

## INFLUENCE OF SALINITY ON GROWTH AND ORGANIC COMPOUNDS CONTENT OF CARROT (*Daucus carota* L.)

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### ABSTRACT

Carrot production of valuable carotenes, carbohydrate and protein are hindered by elevated salinity levels in many parts of the world. To assess this problem, germination and growth of two carrot cultivars (*Daucus carota* cvs Jordan and Napoli) were studied *in vivo* and *in vitro* under different salt stress concentrations (0, 75, and 150 mM NaCl). Seeds were directly or gradually exposed to these salt concentrations. With elevated salinity levels, significant reductions in growth parameters (dry shoot weight, fresh shoot weight, shoot length, root length, and root number) were observed. Also, significant difference in germination percentage was observed at 150 mM NaCl in both cultivars when compared with control treatment (90% germination percentage in Napoli and 71% in Jordan cultivar). Growth rate, tolerant index, and relative water content (RWC) declined as salinity increased. The 150 mM NaCl salinity treatment significantly reduced the shoot chlorophyll and protein content, but increased carbohydrate content. Lesser impairment by the gradual exposure of seedling to salinity provides an opportunity to study the acquirement of salt tolerance.

**Key words:** NaCl, relative water content, gradual exposure, tolerance index, *in vitro*

### INTRODUCTION

Salinity is considered as a confining element for plant growth and production. It takes place in regions with elevated salt and low precipitation [Neumann 1995]. Globally, around 20% of irrigated land and 2.1% of dry land suffers from salt crisis where NaCl is the predominant salt causing agent [Munns and Tester 2008]. Salt level of 4.5 dS m<sup>-1</sup> (50 mM) can cause osmotic effects and imbalance nutrient ions [Ali et al. 2001] that affect plant metabolism through repression of several enzymes [Greenway and Munns 1980]. By inducing osmotic imbalance, salt stress reduces plant ability to absorb water and dissolved nutrient [Niu et al. 1995]. Salt toxicity induces loss of chloroplast activity which leads to decreased photosynthetic rate [Apel and Hirt 2004]. Plant tolerant to abiotic stress

can be obtained using different agents in the culture media. Carrot is a glycophyte plant and it is considered sensitive [Berstain and Ayers 1953, Gibberd et al. 2002] or moderately sensitive to salinity [Maas and Hoffman 1977, Mangal et al. 1989]. Unlukara et al. [2011] found that electrical conductivity (EC) of 1.5 dS m<sup>-1</sup> declined carrot yield by 35% and 50% when EC reached 2.5 dS m<sup>-1</sup>. As studying of different growth stages under various salt stress conditions has special importance to agriculture management [Dizaji et al. 1998].

The overall hypothesis of our study was to seek the potential application of *Daucus carota* L. to saline soils. However, this will require a series of long period experiments with salted field soils and saline water be-

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fore its application. The objectives of this study were to determine the effect of salinity on germination and growth of two carrot cultivars.

## MATERIALS AND METHODS

**Establishment of *in vitro* seeds cultures.** Carrot (*Daucus carota* L., cvs Jordan and Napoli) seeds were washed with water flow in a flask containing a few drops of detergent for 20 minutes. Under the laminar air-flow cabinet, they were washed with 70% ethanol for 15 seconds, followed by 40% sodium hypochlorite for 20 minutes, and then washed with 70% alcohol for 1 minute. Seeds were rinsed three times with distilled water for 15 min. Sterilized seeds were then allowed to germinate on hormone free solid MS medium. The solution used for the study consisted of zero (control), 75, and 150 mM NaCl and added to the media. For each species 10 seeds for each of the three NaCl treatments were used. Seeds were allowed to germinate in laboratory conditions on MS medium in Petri dishes soaked in a solution of the respective salt concentration. The medium pH was adjusted to 5.8 using 0.1 N HCl or 0.1 N NaOH. Seven g L<sup>-1</sup> plant agar were added to solidify the media, then media was autoclaved for 20 minutes at 121°C and a pressure of 1.15 kg/cm. Inoculated Petri dishes were incubated in growth chamber at 24 ±2°C and 16 h light. Seed germination was investigated after every 2 days. It was started after 3 days (seeds were considered to be germinated with the emergence of the radical). Germinated seeds were recorded daily during 20 days and the appearance of 2 mm or more of radicle was considered as germination. The germinating seeds were counted at regular intervals. Seeds were left to germinate at 25 ±1°C with 8 h photoperiod under controlled environmental conditions in the laboratory of the Department of Horticulture and Agronomy at University of Wisconsin, Madison, WI. Treatments were assessed in factorial arrangement based on a completely randomized design (CRD) with five replications. Each replication includes one Petri dish (ten seeds per Petri dish). Means were separated according to the protected least significant difference (protected-LSD) at error rate of 0.05. Data were analyzed using SAS program (SAS Inc., Cary, NC, USA).

