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Research article

RESPONSE OF VARIABLE TOMATO (*SOLANUM LYCOPERSICUM* MILL.) GENOTYPES TO SALINITY AT GERMINATION AND EARLY SEEDLING GROWTH STAGES

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ABSTRACT: Soil salinity is one of the most important constraints that limit crop production in arid and semi arid regions. Germination is the first stage in a plant's life, which can be affected by stress and as a result salinity reduces germination and delays emergence in most cultivars, however there are cultivars that can tolerate this condition. This study aimed to determine the effect of different level of salinity (0, 50, 100, 150 and 200mM NaCl) on germination and seedling growth of six tomato cultivars. The experiment followed a randomized complete block design with four replications. All the results analyzed by SPSS ver.16 statistical software and comparision of average had done by Duncan test on 5% possible level. Results confirmed that increasing NaCl concentrations reduced germination percentage, radicle and plumule length, radicle and plumule dry weight. Results showed that the highest values for these traits were from the CaljN3 cultivar, and traits in the other cultivars diminished with increasing water salinity. Germination and seedling growth were strongly inhibited by 200mM NaCl. Moreover, tomato shoots appear to be more sensitive than roots. It can be concluded that plumule and radicle elongation may be used as a suitable breeding criteria for the selection of the better salt tolerant cultivars at the seedling stage.

Key words: Abiotic stress; NaCl; seedling; tomato.

INTRODUCTION

Abiotic stress, such as salinity, drought, extreme temperature and heavy metals toxicity is an important cause of limiting plant growth and reducing average yields for most crop plants. However salinity is major problem in arid and semi-arid regions [1]. High salinity includes both an ionic (chemical) and an osmotic (physical) component. Salinity becomes a concern when an excessive amount or concentration of soluble salts occurs in the soil or water. In general NaCl is the predominant salt causing salinization. Plants have mechanisms to regulate its accumulation [2]. Salinity induces specific changes on cell, tissue and organ levels. These changes are physiological, hormonal balance, morphological and anatomical [3]. The establishment stage of the crop consists of three parts: germination, emergence and early seedling growth; that are particularly sensitive to substrate salinity [4,5]. Germination is reactivation of growth triggered by environmental stimuli as simple as availability of water and oxygen, or as complex as temperature, light, endogenous inhibitor and promoter interactions. Successful seedling establishment depends on the frequency and the amount of precipitation as well as on the ability of the seed species to seed germination and grow while soil moisture and osmotic potentials decrease [6,7]. The decrease in germination rate particularly under drought and salt stress conditions may be due to the fact that seeds to prevent germination develop and osmotically enforced dormancy under water stress conditions. This may be an adaptive strategy of seeds to prevent germination under stressful environment thus ensuring proper establishment of the seedling [8], Several investigation of seed germination under salinity stress have indicated that seeds of most species attain their maximum germination in distilled water and are very sensitive to elevated salinity at the germination and seeding phases of development [9,10].

In many crop species seed germination and early seedling growth are the most sensitive developmental stages to salinity stress. Salinity stress greatly delays onset and reduces the rate of germination events. It has been reported that salinity limits plant growth and productivity [11]. There are big changes in morphology and anatomy of plants growing in saline soils. Salinity generally affects growth rate and it results in plants with smaller leaves, shorter stature and roots' structure by reducing their length and mass [12].

