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*Research Paper*

# Effects of direct and gradual salinity exposure on carrot (*Daucus carota* L.) seeds and recovery response

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**ABSTRACT**

Salinity is a major cause of abiotic stress in arid and semi-arid climates that substantially reduces crop yield. This study evaluated the effects of salinity on germination and early seedling growth of two carrot cultivars *in vitro* under varying salinity levels. Salinity was induced by incorporating up to 150 mM of sodium chloride (NaCl) into the culture media. Seeds were either exposed directly to salinity, by planting on MS media containing salt, or gradually exposed by sequential transfer every four days to higher salt concentrations. Salinity caused significant reductions in germination parameters (germination percentage, speed, and energy) with elevated salinity level, but growth was less impaired by gradual exposure to salt. The gradual exposure of seedlings to salinity provides an opportunity to study the development of salt tolerance. The viability of seeds of glycophytes failing to germinate when exposed to salinity has not been previously reported. It was discovered that many carrot seeds that failed to germinate on saline media in this study recovered and grew when transferred to salt-free media.

**Key words:** Salinity, abiotic stress, carrot, germination parameters, seed recovery.

## INTRODUCTION

Abiotic stress leads to a series of morphological, physiological, biochemical, and molecular changes that adversely affect plant growth and productivity (Wang et al., 2001). Since agriculture is increasingly forced to utilize marginal waters to meet its demands, increased risks of soil salinization significantly reduce crop yield and quality, especially in the arid and semi-arid areas (Bolarin et al., 1993; Mohammad et al., 1998; Paranychianakis and Chartzoulakis, 2005), where annual precipitation is inadequate to leach excessive salts and prevent salt accumulation in the root zone (Bolarin et al., 1993). Saline soils have complex ionic profiles and concentrations of dissolved salts (Volkmar et al., 1998) but NaCl is often the predominant salt causing salinization, and plants have mechanisms to regulate its accumulation (Munns, 2002; Munns et al., 2006). Germination and seedling characteristics are among the most 49 robust criteria used for evaluating salt tolerance in plants (Boubaker, 1996). Salinity either creates osmotic pressure that prevents water uptake or induces toxic effects.

While salinity effects have been studied in several crop plants, an improved understanding of the responses of horticultural crop species to salinity is needed to aid in the development of more tolerant cultivars and improved management practices.

Carrot (*Daucus carota* L.) is a glycophyte considered sensitive (Gibberd et al., 2002) or moderately sensitive (Maas and Hoffman, 1977; Matsubara and Tasaka, 1988; Mangal et al., 1989) to salinity. When the electrical conductivity (CE) was 1.5 dS/m, carrot yield declines of

35% were observed and can reach 50% reduction with 2.5 dS/m (Unlukara et al., 2011). Mark et al. (2002) reported that at salinities above 20 mM, carrot growth was reduced by 7% of each 10 mM increment in salinity.

Schmidhalter and Oertli (1991) concluded that germination and seedling growth are affected by both drought stress and salt stress. Kahouli et al. (2014a, b) reported that carrot germination percentage and speed were reduced with increasing NaCl concentrations up to 16g/L (~274 mM), which suppresses all germination, while yield reductions in the field up to 70% were noted when irrigating with water containing 3g/L NaCl.

Most studies evaluating the effects of salinity on plant growth apply the same concentration of salts throughout experiments. Several studies, however, have also evaluated the effects of increasing salinity during growth. For example, the ability of wheat plants to grow under salinity stress was found to increase when the plants were subjected to a gradual increase in NaCl concentration in the growth medium (Nasser et al., 1980). Bolarín et al. (1993) reported germination, growth, and ion concentration in tomato after varying pre- and post-emergence salt treatments. When salt was applied pre-emergence, salt tolerance increased with plant age, whereas when applied post-emergence, 45-day-old plants were the most salt tolerant.

Salinity not only reduces the growth but also reduces percentage of seeds that germinate in both glycophtes and halophytes. The ability of seeds to maintain viability and recovery after an extended period of exposure to salinity has been investigated in several halophytes (Naidoo and Naicker, 1992; Keiffer and Ungar,1995; Khan and Ungar, 1997; Rubio-Casal et al., 2003; Song et al., 2006) but recovery has not been reported for glycophytes. Since no studies have focused on the effect of gradual salinity exposure on carrot growth, or the recovery responses of ungerminated carrot seed, these phenomena were evaluated in this study.