**Seed germination assay.** Petri plates were periodically checked and the number of seeds that sprouted

and germinated was counted daily up to 15 days. Fresh weight and length of seedlings shoot and root were measured and recorded at the time of harvest (15 days after treatment application). For gradual exposure treatments, seeds of both cultivars were transferred sequentially every five days to new NaCl concentrations of 0.0, 75 and 150 mM in 15 days. After final count, germination percentage and germination energy were calculated according to [Ellis and Reberts 1981, Ruan et al. 2002].

Microshoots physiological response to salt stress *in vitro* and in soil. Small microshoots that resulted from germination process were subcultured directly to different salt levels. In another treatment, microshoots were exposed gradually to NaCl salinity stress by subculturing microshoots on solid proliferation media free from NaCl (control). After two weeks, five flasks were maintained and other microshoots were subcultured using 75 mM NaCl. After another two weeks, five flasks were maintained at 75 mM and all others were subcultured using 150 mM NaCl. Fresh and dry shoot weight, shoot height, and root length, were recorded. After producing more shoots and leaves, carrot seedlings of the two cultivars were planted in 1.5 kg of soil in plastic pots and were kept at field capacity moisture. Salted water with 75 and 150 mM NaCl were supplied to the plants and control plant watered with 0 mM of NaCl for two months. The tolerance index was determined as 'FW on NaCl medium/FW on NaCl-free medium × 100'. Most apices of *L. esculentum* had not rooted after 32 days of culture. Relative water content (RWC%) and tolerance index were determined as fresh weight on salt medium/ fresh weight on control ×100 [Cano et al. 1998]. Treatments were analyzed using completely randomized design (CRD) with five replications (a flask or pot in each replicate).

**Determination of total chlorophyll, carbohydrate, and protein.** The total chlorophyll and carotenoid contents were determined according to Lichtenthaler [1988] using pure acetone as the extraction solvent. The absorbance at 662, 645 and 470 nm was measured immediately after extraction. Carbohydrates in the leaves were estimated using Sadasivam and Manikam methods [2005], Murugesan and Rajakumari [2006]. Total soluble protein content was determined according to Lowery et al. [1951] by spectrophotometer at wavelength of 750 nm.

## RESULTS

**Effect of NaCl *in vitro* germination and growth parameters.** Results showed that different levels of salinity have significant effect on carrot seed germination. Germination percentage at 75 mM treatment in comparison with 150 mM showed significant differences in both cultivars (Fig. 1). Data showed that seed germination percentage was higher in Napoli cultivar compared to Jordan cultivar when seeds exposed to direct sodium chloride. The highest germination percent was obtained by Napoli cultivar with 92% in control level and 68% in 150 mM NaCl, while Jordan cultivar with 68% in control level and 46% in 150 mM NaCl. Germination energy (GE%) which is defined as percent of seeds germinating over a given period of time was significantly reduced with an increased salt concentration (Fig. 2). With 75 mM salt, GE% was slightly reduced compared with 150 mM NaCl exposure. Similar to GE%, growth rate was reduced as the level of salinity was increased (Fig. 3). Significant reduction was recorded mainly at the higher level of salt concentration compared to control in both cultivars.

**Shoot fresh weight, shoot length, and shoot number.** Shoot fresh weight was affected by increased salinity levels (Fig. 4), the highest reduction rate was recorded at 150 mM of NaCl treatment for both cultivars. Seedling length of both radicle and plumule showed inverse relationship with salt concentration (Fig. 5). Seedling height reduction is a frequent phenomenon of many crop plants grown under salt stress. The most reduction in radicle length and plumule was related to 150 mM. Also, seedling radicle and plumule length decrease. Significant reduction was observed in shoot number at 150 mM NaCl compared to control seedlings which grew without salt (Fig. 6). The reduction in shoots production at 150 mM NaCl was 32% and 42%, for Jordan and Napoli cultivars, respectively. Salt tolerance, as depicted by tolerance index was decreased significantly with increased salinity level (Fig. 7). Microshoots showed significantly higher tolerance index when gradually salinized at 75 and 150 mM NaCl than when directly exposed to salt stress. Also, RWC % was generally reduced with elevated salt level (Fig. 8).

