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PHYSIOLOGICAL RESPONSES TO SALINITY IN *SOLANUM LYCOPERSICUM* L. VARIETIES

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Abstract

Worldwide over 30% of irrigated and 7% of rainfed agriculture has been limited by salinity stress. Tolerance of crops to salinity varies and negatively affects agricultural productivity. Despite the plethora of information on NaCl tolerance mechanisms, it is still not completely elucidated. The purpose of this research was to determine NaCl tolerance of eight tomato varieties (Tropic, Feroz, Ace, Super Rio Grande, Yaqui, Missouri, Vita and Floradade) by evaluating their physiological traits. These varieties were exposed to salinity stress by the addition of NaCl (0, 50, 100, 150 and 200 mM). The physiological variables measured were stomatal conductance, water potential, chlorophyll a, b, total, indirect chlorophyll content, leaf temperature, transpiration and relative water content. The results showed differences in tolerance between varieties in terms of NaCl concentrations and there was interaction between varieties × NaCl in the majority of physiological variables. Symptoms of NaCl stress in the tomato plants were leaf wilting, desiccation, necrosis, and death. All measured variables decreased as salinity increased, except for relative water content and leaf temperature, values of both these variables increased with higher concentrations of NaCl. Physiological traits may be used as an effective means for screening for salinity tolerance in tomato varieties. Amongst the tomato varieties evaluated were Missouri the most tolerant, and Rio Grande the least tolerant. The results indicate that the varieties best tolerant to NaCl conditions from most to least tolerant in successive orderare: Missouri, followed by Ace, Yaqui, Tropic, Floradade, Feroz, Vita and Rio Grande.

Key words: Salinity tolerance, Arid zones, Relative water content, Stomatal conductance, Tomatoes.

Introduction

Our planet is a brackish biosphere with the majority of its water in its oceans with around 30-35g of NaCl L^{-1} (Flowers, 2004). Worldwide NaCl is a ubiquitous chemical plant stressor. Salinity stress affects around 20% of all agricultural areas cultivated in the world. In terms of percentages, 30-50% of the irrigated crops and 7% of rainfed agriculture (Kaya *et al*., 2002; Schroeder *et al*., 2013). NaCl effects agricultural soils throughout the world (~45 million ha) to some degree and this is estimated to increase because of global climate changes, irrigation practices, as well as sea water intrusion to aquifers from coastal areas. NaCl can cause negative impacts in plant development, growth, production, yield and the overall health. According with Munns & Tester (2008), NaCl inhibits growth by first affecting its osmotic phase and later as salinity increases, accelerating senescence. How plants reply to NaCl stress is different according to the developmental plant stage (Rzepka-Plevneš *et al*., 2008) and generally is more sensitive during the younger seedling phase (Cuartero & Fernández-Muñoz, 1999; Alian *et al*., 2000; Rzepka-Plevneš *et al*., 2007). Some plants with tolerance in early growth stages demonstrate enhanced NaCl tolerance when fully developed (Akinci *et al*., 2004). Undoubtedly, plants have undergone a complex evolutionary response to salinity through cell, organ and whole-plant processes. These adaptations use metabolic and signaling pathways and networks that at present are not completely understood. In general terms, the processes of plant NaCl tolerance involve balancing ionic and osmotic components to permit water flow even under these conditions.

Tomatoes are a valuable worldwide cultivated horticultural crop because of its nutritional and commercial value, as well as its great yield, playing an important role in the human diet, since it is consumed in variety of forms: consumed fresh or modified and preserved (*i.e*. tomato sauce and ketchup).

Tomatoes cultivation can be found in arid zones that have water scarcity and high NaCl content. Due to its value in human diet many tomato genotypes have been studied for its tolerances to NaCl stress; results have shown that a great genetic variation of NaCl tolerance occurs among tomato cultivars. As in many other crops, NaCl affected soils or waters alters tomato development, production and yield around the world. To counteract NaCl stress, plant breeding programs have been initiated. However, in general they have not generated a sufficient number of NaCl tolerant varieties (Foolad & Lin, 1998).

Thus, finding NaCl tolerant tomato genotypes in the germplasm is desired to improve production (Foolad, 1996; Kaveh *et al*., 2011). Many tomato genotypes are able to grow with normal yield even if NaCl is present; this is known to be true since tomato has been long cultivated successfully in some regions although impacted by NaCl (Cuartero *et al*., 2006; Frary *et al*., 2010). It would be useful to find NaCl tolerant varieties, in order to use somewhat brackish water to grow tomatoes. To do this it is critical to understand the biochemical, genetic and physiological mechanisms of NaCl tolerance, and as a first step it is necessary to find the tolerant varieties.

Hence, a wide variety of research has been done to search for NaCl tolerant tomatoesgrown in moderate salinity soils or watered by brackish water that produce appropriate vegetative growth in a temporal scale with good yields. According to Hartz (1990) some commercial genotypes of tomato show no or slight productivity losses and can tolerate modest NaCl levels as high as 2.5 dS m^{-1}. There are quite limited numbers of studies of tomato genotypes at higher dosages of salinity. In this sense, more research is needed to evaluate tomato genotypes at higher salinity concentrations in order to produce plants that maintain vegetative growth and yield. Research with the tomato should focus on improving yield while examining genetic, cellular, molecular, biochemical, morphometric and physiological responses.

Based on these factors, the purpose of this research was to take the first step in searching for tomato varieties that are NaCl tolerant at higher dosages than previously reported, by evaluating physiological traits in eight tomato varieties. The varieties that were stressed with different concentrations of NaCl were Tropic, Feroz, Ace, Super Rio Grande, Yaqui, Missouri, Vita and Floradade. This research was done to provide the data for selecting tomato varieties that are less impacted or if possible perform better with NaCl, especially with respect to early vegetative growth and its physiological traits, with the ultimate goal of finding a variety or varieties with higher production yields.

