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Response of Tomato (*Lycopersicon esculentum* Mill.) Cultivars to MS, Water Agar and Salt Stress in *in vitro* Culture

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Abstract: The effect of MS and water agar medium, containing NaCl and sucrose on germination percentage, seedling growth, chlorophyll content, acid phosphates activity and soluble proteins were studied in different cultivars of *Lycopersicon esculentum* Mill. (Cv. Isfahani, Shirazy, Khozestani and Khorasani). Seeds were germinated under various mediums, MS with and without sucrose, water agar with and without sucrose with different concentration of NaCl (0, 40, 80, 120 and 160 mM). Increasing of salinity decreased the germination percentage and seedling dry weight. The highest germination percentage was found in Cv. Isfahani and the lowest in Cv. Shirazy. Chlorophyll content (Chl a, Chl b and total Chl) were decreased with increasing of salinity in both Cv. Isfahani and Shirazy. In Cv. Shirazy. Acid phosphates (Apase) activity was decreased in stem-leaf while it was increased in roots. Soluble protein was changed in different salt concentration. Enzyme activity was decreased in stem-leaf in Cv. Shirazy but was increased in Cv. Isfahani. Soluble proteins in roots of both Cv. showed variation. Finally, Water Agar (WA) medium in comparison with MS medium resulted in higher tomato seed germination in different NaCl concentration.

Key words: Seed germination, tomato, NaCl stress, acid phosphatase, soluble proteins

INTRODUCTION

Plants are responded to salinity stress through morphological, physiological and metabolic occurring in all plant organs. NaCl salinity is known to decrease seed germination and also affect other metabolic process[1]. In many crop plants, including tomato (*Lycopersicon esculentum* Mill.), seed germination and early seedling growth are the most sensitive stages to environmental stresses[2]. The response to salinity during germination has been reported to be more complex than during plant growth because it depends on the availability of stored compounds[3]. During imbibitions phase, one of the important processes is the quick re-establishment of the membrane integrity that may be indicated by development of ion uptake capacity. Reduced growth of the seedlings may result from reduced mobilization of reserves from the cotyledons or from their reduced utilization by the embryo axis[4].

Tomato is an important greenhouse crop in semi-arid regions of Mediterranean countries[5]. Under optimal germination conditions, most tomato seeds germinate within 36 to 72 h after seed imbibitions.

However, germination is delayed or completely inhibited, depending on the stress intensity and the genetic background of the seed[6]. It has been found that germination and growth of young seedlings are reduced with a high NaCl concentration in beetroot[7]. Research has shown that tomato salt tolerance during seed germination is genetically and physiologically controlled and suggested that this trait could be improved by *in vitro* culture manipulation or directional phenotypic selection[8].

Some enzymes are affected by stress for example; acid phosphatase activity of roots in *Medicago polymorpha* is increased under phosphate deficiency[9]. Barrett-Lennared *et al.*[10] also demonstrated that salt and water stress increase acid phosphatase activity in *Pisum sativum*.

Several salt-induced proteins have been identified in plant species[11]. Pareek *et al.*[12] suggested that stress proteins could be used as an important molecular marker for improvement of salt tolerance using genetic engineering techniques, in many studies. 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 general, most of the research on salt tolerance in tomato has been developed in wild versus domesticated species[13] and very few reports on commercial cultivars are available[14].

The aim of this study was comparison of MS and WA (water agar) medium containing NaCl on tomato seeds germination and some other growth parameters.

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MATERIALS AND METHODS

Four tomato (*Lycopersicon esculentum* Mill.) cultivars, Isfahani, Shirazy, Khozestani and Khorasani 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 times. *In vitro* germination was accomplished in 8 cm petri dishes containing sterile MS[15] and Water Agar medium (Table 1). The pH of the medium was adjusted to 5.8 with NaOH then Agar (0.9%) was added. Ten seeds were placed in each petri dish (total of 100) and were incubated in the culture room under flourcent light (90 mol m^{-2} s^{-1}), with 16 h photoperiod and temperature of 25±2°C for 8 days and then percentage of seed germination was calculated.