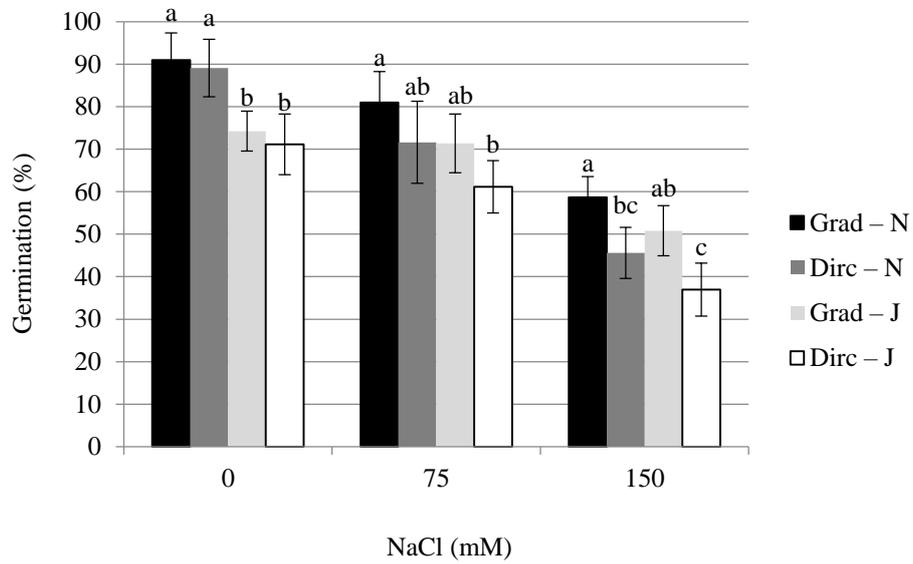
## Plant physiological responses to salt stress

**Leaf chlorophyll, carbohydrate, and protein content.** A statistically significant relationship between leaves chlorophyll content and stress treatments was observed (Fig. 9). Jordan cultivar showed the highest reduction in total chlorophyll concentration and was considered as salinity sensitive cultivar. While, Napoli cultivar showed the highest reduction in total chlorophyll concentration and was considered as salt tolerant. The influence of different salinity levels on carbohydrate content of carrot plant is shown in Figure 10. Leaf sugar concentration increased with increased NaCl level. Total carbohydrate content decreased at 0 and 75 mM NaCl and increased at 150 mM NaCl in Napoli cultivar, however, it increased in Jordan cultivar at 75 and 150 mM NaCl. It was observed that 150 mM NaCl induced a significant decrease in protein contents. NaCl significantly reduced microshoot content of crude protein at 75 and 100 mM NaCl in both cultivars (Fig. 11). The reduction in salinized microshoot crude protein content was less aggravated in the gradual salt shock compared to the direct salt shock treatment.

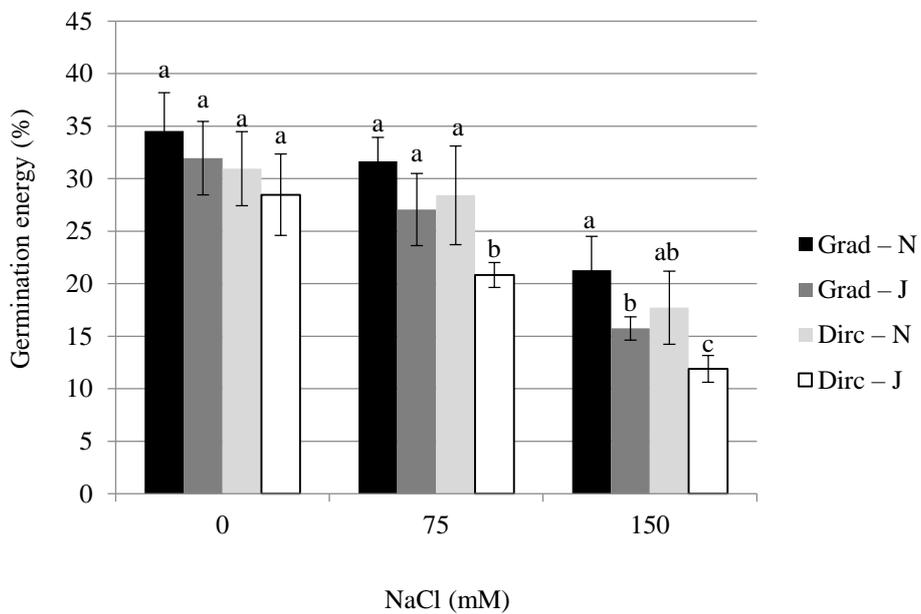
## DISCUSSION

Salinity stress has a significant effect on the plant growth and development [Munns and Tester 2008, Rasool et al. 2013]. It inhibits germination and growth of plants at early seedling and growth stages [Ferdose et al. 2009, Silva et al. 2014]. Salt tolerance, as depicted by tolerance index was decreased significantly with increased salinity level in both cultivars; this reduction was less aggravated in Napoli cultivar. Othman [2006] argued that the expected causes of the reduction in carrot yield, and its components could be the reduction of the cell contents, reduced tissues development and differentiation, membrane damage and disturbed avoidance mechanism.

