Full Length Research Paper

# Effects of salinity stress on seedlings growth, mineral nutrients and total chlorophyll of some tomato (*Lycopersicum esculentum* L.) cultivars

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Accepted 22 July, 2010

In this study, six cultivars of tomato (Lycopersicum esculentum L. var. Jaguar, Xewel, Nadira, Lindo, Mongal and Ninja) were evaluated. They were subjected to salt stress during vegetative growth. Three concentrations of salt solution 50, 100 and 200 mM NaCl and the control (Wacquant nutrient solution) were used in irrigation. The total chlorophyll, the dry weight of seedlings (roots dry weight, stems dry weight and leaf dry weight), the plant height and the mineral nutrient concentrations (Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>2+</sup>) were determined. The results showed that the salt treatments increased significantly Na<sup>+</sup> concentrations in roots, stems and leaves of plants, whereas K<sup>+</sup> and Ca<sup>2+</sup> concentrations and K<sup>+</sup>/Na<sup>+</sup> selectivity ratio of plants were decreased in all tomato cultivars. The results also revealed after six weeks of salt treatments that the dry weight partitioning and the plant height decreased significantly in Jaguar, Xewel, Nadira and Mongal with increasing salinity. Jaguar, Xewel, Nadira and Mongal can therefore be considered as salt-sensitive cultivars which tolerance level ranges from 0 to 50 mM NaCl. The Lindo and Ninja plant height was less affected by salt stress than the four other cultivars. In Ninja, the moderately salt-tolerant cultivar, the growth parameters were significantly reduced at 100 mM NaCl. The supply of mineral nutrient solution with NaCl did not affect significantly leaf total chlorophyll content and plant organs dry weight of Lindo at 100 mM NaCl suggesting that it was relatively more tolerant in saline medium than other cultivars studied. The Lindo cultivar could be cultivated in environments with relatively moderate salinity.

Key words: Growth, Lycopersicum esculentum, tolerance, mineral nutrients, plant organs.

# INTRODUCTION

The soil salinization is one of the main factors especially limiting the agricultural productions in arid and semi arid regions (Munns, 2002). Worldwide, more than 60 million hectares of irrigated land (representing some 25% of the total irrigated land in the world) have been damaged by salt (Cuartero and Fernandez-Munoz, 1999; Mekhaldi et al., 2008). The detrimental effects of salt on plants are the consequence of both a water deficit that results from the relatively high solute concentrations in the soil as well as a stress specific to Cl<sup>-</sup> and Na<sup>+</sup>, resulting in a wide variety of physiological and biochemical changes that inhibit plant growth, development and proteins synthesis (Alam et al., 2004; Le Rudulier, 2005; Zadeh and Naeini, 2007; Taffouo et al., 2008, 2009, 2010a,b). The responses of plants to salt stress have long been investigated, since a better knowledge of the effect of NaCl on plants is critical for land management in saline areas (Munns, 2002, 2005). Salinity can inhibit plant growth by a range of mechanisms, including low external water potential, ion toxicity and interference with the uptake of nutrients, particulary K<sup>+</sup> (Tester and Davenport, 2003). The degree to which each of these factors affects growth depends on the plant genotype and environmental conditions (Zadeh et al., 2008). In saline soil, salt induced water deficit is one of the major constraints for plant growth (Zadeh et al., 2008; Taffouo et al., 2010a).

Tomato (*Lycopersicum esculentum* L.) is an important vegetable crop in Cameroon where it is used in soup preparation and salad in homes and hotels. Despite the importance of tomato in the nutrition of people, its

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production is very low as most farmers depend mainly on natural fertility of the soil (Olaniyan et al., 2007). Most cultivated plants, tomato inclusive, are sensitive to salt stress (Agong et al., 2003). However, tomato production has been gradually extended into the more marginal lands, thus, exposing the crop to a greater risk of salt stress (Agong et al., 1997). Tomato is a popular vegetable necessitating its improvement to fit in the environments with varying salinity. For increased production of the tomato crop under saline environment, suitable cultivars are required to overcome the soil salinization in semi and arid areas (Agong et al., 2003). The quest for better tomato yielding varieties for the marginal areas continues to receive global attention with limited break-through in producing salt tolerant tomato cultivars (Agong et al., 1997; Foolad and Chen, 1998; Cuartero and Fernandez-Munoz, 1999; Agong et al., 2003). Byari and Al-Maghrabi (1991) found that tomato cultivars varied greatly in their response to different salinity levels. Increasing NaCl concentration in nutrient solution adversely affected tomato shoot and roots, plant height, K<sup>+</sup> concentration, and K/Na ratio (Al-Karaki, 2000).