Further growth analysis was conducted on plants with the highest and lowest germination rate. Dry weight of salt treated seedlings was measured after 21 day. Chlorophyll content (Chl.a, Chl.b and Chl. total) of leaves was extracted from 1g of fresh tissue by 80% acetone and was determined by spectrophotometer according to Lichtenthaler *et al.*[16].

Table 1: Salt treated media used for *in vitro* germination of tomato (*Lycopersicon esculentum* Mill.). (WA: water agar, MS: Murashige and Skoog)

Medium No.	Combination
1	WA + 0, 40, 80, 120, 160 mM NaCl
2	WA + Sucrose (3%) + 0, 40, 80, 120, 160 mM NaCl
3	MS + 0, 40, 80, 120, 160 mM NaCl
4	MS + Sucrose (3%) + 0, 40, 80, 120, 160 mM NaCl

Soluble proteins and acid phosphatase enzymes 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) at 4°C. Extracted solution was used for proteins assay according to method of Bradford[17] and acid phosphatase activity according to Julie *et al.*[9].

RESULTS

Result shows that seed germination in these tomato cultivars was decreased with increasing of NaCl concentration. However, seed germination in media numbers 2 and 4 (WA+sucrose and MS+sucrose) was decreased significantly (Fig. 1, B and D). In medium No. 1 (WA) tomato seeds resulted in higher percentage of germination in different concentration of NaCl. Cultivars Shirazy and Isfahani showed highest and