Materials and Methods

Study area: The research was conducted in a shade-enclosure located in the city of La Paz, Baja California Sur, Mexico (24°08′ 09.73” N, 110°25′ 41.73” W) at 7 m.a.s.l. (Fig. 1), with fabric made of monofilament stabilized polyethylene, with a filament density of 160 filaments cm^{-2} , with a square aperture of 0.4 × 0.8 mm (model 1610 PME CR). The mean temperature in shade-enclosure was 29.0°C, with average maximum and minimum 29.0°C and 40.0°C, respectively with 60% relative humidity during tomato early vegetative growth stage (May to July). All weather-related measurement data was captured with a weather station located at the study area (Vantage Pro2® Davis Instruments, USA). The site Köppen climate classification is Bw (h´) hw (e), i.e. semiarid with xerophytic vegetation (García, 2004). The water retention in the ground was low to medium, at high sand content (< 1% organic matter), with neutral to alkaline pH, good permeability and aeration.

Plant material and experimental conditions: The physiological traits in the early vegetative growth phase of eight tomato varieties of *Solanum lycopersicum* L. were evaluated: Missouri, Super Rio Grande, Yaqui (Saladette type), Tropic, Feroz, Ace, Vita and Floradade (Ball type). On May 1, seeds of each tomato variety were placed in shade-enclosure for about 20 days in a peat moss based medium. Watering was carried out as required to maintain moisture of soil and fertilization was done once every 5 days with a Hoagland solution (Hoagland & Arnon, 1950).

On May 22, plants were transplanted (seedlings with approximately 4 or 5 leaves and 10-15 cm of height) into plastic pots with drainage holes where 25 cm high, 20 cm wide on the upper surface with approximately 4 kg of capacity, containing a blend of peat-moss (Sunshine® Peat Moss Grower Grade White, Sun Gro Horticulture Canada Ltd.) and sand(1:1). These seedlings were then placed in a shade-enclosure with mesh which allowed filtered sunlight. To allow for plant root establishment the seedlings were irrigated for 10 days with 1 L of tap water every second day. Uniform plants were chosen to be treated with NaCl. Watering was carried out daily for all plants with tap water that was mixed with one solution with concentrate nutrients (stock solution) containing (g in 3 L^{-1} of distilled water): 168 of KNO_3, 30.6 of (NH_4) (NO_3), 44.4 of (NH_4) H_2PO_4, 180.6 of Ca $(NO_3)_2$, 126 of $MgSO_4$, 6.0 of $FeSO_4$, 1.5 of $MnSO_4$, 0.3 of $ZnSO_4$, 0.3 of $CuSO_4$ and 0.3 of H_3BO_3 according to the recommendation of Samperio-Ruiz (1997) for tomatoes. A methodology by Murillo-Amador *et al*. (2007) was followed during the second week that implemented NaCl treatments gradually to elude sudden change in solute concentration and its negative impacts (osmotic stress). Briefly, the Murillo-Amador *et al*. (2007) methodology involves flushing the soil with a surplus of solution (500 mL) at the NaCl level of interest. The NaCl treatments applied were as follows: 0, 50, 100, 150 and 200 mM of NaCl (0.3, 2.6, 5.1, 7.6 and 10.3 dS m^{-1}, respectively). Water draining from the pots was collected and the pH and electrical conductivity was measured for all treatments. This was done to verify that drained water was similar to applied water. By addition of H_2SO_4 or KOH the pH was maintained at 6.0. The plants received a calculated average a daily dose of 393 ± 65 µmol m^{-2} s^{-1} of sunlight.

Stomatal conductance, transpiration rate and leaf temperature: All variables were measured twice in the shade-enclosure with saturated light (June and July) using a handy porometer sensor (Model Li-1600, Li-COR Inc.) with typical cuvette temperature range of 35.9 ± 1.5°C, with a flux of 1.4 ± 1.5 cm^3 s^{-1} and a relative humidity of 37.0 ± 5.6%. Stomatal conductance (Gs= cm^{-2} s^{-1}), transpiration rate (E= µg cm^{-2} s^{-1}) and leaf temperature (°C) were measured at 10:00-13:00 hours (4 replicates) on a clear, cloud-less day in fully expanded, healthy, turgid, flat and uniform in color and size leaves of each tomato variety and NaCl treatment, respectively.

SPAD-readings: SPAD readings of leaves were taken twice (June and July) using a handy SPAD 502 chlorophyll meter (Minolta Camera Co., Ltd., Japan). The methodology followed was that of Ruiz-Espinoza *et al*. (2010). Briefly, on the left and right of the midrib three measurements are made |from uniform healthy leaf for a total of 6 measurements and the samples were gathered from three different plants per plot treatment and the control plot in the early morning between 08:00 and 10:00 h. One youngest, fully expanded, healthy, turgid, flat and uniform in color and size leaf from three different plants per plot was selected. A total of 720 leaves were measured.

