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Research Article

Growth, Biochemical Constituents, Micronutrient Uptake and Yield Response of Six Tomato (*Lycopersicum esculentum* L.) Cultivars Grown under Salinity Stress

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Abstract

The impact of stress caused by NaCl on the growth, yield, micronutrient acquisition and the biochemical constituents of tomato (*Lycopersicum esculentum* L.) cultivars exhibiting differences in salt-tolerance was examined in a greenhouse and field conditions. Plants were subjected to four levels of NaCl (0, 50, 100 and 200 mM). Results showed a significant reduction ($p < 0.05$) of dry weights of roots, shoots and whole plants, number of fruit per plant, flowering time, fruit yield, fruit weight per plant, number of flowers per plant and harvest index in cvs. Xewel, Mongal, Jaquar and Nadira (salt-sensitive) at 50 mM NaCl while those parameters were drastically decreased by salinity in salt-moderately tolerant cv. Ninja and salt-tolerant cv. Lindo at 100 and 200 mM NaCl, respectively. The NaCl addition leads to a decrease of Cu, Zn and Fe contents in leaves of all cultivars while soluble proteins (PR), carbohydrates (CH), total Free Amino Acids (FAA) especially proline (PRO) contents significantly ($p < 0.05$) increased in leaves of cv. Lindo than others. The main strategy of salt-tolerance in cv. Lindo seems to be increased osmotic adjustment through the strongly accumulation of PR, CH and PRO in leaves. The PR, CH and PRO could be used as potential biochemical indicators of early selection and osmotic adjustment ability for salt-tolerant plants. Results also showed a relatively higher tolerance of cv. Lindo to all yield components and micronutrient uptake than others, suggesting that Lindo cultivar could increase tomato production on salt affected soils.

Key words: *Lycopersicum esculentum*, compatible solutes, growth, yield, micronutrient, salinity

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The salinity of soil is among the most important abiotic stresses and this environmental stress limits agricultural productivity worldwide (Ashraf and Foolad, 2007). There are two main sources that contribute to soil salinity; primary or natural cause resulting from mineral degradation and the saline bedrocks (Ashraf and Wu, 1994) and secondary source resulting from the use of saline water irrigation (Rais *et al.*, 2013). Numerous studies showed that more than 60 million ha of irrigated land have been damaged by salt (Cuartero and Fernandez-Munoz, 1998). The amount of nutrients significantly decreased in stressed plants by increasing soil salinity (Shahriaripour *et al.*, 2010). Soil salinity supply alters ions transport and plant organs content (Cramer, 1997). Being toxic, sodium has a negative impact on cell metabolism and deleterious effects on the functioning of some cellular enzymes. A high level of sodium leads to an osmotic imbalance, a membrane disorganization, a reduction in growth and an inhibition of cell division and expansion (Alam *et al.*, 2004; Rais *et al.*, 2013).

Solubility of microelements such as copper (Cu), iron (Fe) and zinc (Zn) is notably low in saline soils. Plants cultivated in these areas often show deficiency symptoms (Page *et al.*, 1990). The Cu has been recently associated to an enhancing effect on plant cell metabolic processes. It can also act strongly on chromatin, the photosynthetic apparatus, growth and senescence processes when present in high amounts (Maksymiec, 1998). One of the Cu accumulation sites in higher plants is the chloroplasts. This metal is directly involved as a component of plastocyanin in the photosynthetic electron transport chain (Kato, 1977). The mechanism of Cu toxicity depends on the growth stage of the treated plants (Maksymiec, 1998). Zn deficiency decreases the production and the nutritive value of grain from cereal grown in all regions of the world (Velu *et al.*, 2014). The Zn level and the genotypes have a valuable effect on the biomass production in the plants (Maqsood *et al.*, 2009). The main limiting factor for most field crop variety all over the world is Fe deficiency and generally arises from the interaction of limited soil Fe bioavailability with cultivated genotype (Neil *et al.*, 2006). Numerous studies have shown that salinity reduces the transport of Fe from seed to seedling in sunflower and also Zn transport to the aerial parts (Sanchez-Raya and Delgado, 1996).

The tolerance to salt stress is determined by the osmotic adjustment, the specific protein and free radical enzymes involved in the protection of protoplast functions, the maintenance of ion homeostasis and the control of ion and water flux (Parida and Dos, 2005). In glycophyte plants submitted to salt stress the level of sugars is approximately