Therefore, the objective of this research was to examine the influence of soil salinity on the growth, total chlorophyll and some mineral nutrients of tomato cultivars with an aim to identify salt-tolerant ones for semi and arid areas.

### MATERIALS AND METHODS

#### Plant material and growth conditions

The seeds of Lycopersicum esculentum L. var. Jaguar, Xewel, Nadira, Lindo, Mongal and Ninja were obtained from the Agronomic Institute for Research and Development (IRA, Foumbot), Cameroon. Tomato seeds were sterilized for 20 min using 3% sodium hypochlorite then washed with distilled water. Seeds were planted in pots. Each pot was filled with 1000 g of sand previously cleaned and rinsed, respectively, in HCl and distilled water. Pots were kept in laboratory (temperature: 26 ± 3°C, light: 5000 lux for 12 h photoperiod and relative humidity of 51 - 70%) and supplied every three days with nutrient solution containing 0.4 mM of KNO<sub>3</sub>, 0.2 mM of KH<sub>2</sub>PO<sub>4</sub>, 1.0 mM of Ca<sub>2</sub>NO<sub>3</sub> and 0.4 mM of MgSO<sub>4</sub> (Wacquant, 1974). The pH of the nutrient solution was  $6.1 \pm 0.1$  and the nutrient solution was changed weekly. Five plants were let to grow in each pot. Three replicate pots were kept for each treatment. Treatments including 0, 50, 100 and 200 mM NaCl were daily supplied and started 2 weeks after sowing. Five randomly chosen plants from each variety and treatment were harvested after 6 weeks of culture in treatment solutions and used for subsequent physiological analyses.

#### Growth parameters

Dry weight of plant partitioning (roots dry weight, stems dry weight and leaves dry weight) and plant height were determined.

#### Mineral nutrients analysis

Roots, stems and leaves of harvested plants were separated and then divided into two parts: one was dried for 24 h at 70 °C, powdered

and analyzed for sodium, potassium and calcium concentrations. For the extraction of these three elements, five samples each of 0.5 g of dry materials (roots, stems and leaves) were thoroughly mixed with 20 mL of HCL 1/10 for 24 h. Sodium, potassium and calcium concentrations were determined through Flame photometer (Jenway) (Taffouo et al., 2010a). The other (leaves) was used to determine total chlorophyll concentration. Total chlorophyll of plants was extracted in 80% (v/v) aqueous acetone and absorption was measured in Thermospertronic Heλios  $\beta$  model spectrophotometer at 645 and 663 nm (Arnon, 1949). Chlorophyll content (mg I<sup>-1</sup> fresh leaf weight) was calculated using the following formula:

Total chlorophyll =  $(20.2 \times D_{645} + 8.02 \times D_{663}) \times (50/1000) \times (100/5) \times 1/2$ 

where D = absorbance.

#### Statistical analysis

The experiment was carried out in a completely randomized design. Data are presented in term of mean ( $\pm$  standard deviation). All data were subjected to analysis of variance (ANOVA). Multiple comparisons of several means were set up using the ANOVA method following by all pairwise analysis using the student-Newman-keuls procedure when the normality and equal variance conditions passed. The Dunnett's procedure (Sigma Stat software 2.03) was also used to compare data noted in experimental groups to those recorded in the single control group.

