

## Using Growth Parameters for *In-vitro* Screening of Potato Varieties Tolerant to Salt Stress

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### ABSTRACT

Salt stress negatively impacts crops yield throughout the world. Nine varieties of potato (*Solanum tuberosum* L.) were screened for salt stress tolerance by measuring *in-vitro* growth of the aerial plant parts, as well as roots. Salt stress was evaluated by adding 25, 50, 75, 100, 125, 150 and 200 mM of NaCl to Murashige- Skoog (MS) medium and compared to MS medium without NaCl. Plant length and stem thickness, leaf area, roots number, length, and thickness, and plant fresh and dry weights were measured. Osmotic pressure ( $\Psi_{\text{medium}}$ , MPa) and electrical conductivity ( $EC_{\text{medium}}$ , mS cm<sup>-1</sup>) of media ranged from -0.2 to -0.91 MPa and 5.8 to 24 mS cm<sup>-1</sup>, respectively. Salt stress adversely affected the plant growth, and varieties differed in their responses. Progressive reduction in the studied parameters occurred as NaCl levels increased. Grouping all the varieties by cluster analysis, based on the growth parameters response to salt stress, resulted in three distinct groups: (1) salt tolerant group of two varieties, namely, Taurus and Sultana; (2) moderately salt tolerant group of four varieties, namely, Loane, Diamant, Amarin, and Sylvana; and (3) salt sensitive group of three varieties, namely, Toscana, Soraya, and Kenita. The response variation of these potato varieties under NaCl indicated the possibility of using them for developing salt tolerant varieties for production in Syria.

**Keywords:** *In-vitro* culture, NaCl, Salinity tolerance, *Solanum tuberosum*.

### INTRODUCTION

Salinity stress is a critical environmental constraint to crop productivity, especially in arid and semiarid regions (Munns, 2005; Witzel *et al.* 2009). Potato (*Solanum tuberosum* L.) has threshold salinity levels from 1.6 to 2.5 dS m<sup>-1</sup> and is considered as moderately salt sensitive compared with other crops (Maas and Hoffman, 1977; Backhosen *et al.*, 2005; Shaterian *et al.*, 2005). High salinity levels cause a diverse set of physiological, morphological, and developmental changes in plants (Bohnert *et al.*, 1995). Although Potluri and Prasad (1993) found that low concentrations of sea salt actually improved *in-vitro* growth of some potato cultivars, many studies reported

that salinity reduced the dry matter of tubers (Ghosh *et al.*, 2001).

Much of constrains in salinity stress is related to water stress arising from excessive uptake of salts by the potato plant and the resulting reduction in water potential (Gandar and Tanner, 1976; Shaterian *et al.*, 2005). Excessive amounts of salt in plants can become toxic in older leaves, causing premature senescence and a reduction in total photosynthetic leaf area (Munns, 2002). Toxic accumulation of Na<sup>+</sup> and Cl<sup>-</sup> in the leaves has also been correlated with stomatal closure and reduction of total chlorophyll content in leaves (Greenway and Munns, 1980; Romero-Aranda and Syvertsen, 1996; Schapendonk *et al.*, 1989; Tavakkoli *et al.*, 2010). Moreover, it was found that salinity

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changes photosynthetic parameters, including osmotic and leaf water potential, pigment compositions, and relative leaf water content (James *et al.*, 2002; Munns, 2005). Plant height and leaf elongation also decrease with increasing NaCl in the *in-vitro* nutrient solution (Potluri and Prasad, 1994; Khenifi *et al.*, 2011). Salinity not only decreases the area of individual leaves, but also the total leaf area of plants (Aghaei *et al.*, 2008). Such reductions in leaf area are likely to decrease whole plant photosynthesis and, thus, growth.

The selection of salt-tolerant potato lines is difficult due to the complexity of polygenic control for salt tolerance (Greenway and Munns, 1980; Flowers and Yeo, 1995; Shannon, 1997). A small number of potato genotypes has been reported in salinity tolerance under outdoor, greenhouse, or *in-vitro* conditions. Field and greenhouse trials (Levy, 1992; Naik and Widholm, 1993; Nadler and Heuer, 1995; Houshmand *et al.*, 2005) were used to examine and screen the genotype salinity tolerance under sodium salt irrigation solutions. The *in-vitro* evaluations of NaCl effects on potato genotypes were recently proposed as alternatives to the costly, labor intensive, and sometimes problematic field traits. Moreover, a correlation among salt stress responses of *in-vitro*, field, and greenhouse potatoes (Morpurgo, 1991; Naik and Widholm, 1993) was reported. The successful *in-vitro* screening has stimulated many attempts for the development of salt-tolerant plant (Rahnama and Ebrahimzadeh, 2005; Zhang *et al.*, 2005; Rahman *et al.*, 2008; Soleimani *et al.*, 2010; Homayoun *et al.*, 2011; Daneshmand *et al.*, 2012) and other types of stress (Gopal and Iwama, 2007; Arvin and Donnelly, 2008).

Thus, the objective of this study was to find out the effect of different NaCl levels on *in-vitro* growth traits of nine potato varieties, to assess a growth classification of varieties according to their salt tolerance and to evaluate the most promising salt-tolerant varieties for future use in potato breeding program in Syria.