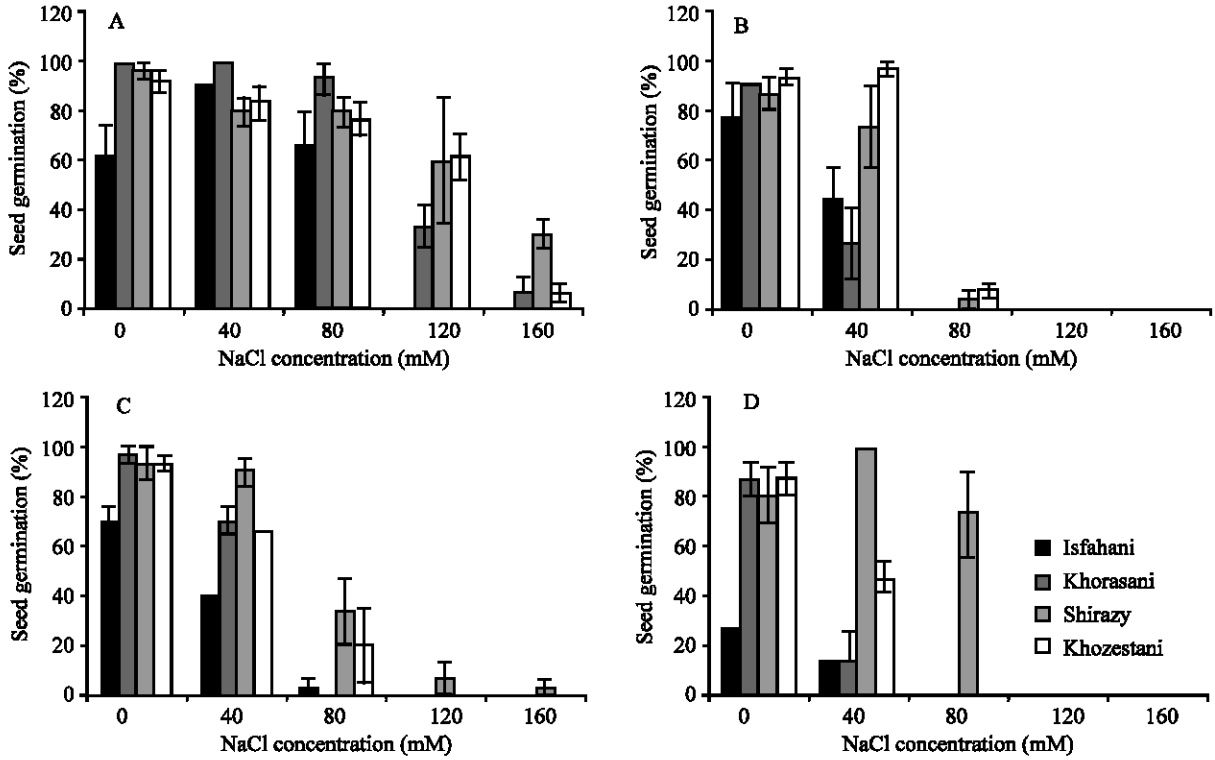

Fig. 1: Effect of NaCl and media on seed germination of tomato (*Lycopersicon esculentum* Mill.) Cv. Isfahani, Khorasani, Khozestani and Shirazy. A: WA, B: WA + 3%sucrose, C: MS, D: MS+3% sucrose

Table 2: Stem-leaf and root dry weight and the ratio of root/stem-leaf dry weight in two tomato cultivars under NaCl stress

	Stem-leaf DW(g)					Root DW (g)					Root/Stem-leaf DW (g)				
	NaCl (mM)					NaCl (mM)					NaCl (mM)				
Cultivars	0	40	80	120	160	0	40	80	120	160	0	40	80	120	160
Isfahani	0.217 a	0.127 b	0.127 b	0.01 c	0.01 c	0.110 a	0.030 b	0.031 b	-	-	0.507 a	0.236 b	0.226 b	-	-
Shirazy	0.212 a	0.208 a	0.165 a	0.03 b	0.045 b	0.142 a	0.092 b	0.052 b	-	-	0.670 a	0.442 b	0.315 b	-	-

DW: dry weight, common letter are not significant $p<0.5$

Table 3: Effect of NaCl on chlorophyll content of two tomato cultivars

			NaCl (mM)				
Cultivar			0	40	80	120	160
Isfahani	Stem-Leaf (FW, mg/g)	Chla	1.027±.212a	0.970±0.099a	0.727±0.018ac	0.446±0.149bc	0.465±0.088bc
		Chlb	0.464±0.101a	0.417±0.046ac	0.349±0.057ac	0.236±0.036bc	0.30±0.002bc
		Total	1.513±0.317a	1.400±0155a	1.092±0.047ac	0.670±0.168bc	0.785±0.091bc
Shirazy	Stem-Leaf (FW, mg/g)	Chla	1.420±0.027a	1.295±0.084a	0.572±0.131b	0.570±0.066b	0.239±0.054c
		Chlb	0.754±0.02a	0.595±0.063b	0.286±0.041c	0.245±0.042ce	0.121±0.026de
		Total	2.209±0.043a	1.919±0.149a	0.871±0.174b	0.827±0.105b	0.366±0.082c

(Chl: chlorophyll, FW: fresh weight, common letters are not significant $p<0.5$)