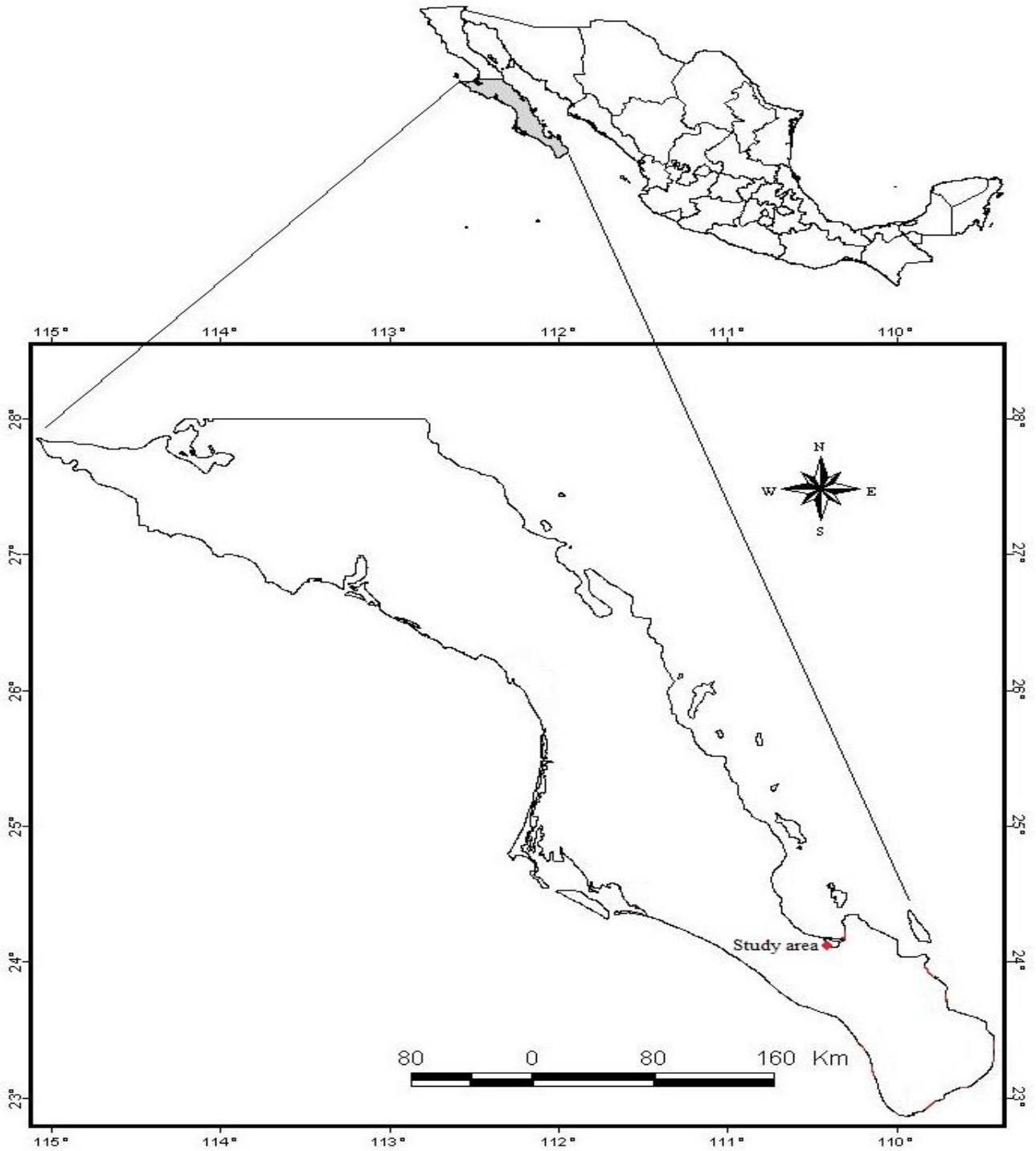

Fig. 1. Study area (red point) located at Centro de Investigaciones Biológicas del Noroeste, S.C. in La Paz, Baja California Sur, Mexico.

Leaf water potential (LWP): A dew-point psychrometer (WP4-T, Decagon Devices, Pullman, Washington, USA) was used to measure the leaf water potential. This measurement was carried out twice (June and July) in one plant per variety and NaCl treatment that had been in direct sunlight for a minimum of 1 h before being measured and these were healthy fully developed leaves.

Relative water content (RWC): The mean ofrelative water content was calculated based on averaging two measurements (June and July). Leaves were collected at mid-plant height. Individual leaves were selected and three samples circular in form were hole-punched from the same leaves (total area of 5.10 cm^2). For (fresh mass, FM) leaves were weighed upon removal from plant in the field. Turgid mass (TM) was obtained by floating disk leaf samples in water on a petri dish, removing sample, gently removing excess water and weighing until constant weight was achieved. During this process lead was in dim light (approximately 20 µmol m^{-2}) and laboratory temperature varied between 25±2 °C. At the end of the imbibition period, leaf samples were placed in a pre-heated oven (Shel-Lab®, model FX-5, serie-1000203), at 80 °C until constant weight (approximately 72 h), in order

to obtain the dry mass (DM) (Catsky, 1974; MacNicol *et al*., 1976; Turner, 1979). An analytical scale with precision of 0.0001 g (Mettler Toledo®, model AG204) was used for weighing of all samples. Values of FM, TM, and DM were used to calculate RWC, using the equation:

$$RWC\ (\%) = [(FM\text{-}DM)/(TM\text{-}DM)] \times 100.$$

Chlorophyll a, b and total: Chlorophyll a, b and total content (Chl *a* + Chl *b*) was determined by the method of Arnon (1949) and expressed on a leaf area basis (mg cm^{-2}). The procedure followed is described in detail by Ruiz-Espinoza *et al*. (2010), The procedure involved macerating leaves in aqueous acetone (80 %), centrifuged to transparency (typically 2 to 3 min) and absorbance measured with spectrophotometer (Spectronic Unicom®, Cambridge, UK) at 645 nm and 663 nm. Leaves measured for chlorophyll were the same leaves that were used by the SPAD.

Experimental design: A factorial experimental with two-ways of classification was carried out, with eight tomato varieties (Missouri, Super Rio Grande, Yaqui, Tropic, Feroz, Ace, Vita and Floradade) as a first factor and five NaCl levels (0, 50, 100, 150 and 200 mM NaCl) as a second factor, were arranged in a completely randomized design with four replicates and each replicate consisted of one pot with three plants per pot, that is to say, twenty pots per variety or 160 pots in total.

Statistical analysis: For statistical analysis Statistica version 10 was used. First the homogeneity of variance was confirmed for the data set by employing Bartlett's test. Once it was found that the homogeneity of variance was within adequate ranges a two way ANOVA was carried out for growth parameters with tomato variety as one factor and the other factor being salinity concentrations. In addition, MANOVAs were run for measuring shared/related constructs. Tukey test where run to test for mean differences at $p \leq 0.05$. Relative water content received a special statistical treatment that required that it be arcsine transformed prior to ANOVA (Sokal and Rohlf, 1988).

Results

Analysis of MANOVA revealed that there were significant differences between tomato varieties (Wilks=0.000030; F=18.50; p=0.000001), NaCl treatments (Wilks=0.003943; F=14.8; p=0.000001) and the interaction of varieties × NaCl (Wilks=0.000370; F=2.0; p=0.000001) including all physiological variables. Wilks was significant (0.01) indicating that ANOVA results are not random results of false positive (Johnson, 1988).