## MATERIALS AND METHODS

### Plant Material and Culture Conditions

The experiment was carried out in the laboratories of the National Commission for Biotechnology (NCBT, Damascus, Syria). Nine potato varieties (Toscana, Amarin, Kenita, Sultana, Soraya, Taurus, Diamant, Sylvana, and Loane) were used for this study. The sprouted healthy tubers of these varieties were planted in 50 × 80 cm slabs divided into holes containing steamy disinfected compost. The stems of grown plants were cut into nodal parts consisting of a single node and leaf, in order to be used as primary explants. Nodal parts were disinfected in a solution of 0.5% (v/v) sodium hypochlorite for 5 minutes. Then, they were rinsed with distilled water three times and transferred to 15 mL of MS medium (Murashige and Skoog, 1962), supplemented with 20 g L<sup>-1</sup> sucrose and 7 g L<sup>-1</sup> agar (pH 5.7±0.1). Cultures were maintained under a 16/8 hour photoperiod with 150 μmol m<sup>-1</sup> s<sup>-1</sup> natural light intensity supplemented with sodium vapour pressure lamps at 25±1°C. The *in-vitro* grown plants were propagated by 4 weeks sub-culturing interval. The third subculture was used in the experiment.

### Salt Stress Treatments

Salt stress was assessed by transferring single nodes to MS medium containing 0, 25, 50, 75, 100, 125, 150, and 200 mM of NaCl (C, T1, T2, T3, T4, T5, T6 and T7, respectively) with ten replicates per treatment. The *in-vitro* grown plants were harvested after 6 weeks (salt stress period) in order to do the measurements.

### Measurements

The electrical conductivity (EC<sub>medium</sub>) and osmotic pressure (Ψ<sub>medium</sub>) of MS medium

containing different NaCl concentrations, were measured using Microprocessor Precision Conductivity Meter (LF 539, Electronics India Co., India) and osmometer (OM 815, Vogel GmbH and Co. KG, Germany), respectively. After salt stress period, ten plants per variety were rinsed in distilled water and separated into leaves, stems, and roots. Leaves and roots number were recorded. Roots length and diameter, as well as stem length and diameter, were assessed using digital caliper (500-181U Mitutoyo, precision 1/100th). Leaves area was measured using a Li-Cor 3100 area meter (Li-Cor, Lincoln, NE, USA). The plant fresh and dry weights (oven-dried at 70°C for at least 72 hours) were determined according to Schafleitner *et al.* (2007).

#### Experimental Design and Statistical Analysis

The experiment was designed as completely randomized design (CRD). Each variety had ten replicates for each treatment. Significant differences between varieties were assessed according to the LSD test at 1% level, using the R-version 2.5.3 statistical software (The R Project for Statistical Computing, <http://www.r-project.org/>). To establish the cluster analysis, the sum of relative values for all growth parameters was calculated as the following equation:

$$\text{Relative value} = \sum_{p_1}^{p_9} \frac{\text{Stressed} * 100}{\text{control}}$$

Where the relative value for each variety was calculated as the sum of differences between control and stressed plants for the nine parameters ( $p_1 \rightarrow p_9$ ).

## RESULTS

### Electrical Conductivity and Osmotic Pressure of Culture Medium

The medium electrical conductivity ( $EC_{\text{medium}}$ ,  $\mu\text{S}$ ) increased regularly as NaCl concentration was increased (Table 1). The EC of MS medium of THE control *i.e.* 0

mM of NaCl, was 5.8  $\text{mS cm}^{-1}$ . The highest EC value (24  $\text{mS cm}^{-1}$ ) was observed at 200 mM NaCl treatment. On the other hand, a linear pattern was observed for the medium osmotic pressure ( $\Psi_{\text{medium}}$ , MPa), which decreased with increase in NaCl concentration. The  $\Psi_{\text{medium}}$  of the control was -0.2 MPa and it decreased until it reached the lowest value of -0.91 MPa in a medium supplemented with 200 mM NaCl.

### Plant Length and Number of Leaves per Plant

The general tendency of plant length and number of leaves was to decrease with the NaCl concentration increase (Table 2). Concerning the plant length, significant differences were observed between the control and NaCl treatments (Table 2). These significant differences were observed from T1 treatment in most of the varieties such as Sultana, Soraya, Taurus, Kentia, Diamant, Sylvana and Amarin. For example, Sultana plant length decreased regularly from 11.38 cm for the control to 2.33 cm for T7. However, the significant decrease in Toscana plant length was noted starting from T2 (4.33 cm) as compared to the control (9.25 cm), and Loane plant length did not show significant difference as compared to the control (8.63 cm) until T5 (2.17 cm).

**Table 1.** NaCl concentrations (mM), electrical conductivities ( $EC_{\text{medium}}$ ,  $\text{mS cm}^{-1}$ ) and osmotic pressures ( $\Psi_{\text{medium}}$ , MPa) of MS medium.<sup>a</sup>

Treatment	NaCl	$EC_{\text{medium}}$	$\Psi_{\text{medium}}$
C	0	5.8	-0.2
T1	25	8	-0.3
T2	50	10.2	-0.4
T3	75	12.8	-0.5
T4	100	14.8	-0.55
T5	125	17	-0.64
T6	150	20	-0.73
T7	200	24	-0.91

<sup>a</sup> Values are means of ten replicates (n= 10).