## **RESULTS AND DISCUSSION**

#### Seedlings growth

The results of this study showed that the seedlings growth was affected by salinity and the effect was varied depending on salinity level and cultivar. After 6 weeks of salt treatments, the dry weight decreased significantly in Jaguar, Xewel, Nadira and Mongal plant organs with increasing salinity (Table 1). These tomato cultivars can therefore be described as "sensitive glycophytes" which tolerance level was below 50 mM NaCl (Levitt, 1980). Similar observations were reported by Alam et al. (2004) and Hajer et al. (2006) on growth of some modern rice and three tomato cultivars. The reduction of the plant organs dry weight due to increased salinity may be a result of a combination of osmotic and specific ion effects of  $Cl^{-}$  and  $Na^{+}$  (Turan et al., 2007; Taffouo et al., 2010a). According to Alam et al. (2004) many nutrients have an essential role in the process of cell division and cell extension and those would cease soon after the supply were halted, especially in tissues with little nutrient storage. Therefore, the dominant specific reason for reduced tomato plant growth in the present study under salt stress could be due to disturbed/imbalance nutrition. The results also indicated that the roots, stems and leaves' dry weights of Lindo cultivar decreased only at high salttreatment (200 mM NaCl). Similar outcome were obtained earlier by Taffouo et al. (2008, 2010a) in bambara groundnut landrace (White Seed Coat) and tropical curcubit species (Lagenaria siceraria), salt tolerant species.

The plant height decreased with increasing salinity in

Varieties	NaCl	Plant organs						
	Treatments (mM)	Roots	stems	leaves				
Jaguar	0	132.4±1.6	168.2±2.2	193.0±2.0				
	50	112.7±1.7*	142.3±1.7*	172.3±1.5*				
	100	99.4±1.5**	103.4±2.0**	153.4±1.8**				
	200	53.3±1.7***	83.4±1.9***	132.4±1.5***				
Xewel	0	131.3±2.1	172.5±1.7	202.6±1.7				
	50	112.3±1.6 *	170.4±1.2 ns	200.6±2.7 ns				
	100	101.5±1.7**	121.7±2.8**	170.5±1.4*				
	200	70.6±1.3***	94.4±2.1***	156.4±1.4**				
Nadira	0	137.3±1.8	161.3±2.2	198.1±2.4				
	50	110.5±1.5*	141.6±1.6*	173.4±1.2*				
	100	98.4±1.2**	110.4±2.6**	152.3±1.7**				
	200	50.5±1.9***	84.5±2.2***	133.4±2.6***				
	0	146.3±0.9	198.4±1.3	235.3±1.3				
Lindo	50	140.5±3.4 ns	195.2±1.2 ns	230.4±1.8 ns				
	100	139.8±3.2 ns	190.3±2.1 ns	228.4±1.5 ns				
	200	100.2±1.3*	112.3±1.7*	185.42±1.7*				
Mongal	0	131.6±1.2	170.5±2.8	204.6±2.3				
	50	120.3±1.5 *	149.2±1.0 *	175.0.3±2.3*				
	100	106.5±1.1*	130.4±1.7**	171.3±1.7*				
	200	69.4±1.6***	95.3±1.5***	150.2±1.5*				
Ninja	0	141.5±2.1	183.4±1.9	228.2±2.5				
	50	139.1±1.6 ns	179.5±1.8 ns	225.6±0.2 ns				
	100	126.2±0.9 *	152.2±0.6 *	201.3±2.6 *				
	200	99.6±2.5*	101.4±1.7***	175.4±0.8*				

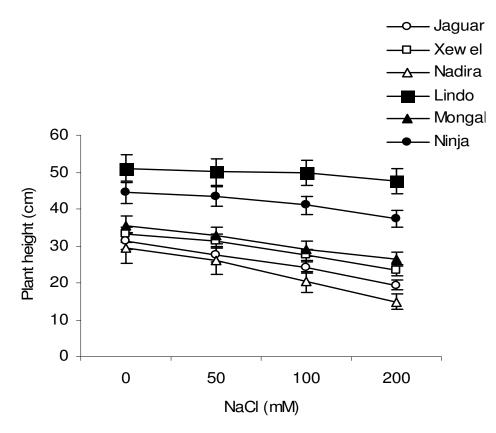
 Table 1. Dry weight partitioning (mg) in six tomato varieties after 6 weeks culture under salt stress on Wacquant medium (control), media with 50, 100 and 200 mM NaCl.