Stomatal conductance, transpiration rate and leaf temperature: After one week of NaCl treatments, the leaves of of some varieties that were subjected to 200 mM NaCl had signs of necrosis and wilting, while other genotypes took longer than two weeks to reach necrosis and wilting (at 150 or 200 mM NaCl), while other varieties simply dried up and died in less than a week. In general, the primary leaves had more damage than the first and second trifoliate leaves. Stomatal conductance displayed significant differences between NaCl treatments ($F_{4,80}$=64.83; $p \leq 0.0001$) but did not show differences between varieties ($F_{7,80}$=1.47; p=0.18) and the interaction of varieties × NaCl ($F_{28,80}$=1.14; p=0.32). The *Gs* values decreased as NaCl levels increased and although varieties as factor did not show significant differences, the values of *Gs* varied by tomato cultivar from 1.14 to 2.37 $cm^{-2}\ s^{-1}$, where Feroz was the variety with highest *Gs* (Table 1). Although *Gs* did not show significant statistical interaction differences between varieties × NaCl, all varieties had a trend of decreasing *Gs* as NaCl levels increased from 0 mM to 100 mM, while Gs differences at higher values (100mM, 150 mM and 200 mM) were virtually identical in the eight cultivars (Table 2). Transpiration rate exhibited significant differences between varieties ($F_{7,80}$=5.74; $p \leq 0.0001$), NaCl treatments ($F_{4,80}$=247.08; $p \leq 0.0001$) and the interaction of varieties × NaCl ($F_{28,80}$=2.73; $p \leq 0.0001$). As expected, Feroz had the highest values of *E* because of this variety had higher values of *Gs*, while Tropic had the lowest *E* (Table 1). Transpiration decreased as NaCl levels increased (Table 1). When the interaction varieties × NaCl was analyzed, all varieties displayed a similar trend when NaCl levels increased, although some varieties such as Tropic, Floradade and Rio Grande had *E* values that were very similar at 150 or 200 mM or with just slightly higher valuesat 200 mM NaCl (Table 2). Leaf temperature had significant differences between varieties ($F_{7,80}$=44.5; $p \leq 0.0001$) and NaCl treatments ($F_{4,80}$=11.00; $p \leq 0.0001$) while the interaction of varieties × NaCl did not show significant differences ($F_{28,80}$=1.40; $p \geq 0.12$). The variety Vita followed by Rio Grande and Feroz had higher *LT* while Ace had the lowest leaf temperature (Table 1). Leaf temperature increased as NaCl levels increased (Table 1). Even though *LT* did not show significant differences between the interaction varieties × NaCl, all varieties showed a similar trend of increasing values of *LT* as NaCl levels increased, however, different responses are observed at each NaCl levels of every variety, e.g. Missouri had the lowest *LT* at 100 mM NaCl, while the highest *LT* was for Vita at 150 mM NaCl (Table 2).

SPAD readings:SPAD readings exhibited significant differences between varieties ($F_{7,80}$=6.61; $p \leq 0.0001$) revealing higher and similar values the varieties Floradade, Ace, Tropic, Missouri and Vita, while Rio Grande had the lowest values of this variable (Table 1). Also, SPAD displayed significant differences between NaCl treatments ($F_{4,80}$=48.35; $p \leq 0.0001$), showing a decrease of SPAD values as NaCl levels increased (Table 1). This variable revealed significant differences between the interaction of varieties × NaCl ($F_{28,80}$=1.95; $p \leq 0.01$), displaying differential response of varieties at the NaCl levels, e.g. SPAD values generally decreased with increasing concentrations of NaCl for all cultivars, at the highest concentration of 200 mM NaCl all cultivars had the lowest SPAD reading, except Rio Grande which had the lowest at 150 mM NaCl, but statistically insignificant since the SPAD value is very close to the value of 200 mM NaCl (Table 2).

Table 1. Physiological response of tomato varieties under NaCl stress.

Varieties	Chl a	Chl b (µg cm^2)	Chl total	SPAD readings	RWC (%)	LWP (MPa)	LT (° C)	E (µg cm^{-2} s^{-1})	Gs (cm^{-2} s^{-1})
Missouri	3.48 b	1.25 ab	4.73 b	30.32 a	80.21 bc	-2.32 a	34.04 c	25.80 bcd	1.60 a
Ace	3.63 ab	1.28 ab	4.91 ab	30.83 a	78.18 bc	-2.38 a	32.31 d	27.04 bcd	1.94 a
Yaqui	3.61 ab	1.25 ab	4.86 ab	29.17 ab	79.19 bc	-2.50 a	35.82 b	23.51 cd	1.35 a
Feroz	3.64 ab	1.24 ab	4.89 ab	28.96 ab	76.13 bc	-2.60 ab	36.64 ab	35.57 a	2.37 a
Tropic	3.94 a	1.37 a	5.31 a	30.80 a	75.19 c	-2.61 ab	33.31 c	22.23 d	1.46 a
Rio Grande	2.82 b	1.03 c	3.85 c	27.00 b	81.42 a	-2.72 ab	36.68 ab	27.90 abcd	1.47 a
Floradade	2.75 b	1.01 c	3.77 c	31.15 a	80.47 bc	-2.91 b	35.83 b	30.58 abc	1.14 a
Vita	3.47 b	1.20 b	4.68 b	30.02 a	77.48 bc	-3.55 c	37.17 a	31.78 ab	1.63 a
Salinity (mM NaCl)	**Chl a**	**Chl b (µg cm^2)**	**Chl total**	**SPAD readings**	**RWC (%)**	**LWP (MPa)**	**LT (° C)**	**E (µg cm^{-2} s^{-1})**	**Gs (cm^{-2} s^{-1})**
0	3.61 a	1.26 ab	4.87 a	33.54 a	81.68 a	-2.01 a	34.20 d	66.27 a	5.11 a
50	3.50 ab	1.23 ab	4.73 ab	31.51 b	75.15 b	-2.20 a	35.01 cd	32.57 b	1.51 b
100	3.64 a	1.28 a	4.92 a	29.55 c	73.75 b	-2.52 b	35.15 bc	17.50 c	0.61 bc
150	3.24 ab	1.16 bc	4.40 bc	28.45 c	79.53 a	-3.00 c	35.92 ab	12.68 cd	0.43 c
200	3.11 b	1.09 c	4.20 c	25.85 c	82.56 a	-3.77 d	36.09 a	11.24 d	0.43 c

Chl a= chlorophyll a; Chl b= chlorophyll b; Chl total= chlorophyll total; RWC= relative water content; LWP= leaf water potential; LT= leaf temperature; E= transpiration; Gs= stomatal conductance. Values within the same column with same letter(s) are not significantly different at $p = 0.05$ (Tukey´s HSD multiple range test)

Table 2. Effects of the interaction varieties × NaCl in the physiological variables of tomato varieties.