**Table 2.** Plant length (cm) and number of leaves per plant of the nine potato varieties according to NaCl treatments.<sup>a</sup>

	Variety	0 mM	25 mM	50 mM	100 mM	125 mM	150 mM	175 mM	200 mM	LSD <sub>1%</sub>
Plant length	Sultana	11.38 <sup>a</sup>	7.75 <sup>b</sup>	6.12 <sup>b</sup>	5 <sup>bc</sup>	3 <sup>c</sup>	2.67 <sup>c</sup>	2.42 <sup>c</sup>	2.33 <sup>c</sup>	2.99
	Loane	8.63 <sup>a</sup>	8.50 <sup>a</sup>	7.75 <sup>a</sup>	7.65 <sup>a</sup>	6.13 <sup>ab</sup>	2.17 <sup>c</sup>	3.25 <sup>bc</sup>	1.17 <sup>c</sup>	2.89
	Soraya	12.25 <sup>a</sup>	6.75 <sup>b</sup>	6.33 <sup>b</sup>	5.00 <sup>bc</sup>	2.33 <sup>cd</sup>	2.17 <sup>cd</sup>	1.83 <sup>cd</sup>	0.75 <sup>d</sup>	3.24
	Toscana	9.25 <sup>a</sup>	8.38 <sup>a</sup>	4.33 <sup>b</sup>	1.92 <sup>bc</sup>	1.38 <sup>bc</sup>	2.33 <sup>bc</sup>	1.51 <sup>bc</sup>	1.33 <sup>c</sup>	2.99
	Taurus	12.25 <sup>a</sup>	7.88 <sup>b</sup>	6.83 <sup>b</sup>	2.69 <sup>c</sup>	3.83 <sup>c</sup>	3.50 <sup>c</sup>	3.33 <sup>c</sup>	1 <sup>c</sup>	2.94
	Kenita	10.67 <sup>a</sup>	5.88 <sup>b</sup>	3.33 <sup>bc</sup>	2.67 <sup>c</sup>	2.00 <sup>c</sup>	1.92 <sup>c</sup>	1.83 <sup>c</sup>	1.74 <sup>c</sup>	3.09
	Diamant	9.63 <sup>a</sup>	7.75 <sup>ab</sup>	6.00 <sup>b</sup>	5.50 <sup>bc</sup>	2.75 <sup>cd</sup>	3.00 <sup>cd</sup>	2.83 <sup>cd</sup>	2.00 <sup>d</sup>	2.94
	Sylvana	11.25 <sup>a</sup>	9.38 <sup>a</sup>	5.83 <sup>b</sup>	3.75 <sup>bc</sup>	4.33 <sup>bc</sup>	3.50 <sup>bc</sup>	2.83 <sup>c</sup>	1.50 <sup>c</sup>	2.97
	Amarin	15.38 <sup>a</sup>	10.38 <sup>b</sup>	10.75 <sup>b</sup>	7.00 <sup>c</sup>	5.25 <sup>c</sup>	2 <sup>d</sup>	2.08 <sup>d</sup>	2.00 <sup>d</sup>	3.09
Number of leaves	Sultana	11.75 <sup>a</sup>	11 <sup>ab</sup>	10.25 <sup>abc</sup>	10 <sup>abc</sup>	5.67 <sup>cd</sup>	6.67 <sup>bcd</sup>	6.33 <sup>bcd</sup>	4.33 <sup>d</sup>	4.74
	Loane	12.5 <sup>a</sup>	12.5 <sup>a</sup>	11.75 <sup>a</sup>	11.75 <sup>a</sup>	11.25 <sup>a</sup>	9.67 <sup>a</sup>	4.67 <sup>b</sup>	2.33 <sup>b</sup>	4.58
	Soraya	13.5 <sup>a</sup>	10.75 <sup>ab</sup>	9 <sup>abc</sup>	9.5 <sup>abc</sup>	5.33 <sup>cd</sup>	5.67 <sup>bcd</sup>	2 <sup>d</sup>	1 <sup>d</sup>	5.14
	Toscana	13.25 <sup>a</sup>	10.25 <sup>ab</sup>	9.33 <sup>abc</sup>	5 <sup>cde</sup>	7 <sup>bcd</sup>	3.33 <sup>de</sup>	3.33 <sup>de</sup>	1.75 <sup>e</sup>	4.74
	Taurus	13.25 <sup>a</sup>	11.75 <sup>a</sup>	11.67 <sup>a</sup>	11 <sup>a</sup>	10.33 <sup>ab</sup>	10 <sup>ab</sup>	6 <sup>bc</sup>	3 <sup>c</sup>	4.75
	Kenita	13.67 <sup>a</sup>	13.25 <sup>a</sup>	9.33 <sup>ab</sup>	5.33 <sup>bc</sup>	5 <sup>bc</sup>	5 <sup>bc</sup>	3.33 <sup>c</sup>	3 <sup>c</sup>	4.90
	Diamant	11.5 <sup>a</sup>	11.5 <sup>a</sup>	10.67 <sup>a</sup>	10 <sup>a</sup>	8 <sup>ab</sup>	7.5 <sup>ab</sup>	7.67 <sup>ab</sup>	4.33 <sup>b</sup>	4.66
	Sylvana	14 <sup>a</sup>	11.25 <sup>ab</sup>	10.5 <sup>abc</sup>	9.33 <sup>abc</sup>	9.33 <sup>abc</sup>	8.25 <sup>bcd</sup>	6.33 <sup>cd</sup>	3.67 <sup>d</sup>	4.74
	Amarin	13 <sup>a</sup>	13 <sup>a</sup>	11.75 <sup>a</sup>	10.67 <sup>a</sup>	10.5 <sup>a</sup>	5 <sup>b</sup>	4.33 <sup>b</sup>	3.67 <sup>b</sup>	4.90

<sup>a</sup> Values are means of ten replicates (n= 10) and means within row having different letters are significantly different according to the LSD at  $P<0.01$ .