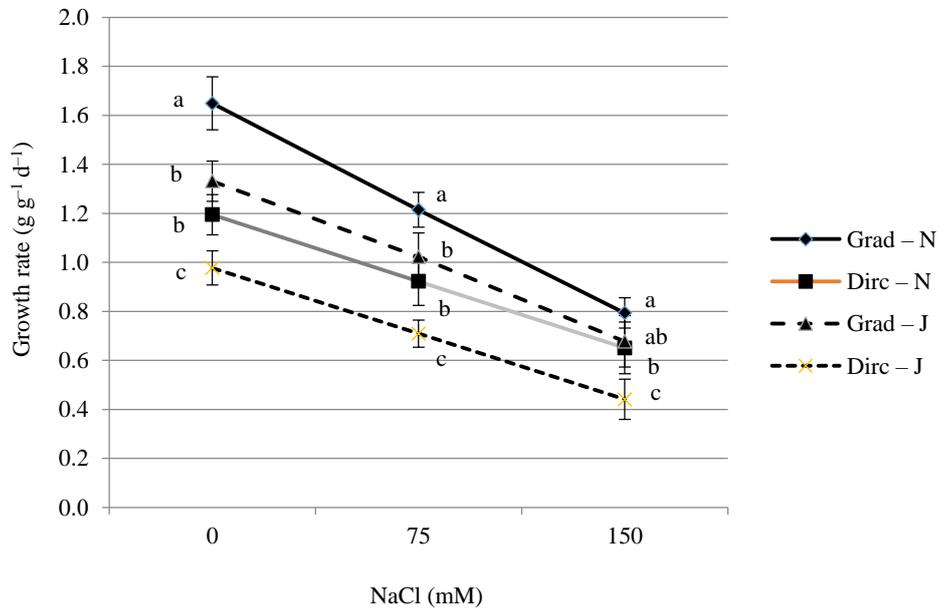
Different studies showed that the higher salinity levels had significant reduction on germination percentage in many plant species such as *Elymus junceus* [Askarian 2004], *Panicum miliaceum* [Alizadeh Banat et al. 2007] and Spanish cultivars [Turhan et al. 2011]. The effect of salinity on plant was expressed as reduced shoot biomass; therefore vegetative growth is the most widely used index in studies on salt tolerance.



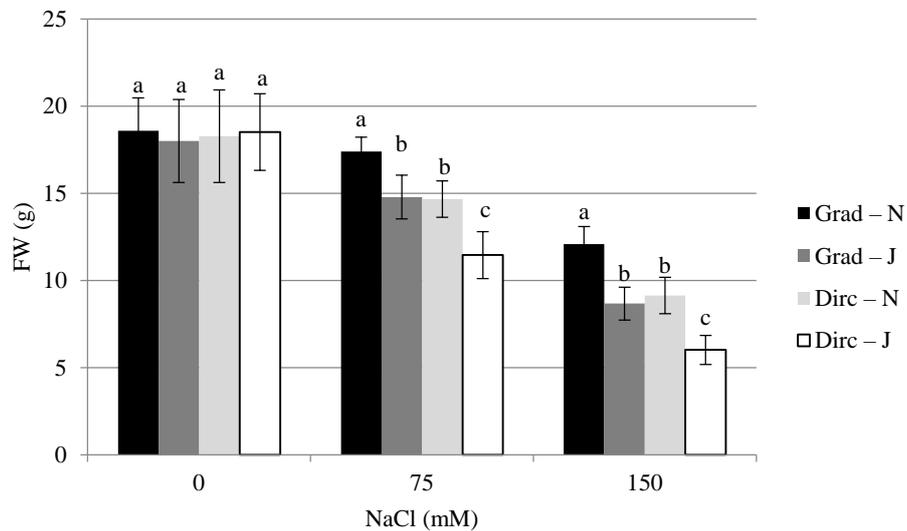
**Fig. 1.** Effect of different concentrations of (NaCl) on carrot germination percentage. Means followed by the same letter within each column are not significantly different at the 0.05 level, according to protected-LSD test