Varieties	Leaf water potential (MPa)					Relative water content (%)					Leaf temperature (°C)				
	0	50	100	150	200	0	50	100	150	200	0	50	100	150	200
Missouri	-1.84a	-2.14a	-2.08a	-2.38a	-3.17a	84.55a	75.99a	77.81a	79.03ab	83.67a	33.22bc	34.19c	31.41a	35.41bc	35.99d
Ace	-1.85a	-2.15a	-2.34ab	-2.42a	-3.15a	81.87ab	75.35a	73.59a	78.85ab	81.32a	31.16d	32.02e	32.53a	31.89d	33.98e
Yaqui	-2.03ab	-2.37a	-2.37ab	-2.74ab	-2.98a	82.92ab	75.14a	70.48a	82.29ab	85.14a	34.63ab	35.78b	35.88a	36.21abc	36.61c
Feroz	-2.05ab	-2.25a	-2.73b	-2.88ab	-3.08a	82.96ab	73.69a	70.61a	75.58ab	77.81a	35.63a	35.64b	36.43a	37.41ab	38.10a
Tropic	-1.85a	-2.11a	-2.55ab	-2.94ab	-3.63a	79.55bc	73.47a	71.35a	73.00b	78.57a	32.94c	33.41d	33.86a	34.29bc	34.07e
Rio Grande	-2.17b	-2.05a	-2.59ab	-3.14ab	-3.65a	83.98a	77.53a	80.65a	82.57ab	82.38a	34.82ab	36.83a	37.37a	37.12ab	37.26b
Floradade	-2.24b	-2.38a	-2.18b	-3.34ab	-3.77a	81.27ab	77.29a	76.06a	79.05ab	88.70a	35.12a	35.55b	36.20a	36.70ab	35.60d
Vita	-2.07ab	-2.16a	-2.66b	-4.14b	-6.74b	76.41c	72.78a	69.44a	85.85a	82.92a	36.13a	36.71a	37.58a	38.34a	37.11b
	Stomatal conductance (cm^{-2} s^{-1})					Transpiration (µg cm^{-2} s^{-1})					SPAD readings				
	0	50	100	150	200	0	50	100	150	200	0	50	100	150	200
Missouri	4.93a	1.86a	0.55ab	0.36bc	0.33ab	60.72a	34.36abc	14.48ab	10.57b	8.91b	34.09a	31.60ab	31.28a	27.65ab	26.96a
Ace	5.01a	2.30a	0.89a	0.70a	0.82a	50.43a	33.22abc	20.70ab	19.21a	11.64b	33.54a	34.15a	30.63a	29.85ab	26.00a
Yaqui	4.82a	0.76a	0.44b	0.43bc	0.30b	62.18a	20.29c	12.87b	12.58b	9.66b	33.01a	28.91b	30.65a	26.96ab	26.34a
Feroz	8.65a	1.74a	0.85a	0.34bc	0.28b	84.41a	46.75a	25.09a	11.81b	9.81b	31.04a	29.28b	30.78a	27.52ab	26.19a
Tropic	4.29a	1.33a	0.67ab	0.50b	0.53ab	48.73a	24.03c	15.44ab	11.53b	11.45b	34.56a	31.76ab	28.75ab	32.47a	26.46a
Rio grande	5.34a	0.93a	0.41b	0.32c	0.36ab	73.40a	30.76bc	12.22b	10.70b	12.45ab	32.33a	29.56b	25.08b	23.69b	24.33a
Floradade	2.93a	1.16a	0.54ab	0.51b	0.56ab	72.28a	28.36bc	18.88ab	15.21ab	18.15a	35.11a	31.75ab	31.79a	31.27ab	25.85a
Vita	4.96a	2.06a	0.55ab	0.30c	0.28c	78.05a	42.80ab	20.37ab	9.86b	7.81b	34.65a	35.11a	27.48ab	28.18ab	24.67a
	Chlorophyll a (µg cm^2)					Chlorophyll b (µg cm^2)					Chlorophyll total (µg cm^2)				
	0	50	100	150	200	0	50	100	150	200	0	50	100	150	200
Missouri	3.43abc	3.38abc	3.55a	3.41ab	3.62a	1.24ab	1.24abc	1.26a	1.19ab	1.31a	4.68abc	4.63abc	4.81a	4.61abc	4.94a
Ace	3.89ab	3.90a	3.89a	3.23abc	3.24a	1.36ab	1.35a	1.36a	1.14ab	1.18ab	5.26ab	5.26a	5.25a	4.37abc	4.43ab
Yaqui	4.04a	3.60ab	3.91a	3.18abc	3.34a	1.40a	1.26ab	1.37a	1.15ab	1.07ab	5.45a	4.87ab	5.28a	4.32abc	4.41ab
Feroz	3.63abc	3.84a	4.06a	3.51ab	3.17a	1.24ab	1.28ab	1.42a	1.20ab	1.06ab	4.87abc	5.12a	5.49a	4.72ab	4.24ab
Tropic	4.27a	4.02a	4.10a	4.14a	3.17a	1.43a	1.38a	1.43a	1.49a	1.12ab	5.70a	5.40a	5.54a	5.63a	4.30ab
Rio grande	3.01bc	2.83bc	2.98a	2.77bc	2.52a	1.07b	1.06bc	1.05a	1.06b	0.92b	4.08bc	3.89bc	4.04a	3.83bc	3.45b
Floradade	2.84c	2.66c	3.20a	2.40c	2.67a	1.04b	0.97c	1.18a	0.89b	0.98ab	3.88c	3.63c	4.38a	3.30c	3.65ab
Vita	3.82abc	3.74a	3.42a	3.26abc	3.14a	1.28ab	1.30ab	1.19a	1.14ab	1.09ab	5.11abc	5.04a	4.62a	4.40abc	4.23ab

Values within the same column with same letter(s) are not significantly different at $p = 0.05$ (Tukey´s HSD multiple range test)

Relative water content (RWC): Significant differences between varieties ($F_{7,80}$=4.25; p≤0.001) were had for this variable, displaying Rio Grande the higher values while Tropic had the lowest values (Table 1). This variable exhibited significant differences between NaCl treatments ($F_{4,80}$=21.78; p≤0.0001) but contrary to expected the higher values were at 200 mM NaCl, followed by 0 and 150 mM NaCl and the lowest values were observed at 100 mM NaCl (Table 1). Despite no significant differences between the interaction of varieties × NaCl ($F_{28,80}$=1.39; p≥0.13), the response of the varieties to NaCl varied and did not follow a linear trend of either increasing or decreasing NaCl increased, it varied as function of concentration. For example, of all cultivars Floradade had the highest RWC at 200 mM NaCl, followed by Vita and Yaqui at 150 and 200 mM NaCl, respectively, while from all varieties, Vita had the lowest RWC at 100 mM NaCl followed by Yaqui, Feroz and Tropic at the same 100 mM NaCl (Table 2).