In most of the varieties, number of leaves was affected significantly only at higher concentration of NaCl, where it was reduced compared to the control (Table 2). The significant differences were observed starting from T3 in Kentia and Toscana, from T4 in Soraya and Sultana, from T5 in Amarin and Sylvana, from T6 in Taurus and Loane, and from T7 in Diamant.

### Plant Diameter and Leaf Area

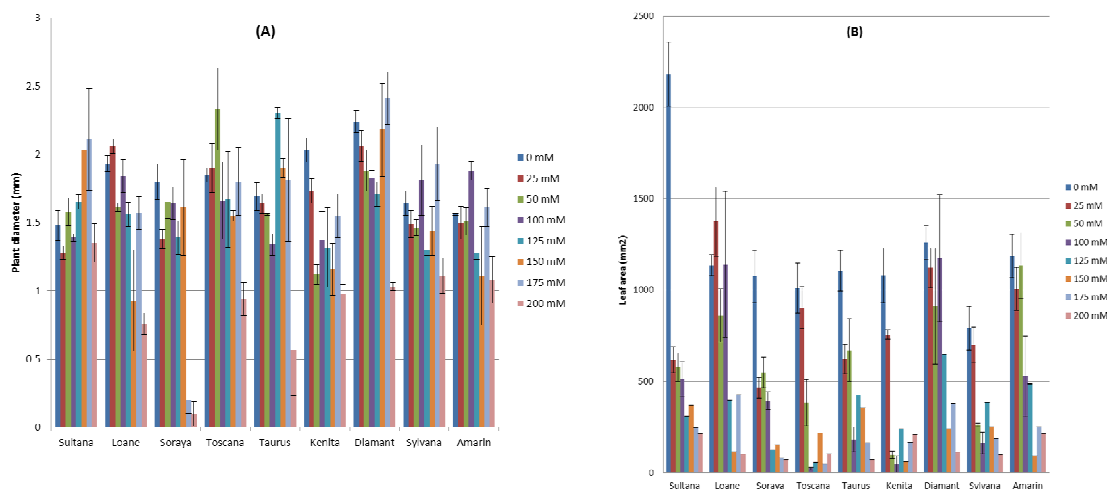
Figure 1 represents the variation in plant diameter and leaf area under NaCl stress. Variation in plant diameter under the NaCl stress depended on the potato variety (Figure 1-A). As a general trend, the plant diameter decreased with the increase of the NaCl concentration, however, some of the varieties showed various changes following the NaCl treatment. Compared to the control, plant diameter increased with T1 in Loane and Toscana, with T2 in Sultana and Toscana, with T3 in Sylvana and Amarin, with T4 and T5 in

Sultana and Taurus, and with T6 in most of varieties, except Loane, Soraya, Toscana, and Kenita (Figure 1-A).

Leaf area of the control plants ranged between 790.75 mm<sup>2</sup> for Sylvana and 2181.75 mm<sup>2</sup> for Sultana (Figure 1-B). The NaCl treatments significantly decreased leaf area in all varieties. Maximum effect was observed with T7 in Soraya and Taurus with a decrease of 94%, followed by a decrease of 91% in Loane, Diamant, Sultana and Toscana, then a decrease of 87%, 82%, and 81% in Sylvana, Amarin and Kenita, respectively (Figure 1-B).

### Roots Morphology

Most varieties showed a regular growth pattern up to T4, though the overall growth was reduced. High NaCl concentration (T6 and T7) affected the rooting in most varieties, as no roots were formed (Figure 2-A). Only Sultana, Sylvana, Taurus and Toscana had roots at T6; however, the roots number was considerably reduced from 5.5, 5.25, 4.75 and



**Figure 1.** Average of plant diameter (A, mm) and leaf area (B, mm<sup>2</sup>) for the nine potato varieties. Values are mean±stander error (n= 10).

6 roots per plant, respectively, in the control to 1.5, 2.33, 1 and 0.67 roots per plant, respectively, at this NaCl concentration. At T5, all varieties formed a certain number of roots, except Diamant and Kenita. At T2, T3, and T4, all varieties showed a regular reduction of roots number as compared to the control, except Kenita which revealed a great sensitivity to NaCl stress starting from T2 (Figure 2-A).