Tomatoes (*Solanum lycopersicum*) are one of the most important vegetable crops. However, it is moderately sensitive to salinity [13], extensive research is necessary to develop growing conditions in moderate salinity to produce good vegetative growth. The effect of salinity concentration in plant growth has been studied in different tomato cultivars. Salinity reduces tomato seed germination and lengthens the time required for germination. Adler and Wilcor (1987) found that salinity adversely affected the vegetative growth of the tomato, and it reduced plant length and dry weight. Salinity also reduced the fresh and dry shoot and root weight of tomato [15]. [15]. Increased salinity over 4000 ppm led to reduction in dry weight, leaf area, plant stem, and roots of tomatoes [16]. The reduction of dry weights due to increased salinity may be a result of a combination of osmotic and specific ion effects of Cl and Na [17]. The leaf and stem dry weights of tomato were also reduced significantly in plants irrigated with saline nutrient solution in contrast with control plants [18]. Byari and Almaghrabi (1991) found that tomato cultivars varied greatly in their response to different salinity levels.
The present study was undertaken to study the response of six tomato cultivars to different levels of salinity and to determine the genotypic variability in their tolerance to salinity both at germination and seedling stages and selecting cultivars for rapid and uniform germination under saline conditions can contribute towards early seeding establishment.

MATERIAL AND METHODS

In order to study the effect of salinity on germination and seedling growth of six tomato cultivars, an experiment is conducted as factorial with completely randomized design with four replications in biotechnology lab of Azerbaijan University. Experimental factors including: cultivar (laleh, urmia, Falat CH, Falat Vana, CaljN3 and Super Strain B) and salinity levels (0, 50, 100, 150 and 200 mM NaCl). Seed germination: seeds were surface sterilized in 2% sodium hypochlorite solution for 15 min and rinsed with distilled water three times, and sown on two layers of No. 1 Whatman filter paper in petri dishes moistened with 6ml of 0, 50, 100, 150 and 200mM NaCl solutions. Eventually, their lids were closed by parafilm and had been located in growth room. The tempreture adjusted in 25°C. This experiment took 10 days. The following characteristics were studied:

Germination percentage (GP):
From second day, the germinated seeds were counted daily in specific time. The seed being considered as germinate when its radicle had emerged. Counting continued till 10 days and the resulted final counting considered as final germination percentage.
Gp: Ni/N x 100
Ni: number of germinated seed till i^{th} day.
N: total number of seeds.
Germination Race (GR):
In order that, from the second day to 10^{th} once a 24 hours we counted germinated seeds and its race was determined by Maguire equation (1962):

$$GR = \sum_{i}^{n} \frac{Si}{Di}$$

GR: Germination Race (number of germinated seed in each day)

Si: number of germination seeds in each numeration

Di: number of days till n numeration.

n: number of numeration times.
At the end of experiment we chose 10 plants from each Petri dish, separated their radicle and plumule and measure each plant's radicle and plumule length separately. Then we put each repetition on the filter separately. In order to make them dry and measure its dry weight, we put them in oven with 75°C temperature for 24 hours
Analysis of variance was performed using standard techniques, differences of means were compared through Duncan's multiple range test (p<0.05) using the SPSS V. 16.0 program.

RESULTS AND DISCUSSION

Means comparison results for different salinity levels indicated that all germination indices, dry weight and lenght of radical and plumel decreased under an increased stress level, so the control had the highest value and the level 200mM had the lowest value for each index.

Germination Percentage (%)

Results demonstrated that germination of tomato was strongly affected by all salinity treatments. Increased salt concentration caused a decrease in germination, so a strong reduction was observed generally at the highest level of salt concentration compared to the control. Averaged across stress levels, CaljN3 had the highest germination percentage (98.89%) and the genotype Urmia had the lowest (84.44%). At the highest level of stress, CaljN3 best maintained that's germination percentages (52.22%), while the other cultivars showed a significant reduction in germination percentages. so that was considered as the most salinity-tolerant cultivar in relation to germination percentage. Precocious Urmia showed the highest reduction from 84.44% (control) to 0.05 (200mM) and was the most salinity-sensitive cultivar in terms of germination percentage (Table 1). Salinity decreased germination percentage of all the cultivars (Table 1). Even at the lowest salinity treatment (50mM). These experimental results showed that salinity stress significantly reduced germination and seedling growth of tomatoes seeds and these results demonstrated clear differences between the genotypes. Germination and early seeding growth of many crop plants are the most sensitive stages to environmental stresses [21]. These results agree with the findings of other researchers such as Abdul Jaleel *et al*. studies (2007) on *Catharanthus roseus* seedlings, Othman *et al*. (2006) on twelve barley genotypes, Maghsoudi Moud A, Maghsoudi K (2008) on thirty-three wheat cultivars. Reduction in germination due to salinity increas can be attributed to the nature of salinity that reduces absorption of water due to lowered osmotic potential of the medium and changes in metabolic activity [25].