Leaf water potential (LWP): Significant differences of LWP were exhibited between varieties ($F_{7,80}$=18.26; p≤0.0001), with Vita having the most negative values and Missouri, Yaqui and Ace the less negative values (Table 1). Also, this variable had significant differences between NaCl treatments ($F_{4,80}$=95.02; p≤0.0001) and the LWP values were most negative as NaCl levels increased (Table 1). Furthermore, LWP revealed significant differences between the interaction of varieties × NaCl ($F_{28,80}$=7.01; p≤0.0001) and when this interaction was analyzed, the varieties Missouri, Tropic and Ace displayed less negative values at 0 mM NaCl, while the most negative values were for the Vita variety at 150 and 200 mM NaCl (Table 2).

Chlorophyll a, b and total: For *Chl a* ANOVA shows significant differences between varieties ($F_{7,80}$=19.49; p≤0.0001). Tropic had the highest value followed by Feroz, Ace and Yaqui, while the rest of varieties had lowest values (Table 1). This variable exhibited significant differences between NaCl treatments ($F_{4,80}$=10.06; p≤0.0001) but contrary to expected, the higher values of *Chl a*were at 100 mM NaCl followed by control (0 mM NaCl), while the lowest values were at 200 mM NaCl (Table 1). Even though this variable did not display significant differences between the interaction of varieties × NaCl ($F_{28,80}$=1.13; p≥0.32) the higher values were exhibited by Tropic at 0 mM NaCl, Tropic at 100 or 150 mM NaCl, while Floradade at 150 mM NaCl had the lowest *Chl a* values. Table 2 shows that the varieties did not follow a specific monotonic linear trend of decreasing *Chl a* as NaCl dosages increase, even varieties such as Missouri had higher values with respect to the control at 200, 150 or 100 mM NaCl, Feroz had higher *Chl a* values at 50 or 100 mM NaCl or Yaqui with the highest values at 200 mM NaCl than at 150 mM NaCl. For *Chl b*, ANOVA revealed significant differences between varieties ($F_{7,80}$=14.43; p≤0.0001), displaying higher values were Tropic, while Rio Grande and Floradade had the lowest values (Table 1). This variable shows significant differences between NaCl treatments ($F_{4,80}$=9.15; p≤0.0001) finding higher *Chl b* values at 100 mM NaCl followed by the control and from this NaCl level, the *Chl b* values decreased as NaCl increased (Table 1). This variable did not show significant differences between the interaction of varieties × NaCl ($F_{28,80}$=1.23; p≥0.23), despite this, exhibited higher values Tropic at 150 mM NaCl, followed by Tropic, Feroz and Yaqui at 0 and 100 mM NaCl, while Floradade at 150 mM NaCl had the lowest values (Table 2). In general terms, the varieties do not follow a specific linear trend of decrease *Chl b* as NaCl increase. For total Chl content, ANOVA revealed significant differences between varieties ($F_{7,80}$=18.42; p≤0.0001), displaying higher values Tropic while Rio Grande and Floradade had the lowest values (Table 1). This variable displays significant differences between NaCl treatments ($F_{4,80}$=9.99; p≤0.0001) with higher total *Chl* content at 100 mM NaCl followed by the control and from this NaCl level, the total *Chl* content values decreased as NaCl increased (Table 1). Notwithstanding, this variable failed to show any significant differences between the interaction of varieties × NaCl ($F_{28,80}$=1.15; p≥0.30). This variable exhibited similar response to *Chl b* with higher values Tropic at 0 mM NaCl followed by Tropic and Feroz at 100 or 150 mM NaCl, while Floradade had the lowest total *Chl* content of tomato plants under NaCl treatments. This variable do not shows a trend to exhibit lowest values as NaCl levels increased (Table 2).

Discussion

Because salinity in soils and water is a common environmental condition worldwide, salinity studies in all types of plants have been carried out. One of the main goals is to understand the changes induced in plant physiology related to salinity tolerance in plants. Another goal is the development of tolerant genotypes to salinity by plant breeders. To date, a number of tomato genotypes partially tolerant to salinity have been developed (Cuartero *et al*., 2006), but none that are highly tolerant. To find a more tolerant tomato variety or varieties, the morphological variables selected to be measured were plant height, number of leaves, chlorophyll content, chlorophyll fluorescence, days to flowering, days to fructification, days to ripening, number of flowers, fruit sets of the second and sixty cluster, weight of fruit, diameter of fruit, and total production (Ezin *et al*., 2010). While for changes in physiological parameters in the varieties due to salinity the following variables were measured: leaf water potential (Ψw) and osmotic (Ψπ), gas exchange, stomatal density, the Na content (Romero-Aranda *et al*., 2001), water consumption, efficiency of water use (Reina-Sánchez *et al*., 2005) as well as other physiological variables have been used to characterize plant stress due to lack of water or salinity (Alian *et al*., 2000; Bahaji *et al*., 2002; Rzepka-Plevneš *et al*., 2007; Rzepka-Plevneš *et al*., 2008). The present study had significant differences between tomato varieties, salinity and the interaction of varieties × NaCl (salinity) for the majority of physiological variables measured. Although the growth was not reported here, after one week of NaCl treatments application, the leaves of certain plants of some varieties subjected to 200 mM NaCl started showing stunted growth, necrosis and even death compared to other genotype varieties at 150 or 200 mM of NaCl.