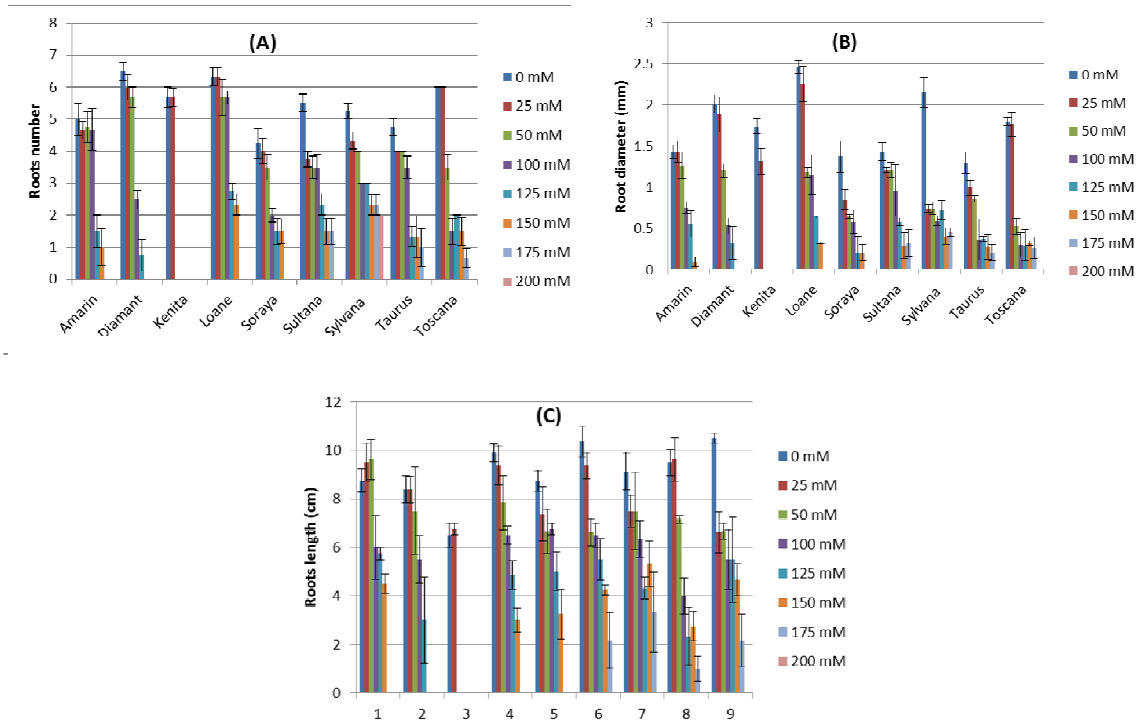
Roots diameter of the control varied among the varieties (Figure 2-B). Loane and Sylvana had the largest roots diameter i.e. 2.46 and 2.15 mm, respectively, while the remaining varieties had smaller diameter i.e. between 1.29 and 1.80 mm. The roots diameter decreased with NaCl concentration depending on the variety. The roots diameter of Sultana, Sylvana, Taurus, and Toscana considerably decreased by 4.48, 4.78, 6.45 and 6.75 times, respectively, as compared to the control (1.43, 2.15, 1.29 and 1.80 mm, respectively) at T6. At T5, most varieties showed a sharp reduction of roots diameter (Figure 2-B).

Concerning roots length, the control treatment of Sultana, Toscana, and Loane showed the longest roots (10.38, 10.50 and 9.88 cm, respectively) (Figure 2-C). The roots length was reduced by NaCl treatments in all varieties. The roots length was considerably reduced by 79.12, 63.47, 89.47, and 79.37% as

compared to the control (10.38, 9.13, 9.5 and 10.5 cm) in Sultana, Sylvana, Taurus, and Toscana, respectively, at T6. At T5, a reduction of 48.57%, 69.62%, 62.86%, 59.04%, 41.55%, 71.05%, and 55.55% was observed in Amarin, Loane, Soraya, Sultan, Sylvana, Taurus, and Toscana, respectively, as compared to the controls. At T4, a reduction of 34.29%, 65.71%, 50.63%, 42.86%, 46.99%, 52.51%, 75.44%, and 47.62% was observed in Amarin, Diamant, Loane, Soraya, Sultan, Sylvan, Taurus and Toscana, respectively, as compared to the control (Figure 2-C).

### Fresh and Dry Weight of Plant

The plant fresh and dry weight varied among the nine varieties studied (Table 3). Diamant had the higher fresh weight (1.39 g) as compared to the other varieties, while Sultana had the lowest fresh weight (0.61 g). As a general trend, the plant fresh weight decreased with NaCl concentration. For example, the plant fresh weight decreased considerably at the highest NaCl concentration (T7) by 3.8, 8.3, 12, 10.3, 3, 15.3, 6.3, 7.9, and 8.5 times in Sultana, Loane, Soraya, Toscana, Taurus, Kenita, Diamant, Sylvana and Amarin, respectively, as compared to the control.



**Figure 2.** Number (A), diameter (B), and length (C) of roots according to the nine potato varieties and salinity treatments. Values are mean±stander error (n= 10).