50% of the total osmotic potential (Mohamed and Ismail, 2011). Furthermore, the salt tolerance of plant might be improved by the synthesis of soluble carbohydrates (CH). Henceforth, the CH could be used as an indicator to identify drought and/or salt-tolerant genotypes (Kerepesi and Galiba, 2000). The accumulation of soluble CH depends on species and genotypes. Numerous studies showed that salt-tolerant genotypes store more soluble CH (Khosravinejad *et al.*, 2009). In plants, proteins (PR) are involved in osmotic adjustment. They are stored as nitrogen under salt-stress and re-used when the stress is removed (Singh *et al.*, 1987). Under saline conditions, the response of plants to pile of proteins relies on plants species and cultivars. The quantities of soluble PR were higher in salt-tolerant and lower in salt-sensitive cultivar of barley (Hurkman *et al.*, 1989). On the contrary, the amount of soluble PR was higher in salt sensitive wheat cultivar and lower in the tolerant line (Ashraf and O'Leary, 1999). The PR content also decreased in barley varieties under salt stress (Khosravinejad *et al.*, 2009). An increase in salinity level in the culture media leads to an increase in soluble PR pile in cv. isfahani and to a decrease in cv. shirazy (Amini and Ehsanpour, 2005). Proline (PRO) is the most appropriate amino acid in the cytoplasm that contributes to the stability of the osmotic pressure of ions in the vacuoles. Under saline conditions, PRO is highly stored and has a positive effect in the process of adaptation of cells to salt and water stress (Kaviani, 2008). The PRO is involved in protein storage (El-Enany, 1995) and may be related to osmotic and saline stress tolerance (Watanabe *et al.*, 2000; Somayeh *et al.*, 2012; Rais *et al.*, 2013).

Tomato (*Lycopersicon esculentum* L.) is an important vegetable crop in Cameroon (Goufo *et al.*, 2010). There is a growing interest for the cultivation of tomato in semi-arid region of Cameroon, where soils contain high levels of salts and low available Cu, Fe and Zn. In spite of their great importance in nutrition of plants, those micronutrients are much less studied in tomato cultivars under saline conditions. The aim of this study was to compare the responses of tomato cultivars exhibiting differences in salt-tolerance on the growth, yield, micronutrients uptake and biochemical characteristics under saline conditions in order to identify a potential biochemical indicator of early selection of salt-tolerant plants and discuss the physiological responses and adaptive strategies to salt stress.

MATERIALS AND METHODS

The study was conducted in greenhouse and field conditions in Faculty of Science, University of Douala, Cameroon, between September, 2011 to August, 2013. Seeds of tomato (*Lycopersicon esculentum* L.) cultivars: Lindo

(salt-tolerant), Ninja (moderately-tolerant), Jaguar, Xewel, Nadira and Mongal (salt-sensitive) were provided by the IRAD breeding program of Cameroon. Seeds were treated with 3% sodium hypochlorite for 10 min and washed with deionized water three times. Three days after germination, when the first leaves appeared, seedlings were transferred to 2 L plastic pots (Teku Container) filled with 2 kg of sterilized quartz sand and placed in a greenhouse. The experiment was a completely randomized design with one plant per pot and five replicates per treatment. All plants were fertilized daily with a modified nutrient solution (in g L⁻¹) made of 150 g Ca(NO₃)₂, 70 g KNO₃, 15 g Fe-EDTA, 0.14 g KH₂PO₄, 1.60 g K₂SO₄, 11 g MgSO₄, 2.5 g CaSO₄, 1.18 g MnSO₄, 0.16 g ZnSO₄, 3.10 g H₃BO₄, 0.17 g CuSO₄ and 0.08 g MoO₃ (Hoagland and Arnon, 1950). The pH of the nutrient solution was adjusted to 7.0 by adding HNO₃ 0.1 mM. For the determination of physiological and biochemical responses of cultivars to salt stress, tomato cultivars were subjected to 0, 50, 100 and 200 mM NaCl. The average day and night temperatures in the greenhouse was 26 and 20°C, respectively during the growth period with an average relative air humidity of 67.5%. Five seedlings were picked randomly for each cultivar and treated and harvest was done 6 Weeks After Sowing (WAS) and used for subsequent physiological and biochemical analysis.

Under greenhouse condition, parameters evaluated were growth parameters (dry weights of roots, shoots and whole plants, biochemical constituents (soluble proteins, carbohydrates, total free amino acids and proline content) as well as micronutrient (Cu, Zn and Fe contents).

Growth characteristics

Dry weights of tomato plants organs: The different parts of the plant were dried at 65°C in an oven for 3 days. The dry weights were then determined. Plant growth was evaluated as the Root Dry Weight (RDW) and Shoot Dry Weight (SDW) using twenty plants from each variety 6 WAS.

Compatible solutes determination

Soluble carbohydrate content: Soluble carbohydrate (CH) content was obtained using phenol-sulphuric acid method (DuBois *et al.*, 1956). The fresh leaves (1 g) were ground in 5 mL of 80% ethanol twice and filtered by the Whatman No. 2 filter paper. The collected extracts were diluted by deionized water to 50 mL. One milliliter of each sample was poured in test tube, then 1 mL of phenol solution and 5 mL of sulphuric acid were added. The mixture was then swirled. The wavelength was read at 490 nm by a spectrophotometer (Pharmaspec UV-1700 model). The quantity of CH was deduced from the glucose standard curve.