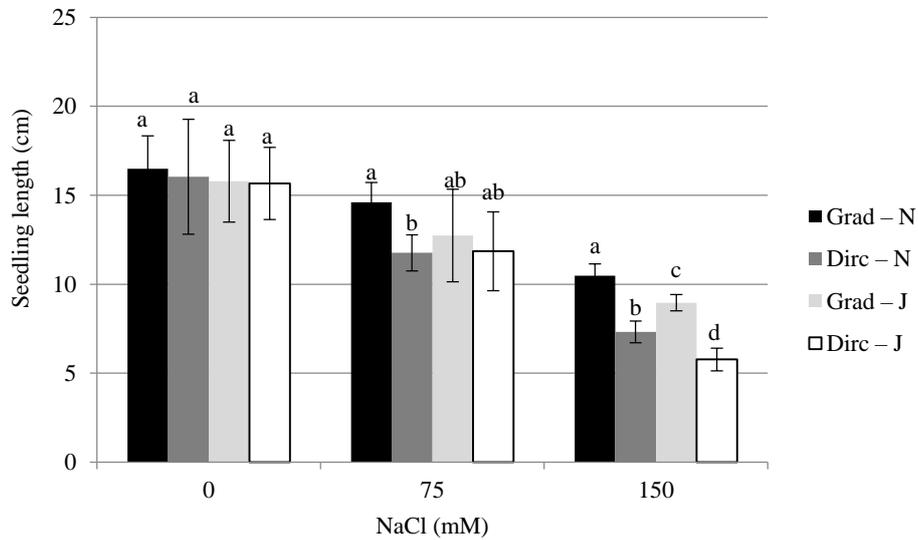
**Fig. 2.** Effect of different concentrations of (NaCl) on carrot germination energy percentage. Means followed by the same letter within each column are not significantly different at the 0.05 level, according to protected-LSD test



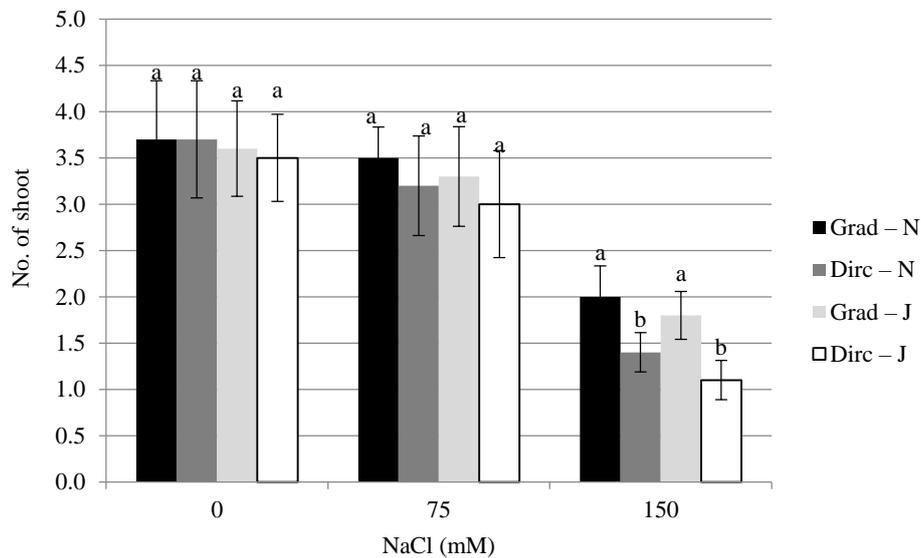
**Fig. 3.** Effect of different concentrations of (NaCl) on carrot growth rate. Means followed by the same letter within each column are not significantly different at the 0.05 level, according to protected-LSD test