**Table 3.** Fresh and dry weight (g) of the nine potato varieties according to salinity treatments.<sup>a</sup>

	Variety	0 mM	25 mM	50 mM	100 mM	125 mM	150 mM	175 mM	200 mM	LSD <sub>1%</sub>
Fresh weight	Sultana	0.61 <sup>a</sup>	0.63 <sup>a</sup>	0.49 <sup>ab</sup>	0.43 <sup>ab</sup>	0.29 <sup>ab</sup>	0.30 <sup>ab</sup>	0.23 <sup>ab</sup>	0.16 <sup>b</sup>	0.42
	Loane	1.48 <sup>a</sup>	1.25 <sup>ab</sup>	0.98 <sup>b</sup>	0.89 <sup>b</sup>	0.47 <sup>c</sup>	0.39 <sup>c</sup>	0.18 <sup>c</sup>	0.15 <sup>c</sup>	0.41
	Soraya	0.97 <sup>a</sup>	0.63 <sup>ab</sup>	0.62 <sup>ab</sup>	0.36 <sup>bc</sup>	0.22 <sup>bc</sup>	0.19 <sup>bc</sup>	0.08 <sup>c</sup>	0.08 <sup>c</sup>	0.45
	Toscana	1.13 <sup>a</sup>	0.89 <sup>ab</sup>	0.46 <sup>bc</sup>	0.11 <sup>c</sup>	0.10 <sup>c</sup>	0.20 <sup>c</sup>	0.09 <sup>c</sup>	0.11 <sup>c</sup>	0.42
	Taurus	0.82 <sup>a</sup>	0.61 <sup>abc</sup>	0.67 <sup>ab</sup>	0.66 <sup>ab</sup>	0.57 <sup>abc</sup>	0.22 <sup>c</sup>	0.35 <sup>bc</sup>	0.27 <sup>bc</sup>	0.41
	Kenita	1.07 <sup>a</sup>	0.72 <sup>ab</sup>	0.44 <sup>bc</sup>	0.33 <sup>bc</sup>	0.27 <sup>c</sup>	0.18 <sup>c</sup>	0.10 <sup>c</sup>	0.07 <sup>c</sup>	0.44
	Diamant	1.39 <sup>a</sup>	1.10 <sup>ab</sup>	1.01 <sup>ab</sup>	0.85 <sup>bc</sup>	0.53 <sup>cd</sup>	0.48 <sup>cd</sup>	0.38 <sup>d</sup>	0.22 <sup>d</sup>	0.41
	Sylvana	1.10 <sup>a</sup>	0.57 <sup>b</sup>	0.33 <sup>bc</sup>	0.31 <sup>bc</sup>	0.22 <sup>bc</sup>	0.22 <sup>bc</sup>	0.19 <sup>bc</sup>	0.14 <sup>c</sup>	0.42
	Amarin	0.93 <sup>a</sup>	0.81 <sup>ab</sup>	0.80 <sup>ab</sup>	0.49 <sup>bc</sup>	0.38 <sup>bc</sup>	0.20 <sup>c</sup>	0.17 <sup>c</sup>	0.11 <sup>c</sup>	0.44
Dry weight	Sultana	0.05 <sup>a</sup>	0.04 <sup>a</sup>	0.05 <sup>a</sup>	0.04 <sup>a</sup>	0.03 <sup>a</sup>	0.02 <sup>a</sup>	0.02 <sup>a</sup>	0.02 <sup>a</sup>	0.04
	Loane	0.10 <sup>ab</sup>	0.12 <sup>a</sup>	0.07 <sup>bc</sup>	0.07 <sup>bc</sup>	0.04 <sup>c</sup>	0.04 <sup>c</sup>	0.02 <sup>c</sup>	0.02 <sup>c</sup>	0.03
	Soraya	0.07 <sup>a</sup>	0.05 <sup>ab</sup>	0.04 <sup>ab</sup>	0.03 <sup>ab</sup>	0.02 <sup>ab</sup>	0.02 <sup>ab</sup>	0.01 <sup>b</sup>	0.01 <sup>b</sup>	0.05
	Toscana	0.11 <sup>a</sup>	0.09 <sup>a</sup>	0.04 <sup>b</sup>	0.01 <sup>b</sup>	0.01 <sup>b</sup>	0.02 <sup>b</sup>	0.01 <sup>b</sup>	0.01 <sup>b</sup>	0.04
	Taurus	0.08 <sup>a</sup>	0.05 <sup>ab</sup>	0.05 <sup>ab</sup>	0.05 <sup>ab</sup>	0.05 <sup>ab</sup>	0.05 <sup>ab</sup>	0.02 <sup>b</sup>	0.03 <sup>b</sup>	0.04
	Kenita	0.10 <sup>a</sup>	0.07 <sup>ab</sup>	0.04 <sup>b</sup>	0.03 <sup>b</sup>	0.03 <sup>b</sup>	0.02 <sup>b</sup>	0.01 <sup>b</sup>	0.01 <sup>b</sup>	0.04
	Diamant	0.13 <sup>a</sup>	0.07 <sup>b</sup>	0.05 <sup>bc</sup>	0.04 <sup>bc</sup>	0.05 <sup>bc</sup>	0.04 <sup>bc</sup>	0.04 <sup>bc</sup>	0.03 <sup>c</sup>	0.03
	Sylvana	0.09 <sup>a</sup>	0.06 <sup>ab</sup>	0.03 <sup>b</sup>	0.02 <sup>b</sup>	0.03 <sup>b</sup>	0.02 <sup>b</sup>	0.02 <sup>b</sup>	0.02 <sup>b</sup>	0.04
	Amarin	0.14 <sup>a</sup>	0.07 <sup>b</sup>	0.07 <sup>b</sup>	0.04 <sup>b</sup>	0.03 <sup>b</sup>	0.02 <sup>b</sup>	0.02 <sup>b</sup>	0.02 <sup>b</sup>	0.05

<sup>a</sup> Values are means of ten replicates (n= 10) and means within row having different letters are significantly different according to the LSD at  $P<0.01$ .