Soluble proteins content: Soluble proteins content (PR) was evaluated using the Bradford (1976) method. The protein standard used was the Bovine Serum Albumin (BSA). The 0.1 g of fresh leaves was homogenized with 4 mL of an already prepared sodium-phosphate buffer, pH 7.2. The mixture was then centrifuged at 13000 rpm for 4.5 min at 4°C. One milliliter of the supernatant was poured into a tube containing 5 mL of the Bradford reagent. The mixture was then shaken and incubated in the dark for 15 min. The absorbance of the resulting blue complex was read at 595 nm with a spectrophotometer UV (PG instruments T60). The standard curve was obtained using BSA 1 mg mL⁻¹.

Proline content: Proline content (PRO) was estimated by Bates *et al.* (1973) method. 0.5 g of fresh leaves was weighed and put inside a flask. Ten milliliters of 3% aqueous sulphosalicylic acid was poured in the same flask. The mixture was homogenized and then filtered with a Whatman No. 2 filter paper. Two milliliters of filtered solution was poured into a test tube and then 2 mL of glacial acetic acid and ninhydrin acid were respectively added into the same tube. The test tube was heated in a warm bath for 1 h. The reaction was quenched by placing the test tube in an ice bath. Four milliliters of toluene was added to the test tube and stirred. The toluene layer was separated at room temperature and the mixture purple color was read at 520 nm by spectrophotometer UV (Pharmaspec model UV-1700). At 520 nm, the absorbance was recorded and the concentration of PRO was determined using a standard curve as µg g⁻¹ FW.

Total free amino acids content: Total free amino acids content (FAA) was determined by the ninhydrin method (Yemm *et al.*, 1955). Fresh leaves (1 g) were ground in 5 mL of ethanol 80%, amino acids were then extracted using reflux technique in boiling ethanol for 30 min. After decanting, the supernatant was filtered using Whatman No. 3 filter paper. The filtrate was collected and the residue used to repeat the extraction. The two mixed filtrates constituted the raw extract of amino acids that were measured using ninhydrin method. The absorbance of purplish bruise complex was read at 570 nm. The standard curve was established using 0.1 mg mL⁻¹ of glycine.

Micronutrients determination

Micronutrient content of tomato plants: The analysis of leaves harvested 6 WAS was performed in order to determine the nutritional status of plants. The fully-developed fourth leaf from the growing point was collected for mineral analysis. The roots, stems and leaves were dried in an oven at 65°C for 3 days after being separated from the main plant. Powders

previously gotten from these parts were analyzed for Zn, Fe and Cu concentrations determination. In order to extract these elements, 0.5 g of dried roots, stems and leaves were independently added to 20 mL of HCl 1/10 for 24 h and then their concentrations were determined by atomic absorption spectrophotometer (Rayleigh WFX-100) method (Pauwels *et al.*, 1992).

Parameters assessed under field conditions were yield components (number of fruit per plant, flowering time, fruit yield, fruit weight per plant, number of flowers per plant and harvest index).

Yield components

Number of fruit per plant, fruit yield, fruit weight per plant, number of flowers per plant and harvest index of tomato plants:

The field experiment was performed at the University of Douala agricultural research farm (4°01'N, 9°44'E) from March, 2011 to August, 2013. The climate is a specific equatorial one named the Cameroonian type. With a lengthy rainy season of approximately 9 months, rainfalls are abundant about 3597 mm per year, the average temperature is about 26.7°C and the relative humidity is 81.4%. Table 1 shows the soil type as predominantly silty sandy soil. A randomized complete block design within a split plot layout with two treatments (0 or 50 mM NaCl) and three replicates was used in this investigation. Plots were 5×4 m surface and intra spacing was 1.5 m and inside the plots the cultivars were 0.50 m spaced. Plants were harvested 12 WAS and the number of fruits per plant, flowering time, fruit yield, weight of fruits per plant, number of flowers per plant and harvest index

Table 1: Physico-chemical properties of the soil taken from 0-20 cm depth of the experimental site in Douala, Cameroon

Properties	Values
Clay (%)	14.20 (1.2) ^a
Coarse sand (%)	27.90 (2.1)
Fine sand (%)	25.60 (1.8)
Coarse silt (%)	26.00 (1.6)
Fine silt (%)	6.30 (0.5)
Nitrogen (%)	0.32 (0.01)
Organic C (%)	0.75 (0.05)
Ratio C/N	2.34 (0.02)
Phosphorus (ppm)	4.60 (0.1)
Potassium (g kg ⁻¹)	0.25 (0.02)
Sodium (g kg ⁻¹)	0.07 (0.01)
Calcium (g kg ⁻¹)	0.23 (0.01)
Magnesium (g kg ⁻¹)	0.17 (0.01)
Zinc (mg kg ⁻¹)	0.29 (0.02)
Cu (mg kg ⁻¹)	1.42 (0.01)
Fe (mg kg ⁻¹)	3.26 (0.1)
pH-water	6.45 (0.1) ^a

a: Values in parenthesis represent the standard error of the mean

were determined. Yield data were collected from twelve plants per repetition for each variant of the experiment.