It appears that a decrease in germination is related to salinity- induced disturbance of the metabolic process leading to an increase of phenolic compounds [11]. Moreover, salinity perturbs a plant's hormone balance [26], and reduces utilization of seed reserves [27]. Shokohifard *et al*. (1989) reported that salt stress negatively affected seed germination; either osmotically through reduced water absorption or ionically through accumulation of Na and CI causing an imbalance in nutrient uptake and a toxic effect. Bewley and Black (1982) suggested that the inhibition of the radicle under salt stress is due to a reduction in the turgor of the radicle cells.

Furthermore, Younis *et al*. (1991) reported that low moisture content under salt stress caused a cessation of metabolism or inhibition of certain steps in metabolic sequences of germination. Conversely, salt stress increased the intake of toxic ions that may have altered certain enzymatic or hormonal activity in seeds during germination [30]. Salt induced inhibition of seed germination could be attributed to osmotic stress or to specific ion toxicity [31].

Table 1. Effects of different salinity levels on germination percentage (%) of six tomato cultivars

	0	50	100	150	200mM	
Cultivars						Mean
CaljN3	98.89^{a}	95.55^{a}	90.00^{ab}	$56.67^{a\text{-}g}$	$52.22^{a\text{-}g}$	78.66^{A}
Falat CH	96.67^{a}	92.22^{ab}	83.33^{ab}	$57.78^{a\text{-}g}$	$46.67^{b\text{-}h}$	75.33^{AB}
Laleh	95.55^{a}	93.32^{a}	$73.33^{a\text{-}e}$	$35.56^{c\text{-}h}$	13.33^{gh}	62.21^{AB}
Falat vana	91.11^{ab}	87.78^{ab}	$67.78^{a\text{-}e}$	$34.44^{d\text{-}h}$	$31.11^{e\text{-}h}$	62.44^{AB}
Super Strain B	86.67^{ab}	81.67^{abc}	$60.00^{a\text{-}f}$	$34.20^{d\text{-}h}$	$30.10^{e\text{-}h}$	58.52^{BC}
Urmia	84.44^{ab}	$80.00^{a\text{-}d}$	$58.92^{a\text{-}g}$	$20.00^{f\text{-}h}$	0.05^{h}	48.68^{C}
Mean	92.22^{A}	88.42^{A}	72.23^{B}	39.77^{C}	28.91^{D}	

Note: Means within columns or treatments with the same letters are not significantly different at the 5% level

Germination race

Data in (Table 2) indicated that there is significant difference between cultivars and levels of salinity. Germination rate was inversely related to salt concentration with increasing salinity, germination rate decreased by 50-100% in all cultivars. CaljN3 regularly had greater germination rate at all salinity levels but the Urmia had strongly reduction in germination rate at all salinity levels. There are reports suggesting that salt may affect the germination rate to a greater extent than the germination percentage [15], these results are also similar to Jamil and Rha (2004), they reported that germination of sugar beet and cabbage decreased as salinity concentration increased and salinity also delayed germination rate.

Similar kinds of results were reported by Jeannette *et al.* (2002), they found that the mean time to germination of almost all Phaseolus species increased with the addition of NaCl and this increase in median germination time was greater in higher concentration as compared to low concentration. It assumed that germination rate and the final seed germination decreased with the decrease of the water movement into the seed during imbibitions [34], similar declines in seed germination rate have been reported in the literature [35]. We also can say that this reduction in germination race relies on salinity bad effect on some physiological processes which are effective on seed germination [36].