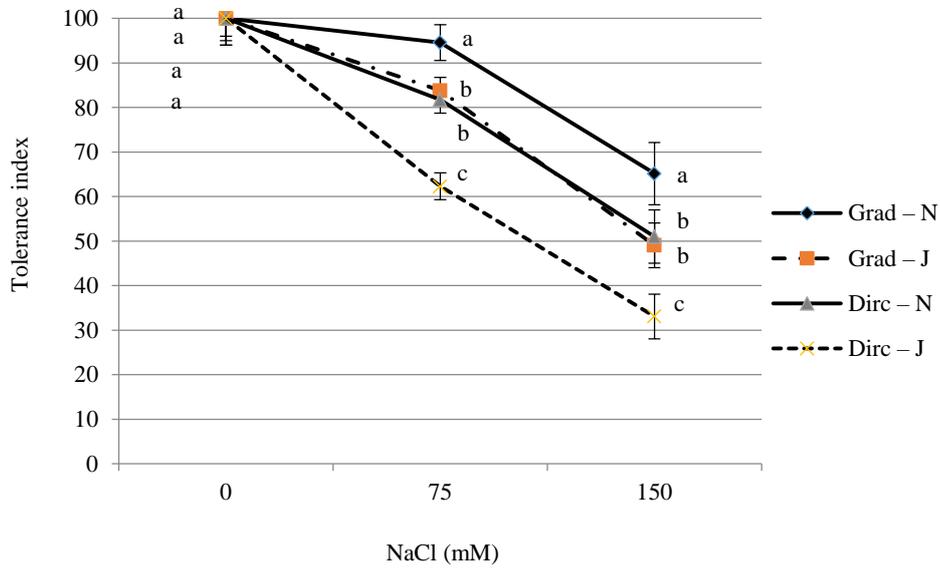
**Fig. 4.** Effect of different concentrations of NaCl on shoot fresh weight of *in vitro* carrot plantlets after 50 days in culture. Means followed by the same letter within each column are not significantly different at the 0.05 level, according to protected-LSD test



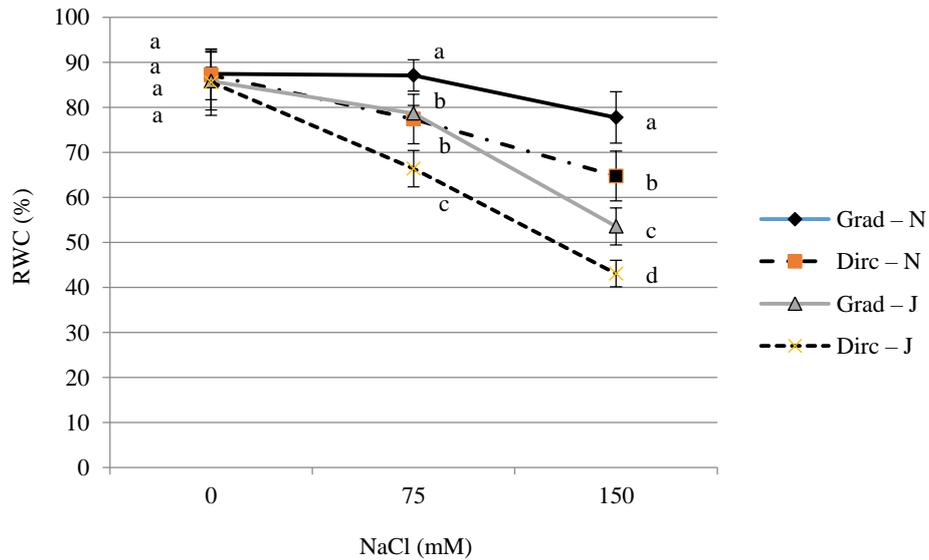
**Fig. 5.** Effect of different concentrations of NaCl on seedling length of *in vitro* carrot plantlets after 50 days in culture. Means followed by the same letter within each column are not significantly different at the 0.05 level, according to protected-LSD test



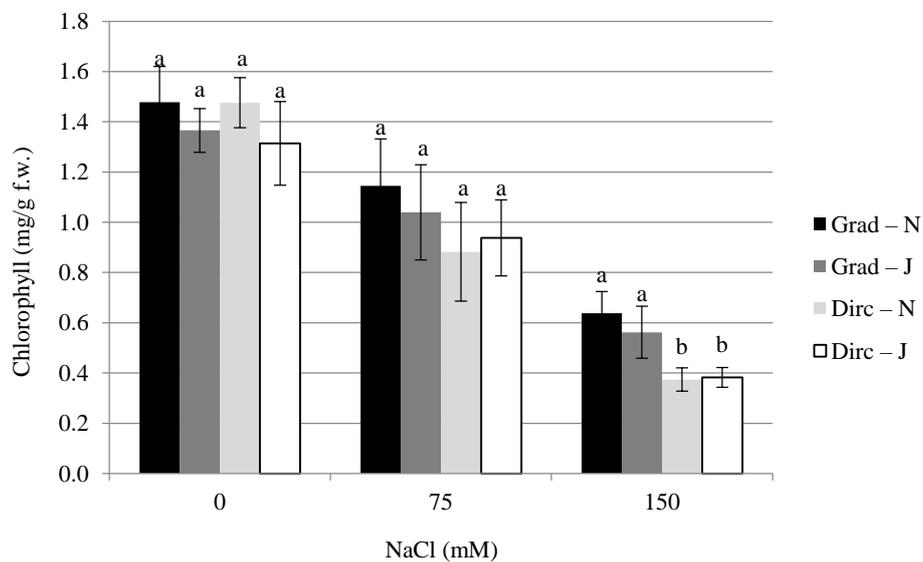
**Fig. 6.** Effect of different concentrations of NaCl on shoot number of *in vitro* carrot plantlets after 50 days in culture. Means followed by the same letter within each column are not significantly different at the 0.05 level, according to protected-LSD test