Statistical analysis: The experiment was performed in a completely randomized design. Data were presented in terms of Mean ± Standard deviation. All the crop data collected was subjected to analysis of variance (ANOVA) and where the F-values were found to be significant, the treatment means were separated by Least Significant Difference (LSD) at 5% probability level using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Growth characteristics: Dry weights of tomato plants organs:

This study showed a significant reduction ($p < 0.05$) of Root Dry Weight (RDW), Shoot Dry Weight (SDW) and total Plant Dry Weight (PDW) in cvs. Xewel, Mongal, Jaquar and Nadira under salinity stress (Table 2). The trend of its reduction was different

Table 2: Changes in plant growth measured as root, shoot and plant dry weights of six tomato cultivars grown at different salinity levels 6 WAS

Cultivar	Salinity level (mM NaCl)	Plant DW (g plant ⁻¹)		
		Root d.wt.	Shoot d.wt.	Total plant d.wt.
Jaquar	0	132.40 ± 1.54 ^b	361.24 ± 3.34 ^d	493.64 ± 4.88 ^d
	50	112.70 ± 1.78 ^d	314.60 ± 5.42 ^e	427.30 ± 7.20 ^g
	100	99.40 ± 1.65 ^f	256.80 ± 2.26 ^h	356.20 ± 3.91 ^k
	200	53.30 ± 1.70 ⁱ	215.81 ± 1.25 ^j	269.11 ± 2.95 ^m
Xewel	0	131.30 ± 2.10 ^b	375.10 ± 3.31 ^c	506.40 ± 5.41 ^c
	50	112.30 ± 1.69 ^d	321.01 ± 1.28 ^e	433.31 ± 2.97 ^g
	100	101.50 ± 1.70 ^f	292.20 ± 2.26 ^f	393.70 ± 3.96 ⁱ
	200	70.60 ± 1.34 ^h	250.84 ± 3.38 ^{hi}	321.44 ± 4.72 ⁱ
Nadira	0	137.30 ± 2.10 ^a	359.40 ± 2.61 ^d	496.70 ± 4.71 ^d
	50	110.50 ± 1.40 ^d	315.01 ± 2.07 ^e	425.51 ± 3.47 ^g
	100	98.40 ± 1.27 ^f	262.70 ± 2.04 ^h	361.10 ± 3.31 ^k
	200	50.50 ± 1.97 ^j	217.91 ± 1.07 ^j	268.41 ± 3.04 ^m
Mongal	0	131.60 ± 1.26 ^b	375.10 ± 2.27 ^c	506.70 ± 4.79 ^c
	50	120.30 ± 1.53 ^c	324.23 ± 3.18 ^e	444.53 ± 4.71 ^f
	100	106.50 ± 1.18 ^e	301.70 ± 3.87 ^f	408.20 ± 5.05 ^h
	200	69.50 ± 1.46 ^h	245.61 ± 2.32 ⁱ	315.11 ± 3.78 ^l
Lindo	0	142.31 ± 0.90 ^a	423.71 ± 1.87 ^a	566.02 ± 2.77 ^a
	50	140.50 ± 3.37 ^a	425.60 ± 2.44 ^a	566.10 ± 5.81 ^a
	100	139.84 ± 3.18 ^a	418.70 ± 3.39 ^a	558.54 ± 6.57 ^a
Ninja	0	135.25 ± 1.30 ^a	417.73 ± 2.83 ^a	552.98 ± 4.13 ^{ab}
	50	141.50 ± 2.13 ^a	411.60 ± 3.33 ^b	553.10 ± 5.46 ^{ab}
	100	139.10 ± 1.64 ^a	407.11 ± 1.55 ^b	544.21 ± 3.19 ^b
	100	126.20 ± 0.91 ^b	353.50 ± 2.76 ^d	479.70 ± 3.67 ^e
	200	84.60 ± 2.28 ^g	276.80 ± 2.26 ^g	361.40 ± 4.54 ^j

Two-way ANOVA result

Salinity level (S)	*	**	**
Cultivar (C)	*	*	*
S × C	ns	*	ns

The result of the two-way ANOVA testing the significance levels of plant DW, within columns, values are mean of five replicates and followed by ±SE, Mean followed by the same letter are not significantly different ($p < 0.05$) by Fisher LSD test, *Significant ($p < 0.05$), **Significant ($p < 0.01$), ns: Not significant

depending on susceptibility of cultivars to salinity. The highest decrease in PDW harvested (45.96%) was found in cv. Nadira when plants were supplied with 200 mM while the lowest (4.07%) was recorded in stressed-plants of cv. Lindo. These findings were closely related to earlier studies in others salt-sensitive cultivars of tomato, e.g., cvs. Momo-taro (Hossain and Nonami, 2012), Super Strain B' (Ali and Ismail, 2014) and Cal-ji, Flat Ch irani and Primo Early (Sardoei and Mohammadi, 2014). Under salt stress conditions, SDW showed highest and positively correlation with RDW ($r = 0.63, p < 0.05$) (Table 4). It has been shown that reduction in photosynthetic capacity under salt stress, reduces SDW and RDW and ultimately adversely affects crop growth (Neocleous *et al.*, 2014), linked with the water stress at the level of the root zone and the inhibition of the division and expansion of cells or the salt toxicity in the plants tissue (Ho, 2003). The reduced growth associated with osmotic stress is attributed to the build up of osmotic pressure of developing cells to meet the increasing osmotic pressure in rooting medium and still maintain turgor (Mudgal *et al.*, 2010). Energy expenditure during osmotic adjustment to salinity stress is one of the main factors for reduced growth (Greenway and Munns, 1980). According to Alam *et al.* (2004) the plant growth might be affected by mineral supply in excess or in deficiency and