A possible stunted growth with NaCl is possible caused by lower stomatal conductance (Table 2), and thereby limiting CO_2 plant consumption (Hayat *et al*., 2009; Karlidag *et al*., 2011). Wang *et al*. (2011) indicates that causes stomata closure. This research, has shown that stomatal conductance varies with NaCl (Table 1). The stomatal conductance of genotypes at 200 mM NaCl varied from highest to lowest as follows: Ace > Floradade > Tropic > Rio Grande > Missouri > Yaqui > Feroz > Vita (Table 2), indicating that the varieties with highest stomatal conductance could possibly have a higher relative water content. For example, Rio Grande had higher stomatal conductance, its relative water content was higher than Feroz, which had lower stomatal conductance (Table 1). This result could be due to transpiration or evapotranspiration genetic differences of the varieties in relation to stomatal conductance and the number and distribution of stomata in the leaves (Miglietta *et al*., 2011) and consequently affected relative water content or efficiency of water use.

The tomato species is moderately sensitive to salinity (Katerji *et al*., 2003) and the maximum level of salinity tolerated by the plants without a reduction in production yield has been reported to be 2.5 dS m^{-1}, after this level, a reduction of 10% in production occurs per each unit increase in electrical conductivity (Maas & Hoffman, 1977). When salinity increases, the leaf water potential decreases reducing water availability for the plants, causing a water deficit to the plants, which in turn affects the stomatal conductance, transpiration, relative water content, chlorophyll and leaf water potential (Katerji *et al*., 2003).

This research confirms tomato stomatal closure is apparently the first stress response to NaCl in the varieties tested. The plant carries out this strategy to retain water this causes a decrease in transpiration when NaCl increases. Other studies (Giorio *et al*., 1996; Guerfel *et al*., 2009; Ozfidan *et al*., 2013) have reported a significant correlation between stomatal conductance, osmotic potential and relative water content. The response of stomatal conductance in the present study could be due to the antagonistic influence of Na to that of K, which has been demonstrated in other studies that Na did not directly affect the stomatal conductance if the ratio K/Na decreased (Shahid *et al*., 2011; Sabra *et al*., 2012).

Excess NaCl in the water medium decreases the partial CO_2 pressure, increasing internal CO_2 concentration as a result of stomatal closure (Tiwari *et al*., 2005; Abbruzzese *et al*., 2009). Leaf water potential under different salinity dosages was most negative for Vita at 150 and 200 mM NaCl than all other varieties, while Missouri, Yaqui and Ace had the least negative values. Leaf water potential decreased considerably with increasing NaCl and was directly proportional to the amount of NaCl added, especially in Vita. Leaf water potential of varieties at 200 mM NaCl varied from highest to lowest as follows: Yaqui > Feroz > Missouri > Ace > Rio Grande > Tropic > Floradade> Vita (Table 2). Retention of water as measured by leaf water potential is believed to be one of the important indicators to NaCl stress (Ashraf & Harris, 2004). In the present study, leaf water potential significantly decreased with NaCl stress in Vita, which can be linked to salt-induced water loss that has been reported previously in tomato (Chen *et al*., 2010) and other species (Kav *et al*., 2004).

The mechanisms of NaCl tolerance depend on the ability of the plant to carry out osmotic adjustment which permits growth to continue under NaCl conditions. In this sense, in the present study, variability in terms of transpiration and relative water content in response to different NaCl concentrations was noticeable among the varieties of tomato. Transpiration decreased as salinity increased while relative water content was highest at the highest salinity dosage (200 mM NaCl) follow by 0 and 150 mM NaCl (Table 1).

At higher NaCl concentration transpiration of varieties differed from high to low transpiration (worst to best water retention performance) as follows: Floradade > Rio Grande > Tropic> Ace > Feroz > Yaqui > Missouri > Vita (Table 2). While relative water content of varieties at 200 mM NaCl contrasted from highest to lowest values as follows: Floradade > Yaqui > Missouri > Vita > Rio Grande > Ace > Tropic > Feroz (Table 2).

Efficiency of water use, production of dry biomass and relative water content are usually used to measure the water status of plants, considering that salinity reduce the osmotic potential of the substrate and makes more negative the soil water potential hence limiting water uptake by the plants (Chen *et al*., 2010). The increase of electrical conductivity due to salinity in the substrate induces a reduction in efficiency of water use in tomato hybrids (Mori *et al*., 2008). This is caused by the reduction of osmotic potential of the substrate due to plant stunted growth lowers water demand (Maggio *et al*., 2004). Also, in other studies the reduction in efficiency of water use and relative water content has been correlated with an increase in stomatal conductance as a result of the exposition of tomato plants to salinity and due to the reduction in leaf area and dry biomass production (Mori *et al*., 2008). Furthermore, the change in relative water content and other water storage changes in plants exposed to NaCl are considered to be related to stomata response to changes of electrical conductivity in the substrate (Cuartero & Fernández-Muñoz, 1999).

Physiological disorders produced by salinity in some cases might be attributed to changes in water uptake and storage. This research shows that relative water content maintains the turgor of the leaves occurred not only at 0 mM NaCl but also at 150 and 200 mM NaCl (Table 1). Storey & Walker (1999) when evaluating citrus under moderate NaCl stress (50 mM NaCl) or at high levels (Hasegawa *et al*., 1986) reported similar results.

According to Hasegawa *et al*. (1986) NaCl tolerance appears to be related to the capacity to resist dehydration. As was previously mentioned, in this present study relative water content decreased and it was induced by NaCl at 50 and 100 mM NaCl, however the trend notably inverts and relative water content increases at the higher concentration of 200 mM NaCl.

Moreover, the *Chla, b* and *total* in all varieties did not follow a specific linear trend of decreasing *Chl* as NaCl increases, although the lower values of *Chl a, b* and *total* were found at 200 mM NaCl, revealing that NaCl stress caused a reduction in these variables (Table 1). The decrease of chlorophyll content has been reported to be

related to a chlorophyll-degrading enzyme, i.e., chlorophyllase, and inhibition of chlorophyll synthesis: 5-aminolevulinic acid (ALA) synthase, under NaCl stress conditions (Santos, 2004). The damage in chlorophyll content is caused indirectly by Na^+ in the medium inhibiting activities of certain key enzymes, specifically related to Rubisco and PEP carboxylase (Soussi *et al*., 1988), as well as causing damage to cell membranes (Sabra *et al*., 2012) and the photosynthetic electron transport chain (Sudhir & Murthy, 2004). Also, one of the contributing causes that has been reported in regard to the decrease in the level of photosynthetic pigments is salinity-induced inhibition of chlorophyll biosynthesis which leads to nutrient deficiency (Khan, 2006). It is also known that NaCl injury affects the photosynthetic apparatus at multiple levels such as the production of enzymes, thylakoid membrane performance, pigment biosynthesis, stomatal closing/opening, gas exchange, structure and role of thylakoid membranes (Sudhir & Murthy, 2004).