Values are the means of 5 repetitions  $\pm$  SE. Based on the ANOVA method following by all pairwise analysis using the student-Newman-keuls procedure and Dunnett's test, values headed by \* differ significantly (\* = P < 0.05; \*\* = P < 0.01 and \*\*\* = P < 0.001), ns = P > 0.05.

all tomato plants and the magnitude of reduction varied between the cultivars (Figure 1). The detrimental effects of salts on plants are the consequence of both a water deficit that results from the relatively high solute concentrations in the soil as well as a stress specific to Cl and Na<sup>+</sup>, resulting in a wide variety of physiological and biochemical changes that inhibit plant growth and development and disturb photosynthesis, proteins synthesis and nucleic acid metabolism (Rajest et al., 1998; Sairam et al., 2002; Trinchant et al., 2004). Other workers have reported decreases of plant height in many species (Alam et al., 2004; Zadeh and Naeini, 2007; Taffouo et al., 2008, 2009, 2010a). According to Alam et al. (2004), it is possible that the decrease in the observed plant height in salinized plants were due to several reasons. One possibility is that salinity reduced photosynthesis, which in turn limited the supply of carbohydrate needed for growth. A second possibility is that salinity reduced shoot and roots growth by reducing turgor in expanding tissues resulting from lowered water potential in root growth medium. Third, a disturbance in mineral supply, either an excess or deficiency, induced by changes in concentrations of specific ions in the growth medium, might have directly affected growth (Lazof and Bernstein, 1998; Zhu, 2002).

# Mineral nutrients of tomato plant organs

Salt treatments increased significantly Na<sup>+</sup> concentrations in roots, stems and leaves of plants, whereas K<sup>+</sup> and Ca<sup>2+</sup> concentrations and K<sup>+</sup>/Na<sup>+</sup> ratio of leaves were decreased in all tomato cultivars (Table 2). According to Greenway and Munns (1980), NaCl, the predominant form of salt in most saline soils, enhances the Na<sup>+</sup> and Cl<sup>-</sup> contents and consequently affects the uptake of other minerals elements. Previous workers (Porcelli et al., 1995; Saghir et al., 2002; Hosseini and Thengane, 2007; Taffouo et al., 2010a) found that salinity increases Na<sup>+</sup> and Cl<sup>-</sup> and decreases K<sup>+</sup>, Ca<sup>2+</sup> and K<sup>+</sup>/Na<sup>+</sup> in plant leaves.



**Figure 1.** Plant height (cm) of six tomato varieties after 6 weeks culture under salt stress on Wacquant medium (control), media with 50, 100 and 200 mM NaCl. Values are the means of 10 replicates  $\pm$  SE. The bars represent the mean standard error.

The data have shown that  $K^+$  uptake and transport to the aerial part of the tomato plant leaves were significantly reduced with increased salinity in all cultivars. This implies a competition between Na<sup>+</sup> and K<sup>+</sup> absorption in tomato plant, resulting in a Na<sup>+</sup>/K<sup>+</sup> antagonism (Siegel et al., 1980). The reduction in  $K^+$  uptake cause by Na<sup>+</sup> is likely to be the result of the competitive intracellular influx of both ions (Cerda et al., 1995). It is well established that many K transport systems have significant affinity for Na<sup>+</sup> (Schachtman and Liu, 1999). After 6 weeks of salt treatment, the Na<sup>+</sup> concentrations was much higher in roots of Jaguar, Xewel and Nadira than that of their leaves, whereas Mongal, Lindo and Ninja showed high Na<sup>+</sup> concentrations in their leaves (Table 2). It is generally accepted that increased K<sup>+</sup>/Na<sup>+</sup> selectivity and reduced Na<sup>+</sup> translocation from the root to the leaves contribute to the overall salt tolerance in sensitive glycophytes (Tester and Davenport, 2003; Zadeh et al., 2008; Taffouo et al., 2009, 2010a).

# Total chlorophyll concentration of tomato plants

The total chlorophyll concentration of tomato leaves was significantly reduced under salt stress in all cultivars except for Lindo at 50 and 100 mM NaCl and Ninja at 50

mM NaCl (Figure 2). Similar results were reported for total leaf chlorophyll concentration of curcubit species (Taffouo et al., 2008), bambara groundnut landraces (Taffouo et al., 2010a) and lentil plants (Turan et al., 2007). This effect of NaCl was attributed to salt-induced weakening of protein-pigment-lipid complex (Strogonov et al., 1970) and increasing chlorophyllase (EC: 3.1.1.14) activity (Stivsev et al., 1973). The decrease in chlorophyll content under salt stress is a commonly reported phenolmenon and in various studies, because of its adverse effects on membrane stability (Ashraf and Bhatti, 2000). In contrast, the supply of mineral nutrient solution with NaCl did not affect significantly leaf total chlorophyll of Lindo cultivar from 50 to 100 mM NaCl. These observations corroborated with the results obtained in Lindo dry weight suggesting that it was relatively more tolerant in saline medium than other cultivars studied.