which result from changes in concentrations of specific ions present in the growth medium. The RDW, SDW and PDW were significantly reduced ($p < 0.05$) in salt-moderately tolerant cv. Ninja and salt-tolerant cv. Lindo only at 100 and 200 mM NaCl, respectively (Table 2). These results were in line with those of Taffouo *et al.* (2014) and Tekam *et al.* (2014), who reported a decrease in RDW, SDW and PDW in salt-tolerant Mouola GG and Fleur 11 cultivars subjected to salinity at 200 mM. In this study, the accumulation of Fe, Cu and Zn was higher in root than shoot in all tomato cultivars under salt stress (Fig. 2). The reduction of plant growth may be partly due to the lack of the role of these microelements in metabolic processes of plant cells (Maksymiec, 1998). Similar results were reported by other researchers (Page *et al.*, 1990; Sanchez-Raya and Delgado, 1996; Neil *et al.*, 2006).

Compatibles solutes

Biochemical constituents of tomato plants: In this study, we observed a significant ($p < 0.05$) increase of soluble proteins (PR), soluble carbohydrates (CH), proline (PRO) and total Free Amino Acids (FAA) in cvs. Lindo and Ninja under salt stress (Fig. 1). This research showed that increased in NaCl levels increased substantially PR content in leaves of cvs. Lindo and Ninja (Fig. 1a). Similar results have already been suggested in

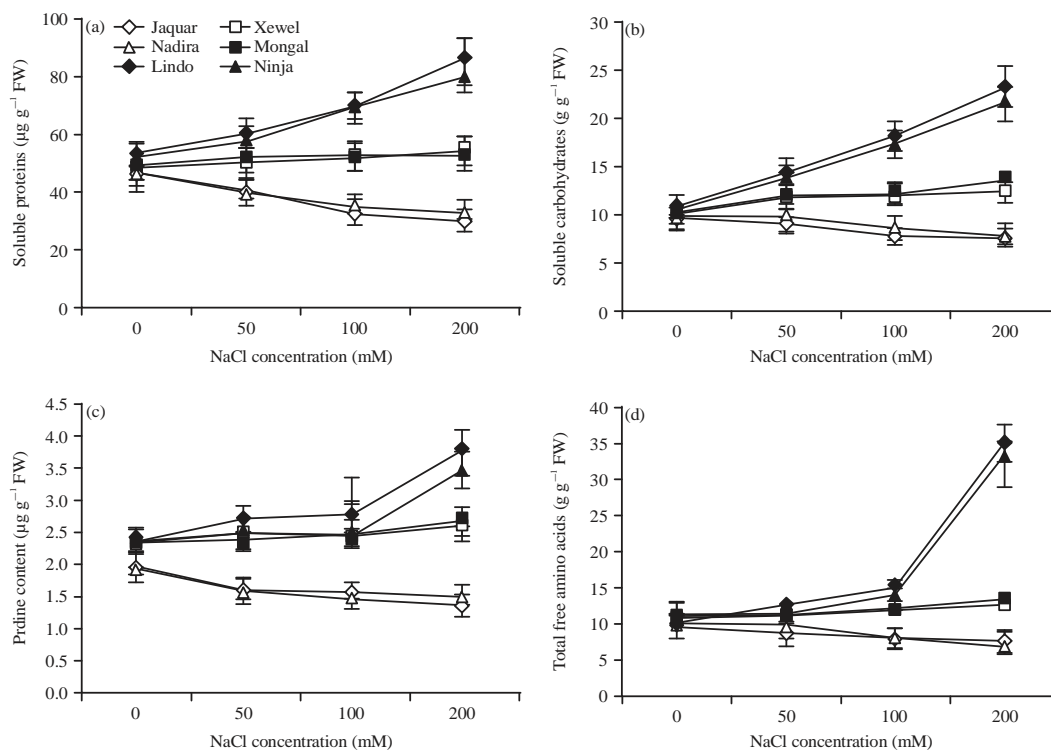


Fig. 1(a-d): Changes in biochemical constituents of tomato cultivars 6 weeks after addition of NaCl at 0, 50, 100 or 200 mM, (a) Soluble proteins, (b) Soluble carbohydrates, (c) Proline content and (d) Total free amino acids, Bars standard errors