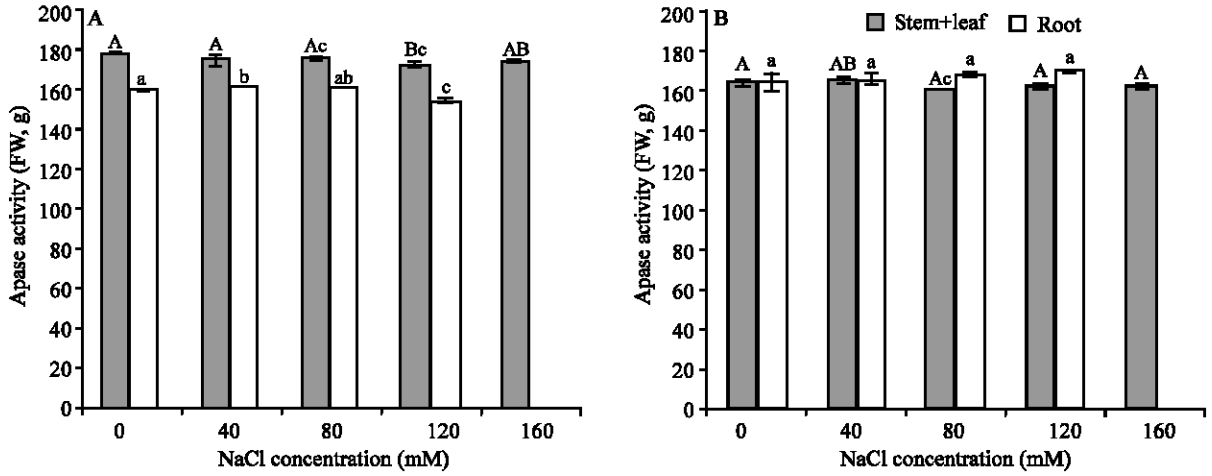

Fig. 2: Acid phosphatase activity of stem-leaf and roots in two tomato cultivars under salt stress. (A: Cv. Isfahani, B: Cv. Shirazy, common letter(s) are not significant $p<0.5$)

lowest percentage of seed germination, respectively. Consequently, these two cultivars (Shirazy and Isfahani) were selected for future analysis.

Stem-leaf and root dry weight were decreased in two Cv. Shirazy and Isfahani with increasing of NaCl concentration. Dry weight of Cv. Isfahani at 80 mM NaCl and Cv. Shirazy at 120 mM showed significant difference ($p<0.5$). In both cultivars root dry weight at 40 mM NaCl was decreased significantly (Table 2).

Chlorophyll content of two tomato cultivars affected by increasing of NaCl concentration. The amount of chlorophyll was decrease when concentration of NaCl was increased. However, at 120 mM NaCl Cv. Isfahani and at 80 mM NaCl Cv. Shirazy showed significant difference (Table 3).

Stem-leaf and roots of two tomato cultivars showed different level of acid phosphatase (Apase) activity in response to increasing of salt concentration in the medium. As a general result level of Apase activity in stem-leaf was slightly higher than roots of cv. Isfahani but it was lower in Cv. Shirazy. In Cv. Isfahani low activity of Apase in stem-leaf at 120 mM NaCl was observed and the difference of Apase activity between stem-leaf and roots was significant at 40 mM NaCl. However, in Cv. Shirazy the difference was not significant (Fig. 2).

Initial level of stem-leaf soluble proteins in Cv. Shirazi was higher than Cv. Isfahani. Protein content of stem-leaf in cultivar Isfahani was significantly increased when concentration of salt was increased up to 40 mM (Fig. 3A). However, in both cvs. Increasing of NaCl