## Conclusion

The results of the present study indicated that the responses of six tomato cultivars to salt stress change with their exposure to salinity. The plant height, the total chlorophyll content and the dry weight decreased significantly in Jaguar, Xewel, Nadira and Mongal plant organs

	NaCl treatments (mM)	Na⁺			K			Ca <sup>2+</sup>			K⁺/Na⁺
Varieties		Roots	Stems	Leaves	Roots	Stems	Leaves	Roots	Stems	Leaves	ix /itu
Jaguar	0	72.2±2.7	58.2±1.5	36.2±1.7	388.1±3.9	528.4±3.1	736.2±4.8	385.1±2.3	510.4±4.2	613.1±2.7	20.34
	50	210.8±4.1**	1812±3.0**	133.2±2.1**	340.2±1.7*	455.3±4.7*	548.2±3.6**	356.4±2.8*	452.4±3.6*	510.3±3.7*	4.12*
	100	312.2±9.9**	210.1±2.2**	163.1±3.7**	310.1±3.5**	422.2±3.0*	455.2±2.0***	312.4±3.5**	340.3±3.1**	422.2±2.5**	2.79**
	200	350.1±6.3***	315.2±4.7***	264.1±2.7**	301.1±0.7**	375.3±2.7**	410.2±3.6***	295.1±2.4***	329.1±2.9***	368.2±2.7***	1.55***
Xewel	0	30.2±1.7	50.4±1.7	66.2±1.7	405.4±3.5	642.3±5.6	995.2±7.7	401.2±3.8	580.1±4.5	760.2±5.9	15.03
	50	70.4±3.7**	170.2±3.0**	185.2±2.2**	380.4±1.2*	595.3±3.7*	800.4±5.9**	379.2±2.3*	530.2±5.1*	743.4±6.7*	4.32*
	100	125.2±4.8**	183.2±2.9*	260.2±2.7**	335.5±3.6**	427.4±2.0**	491.4±3.0***	322.4±2.6**	371.2±2.8**	465.2±4.6**	1.89**
	200	200.1±6.1**	271.2±3.2***	305.2±4.9**	330.4±4.8***	387.2±3.1**	420.3±2.1***	305.2±3.1***	361.2±3.6***	391.1±3.1***	1.38***
Nadira	0	78.2±1.0	60.2±1.9	42.2±1.5	395.2±2.9	546.3±4.3	750.2±4.7	370.3±4.9	505.1±5.4	620.2±4.1	17.78
	50	205.2±3.6**	195.2±3.1**	139.2±2.1**	365.2±3.6*	465.2±5.0*	566.2±3.9**	345.1±2.4*	440.3±4.7*	520.2±4.8*	4.07*
	100	303.2±7.9**	209.1±2.9*	169.1±2.7**	313.5±2.7**	722.2±5.1***	460.3±2.5***	311.4±38**	349.3±4.5**	426.2±3.7**	2.72**
	200	347.2±5.6**	314.2±5.8***	273.4±2.7***	305.2±1.8***	381.3±2.1***	413.2±3.5***	298.2 ±2.1**	345.2±3.0**	375.2±3.8***	1.51***
Lindo	0	34.4±1.0	59.2±1.6	80.3±1.9	680.2±3.6	1050±9.2	1350.2±9.7	681.3±5.1	1090.2±9.3	1230.4±8.5	16.81
	50	90.3±0.7**	194.2±3.5*	217.3±3.0*	610.1±3.6*	981.2±7.8*	1305.4±7.8*	580.1±4.8*	960.2±7.3*	1120.2±6.2*	6.01*
	100	140.3±2.9**	203.3±3.9*	309.5±3.5**	530.4±3.7**	669.4±4.3**	850.2±6.1***	460.5±2.6**	680.2±5.1**	732.4±4.9***	2.75**
	200	250.2±8.0***	305.1±4.9***	340.2±2.4***	510.8±7.8**	646.3±0.1**	842.2±8.1***	430.2±3.5***	672.2±5.9**	720.2±5.7***	2.47***
Mongal	0	31.1±1.9	50.2±1.3	64.2±1.8	475.5±7.1	702.1±2.9	1002.2±7.2	400.3±3.5	577.2±4.8	774.1±4.9	15.61
	50	71.2±1.1**	173.4±2.0*	180.4±1.4*	389.2±4.7*	699.3±7.7ns	810.2±3.2**	380.5±2.3*	539.5±5.6*	750.2±3.5*	4.49*
	100	128.4±2.7**	186.3±1.8*	255.5±2.4**	345.4±3.9**	580.4±4.2*	440.5±2.9***	310.2±3.9**	362.4±2.3**	436.5±2.7**	1.72**
	200	192.7±3.6**	272.2±2.9**	301.2±3.0**	331.2±3.9**	391.1±3.9**	412.5±2.7***	301.3±2.7**	355.2±3.4**	405.2±3.3***	1.37***
Ninja	0	33.2±1.9	51.2±1.4	78.7±1.9	712.4±6.4	1148.2±8.5	1248.3±7.7	690.3±4.9	1005±8.7	1110.4±7.3	15.86
	50	82.2±2.1**	181.2±2.9*	205.3±3.6**	600.2±5.8**	999.2±6.2**	1200.4±8.2*	589.1±3.7*	975.2±7.1*	1010.4±7.1*	5.85*
	100	138.2±2.7**	200.2±3.9*	299.2±3.9***	514.6±3.0***	639.1±3.8***	831.4±6.1***	440.6±2.4**	679.3±6.1**	722.2±5.4***	2.78**
	200	241.2±4.7***	299.4±3.8***	331.2±5.1***	505.0±4.9***	339.2±2.5***	615.5±4.6***	430.2±3.6**	610.2±5.9***	605.2±5.6***	1.86***