recent studies (Cusido *et al.*, 1987; Ashraf and O'Leary, 1999; Amini and Ehsanpour, 2005). In salt-tolerant plants, Na⁺ and Cl⁻ are enclosed in the cells vacuole and the osmotic balance of the cytoplasm depends on soluble compounds (Jones and Storey, 1978). The prominent osmoprotectants in the cytoplasm are K⁺, a few aminoacids (glutamate), sugars (tetrahalose, sucrose) polyols (mannitol) and quaternary ammonium compounds (proline, glycine betaine and choline) (Bourot *et al.*, 2000; Le Rudulier, 2005). The results showed that in salt-tolerant cvs. Lindo and Ninja, an active synthesis of organic compounds as PR contributes to the osmotic balance of the cytoplasm. Salt-tolerant barley to which salinization impaired the growth and uptake of labelled N into proteins also exhibited a pile of PR (Helal *et al.*, 1975). The decline of PR accumulation in leaves of salt-sensitive cvs. Jaquar, Nadira, Mongal and Xewel may be caused by a disturbance in amino acid metabolism, particularly to delay the synthesis of cysteine and methionine or result in an increase in the products of amino acid hydrolysis (Larcher, 1978).

This study showed that NaCl treatment increased markedly the content of CH in leaves of salt-tolerant cv. Lindo and salt-moderately tolerant cv. Ninja compared to those of salt-sensitive cvs. Jaquar, Nadira, Mongal and Xewel (Fig. 1b). These results are similar to those reported on tomato cultivars (Giannakoula and Ilias, 2013). This increase of leaf CH content may be due to an increase in starch hydrolyzes which is required for hydrolytic enzymes activity (Bartels and Sunkar, 2005). The CH accumulation in plant tissues under conditions of environmental stress was due to regulatory and osmotic adjustment in current stress (Dhanapackiam and Ilyas, 2010). Results also showed that in salt-tolerant cv. Lindo and salt-moderately tolerant cv. Ninja the osmotic balance of the cytoplasm relies on an active synthesis of organic compounds as CH. According to Kerepesi and Galiba (2000), the accumulation of CH enhances the plant salt tolerance and may be an important indicator for screening salt-tolerant genotypes. NaCl supply resulted in a significant ($p < 0.05$) increase in leaf PRO content (Fig. 1c) and leaf FAA content (Fig. 1d) of salt-tolerant cv. Lindo and salt-moderately tolerant cv. Ninja compared to those of salt-sensitive cvs. Jaquar, Nadira, Mongal and Xewel. Numerous studies on plant stress responses have some contradictory results regarding the differential responses in PRO between salt-tolerant and salt sensitive cultivars. The magnitude of their accumulation in leaves of salt-tolerant cultivars was either positively (Cusido *et al.*, 1987; Demiral and Turkan, 2006; Babu *et al.*, 2012; Somayeh *et al.*, 2012; Rais *et al.*, 2013; Tekam *et al.*, 2014) or inversely related (Salwa *et al.*, 2010; Kong-Ngern *et al.*, 2012) to salt stress. This build up of the PRO is a method of stress

tolerance because its accumulation contributes to the acquisition of tolerance by maintaining the turgor in cells of many species which is responsible for the osmotic adjustment in tolerant plants grown under saline conditions (Greenway and Munns, 1980). The expression of genes leading to more PRO synthesis on transgenic tobacco and rice resulted in an enhancement of salt tolerance (Kishor *et al.*, 1995). These results obtained in cvs. Lindo and Ninja may be caused by the high accumulation of PRO which has no effect on enzyme functions such that there is continuous water uptake observed even at low soil water potential (Robinson and Jones, 1986) and through maintaining osmotic balance and stabilizing the quaternary structure of complex protein, membranes and many functional units like oxygen evolving PS-II complex (Rajasekaran *et al.*, 1998). The PRO accumulating under salt stress condition also provides energy for survival and growth and allows the plants to tolerate saline conditions (Yokota *et al.*, 2006). The levels of FAA in leaves of treated plants were higher than those of controls in cvs. Lindo and Ninja (Fig. 1d). Similarly, Cusido *et al.* (1987) reported that salinity increased the levels of FAA, especially of aspartic acid, glutamic acid and PRO. Thus, FAA accumulation in leaves, especially of PRO may be a good indicator for screening salt-tolerant genotypes.

Micronutrient concentrations

Micronutrient content of tomato plants: NaCl addition significantly ($p < 0.05$) decreased the content of Cu, Zn and Fe of plant roots and shoots in all cultivars but the magnitude varied according to their salt-tolerance (Fig. 2). Among the effects of salt stress in plant, worthy of note is the induction of nutritional disorders as a result of the effect of salinity on nutrient availability, competitive uptake and transport or partitioning within the plant (Munns and Tester, 2008). The NaCl supply may change through an increase in the solubility of micronutrients under saline conditions and the available concentration of these elements in soils (Sharply *et al.*, 1992). On the other hand, in this experiment, the content of Cu, Zn and Fe in shoots were higher in salt-tolerant cv. Lindo than others under salt stress (Fig. 2b, d and f). Similarly, Marschner (1995) observed a variation in the genotypes of plant in relation to their response and ability to metabolize micronutrient efficiently under saline conditions. In the present study, the highest accumulation of Fe, Cu and Zn was found in roots than shoots in all tomato cultivars under salt stress. Previous studies showed that under salt stress, Fe transport decreases from seed to seedling in sunflower and the Zn transport to the aerial parts also reduced (Sanchez-Raya and Delgado, 1996).