## MATERIALS AND METHODS

### Preparation and treatment of seeds

Carrot (*Daucus carota* L.) cv 'Jordan' and 'Napoli' from a seed market in Amman, Jordan and from Bejo seeds, respectively, were evaluated. Seeds were rinsed with water, surface sterilized with 20% sodium hypochlorite for 15 min, and followed by a 75% ethanol wash for 3 min. Seeds were then rinsed with sterile distilled water three times (15 min each). For studies examining direct exposure to salt, sterilized seeds were germinated on hormone free solid (Murashige and Skoog, 1961) medium containing varying NaCl concentrations (0.0, 50, 100, 150 mM). After sterilization, seeds were transferred into sterile Petri dishes (100 mm). Seeds of both cultivars were allowed to germinate in laboratory conditions at 25 ± 1°C with 8 h photoperiod. Treatments were assessed as a factorial experiment with a completely randomized design at 5 replications. Each replication included one Petri dish with ten seeds. Data were subjected to the analysis of variance (ANOVA) (SAS Inc., Cary, NC, USA). Mean LSDs were considered significant at the 0.05 level of probability.

### Seed germination assay

Petri plates were checked every 2 days and the number of seeds that germinated was recorded up to 16 days. Seedling length and number of leaves (including cotyledons) were measured and recorded at the time of harvest (16 days). Germination percentage, speed of germination, and germination energy were calculated (Ellis and Roberts, 1981; Ruan et al., 2002). Vigor index was calculated according to Vashisth and Nagarajan, (2010). The relative water content (RWC) was estimated using the following equation:

$$RWC\ (\%) = [(W-DW) / (TW-DW)] \times 100,$$

Where, W: Sample fresh weight, TW: Sample turgid weight, DW: Sample dry weight.

### Gradual sodium chloride exposure

For gradual exposure treatments, seeds of both cultivars were planted in medium with no NaCl added and transferred sequentially every four days to increasing NaCl concentrations of 50 mM after four days, 100 mM after eight days, and 150 mM after 12 days, were evaluated.

### Germination recovery

To evaluate the ability for plants to recover from salinity effects, all ungerminated seeds which were directly exposed to 150 mM of NaCl for 2, 4, 6, 8, 10, 12, and 14 days were transferred to salt free medium up to 16 additional days.

## RESULTS

### Effect of NaCl on *in vitro* germination parameters

***Total germination percentage***

Salinity effects on germination of carrot seed were significant and germination was reduced by both direct and gradual exposure to salt, but higher germination rates were observed with gradual exposure. Cultivar effects were not significant but all germination parameters were somewhat higher in 'Napoli', a European hybrid, than in 'Jordan', a Jordanian open-pollinated cultivar. Intra-

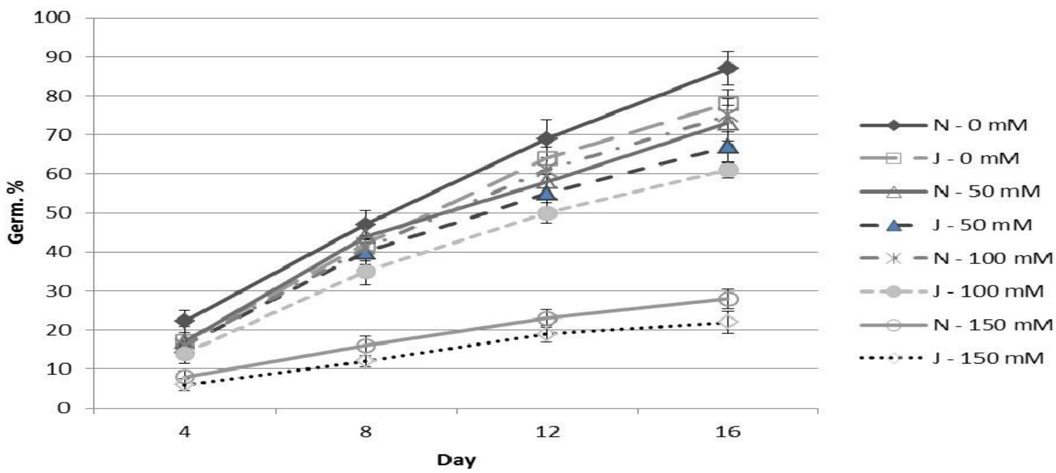


**Figure 1.** Germination percentage (+SD) of 'Jordan' (J) and 'Napoli' (N) carrot seeds exposed to up to 150mM NaCl for 16 days.