Table 2. Effects of different salinity levels on germination race of six tomato cultivars

Cultivars	0	50	100	150	200mM	Mean
CaljN3	32.96^{a}	31.66^{a}	23.16^{a-d}	11.95^{b-f}	7.53^{c-f}	21.45^{A}
Falat CH	31.85^{a}	26.66^{abc}	17.99^{a-f}	10.07^{b-f}	5.69^{d-f}	18.45^{AB}
Laleh	28.85^{ab}	22.11^{a-e}	14.44^{a-f}	5.04^{d-f}	1.67^{f}	14.42^{BC}
Falat vana	28.31^{ab}	23.80^{ab}	15.04^{a-f}	5.81^{d-f}	3.80^{de}	15.35^{ABC}
Super Strain B	18.70^{a-f}	16.00^{a-f}	10.00^{b-f}	6.52^{d-f}	4.16^{d-f}	11.07^{C}
Urmia	26.11^{abc}	21.16^{a-e}	13.13^{a-f}	3.24^{ef}	0^{f}	12.72^{BC}
Mean	27.79	23.56^{B}	15.62^{C}	7.10^{D}	3.80^{E}	

Note: Means within columns and treatments with the same letters are not significantly different at the 5% level

Radicle and plunule Length (centimeter)

Radicle length is one of the most important characters in salt stress experiments because roots are in contact with the soil and absorb water from the soil. For this reason, radicle length provides an important indication of a plant's response to salt stress. Averaged across cultivars, radicle length decreased from 8.76 cm (control) to 1.31 cm (200mM). Among the cultivars, in the control treatment Urmia had the longest radicle length with 10.5 cm; The longest radicle length at high saline level was determined in CaljN3 at 200mM with 2.86 cm, while Precocious Urmia had the shortest radicle lengths with 0 cm at 200mM. Generally, with increasing salinity stress levels, radicle length significantly decreased in all cultivars and CaljN3 and Falat CH exhibited the lowest reduction in radicle length compared to the control level and can be considered as the most salinity-tolerant cultivar for radicle length. Precocious Urmia cultivar with higher reductions in radicle lengths (100%) from control level to 200mM level were the most sensitive cultivar to salinity stress for radicle length (Table 3).

According to (Table 4) plumule length was more sensitive than radicle length and under increasing stress levels, plumule length significantly decreased for all cultivars. Urmia had the significantly longer plumule than the other cultivars. It had the longest plumules in the control level (12.50 cm), but with increasing stress levels it showed 100% reduction in the highest level of stress (0.0), contrary to Falat CH with a 76.1% reduction (from 9.46 to 2.26 cm), which was the least sensitive cultivar to salinity stress for plumule length, in other words, the Falat CH cultivar was the most tolerant to water salinity at the early development stages in terms of plumule length. Other cultivars were evidently more sensitive to salinity stress and showed higher reductions from increased stress levels (Table 3). Root and shoot lengths are the most important parameters for salt stress because roots are in direct contact with the soil and absorb water from soil and then shoots enable its supply to the rest of the plant. For this reason, root and shoot lengths provide important indications of a plant's response to salt stress [32]. Shoot and root growth was restrained by salinity stress. The amount of decrease under higher salinity levels was more or less equal in the most of the cultivars. Werner and Finkelstein (1995) demonstrated that elevated salinity slowed down water uptake by seeds, thereby inhibiting germination and root elongation. Xiong and Zhu (2002) explained that salt stress inhibited the efficiency of translocation and assimilation of stored materials and might have caused a reduction in shoot growth. The reduction in root and shoot development may be due to toxic effects of the NaCl used as well as unbalanced nutrient uptake by the seedlings. It may be due to the ability of a root system to control entry of ions to the shoot, which is of crucial importance to plant survival in the presence of NaCl [39]. In earlier studies, Hussain and Rehman (1995, 1997) and Ghorashy *et al*. (1972) found that roots of seedlings were more sensitive than shoots. But this study demonstrated that the reduction percentages of plumule and radicle lengths in tomato were different, plumule length with 90% was more sensitive than radicle length with an 85% reduction from control to 200mM. Salt stress inhibited the growth of shoot more than root in *Brassica* species [5]. Similar observations have been reported in barley *(Hordeum vulgare* L.), bean *(Phaseolus acutifolius* L.) and tomato [3,43].