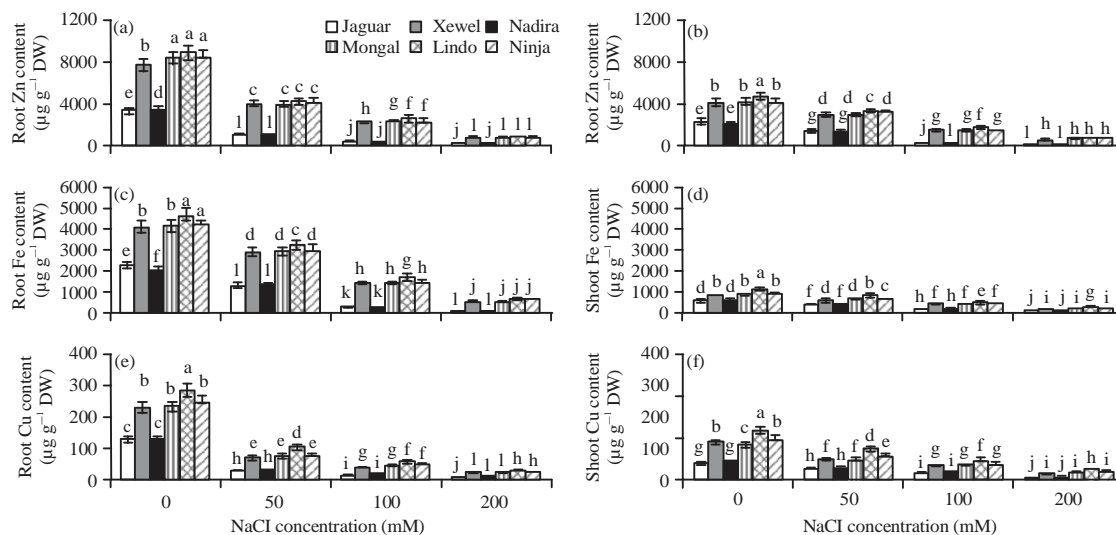


Fig. 2(a-f): Changes in the content of (a) Zn in roots and (b) Zn in shoots, contents of (c) Fe in roots and (d) Fe in shoots and (e) Cu in roots and (f) Cu in shoots of tomato cultivars 6 weeks after addition of NaCl at 0, 50, 100 or 200 mM, bars with the same letters are not significantly different at $p < 0.05$

Table 3: Changes in yield components measured as number of fruit per plant, flowering time, fruit yield, fruit weight, number of flowers and harvest index of tomato cultivars 12 weeks after addition of NaCl at 0 (control) or 50 mM

Cultivar	Salinity levels (mM)	Yield components					
		No. of fruit per plant	Flowering time (days)	Fruit yield (kg ha^{-1})	Fruit weight per plant (g)	No. of flowers per plant	Harvest index (%)
Jaquar	0	23.94 ± 0.20 ^b	39.46 ± 3.62 ^a	91.64 ± 0.80 ^b	95.66 ± 1.02 ^b	26.91 ± 0.91 ^c	0.45 ^c
	50	12.31 ± 0.27 ^d	44.86 ± 3.51 ^a	39.67 ± 0.88 ^b	80.53 ± 1.13 ^c	17.71 ± 0.22 ^d	0.60 ^a
Xewel	0	42.29 ± 0.14 ^a	31.73 ± 1.28 ^b	95.62 ± 0.32 ^{bc}	56.53 ± 1.33 ^d	48.26 ± 0.63 ^a	0.51 ^b
	50	36.70 ± 0.21 ^{ab}	28.53 ± 1.62 ^b	74.97 ± 0.43 ^e	51.06 ± 0.75 ^d	43.35 ± 2.14 ^a	0.67 ^a
Nadira	0	26.55 ± 0.19 ^b	40.60 ± 0.91 ^a	99.70 ± 0.74 ^{ab}	93.86 ± 0.30 ^b	27.74 ± 0.26 ^c	0.54 ^b
	50	15.23 ± 0.17 ^c	45.33 ± 1.36 ^a	47.88 ± 0.56 ^f	78.60 ± 1.05 ^c	18.58 ± 0.44 ^d	0.69 ^a
Mongal	0	33.32 ± 0.10 ^b	42.33 ± 2.20 ^a	101.60 ± 0.40 ^a	98.73 ± 0.61 ^b	41.53 ± 1.88 ^{ab}	0.43 ^c
	50	29.35 ± 0.15 ^b	39.53 ± 0.80 ^a	82.00 ± 0.57 ^d	95.40 ± 0.72 ^b	35.90 ± 0.23 ^b	0.50 ^b
Lindo	0	30.40 ± 0.06 ^b	41.20 ± 2.02 ^a	102.38 ± 0.24 ^a	96.53 ± 0.75 ^b	35.58 ± 0.62 ^b	0.42 ^c
	50	27.18 ± 0.03 ^b	38.80 ± 1.70 ^a	98.31 ± 0.14 ^a	90.40 ± 1.20 ^b	32.68 ± 0.51 ^{bc}	0.69 ^a
Ninja	0	19.47 ± 0.10 ^c	45.73 ± 1.52 ^a	104.37 ± 0.62 ^a	136.80 ± 1.20 ^a	25.84 ± 0.75 ^c	0.44 ^c
	50	16.49 ± 0.15 ^c	41.80 ± 1.4 ^a	90.13 ± 0.84 ^c	128.60 ± 1.11 ^a	23.96 ± 0.24 ^c	0.66 ^a