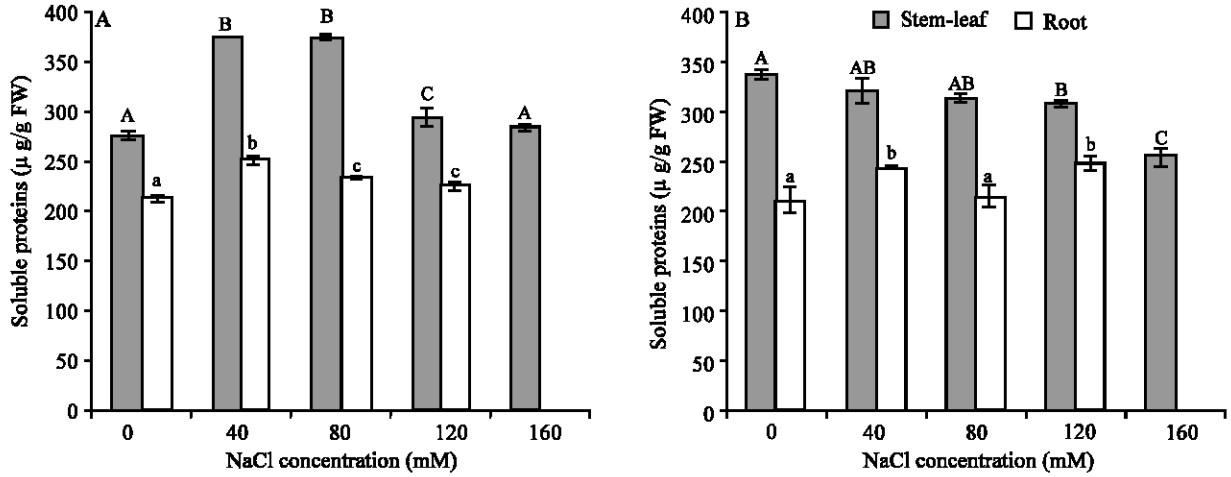

Fig. 3: Soluble proteins of stem-leaf and roots of two tomato cultivars under salt stress. (A: Cv. Isfahani, B: Cv. Shirazy, common letter(s) are not significant $p<0.5$)

concentration resulted in high level of protein in roots. The soluble proteins changes in roots of both cvs were mostly significant (Fig. 3A and B).

DISCUSSION

Salt tolerance during seed germination is a measure of the seeds ability to withstand effects of high concentrations of salts in the medium and germinate rapidly. Excessive salt depresses the water potential of the germination medium, making water less available to the seed and thus lowers the rate of, or completely inhibits germination. However, low rate of seed germination under salt stress could be due to osmotic and/or ionic effects of the saline germination medium[6]. When WA (water agar) was used as a germination medium the percentage of seed germination increased significantly, the low level of osmotic stress (compare with MS medium) had better influence on seed germination of tomato. The difference among seed germination of tomato cultivars might be due to genotype variation. Medium containing sugar and MS component decreased seed germination when the concentration of salt was increased. It can be interpreted as a result of low water potential rather than ion toxicity. Available water seems to be the major limiting factor for tomato seed germination under salt stress[18-20].

It has been well documented that, as a general pattern dry weight of different plant organs are affected by salt stress. Comparison of the response of stem-leaf and root in two tomato Cv. (Isfahani and Shirazy) grown under various concentration of NaCl stress showed that root is more sensitive than stem-leaf. In contrast to the present data in this study, Dasgan *et al.*[21] and Salim[22] found that tomato leaf is more sensitive to salinity than root. It might be due to the cell wall component, cell membrane structure, salt tolerance mechanism and genetic background in different plant species.

In the pervious study on calluses of *Medicago sativa* Cv. Yazdi and Hamedani, acid phosphatase activity are increased under salt and drought stress. However, activity of this enzyme for tomato stem-leaf and roots like other plant species under salt stress condition has no similarity pattern with alfalfa calluses[23].

When NaCl concentration in the medium was increased, soluble proteins in two cvs. Isfahani and Shirazy was changed. Wimmer *et al.*[24] reported that salt stress induce quantitative and qualitative changes in protein composition of the cells. Soluble proteins were higher in Cv. Shirazy than Cv. Isfahani in non-saline medium. A higher content of soluble proteins has been reported in salt tolerant cultivars of barley, sunflower, rice and finger millet[25-28]. In our study, increasing of NaCl concentration resulted in decreasing of soluble proteins in stem-leaf of cv. Shirazy but increasing in Cv. Isfahani at 40 mM NaCl. Ashraf and Oleary[29] reported that stress condition is not always associated with a balance increasing of protein content of the cells. For example, Ashraf and Waheed[30] indicated that leaf soluble proteins decrease due to salt stress in all lines of wheats irrespective of their salt tolerance. Ashraf and Fatima[31] 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[32].

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