The plant dry weight was generally reduced by NaCl treatments in all varieties, except Sultana, where the plant dry weight did not show any change by NaCl stress (Table 3). For example, the plant dry weight was considerably lowered at the highest NaCl concentration (T7) by 80%, 85.71%, 90.91%, 62.5%, 90%, 76.92%, 77.78%, and 85.71% as compared to the control in Loane, Soraya, Toscana, Taurus, Kenita, Diamant, Sylvana, and Amarin, respectively.

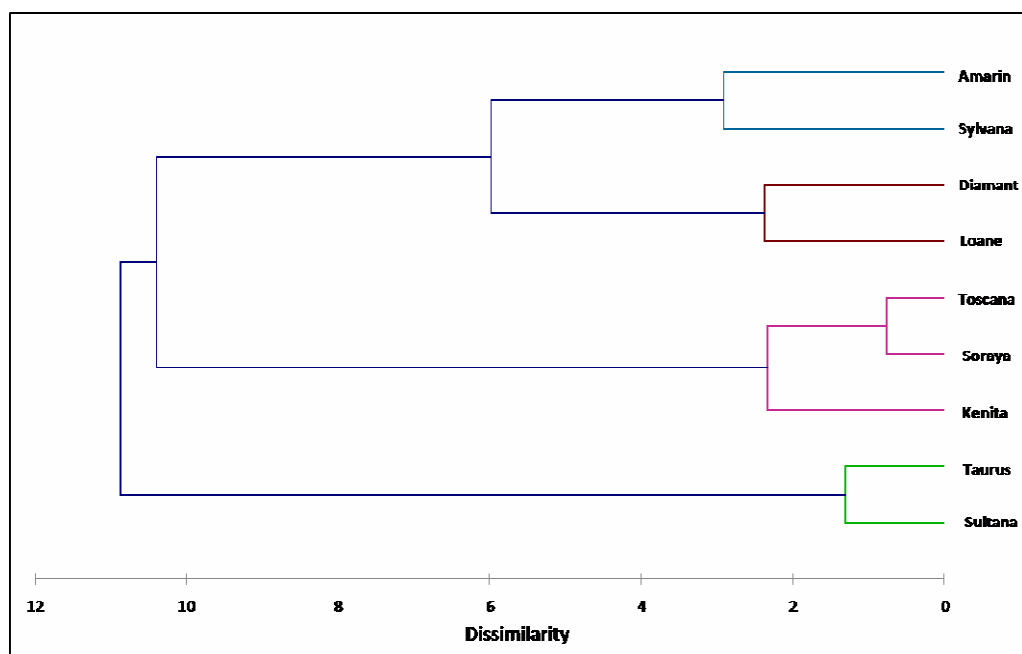
### Cluster Analyses

The cluster analysis, based on the sum of relative values of the differences between the control and stressed plants for growth parameters, resulted in three distinct groups: (1) salt tolerant group consisting of two varieties, namely, Taurus and Sultana; (2) a moderately salt tolerant group consisting of four varieties, namely, Loane, Diamant, Amarin and Sylvana, (3) a salt sensitive group consisting of three varieties, namely, Toscana, Soraya and Kenita (Figure 3).

### DISCUSSION

Plant species and cultivars within a crop species vary greatly in their response to salinity (Marschner, 1995). Moreover, screening a large number of genotypes for salinity tolerance in the field is very difficult, due to spatial heterogeneity of soil chemical and physical properties. The effect of salt stress on *in-vitro* potato growth has been reported to be similar to that observed under field conditions (Zhang and Donnelly, 1997; Aghaei *et al.*, 2008). In view of the significant correlation found between *in-vitro* growth and field performance, Morpurgo (1991) suggested *in-vitro* screening of potato parental material for tolerance to salinity. Many other studies have proposed the *in-vitro* screening of potato genotypes for salt stress tolerance as an alternative approach to costly, labor-intensive, and sometimes problematic field-based screening (Aghaei *et al.*, 2008; Rahman *et al.*, 2008).

In this study, the electrical conductivity increase while the osmotic pressure decrease



**Figure 3.** Dendrogram based on relative values of growth parameters of the nine potato varieties under different salinity treatments.



with the increase of NaCl in medium (Table 1). the increase of  $EC_{medium}$  could be attributed to the accumulation of salts, consequently, decreased osmotic pressure (Pierik, 1987). The osmotic pressure of the medium is the sum of the osmotic pressure of the component (mineral, sugars, etc.) (Lipvaska and Vergudenhil, 1996). Several studies showed the effects of decreased osmotic pressure on the rate of growth and development of *in-vitro* tissues (George and Sherrington, 1984; Shibli *et al.*, 1992).