Two-way ANOVA result

Salinity level (S)	*	*	**	*	*	*
Cultivar (C)	*	*	*	*	*	*
S × C	ns	ns	*	ns	ns	ns

The result of the two-way ANOVA testing the significance levels of agronomic parameters, Data represent Mean ± SE, n = 12, Within columns, values are mean of five replications, Mean followed by the same letter are not significantly different ($p < 0.05$) by Fisher LSD test, *Significant ($p < 0.05$), **Significant ($p < 0.01$), ns: Not significant

Yield components: The number of fruits per plant, fruit yield, fruit weight per plant, number of flowers per plant and harvest index was drastically decreased by salinity, mainly at low salinity level (50 mM NaCl) in cvs. Jaguar, Nadira, Xewel and Mongal (Table 3). These results showed that Jaguar, Nadira and some leguminous plants (e.g., beans), are highly sensitive to salt with yield parameters inhibition at 50 mM NaCl (Levitt, 1980). Other researchers have also reported the reduction of number of seeds, number of flowers, pods yield

as well as 1000 grains weight in salt-sensitive cultivars under saline conditions (Zadeh and Naeini, 2007). Salinity might have reduced the production of crop by overturning water and nutritional balance of plant and loss of photosynthetic capacity, the latter is limiting factor to the supply of carbohydrate for plant grow (Alam *et al.*, 2004). Salinity could also reduce root and shoot development by reducing turgor in growing plant parts as a result of limited water potential in root growth medium (Munns, 2002). Nevertheless, cv. Lindo

Table 4: Correlation between growth and agronomic parameters in tomato cultivars under salt stress

Traits	Root DW (g plant ⁻¹)	Shoot DW (g plant ⁻¹)	No. of flowers	No. of fruit per plant	Fruit yield (t ha ⁻¹)	Fruit weight per plant (g)	Flowering time (days)
Root DW	1						
Shoot DW	0.63*	1					
Number of flowers	0.25	0.75	1				
Number of fruit per plant	0.22	0.66	0.98***	1			
Fruit yield	0.67	0.98***	0.70*	0.60*	1		
Fruit weight per plant	0.49	0.34	-0.33	-0.46	0.38*	1	
Flowering time	-0.04	-0.37	-0.83	-0.89	-0.24	-0.65	1

*Significant (p<0.05), **Significant (p<0.01), ***Significant (p<0.001)

showed a relatively higher tolerance to all yield parameters than others (Table 3). According to Villora *et al.* (2000), the grain yield could not be affected by a low level of salinity (50 mM) even though the leaf area and the shoot biomass are reduced. This is reflected in a harvest index that increases with salinity (Table 3) and the fact that grain yield may not decrease until a given threshold salinity is reached. Differences in flowering or maturity times between the cultivars can lead to differences in yield (Munns and Tester, 2008; Bagheri and Sadeghipour, 2009). There were positive and significant correlations among fruit yield and SDW ($r = 0.98$, $p < 0.001$), number of fruit per plant and number of flowers ($r = 0.98$, $p < 0.001$), fruit yield and number of flowers ($r = 0.70$, $p < 0.05$), fruit yield and number of fruits per plant ($r = 0.60$, $p < 0.05$) and fruit weight per plant and fruit yield ($r = 0.38$, $p < 0.05$) (Table 4). Due to their effects on plant-water relationship and nutritional balance, salinity levels may have an impact on the plant growth and yield (Munns, 2002).

CONCLUSION

The results of this study revealed that the cv. Lindo (salt-tolerant) had significantly different growth and yield components under saline conditions than others. Those parameters were significantly decreased in salt-sensitive cvs. Xewel, Mongal, Jaquar and Nadira at low salinity level (50 mM). The inhibition of plant growth in cvs. Xewel, Mongal, Jaquar and Nadira could be partly due to the lack of the role of Cu, Zn and Fe in metabolic processes of plant cells under salinity stress. The main strategy of salt-tolerance in cv. Lindo seems to be increased osmotic adjustment through the strongly accumulation of PR, CH and PRO in leaves. The PR, CH and PRO could be used as potential biochemical indicator of early selection and osmotic adjustment ability for salt-tolerant plants.

The study also indicates that application of NaCl significantly decreased the content of Cu, Zn and Fe of plant roots and shoots in all cultivars but the magnitude varied according to their salt-tolerance; cv. Lindo showed higher accumulation of those microelements than others.

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