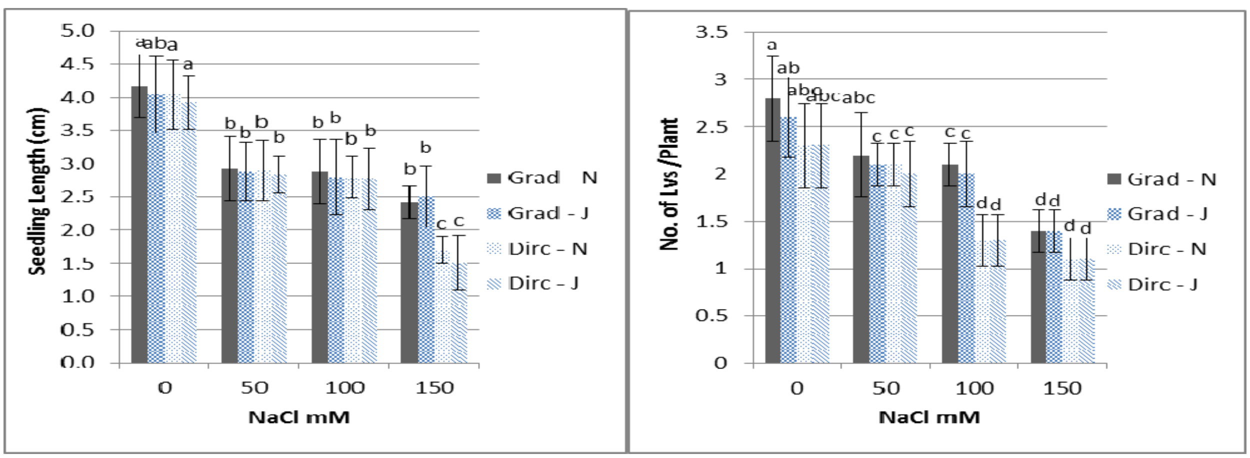


**Figure 2.** Seedling length (A) and number of leaves (B) of 'Jordan' (J) and 'Napoli' (N) carrots after 16 days of direct (Dirc) and gradual (Grad) exposure to NaCl. Vertical bars represent ±SD. Values with the same letter are not significantly different at 5% level.

cultivar variation was similar. Germination response at 50 mM was not significantly different from the control (0 mM), but 100 mM and 150 mM treatments with both direct and gradual exposure to salt were significant (Figure 1).

## Recovery of germination capacity in seeds failing to germinate in saline conditions

Reduction of seedling length is a common phenomenon of several crop plants grown under saline conditions. Radicle and plumule length were reduced with higher salinity for both carrot cultivars, but reduced less in 'Napoli' (Figure 2). Relative to growth in salt-free medium, seedling length was reduced 64% with direct exposure to 150 mM NaCl, and 39% with gradual exposure also, the number of leaves reduced by 56 and 46%, respectively (Figure 2). At the end of the experimental period, leaves of seedlings grown in 150 mM NaCl had a fine layer of small white crystals on their surface. Compared to the control, the relative water content (RWC) in carrot shoots was reduced by 28-30% in direct exposure to 150 mM and 22-24% with exposure (Figure 3).

## DISCUSSION

In halophytes, as in glycophytes, salinity reduces germination capacity and the initiation process of germination (Bayuelo-Jiménez et al., 2002). However, responses are variable and specific for each species (Ungar, 1991). Indeed glycophytes, which includes most species of agronomic interest, have diminished growth in the presence of excessive soil salinity, generally above 100 meq .l -1 NaCl (Marouf and Reynaud, 2007). This is in contrast to halophytes where Naidoo and Naicker (1992) reported that germination was not significantly reduced at

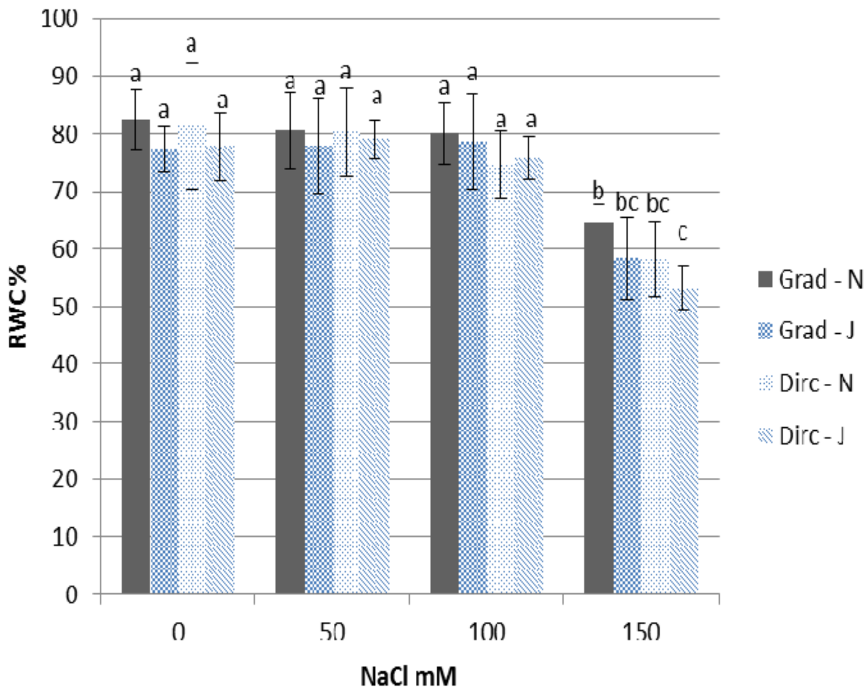


**Figure 3.** Relative water content (RWC) of 'Jordan' (J) and 'Napoli' (N) carrots after 16 days of direct (Dirc) and gradual (Grad) exposure to NaCl. Vertical bars represent ±SD. Values with the same letter are not significantly different at 5% level.