Table 3. Effects of different salinity levels on radicle length (cm) of six tomato cultivars

	0	50	100	150	200mM	
Cultivars						Mean
CaljN3	8.56^{ab}	9.06^{ab}	6.83^{a-f}	6.66^{a-g}	2.86^{c-j}	6.79^{A}
Falat CH	9.16^{ab}	7.40^{a-d}	7.10^{a-e}	5.26^{a-j}	2.50^{d-j}	6.28^{A}
Laleh	8.16^{ab}	7.20^{a-e}	7.10^{a-e}	1.40^{g-j}	0.76^{h-j}	4.92^{A}
Falat vana	7.06^{a-e}	6.10^{a-g}	5.40^{a-i}	3.83^{b-j}	1.33^{g-j}	4.74^{A}
Super Strain B	9.13^{ab}	6.40^{a-g}	6.00^{a-h}	2.06^{e-j}	0.46^{ij}	4.81^{A}
Urmia	10.5^{a}	9.03^{ab}	8.70^{ab}	1.66^{f-j}	0^{j}	5.97^{A}
Mean	8.76^{A}	7.53^{B}	6.85^{B}	3.47^{C}	1.31^{D}	

Note: Means within columns and treatments with the same letters are not significantly different at the 5% level

Table 4. Effects of different salinity levels on plumule length (cm) of six tomato cultivars

	0	50	100	150	200mM	
Cultivars						Mean
CaljN3	9.66^{ab}	7.33^{a-e}	7.21^{a-e}	5.53^{a-e}	1.63^{cde}	6.27^{A}
Falat CH	9.46^{ab}	8.13^{a-d}	7.00^{a-e}	5.53^{a-e}	2.26^{b-e}	6.47^{A}
Laleh	8.00^{a-d}	5.86^{a-e}	4.53^{b-e}	0.93^{de}	0.31^{e}	3.92^{B}
Falat vana	7.50^{a-}	5.10^{a-e}	3.83^{b-e}	0.96^{de}	0.51^{de}	3.58^{B}
Super Strain B	7.00^{a-e}	5.60^{a-e}	3.50^{b-e}	1.56^{cde}	0.21^{e}	3.57^{B}
Urmia	12.50^{a}	9.43^{ab}	8.90^{abc}	1.23^{de}	0^{e}	6.41^{A}
Mean	9.02^{A}	6.90^{B}	5.82^{C}	2.62^{D}	0.82^{E}	

Note: Means within columns and treatments with the same letters are not significantly different at the 5% level

Radicle and plumule Dry Weight (milligram)

Under increasing stress levels, radicle dry weight significantly decreased in all cultivars. Averaged across cultivars, radicle dry weight decreased from 0.66 mgr (control) to 0.15 mgr (200mM). According to these results, in the control level CaljN3 had the heaviest radicles (0.77 mgr), but with increasing stress levels it showed a 79% reduction to 0.16 mgr in the 200mM, while Falat CH with 0.62 mgr in the control level had a 45% reduction to 0.34 mgr under the highest level of stress, therefore it was the least sensitive cultivar for radicle dry weight. However, Urmia with 100% reductions was most sensitive (Table 5). Under increasing stress levels, plumule dry weight significantly decreased in all six cultivars. In the control level, the CaljN3 cultivar, with 6.22 mgr had the heaviest plumule dry weight, and with increasing stress levels it showed 63% reduction, so it was the least sensitive for plumule dry weight. However Urmia showed 100% reduction from control to the highest level of stress and it was most sensitive cultivar (Table 6).