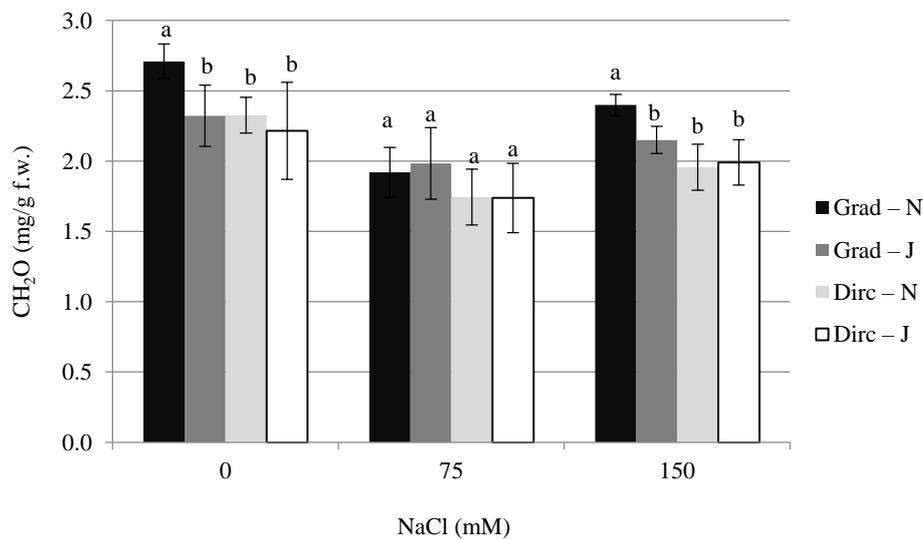
**Fig. 7.** Effect of different concentrations of NaCl on carrot tolerance index. Means followed by the same letter within each column are not significantly different at the 0.05 level, according to protected-LSD test



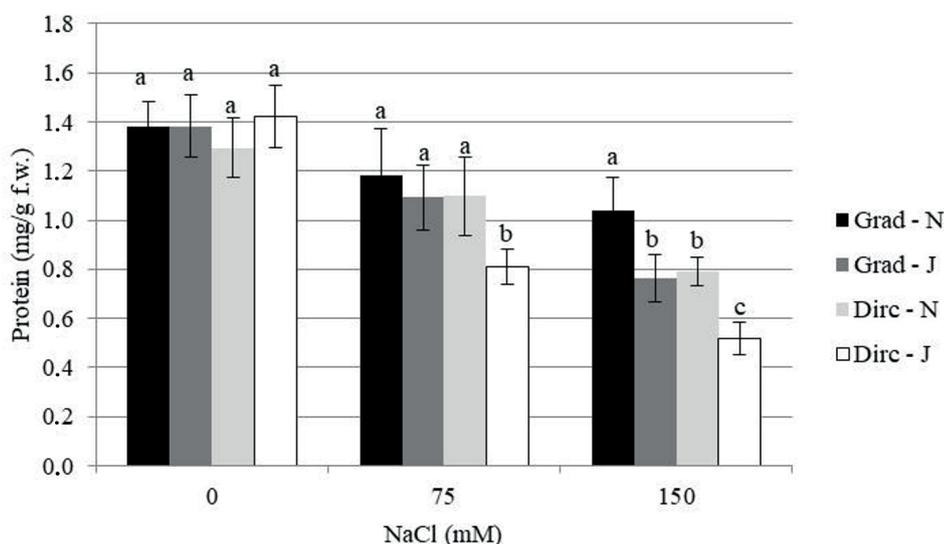
**Fig. 8.** Effect of different concentrations of NaCl on carrot relative water content. Means followed by the same letter within each column are not significantly different at the 0.05 level, according to protected-LSD test



**Fig. 9.** Effect of different concentrations of NaCl on carrot chlorophyll. Means followed by the same letter within each column are not significantly different at the 0.05 level, according to protected-LSD test