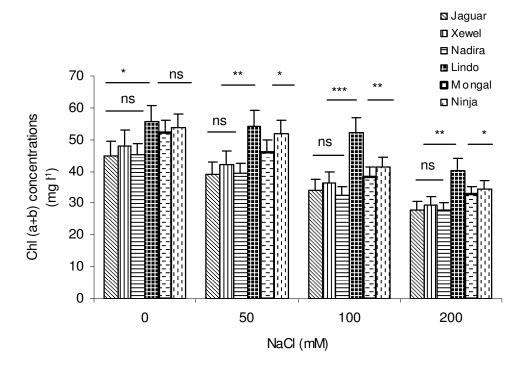
**Table 2.** Distribution of Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> concentrations (µéq g<sup>-1</sup> MS) and K<sup>+</sup>/Na<sup>+</sup> leaves ratio among tomato seedlings organs after 6 weeks culture under salt stress on Wacquant medium (control), media with 50, 100 and 200 mM NaCl.

Values are the means of 5 repetitions  $\pm$  SE. Based on the ANOVA method following by all pairwise analysis using the student-Newman-keuls procedure and Dunnett's test, values headed by \*differ significantly (\* = P < 0.05; \*\* = P < 0.01 and \*\*\* = P < 0.001), ns = P > 0.05.

with increasing salinity. These tomato cultivars can therefore be considered as salt-sensitive cultivars with tolerance level below 50 mM NaCl

whereas in moderately salt-tolerant cultivar (Ninja) the growth parameters were significantly reduced at 100 mM NaCl. The supply of mineral nutrient

solution with NaCl did not affect significantly leaf total chlorophyll content and plant organs dry weight of Lindo cultivar at 100 mM NaCl suggesting



**Figure 2.** Chl (a+b) concentrations (mg  $|^{-1}$ ) of six tomato varieties leaves after 6 weeks culture under salt stress on Wacquant medium (control), media with 50, 100 and 200 mM NaCl. Values are the means of 5 replicates ± SE. ns = P >0.05; \* = P < 0.05; \*\* = P < 0.01, \*\*\* = P < 0.001.

that it was relatively more tolerant in saline medium than other cultivars studied. The Lindo cultivar could be cultivated in environments with relatively moderate salinity.

Results also showed that salt treatments increase significantly  $Na^+$  concentrations in roots, stems and leaves of plants, whereas  $K^+$  and  $Ca^{2+}$  concentrations and  $K^+/Na^+$  ratio of plants were decreased in all tomato cultivars.

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