Table 5. Effects of different salinity levels on radicle dry weight (mg) of six tomato cultivars

	0	50	100	150	200mM	
Cultivars						Mean
CaljN3	0.77^{a}	0.63^{abc}	0.50^{a-d}	0.26^{a-d}	0.16^{bcd}	0.46^{A}
Falat CH	0.62^{ab}	0.50^{a-d}	0.42^{a-d}	0.32^{a-d}	0.34^{a-d}	0.44^{AB}
Laleh	0.67^{ab}	0.62^{abc}	0.32^{a-d}	0.25^{a-d}	0.17^{a-d}	0.40^{AB}
Falat vana	0.71^{ab}	0.56^{a-d}	0.36^{a-d}	0.17^{a-d}	0.06^{cd}	0.37^{AB}
Super Strain B	0.46^{a-d}	0.37^{a-d}	0.36^{a-d}	0.19^{a-d}	0.22^{a-d}	0.32^{B}
Urmia	0.74^{ab}	0.363^{a-d}	0.46^{a-d}	0.28^{a-d}	0^{d}	0.36^{AB}
Mean	0.66^{A}	0.50^{B}	0.40^{C}	0.24^{D}	0.15^{E}	

Note: Means within columns and treatments with the same letters are not significantly different at the 5% level

The response of plants to salt stress is a complex phenomenon that involves biochemical and physiological processes as well as morphological and developmental changes [36,44]. Difference among species and cultivars for salinity tolerance may depend on their differences in salinity tolerance mechanism. Exploitation of these useful genetic variations in salinity tolerance particularly of crop plants is an economical approach for proper utilization of salt- affected agricultural lands [45]. Development of salt tolerant tomato cultivars. Thus, more research for salt tolerance in these cultivars would involve screening a larger range of germplasm.

Table 6. Effects of different salinity levels on plumule dry weight (cm) of six tomato cultivars

	0	50	100	150	200mM	
Cultivars						Mean
CaljN3	6.22[a]	5.13[a]	3.78[ab]	3.23[abc]	2.30 [a-d]	4.13[A]
Falat CH	6.15[a]	4.25[ab]	3.50[abc]	2.73[a-d]	1.85[cd]	3.69[AB]
Laleh	4.44[ab]	3.33[abc]	3.45[abc]	2.53 [a-d]	1.37[cd]	3.04[B]
Falat vana	4.84[ab]	4.69[ab]	3.56[abc]	2.63 [a-d]	1.09[cd]	3.38[AB]
Super Strain B	4.45[ab]	4.47[ab]	4.07[ab]	2.05 [a-d]	1.17[cd]	3.24[AB]
Urmia	5.73[a]	4.90[ab]	4.59[ab]	2.41 [a-d]	0[d]	3.52[AB]
Mean	5.30[A]	4.49[B]	3.82[C]	2.59[D]	1.29[E]	

Note: Means within columns and treatments with the same letters are not significantly different at the 5% level

CONCLUSION

Commonly, tomato reacted to salinity rather like a glycophyte showing a steady decrease in germination with increasing salinity. In the germination and seedling growth stages there were differences among the cultivars for salt tolerance; in comparison with the other cultivars CaljN3 and Falat CH were more resistant to high salt concentrations respectively, however Urmia was the most susceptible. Results of germination tests illustrated that CaljN3 and Falat CH were best adapted to extreme environmental conditions of dry land and highly saline soil. Specially among the cultivars CaljN3 had the lowest reduction compared to the control to a high salinity level in all germination indices, so CaljN3 was the best adapted cultivar and the least sensitive to saline conditions at the germination stage. In this study, seed germination and plumule elongation greatly reduced at 200mM. Moreover, tomato shoots appear to be more sensitive than roots. It can be concluded that plumule and radicle elongation may be used as a suitable breeding criteria for the selection of the better salt tolerant cultivars at the seedling stage. In these terms the more vigorous cultivars like CaljN3 and Falat CH could be considered useful cultivars for breeders for the future.

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