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Soluble Proteins, Proline, Carbohydrates and Na^+/K^+ Changes in Two Tomato (*Lycopersicon esculentum* Mill.) Cultivars under *in vitro* Salt Stress

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Abstract: The effect of NaCl stress on soluble proteins, proline, carbohydrates and Na^+/K^+ of two tomato (*Lycopersicon esculentum* Mill.) cultivars Isfahani and Shirazy were studied. Seeds were germinated on medium containing only water agar, then transferred to MS medium supplemented with different concentrations of NaCl (0, 40, 80, 120 and 160 mM) for 21 days. Increasing of salinity resulted in increasing of soluble proteins in stem & leaf of cv. Isfahani but decreasing in cv. Shirazy. Soluble proteins in roots of both cultivars showed some variations. When concentration of NaCl in the medium was increased proline contents of stem-leaf and roots in both cultivars increased significantly. However, cv. Shirazy showed higher amount of proline level. Proline content in stem-leaf in comparing with roots was higher in two cultivars. In response to increasing of salt concentration of the medium, the average amount of total carbohydrate in stem-leaf of cv. Shirazy increased but, in cv. Isfahani level of carbohydrate decreased. When explants form both cultivars were exposed to the higher concentration of salt the level of carbohydrate in roots increased. High-level salinity decreased the reduced sugars in both cv. either in stem-leaf or roots. Salt stress increased Na^+ and decreased K^+ content in both cultivars significantly.

Key words: tomato, salt stress, soluble proteins, proline, carbohydrates, *in vitro*

INTRODUCTION

Salt stress is certainly one of the most serious environmental factors limiting the productivity of crop plants[1]. This is due to the fact that salinity affects most aspects of plant physiology, growth and development[2]. One metabolic response to salt stress is the synthesis of compatible osmolytes. These organic compounds are thought to mediate osmotic adjustment, protecting sub cellular structures and oxidative damage by their free radical scavenging capacity[3-5].

Tomato (*Lycopersicon esculentum* Mill.), one of the important and widespread crops in the word, is sensitive to moderate levels of salt in the soil. Since many authors have reported large variation among tomato genotypes in their response to salinity, genetic variability within a species is a valuable tool for screening and breeding for higher salt tolerance[6-9].

Salinised tomato plants are able to produce osmotically active organic substances mainly amino acids and sugars, which help to alleviate the salinity, mediated osmotic stress. Selection and breeding of the cultivars that can grow and produce economic yield under the saline conditions are more permanent and complementary solutions to minimize the detrimental effects of the salinity[6,10].

Several salt-induced proteins have been identified in plant species[11]. Pareek *et al*.[12] suggested that stress proteins could be used as important molecular markers for improvement of salt tolerance using genetic engineering techniques. However, proteins produced under salt stress are not always associated with salt tolerance. Using proteins as a salt tolerance indicator depends on the nature of the plant species or cultivar. In this regard, proline is a compatible solute known to accumulate in plants subjected to unfavorable environmental conditions. The concentration of this amino acid has been used in experiments as a measure of the stress imposed on tomato plants grown at different NaCl concentration in *in vitro* culture. It protects folded protein structures against denaturation, stabilizes cell membranes by interacting with phospholipids, functions as a hydroxyl radical scavenger, or serves as an energy and nitrogen source[13].

Accumulation of solutes like proline, therefore, are important factors that help the plant systems to adopt in saline environment[14,15]. The physiological significance and the mechanisms leading to proline accumulation in *Lycopersicon* genus have poorly understood . Moreover, according to Cram[16], from the various organic osmotica, sugars contribute up to 50% of the total osmotic potential in glycophytes subject to saline conditions.

Establishment of ion homeostasis is an essential requirenment for plants to survive under salt stress conditions. Plant cells respond to salt stress by increasing Na^+ efflux at the plasma membrane and its accumulation in the vacuole[2]. Salt tolerance requires not only adaptation to Na^+ toxicity but also the acquisition of K^+ whose uptake by the plant cell is affected by high external Na^+ concentration. The uptake

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of K^+ is affected by Na^+ due to the chemical similarities between both ions. Potassium is an essential nutrient being the major cationic inorganic nutrient in most terrestrial plants. Therefore, K^+ transport systems involving good selectivity of K^+ over Na^+ can also be considered as an important salt tolerant determinant[17].