**Fig. 10.** Effect of different concentrations of NaCl on carrot carbohydrate. Means followed by the same letter within each column are not significantly different at the 0.05 level, according to protected-LSD test



**Fig. 11.** Effect of different concentrations of NaCl on carrot protein. Means followed by the same letter within each column are not significantly different at the 0.05 level, according to protected-LSD test

Cano et al. [1998] reported that the different degree of salt tolerance between *Lycopersicon esculentum* and *L. pennellii* was not clearly shown on the basis of shoot growth of plantlets. Indeed, Maas [1986] reported that root yield declined 14% for each increase unit of salinity beyond the threshold of 1.0 dS m<sup>-1</sup>. Matsubara and Tasaka [1988] noted a reduction of 50% of the fresh weight root of carrot with NaCl concentrations ranging from 68 to 102 mM, same Mangal et al. [1989] reported the same reduction with a salinity of 78 mM.

Chlorophyll content is one of the main cell components which are sensitive to salt stress [Ahmad et al. 1978, Hajar et al. 1993]. In this study, chlorophyll content in salt treated plants was much lesser than non-treated plants especially higher salt concentration. As a result of salt stress, chlorophyll content decreased significantly in both carrot cultivars. The reduction in chlorophyll content may be as a result of the increasing in the activity of chlorophylase [Rao 1981, Noreen and Ashraf 2009]. The decline in chlorophyll content under salt stress was also stated by Yasar et al. [2008] and Kusvuran [2010]. The increase in salinity level resulted in a significant decrease in leaf soluble protein. This agrees with other results obtained in cucumber seedling [Huan-Wen et al. 1999], rice leaf [Luttus et al.

1996], and cowpea leaf [Silveira et al. 2001]. Similarly, Abu-Khadijeh [2002] reported that the leaf soluble protein content was significantly reduced as salinity level increased in hydroponic and *in vitro* grown tomato.

Lower protein levels in salt stressed plant parts were an outcome of the decreased protein synthesis and increased activities of protein hydrolyzing enzymes [Pessaraki and Tucker 1988]. Reduction in crude protein content, in response to salinity, was also reported in rice leaf [Luttus et al. 1996], rice grain [Sultana et al. 1999] and in *Coleus blume* tissues [Gilbert et al. 1998]. The increase in total carbohydrate was reported recently in salt stressed wheat seedling grown in hydroponic culture [Kerepesi and Galiba 2002]. Carbohydrates are the energy source and carbons needed for adaptive and/or defensive reactions to various stresses [Todaka et al. 2000]. Noiraud et al. [2000] reported that carbohydrate accumulation under salt stress was the result of an increase of carbohydrate synthesis and a decrease in carbohydrate catabolism.

Although salinity tolerance is a complex trait, it can be improved through incorporation between molecular physiologists and geneticists [Munns et al. 2006]. Many researchers have explored the physiological and molecular mechanism of salinity, however, we

still far away from truly understanding the whole picture of such tolerance [Bartels and Sunkar 2005, Silva et al. 2014]. Our results indicate that Jordan and Napoli cultivars stayed healthy and unchanged in their nutritive properties up to 75 mM NaCl as this concentration caused a 29% and 23% reduction in Jordan and Napoli cultivars, respectively. These percentages are an economically acceptable reduction in semi arid areas where only low quality irrigation water is available. Doubling the salinity of irrigation water to 150 mM NaCl showed growth reduction of 69% in Jordan cultivar and 57% in Napoli cultivar which is considered economically unacceptable. The acclimatization through gradual stress treatment proved that adaptation to saline condition cause a readjustment in the activities of certain key metabolic enzymes.

Results indicated that growth of carrot microshoots were adversely affected by gradual and direct salinity shock. This reduction was less aggravated in the gradual shock treatment especially in Napoli cultivar which showed a better salinity tolerance compared to Jordan cultivar. Generally, Napoli cultivar showed better growth responses compared to Jordan cultivar under salinity stress, especially when exposed to gradual shock.

## CONCLUSIONS

1. Jordan and Napoli cultivars stayed healthy and unchanged in their nutritive properties up to 75 mM NaCl, this level of salinity is an economically acceptable reduction in semi arid areas where only low quality irrigation water is available. The 150 mM NaCl salinity treatment significantly reduced all growth parameters. The less impaired by the gradual exposure of seedling to salinity provides an opportunity to study the acquirement of salt tolerance.

2. By inducing osmotic imbalance, salt stress reduces the ability of both carrot cultivars to absorb water and dissolved nutrient. But, Napoli cultivar which showed a better salinity tolerance compared to Jordan cultivar, especially when exposed to gradual shock.

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