There are two ways in which salinity affects plant growth. Firstly, ions of dissociated salts can become toxic to plants if their concentration in the medium exceeds a certain value. The concentration at which toxicity effect appears depends on the ion and plant species involved (Volkmair *et al.*, 1998; Ashraf and Harris, 2004). Secondly, salts lower the osmotic potential of culture medium (Evers *et al.*, 1998; Zhu, 2001; Daneshmand *et al.*, 2010). Many studies suggested that higher levels of  $Na^+$  resulted in lower levels of  $K^+$  in shoots and roots, leading to damaging effects of NaCl in potato. Although halophytes can actively control their uptake of  $Na^+$  and  $Cl^-$  (Ashraf, 1994; Colmer *et al.*, 1995; Santa-Maria and Epstein, 2001), however, salt-sensitive plants such as potato cannot control the influx of these ions (Flowers and Yeo, 1986).

The varieties used in this study responded differentially to salt stress. According to cluster analyses (Figure 3), Taurus and Sultana were classified as a tolerant variety, while Loane, Diamant, Sylvana and Amarin were classified as moderately tolerant. Toscana, Soraya, and Kenita were classified as sensitive varieties to salt stress. Nevertheless, all of the varieties showed reduction in plant and roots length, plant and roots diameter, number of roots and leaves, leaves area, and plant fresh and dry weight under NaCl stress (Figures 1 and 2, Tables 2 and 3). This decrease in growth caused by the salt stress was also observed in potato variety cv. Cardinal, (Farhatullah and Raziuddin, 2002) and in several other potato

varieties (Shaterian *et al.* 2005). Similar pattern of *in-vitro* potato grown under different NaCl concentrations was reported (Potluri and Prasad 1993; Homayoun *et al.* 2011; Khenifi *et al.* 2011). However, Morpurgo (1991) reported that some cultivar such as cv. Serrana produced the greatest roots growth *in-vitro* in MS liquid medium containing 154 mM NaCl. It has also been reported that under salt stress, relatively salt-tolerant potato cultivars accumulated more fresh and dry weights than salt-sensitive cultivars (Rahnama and Ebrahimzadeh, 2004).

Little information is available on the salt stress tolerance for the varieties used in this study. According to our results, Diamant is classified as moderately tolerant variety. This result agrees with previous results obtained by Aghaei *et al.* (2008), where Diamant was classified as moderately salt tolerant based on *in-vitro* screening at different concentrations of NaCl (0, 30, 60, 90 and 120 mM) using physiological parameter and random amplification of polymorphic DNA (RAPD) analysis. However, Diamant has been introduced as a salt sensitive by Rahnama and Ebrahimzadeh (2005 and 2006). The difference might be due to the difference in experimental conditions or the range of salt concentrations in the medium.

This study showed that the NaCl stress tolerance of potato genotypes could be easily evaluated by the *in-vitro* screening, based on growth parameters, for identification of suitable lines with improved NaCl tolerance. Salinity still remains the major abiotic stress that limits agricultural production (Altman, 2003). A number of mechanisms related to improved stress adaptation in crops have been suggested (Levy and Veilleux, 2007). Therefore, a well-focused approach combining the molecular, physiological, and metabolic aspects of abiotic stress tolerance is required to establish a screening approach (Bhatnagar-Mathur *et al.*, 2008).



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### استفاده از پارامترهای رشد برای بهگزینی آزمایشگاهی رقم های سیب زمینی متحمل شوری

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#### چکیده

در سراسر جهان تنش شوری تأثیری منفی بر عملکرد محصول دارد. در این پژوهش، به منظور بهگزینی سیب زمینی (*Solanum tuberosum* L.) برای مقاومت به شوری، رشد هوایی و رشد ریشه 9 رقم سیب زمینی در آزمایشگاه بررسی شد. برای ایجاد تنش شوری، کلرور سدیم به مقدار 25، 50، 75، 100، 125، 150، و 200 میلی مول به محیط رشد (Murashige- Skoog (MS) افزوده شد و نتایج با گیاهان شاهد در همان محیط رشد بدون نمک طعام مقایسه شد. طول گیاه و ضخامت ساقه، مساحت برگ ها، تعداد ریشه و طول و قطر آنها، همراه با وزن تر و خشک گیاهان اندازه گیری شد. فشار اسمزی ( $\Psi_{\text{mediu}}$ ) بین 0/2- و 0/91- مگاپاسکال و هدایت الکتریکی ( $EC_{\text{medium}}$ ) بین 5/8 و 24 دسی زیمنس بر متر تغییر میکرد. تنش شوری اثر منفی روی رشد گیاه داشت و رقم های مختلف واکنش های متفاوتی نشان دادند. با افزایش غلظت کلرور سدیم کاهش تدریجی در پارامترهای اندازه گیری شده رخ داد. گروه بندی رقم ها با استفاده از تجزیه خوشه ای و بر مبنای واکنش پارامترهای گیاهی به تنش شوری منجر به ایجاد سه گروه شد: (1) گروه متحمل شوری شامل رقم های Taurus و Sultana، (2) رقم های نسبتاً مقاوم به شوری شامل رقم های Diamant، Loane، و Kenita، و (3) گروه حساس به شوری شامل Soraya، Toscana، و



تغییرات واکنش های این رقم های سیب زمینی نسبت به کلرور سدیم چنین اشاره داشت که ممکن است از آنها برای تولید رقم های متحمل شوری در سوریه استفاده کرد.