In general, most of the research on salt tolerance in tomato has been developed in wild versus domesticated species[7] and very few reports on commercial cultivars are available. The aim of this study is to evaluate the effect of NaCl on soluble proteins, proline, carbohydrates and Na^+, K^+ in the two most popular tomato cultivars grown in Iran.

MATERIALS AND METHODS

Two tomato (*Lycopersicon esculentum* Mill.) cultivars, Isfahani and Shirazy were obtained from Seed and Seedling Resources of, Isfahan, Iran. In order to germinate, seeds were surface sterilized by soaking in 5% (v/v) sodium hypochlorite solution for 15 min and washed with sterile distilled water 3-4 times. *In vitro* germination was accomplished in 8 cm petri dishes containing sterile Water Agar medium. The pH of the medium was adjusted to 5.8 with NaOH then Agar (0.8%) was added. Ten seeds were placed in each petri dish (total of 200) and were incubated in the culture room under flourcent light (90 $molm^{-2} s^{-1}$), with 16-h photoperiod, and temperature of 25±2°C for 6 days, then seedlings transferred to MS, Murashige and Skoog[18] medium supplemented with 0, 40, 80, 120 and 160 mM NaCl for 21 days.

Soluble proteins were extracted from young leaves or roots in an extraction buffer (0.01 M Tris-HCl, 10% glycerol, 5% PVP, 1% Triton X 100, pH = 6.8) and protein assay was carried out according to method of Bradford[19].

For proline determination, 10 mL of 3% (W/V) aqueous sulfosalicylic acid solution was added to 1 g of fresh stem and leaf or root samples and was homogenised and filtered through one layers of filter paper (Whatmann, No. 1,Germany) then proline assay conducted according to method of Bates *et al.*[20].

Carbohydrates were extracted from dry stem and leaf or roots of tomato plantlets in warm water. Concentration of total and reduce sugars determined based on methods of Dubois *et al.*[21] and Jeffries *et al.*[22] respectively.

Na^+ and K^+ content were quantified by Flame Photometer based on Watad *et al.*[23] and reported as mol g^{-1} dry weight.

RESULTS

Soluble proteins: Soluble proteins in stem and leaf of tomato cultivar Isfahani was significantly increased when concentration of NaCl increased from 0-40 and 80 mM, while in cv. Shirazy at the same condition soluble protein slightly decreased. At 160 mM NaCl both cultivars showed the same amount of protein in stem and leaf. Protein content of roots in cv. Shirazy Under salt stress was a little higher than cv. Isfahani. However, at 40 mM NaCl both cultivars showed maximum amount of protein. The difference between protein content of roots in cv. Shirazy at 40 and 80 mM NaCl was not significant (Fig. 1).

Proline content: As a general pattern, salt treated tomato plants resulted in increasing of proline content in stem and leaf much higher than roots in both cultivars. Maximum amount of proline in stem and leaf of cv. Shirazy and Isfahani were observed at 160 mM NaCl respectively (Fig. 2). There was a significant difference between proline content in stem and leaf and roots in both tomato cultivars.

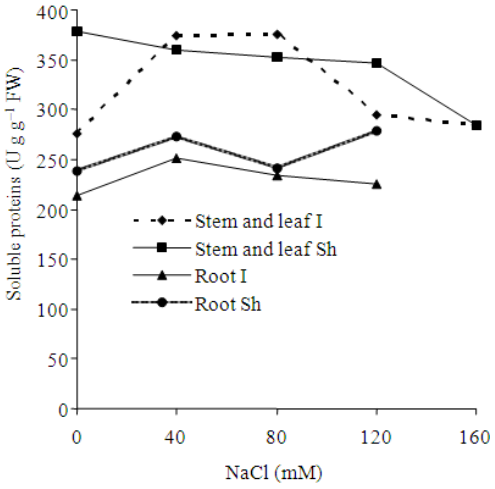

Fig 1: Soluble proteins in stem-leaf and roots of two tomato cultivars under salt stress