Variability in terms of *Chl a, Chl b* and *total Chl* content attributes between the varieties of tomato in response to different NaCl levels was significant (Table 1). *Chl a* content in the varieties at 200 mM NaCl fluctuated from highest to lowest values as follows: Missouri > Yaqui > Feroz > Ace > Tropic > Vita > Floradade> Rio Grande (Table 2). Chlorophyll *b* content of varieties at 200 mM NaCl changed from highest to lowest values as follows: Missouri > Ace > Tropic > Vita > Yaqui > Feroz > Floradade > Rio Grande (Table 2). Similarly, *total chlorophyll* content at 200 mM NaCl varied from highest to lowest values as follows: Missouri > Ace > Yaqui > Tropic > Feroz > Vita > Floradade > Rio Grande (Table 2).

When the chlorophyll was measured indirectly using SPAD-502, *Chl* attributes among the varieties of tomato in response to NaCl was noticeable and decreased as NaCl concentrations increased (Table 1). The response in the varieties when SPAD chlorophyll fluorescence readings were measured at 200 mM NaCl (from highest to lowest values) were: Missouri > Tropic > Yaqui > Feroz > Floradade > Ace > Vita > Rio Grande (Table 2). The effect of NaCl stress on the physiological variables were evident in the present study, in addition to Chl, another variable well documented to change due to salinity effects is leaf temperature, it increases with NaCl stress as a result of stomatal closure (Table 1). The variability of leaf temperature among varieties of tomato in response to different NaCl levels was noticeable. Leaf temperature increased as salinity increased (Table 1). At 200 mM NaCl leaf temperature of varieties changed from highest to lowest values as follows: Feroz > Rio Grande > Vita > Yaqui > Missouri Floradade > Tropic >Ace (Table 2). The higher NaCl concentration caused some varieties to close their stomata more than others causing leaf temperature to increase.

Other studies with cereals under salinity stress have reported the best-accepted effect with the increases in leaf temperature due to stomatal closure (Sirault *et al*., 2009). In addition, temperature increased have been found to be associated with the inhibition of shoot elongation (Munns & Passioura, 1984; Rajendran *et al*., 2009). Characteristics related to yield due to salinity tolerance are important in the plant breeding studies; however they are not the only variable that can be measured when evaluating salinity tolerance. For example, it is been found that for evaluating salinity tolerance it is not only important to measure Na content, but other variables as well such as Na/K ratio, osmotic potential, as well as lipid peroxidation (important in assessing secondary oxidative stress), although this variable is less critical.

Morphological, physiological and biochemical characteristics have been found to be useful for inferring the outcome of plant developmental growth due to NaCl. In this study, to determine the degree of NaCl tolerance both morphological as well as physiological characteristics were measured. These include, not only variables that directly influence the performance of biomass yield or quantity, but quality since biomass quality in terms of biochemical characteristics will also be affected (Cuartero & Fernández-Muñoz, 1999). Based on the aforementioned, the present study, instead of measuring standard Na content and Na/K ratio, and osmotic potential to evaluate salinity tolerance, selected the following physiological characteristics, such as chlorophyll content, relative water content, leaf temperature, leaf water potential, transpiration and stomatal conductance,. These parameters have been used in other studies as a guide to evaluate potential yield of crops under limited water conditions, as well as under abiotic stress such as salinity (Lovelli *et al*., 2012). Based on the measured results, it is possible to infer the varieties tolerant to salinity in the concentrations tested from most to least. This classification was carried out by generating a tolerance indicator and the methodology involved given a score of 1 to 8 for each physiological variable measured, with a score of 8 being the highest and a score of 1 being the lowest. The total score of each variety was summed and the variety with the highest total score was considered to be the most tolerant variety to salinity stress. Based on this methodology the following order of NaCl tolerance in the varieties tested was determined from most to least: Missouri > Ace > Yaqui > Tropic > Floradade > Feroz > Vita > Rio Grande. It is recognized that this methodology is subjective because all nine physiological variables were weighted equally and summed, but nevertheless this indicator provides a first order qualitative approximation of salinity tolerance which will provide guidance to plant breeders growing tomatoes under NaCl conditions.

All tomato varieties evaluated here are commercial and most of them are moderately tolerant to salinity and the degree of tolerance is growth stage dependent. Plant developmental stages involve germination, emergence, vegetative growth, flowering and reproductive stage; however, the variation for NaCl tolerance within these commercial varieties is limited. Future studies related to salinity tolerance in tomato will be carried out to examine wild species, since they represent a potential source of untapped genes for breeding plants tolerant to this abiotic factor.

Conclusions

The reaction of tomato plants to salinity is a complex trait. In the present study, the eight varieties subjected to non-lethal NaCl concentrations had different responses.

Probably in most cases, tomato variety tolerance or higher sensitivity to NaCl stress is due to the genetic variability. As NaCl levels increased in tomato varieties well known signs of NaCl stress were measured, such as necrosis, wilting leaves, plants drying up; decrease of chlorophyll content, chlorophyll SPAD readings, leaf water potential, transpiration, leaf temperature increases and stomatal conductance. Increases of relative water content occurred with NaCl dosages of 150 and 200 mM. All differences were associated with different degrees of NaCl sensitivity tolerance in the varieties studied. Physiological traits are useful for finding tolerant strains. Amongst the tomato varieties evaluated (Missouri, Super Rio Grande, Yaqui, Tropic, Feroz, Ace, Vita and Floradade), Missouri resisted better to salinity stress while Rio Grande was the least tolerant. The variety or varieties suggested for growing in soil or areas where water is affected by NaCl is Missouri followed by Ace and in decreasing order Yaqui, Tropic, Floradade, Feroz, Vita and Rio Grande.

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