment in Tomato Leaf Discs . *Plant Sci.,* 145: 83-91.

Balibrea, M.E., Rus-Alvarez, A.M., Bolarin, M.C. and Perez-Alfocea, F. 1997. Fast Changes in Soluble Carbohydrates and Proline Contents in Tomato Seedlings in Response to Ionic and Non-ionic Iso-osmotic Stresses. *J. Plant Physiol*., 151: 221-226.

Bates, L.S., Waldren, R.P. and Teare, I.D. 1973. Rapid Determination of Free Proline for Water Stress Studies. *Plant Soil.*, 39: 205-207.

Bolarin, M.C., Fernandez, F.G., Cruz, V. and Cuartero, J. 1991. Salinity Tolerance in Four Wild Tomato Species using Vegetative Yield Salinity Response Curves. *J. Amer. Soc. Hort. Sci.*, 116:286-290.

Bolarin, M.C., Perez-Alfocea, F., Cano, E.A., Estan, M.T. and Caro, M. 1993. Growth, Fruit Yield, and Ion Concentration in Tomato Genotypes After Pre-and Post-emergence Salt Treatments. *J. Amer. Soc. Hort. Sci.*, 118: 655-660.

Bolarin, M.C., Santa-Cruz, A., Cayuela, E., Perez-Alfocea, F. 1995. Short-term Solute Changes in Leaves and Root of Cultivated and Wild Tomato Seedlings Under Salinity. *J. Plant Physiol.*, 147:463-468.

Cano, E. A., Perez-Alfocea, F., Moreno, V. and Bolarin, M. C. 1996. Response to NaCl Stress of Cultivated and Wild Tomato Species and Their Hybrids in Callus Cultures. *Plant Cell Reports.*, 15: 791-794.

Caro, M., Cruz, V., Cuartero, J., Estan, M. T. and Bolarin, M. C. 1991. Salinity Tolerance of Normal Fruited and Cherry Tomato Cultivars. *Plant Soil.*, 136: 249-255.

Cruz, V., Cuartero, J., Bolarin, M.C. and Romero, M. 1990. Evaluation of Characters for Ascertaining Salt Stress Responses in *Lycopersicon* Species. *J. Amer. Soc. Hort. Sci.*, 115:1000-1003.

Cuartero, J. and Fernandez-Munoz, R. 1999. Tomato and Salinity. *Scientia Hort.*, 78:83-125.

Delauney, A.J. and Verma, D.P.S. 1993. Proline Biosynthesis and Osmoregulation in Plants. *Plant J.*, 4: 215-223.

Fooland, M.R. 1996. Genetic Analysis of Salt Tolerance During Vegetative Growth in Tomato *Lycopersicon esculentum* Mill. *Plant Breeding.*, 115:245-250.

Franco, J.A., Perez-Saura, P.J., Fernandez, J.A., Parra, M. and Garcia, A.L. 1999. Effect of Two Irrigation Rates on Yield, Incidence of Blossom-end Rot, Mineral Content and Free Amino Acid Levels in tomato Cultivated Under Drip Irrigation Using Saline Water. *J. Hort. Sci. Bio.*, 74: 430-435.

Greenway, H. and Munns, R. 1980. Mechanisms of Salt Tolerance in Nonhalophytes. *Ann. Rev. Plant Physiol.*, 31: 149-190.

Guerrier, G. 1998. Proline Accumulation in Salt-treated Tomato: Different Proline Precursors in *Lycopersicon esculentum* and *Lycopersicon pennellii* . *J. Plant Nutr.*, 21: 505-513.

Liu, J. and Zhu, J. 1997. Proline Accumulation and Salt-stress-induced Gene Expression in a Salt-hypersensitive Mutant of Arabidopsis. *Plant Physiol.*, 114: 591-596.

Marschner, H. 1986. *Mineral Nutrition in Higher Plants.* Academic Press Inc., London.

Mohammad, M., Shibli, R., Ajlouni, M. and Nimri, L. 1998. Tomato Root and Shoot Responses to Salt Stress Under Different Levels of Phosphorus Nutrition. *J. Plant Nutrition.*, 21:1667-1680.

Perez-Alfocea, F., Balibrea, M.E. Santa Cruz, A. and Estan, M.T. 1996. Agronomical and Physiological Characterization of Salinity Tolerance in a Commercial Tomato Hybrid. *Plant Soil.*, 180: 251-257.

Rajasekaran L., Aspinall, D. and Paleg, L.G. 1999. Physiological Mechanism of Tolerance of Lycopersicon spp. Exposed to Salt Stress. *Canadian Jour. Plant Sci.*, 151-158.

Rodriguez, P., DellAmico, J., Morales, D., SanchezBlanco, M.J. and Alarcon, J. J. 1997. Effects of Salinity on Growth, Shoot Water Relations and Root Hydraulic Conductivity in Tomato Plants. *J. Agric. Sci.,* 128:439-444.

Rush, D.W. and Epstein, E. 1981. Comparative Studies on the Sodium, Potassium, and Chloride Relations of a Wild Halophytic and Domestic Salt-sensitive Tomato Species. *Plant Physiol.*, 68: 1308-1313.

Sacher, R.F. and Staples, R.C. and Robinson, R.W. 1983. Ion Regulation and Response of Tomato to Sodium Chloride: A Homeostatic System. *J. Amer. Soc. Hort. Sci.*, 108: 566-569.

Schwarz, D. and Kuchenbuch, R. 1998. Water Uptake by Tomato Plants Grown in Closed Hydroponic System Dependent on the EC-level. *Acta Hort.*, 458: 323-327.

Shannon, M.C. 1985. Principles and Strategies in Breeding for Higher Salt Tolerance. *Plant Soil.*, 89: 227-241.

Shannon, M.C., Gronwald, J.W. and Tal, M. 1987. Effects of Salinity on Growth and Accumulation of Organic and Inorganic Ions in Cultivated and Wild Tomato Species. *J. Amer. Soc. Hort. Sci.*, 112: 416- 423.

Smith, M.A.L., Spomer, L.A., Shibli, R.A. and Knight, S.L. 1992. Effects of NaCl Salinity on Miniature Dwarf Tomato 'Micro-Tom': II. Shoot and Root Growth Responses, Fruit Production, and Osmotic Adjustment. *J. Plant Nutr.*, 15:2329-2341.

Snapp, S.S. and Shennan, C. 1994. Salinity Effects on Root Growth and Senescence in Tomato and the Consequences for Severity of *phytophthora* Root Rot Infection. *J. Amer. Soc. Hort. Sci.*, 119: 458-463.

Taleisnik-Gertel, E., Tal, M. and Shannon, M.C. 1983. The Response to NaCl of Excised Fully Differentiated and Differentiating Tissues of Cultivated Tomato, *Lycopersicon esculentum*, and its Wild Relatives, *L. peruvianum* and *Solanum pennellii*. *Physiol. Plant.*, 59:659-663.

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(/   15.0   8.3   6.0   2.0)
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.   (*L. esculentum var. cerasiforme*) (   )
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