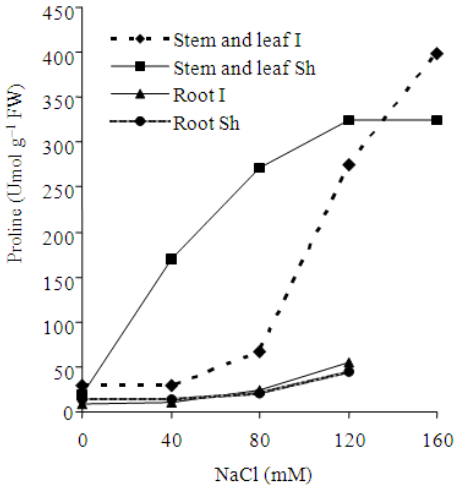

Fig 2: Proline content in stem and leaf and roots of two tomato cultivars under salt stress

Table 1: Carbohydrates (total and reduce) content in stem-leaf and roots of two tomato cultivars under salt stress (common letters in each column are not significant p<0.05)

		Isfahani Carbohydrates mg g^{-1} DW		Shirazy Carbohydrates mg g^{-1} DW	
Organ	NaCl Conc. mM	Total	Reduce	Total	Reduce
Stem-leaf	0	40.33A	28.03A	55.53A	15.9A
	40	16.41B	14.73BC	157.53B	9.2B
	80	14.87B	11.87B	268.87C	8.2B
	120	7.67C	5.61D	298.87CD	8.2B
	160	48.53D	18.03CE	329.5D	14.2A
Root	0	27.85a	12.45a	69.08a	7.12a
	40	35.23a	11.42a	73.75a	5.69ac
	80	38.93b	7.72b	74.89a	4.82bc

*: In 120 and 160 mM NaCl plants no produced enough roots for analysis Na^+ and K^+ content

Table 2: Na^+, K^+ content and Na^+/K^+ ratios of stem & leaf and roots of two tomato cultivars under salt stress (common letters in each column are not significant p<0.01)

		Isfahani mol g^{-1} DW			Shirazy mol g^{-1} DW		
Organ	NaCl Conc. mM	Na^+	K^+	$Na^+:K^+$	Na^+	K^+	$Na^+:K^+$
Stem-leaf	0	0.143A	85.52A	0.017A	0.127A	174.5A	0.007A
	40	4.08B	227.4B	0.018A	4.12B	227.4B	0.018B
	80	5.74C	140.7C	0.041C	6.45C	203.76C	0.031C
	120	7.29D	105.23A	0.070D	6.49C	105.23D	0.062D
	160	7.4D	117.05D	0.064D	6.58C	120.99D	0.054E
Root	0	1.58a	113.11a	0.014a	0.66a	168.29a	0.003a
	40	3.29b	93.41a	0.036b	4.73b	81.58b	0.058b
	80	4.87c	65.82b	0.075c	6.00c	69.76b	0.087c

In 120 and 160 mM NaCl plants no produced enough roots for analysis

Carbohydrate content: We found a significant difference in total and reduce carbohydrate between two tomato cultivars under salt treatment. For example, at 120 mM NaCl total carbohydrate in cv. Isfahani was 7.67 mg g^{-1} DW while in cv. Shirazy was 298.87 mg g^{-1} DW. A similar but moderate pattern of carbohydrate changes was observed in roots. Moreover, salt treated plants showed that the amount of total carbohydrate of stem and leaf in cv. Shirazy significantly increased while, in cv. Isfahani decreased (except 160 mM NaCl). Reduce sugars of stem and leaf in both cultivars decreased when plants exposed to salt stress (Table 1).

Total Na^+ content was greater in NaCl treated than untreated plants in both cultivars. After salt treatments level of Na^+ in stem and leaf as well as roots in both cultivars increased significantly. The highest amount of Na^+ in stem and leaf was observed at 160 mM NaCl, while roots showed the great amount of Na^+ at 80 mM salt. Evaluation of K^+ content in stem and leaf of cv. Isfahani and Shirazy showed that, K^+ content is increased as salt concentration increase in the medium. A similar pattern was found in salt treated roots. The original level (untreated) of K^+ content in stem and leaf and roots of cultivar Shirazy was higher than cv. Isfahani. Maximum amount of K^+ in stem and leaf in both cultivars found at 40mM NaCl (Table 2).