salinities below 250 mM NaCl, with some germination observed in salinity up to 500 nM. In agreement with these general observations for other glycophytes, and previous observations on carrot, the behavior of carrot seed germination at 150 mM NaCl was affected in all germination parameters (G%, GS, GE, VI) and seedling length was reduced. This agrees with earlier studies by Maas (1986) who observed that the root yield declines 14% for every unit increase in salinity (ECe) beyond the threshold of  1.0 dS m -1, and Matsubara and Tasaka (1988) who noted a reduction of 50% of the fresh weight root of carrot with NaCl concentrations ranging from 68 to 102 mM. Mangal et al., (1989) reported a similar reduction with a salinity of 78 mM, while Kahouli et al. (2014b), who demonstrated reductions in germination with rising salt up to 16g/L at which point no germination was observed. Fresh weight of carrot was found to reduce with increasing salinity (Unlukara et al. (2011). Schmidhalter and Oertli (1991) concluded that germination and seedling growth are affected by osmotic stress in carrot.

In recent studies, Kahouli et al. (2014a) demonstrated that salt stress reduced the parameters of growth of ten accessions of carrot. Accession L10 in their studies was the most sensitive with a reduction of 85% haulm dry weight and 89% root dry weight with irrigation water containing 3 g/l NaCl. These results are similar to those obtained by Rode and Nothnagel (2012) on carrot, Kaya and Higgs (2002) on cucumber, Bernstein and Ayers (1953) on onion and Graifenberg et al. (1996) on fennel.

Germination percentage, germination speed, germination energy, and vigor index were reduced in carrot by salinity. Salinity is known to retard or prevent the uptake of water, and to result in a toxic accumulation of ions which damage the embryo and growing plants. Therefore, restriction of water absorption reduces seed germination, growth and development. Maas and Hoffman (1977) suggested that the relationship between salinity and yield might be expressed as a negative linear response function at salinities above a critical threshold, and set the threshold for carrot at 1 mmho $cm^{-1}$ (approx. 10 mM NaCl). Indeed, salinity reduced growth and productivity when it reached a toxic level of 150 mM, as also reported by Chartzoulakis and Klapaki (2000). Similar results were obtained for both halophytes, *Salvia fruticosa* (Khan and Ungar, 1998), and *Suaeda moquinii* (Khan et al., 2001)), grown in high salinity (1000 mM), and the glycophyte wheat (El-Bassiouny and Bekheta, 2005) grown in the presence of salt (14 dS/cm).

Gradual exposure to salinity delayed carrot seed germination as reported for wheat (Nasser et al., 1980). It also impairs physiological process, enzyme activity, and related phenomena to a lesser extent than when plants are directly exposed to salt stress (Naseem et al., 2005; Morais et al., 2012). The less severe responses of carrot seedlings gradually exposed to salt may suggest an opportunity to study an adaptive capacity of carrot to the debilitating influences of salinity. Therefore, gradual exposure may be more effective than direct exposure to study physiological characteristic of plant tolerance imposed by salinity stress, or may reflect a different physiological response than direct exposure.

Interestingly, a recovery of germination was observed with seeds transferred to salt-free medium. The recovery of carrot seed germination in seeds exposed to high salinity (150 mM), followed by a transferred of seeds to salt free medium has not been previously reported for glycophytes. However, several studies reported germination recovery in halophytes (Rubio-Casal et al., 2003; Song et al., 2006). For example, Khan and Ungar (1997) observed recovery of seed germination in desert shrubs (*Haloxylon recurvum* and *Suaeda fruticosa*), and the herbs (*Zygophyllum simplex* and *Triglochin maritima*). Many seeds that failed to germinate on saline media recovered and grew when transferred to salt free media, as we observed in carrot.

The recovery of germination potential in carrot seed exposed to salt suggests that the exposure of this glycophyte to salinity is reversible and not destructive. Neither of these observations on gradual exposure or recovery from salinity effects suggests measures to alleviate salinity effects in carrot production, but may provide insights into new approaches to study the responses of plants to salinity.

## Conclusion

With direct and gradual NaCl stress, increased salinity led to reduced seed germination and plant growth for two carrot cultivars. Gradual exposure may provide an opportunity to study the development of physiological responses of plants to salt stress. Seeds exposed to 150 mM NaCl resulted in significant reduction in germination, but ungerminated seeds transferred to salt free medium were still able to germinate and grew normally.

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