DISCUSSION

Plant species and even different cultivars within the same species differ greatly in their response to salinity[24]. In this investigation, when NaCl concentration in the medium increased, soluble proteins in two cvs. Isfahani and Shirazy was changed. For instance, soluble proteins increased in stem and leaf of cv. Isfahani but decreased in cv. Shirazy. The same results were observed by Wimmer *et al.*[25]. They reported that salt stress induces quantitative and qualitative changes in protein content of the plant cells. A higher content of soluble proteins has also been reported in salt tolerant cultivars of barley, sunflower, rice and finger millet under salt stress condition[26-29]. Our results indicating that increasing or decreasing of protein content in plants exposed to salt stress is relatively genotype dependent. Moreover, Ashraf and Oleary[30] reported that stress condition is not always associated with a balance increasing of protein content of the cells. For example, Ashraf and Waheed[31] showed that leaf soluble proteins decreased due to salt stress in all lines of wheat irrespective of their salt tolerance. Ashraf and Fatima[32] have also found that salt tolerant and salt sensitive accessions of safflower didn't differ significantly in leaf soluble proteins. However, the quantitative changes in polypeptides may be responsible for adjustments in metabolic pathways under saline conditions[33]. In our experiments accumulation of proteins in plants grown under saline condition may provide a storage form of nitrogen that is re-utilized when stress is over and may play a role in osmotic adjustment. Increasing of soluble proteins may be due to synthesis of osmotin like protein or structural protein in particular synthesis of those proteins which are involved in modification of cell wall.

With increasing of NaCl, the proline content of two cultivars increased significantly (Fig. 2). Storey and Wyn Jones[34] reported that the proline concentration was 10-fold in shoots and 18-fold higher in roots of plants grown at 100 mM NaCl than in plants grown in the absence of salt. In our experiments a similar observation in respect to proline content of stem and leaf and roots was found when plants were exposed to salt stress. Higher level of proline content in stem and leaf may be due to expression of genes encoding key enzymes of proline synthesis Pyrroline-5-carboxylate (P5C) and low activity of the oxidising enzymes (proline dehydrogenase) which is controlled by osmotic and salinity stress. Proline accumulation in leaves and, mainly, in roots is considered as a salt sensitive trait in tomato that may be used to select plants with different degrees of tolerance. Finally, proline may act as a signalling/regulatory molecule able to activate multiple responses that are component of the adaptation process[35].

Change in soluble sugars content under salt stress has already been reported for a number of species. For example, Ashraf and Tufail[27] determined the total soluble sugars content in five sunflower accessions differing in salt stress. They found that although sugar content increased significantly in all five lines with increasing salt in the growth medium, the salt tolerant lines had generally greater soluble sugars than the salt sensitive ones. Our data showed that higher amount of total sugars in cv. Shirazy, might be responsible for higher salt tolerance. At the early step of this study we found that cv. Shirazy has higher seed germination and better growth under salt stress conditions (data not shown). One reason for that may be due to higher capacity for sugar accumulation or increase transition of sugar following salinisation from shoot to root[36]. Information regarding the role of sugars in adaptation of plants to salinity is, therefore, insufficient to conclude that they are universally associated with salt tolerance. However, this does not rule out a significant role of soluble sugars in salt tolerance nor a potential role for soluble sugar accumulation as an indicator for salt tolerance in breeding programs for some species[11].

Under salt stress one of the mechanisms of salt tolerance is accomplished by uptake and accumulation of inorganic ions, mainly Na^+, K^+ and Cl^- [37]. In our study under salinity, cv. Isfahani accumulated high level of Na^+ in stem and leaf in comparison with cv. Shirazy. The rise in Na^+ concentrations in the leaves lowers the osmotic potential, so contributing to the maintenance of the water potential difference between the leaves and the medium required to obtain water from the saline solution. Potassium content on the other hand has been reduced markedly in both cultivars. A similar data has been reported previously[38]. Na^+/K^+ ratio may serve as an indicator of crop tolerance to stress as the increase of Na^+ in salt tolerant species is generally associated with a decrease in K^+[39]. In our experiments with increasing of NaCl in the medium the Na^+/K^+ ratio increased in stem and leaf and root up to 120mM NaCl and decreased back again at 160mM NaCl. It is indicating that these cultivars are relatively salt tolerant or they may have potential for salt adaptation.

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