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Effect of saline water on germination and early growth stage of five *Apiaceae* species

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Improving crop tolerance to salinity stress is a major challenge in many regions of the world towards sustaining the global food security. In this study, an interspecific difference in salinity tolerance was examined among five *Apiaceae* species: Caraway, celery, dill, fennel and parsley. Saline water was used at concentrations of 0, 1000, 2000 and 3000 ppm for 15 days from seed sowing (germination experiment) or for 30 days after one month of germination (early growth stage experiment). Germination rate, seedling length and leaf water content were decreased significantly with increase in salinity levels. These were associated with significant increases in Na^+ content and significant decreases in K^+/Na^+ ratio. Ion leakage showed no significant changes. Germination rate and seedling length are associated greatly with relative water content and less Na^+ content. These results suggest that inhibition in germination and early growth stage are associated mainly with osmotic stress than ion toxicity. Mostly, proline content increased significantly under salinity stress conditions. There were significant differences among species in response to salinity stress and Celery species was the most sensitive species to salinity stress compared to others.

Key words: Caraway, celery, dill, fennel, parsley, Na^+, K^+, osmotic stress.

INTRODUCTION

Salinity is one of the major abiotic stress limiting the growth and productivity of plants (Rasool et al., 2013a). It is estimated that about one-third of the irrigated land on earth suffers from salinity (Taiz and Zeiger, 2002; FAO 2011). Given the amount by which food production is likely to be lost as a result of such stress, increased salinity stress tolerance of crops will be an important aspect of plant breeding in the future towards sustaining global food production in many regions of the world, especially in the arid and semi-arid regions (Flowers, 2004). Salinity tolerance is a complex trait that can be improved through integration between molecular geneticists and physiologists (Munns et al., 2006). Enormous amount of researches have explored the molecular and physiological mechanism of salinity stress, however, we are still far from understanding the key traits that confer such tolerance (Bartels and Sunkar, 2005; Silva et al., 2014).

Salinity stress is associated mainly with two types of stresses: osmotic stress and/or ion toxicity (Eisa et al., 2012). Osmotic stress is caused by low water potential in saline soils which inhibit water absorption into plant tissues (Munns, 2005; Koyro et al., 2011). This is associated with stomata closure to avoid water loss, which in turn lowered the photosynthetic rate and CO_2 availability (Huchzermeyer and Koyro, 2005; Flexas et al., 2007). Ion toxicity occurs when salt accumulates to toxic concentration in leaves. Accumulation of Na^+ ion in plant tissues at excessive levels is one of the major factors causing salinity damage (Flowers and Hajibagheri, 2001; Mitsuya et al., 2003a,b). Fortunately, the plant can develop different mechanisms to prevent

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excessive accumulation of toxic ions in their cytoplasm. It was found that Na^+ exclusion ability is significantly correlated with salinity resistance in plants (Munns and James, 2003; Ferdose et al., 2009). Salinity resistance also varies with the growth stage.

Previous studies have revealed that rice plants are highly sensitive to salinity especially at young seedling stages and less so at developed stages (Yeo et al., 1990; Lutts et al., 1995; Shannon, 1998). Comparison of salinity tolerance in different growth stages may present clues to the mechanism of tolerance. However, the causes of differences in salinity tolerance with the growth stage is not well understood yet (Ferdose et al., 2009).

The *Apiaceae* (*Umbelliferae*) is a large family of mostly aromatic plants with 3000-3750 species spread across 300-455 genera (Constance, 1971; Pimenov and Leonov, 1993). This family includes some familiar vegetables, flavorings, or garnishes such as angelica, anise, caraway, carrot, celery, chervil, coriander, cumin, dill, fennel, lovage, parsley, and parsnip. This study was conducted to find the differences in salinity tolerance among five *Apiaceae* species at germination stage and at early growth stage. The inhibitions of germination rate and growth, water status and ion accumulation were examined to obtain insight to salinity tolerance mechanisms.

MATERIALS AND METHODS

Plant materials

Five species belonging to the Family *Apiaceae* (*Umbelliferae*) were used, which includes: Caraway (*Carum carvi*), Celery (*Apium graveolens*), Dill (*Anethum graveolens*), Fennel (*Foeniculum vulgare*) and Parsley (*Petroselinum crispum*). The experiments had been done in a greenhouse starting from the end of November 2012 at the Agricultural Experimental Farm, South Valley University, Qena, Egypt.

Germination rate experiment

Thirty seeds were germinated in 10-cm plastic pots filled with peat moss. Five replicates (pots) per treatment were used. The seeds were irrigated with saline water of 0, 1000, 2000 or 3000 ppm. The germinated seeds were counted after two weeks to calculate the germination rate (GR) as the following $GR(\%) = \frac{g}{30} \times 100$, where *g* is the number of germinated seeds and 30 is the total number of seeds.

Early growth stage experiment

Seeds were sown in 10-cm plastic pots filled with clay and sand (1:1). Water was supplied daily to avoid drought stress. One month after germination, the plants were exposed to the salinity stress treatments for 30 days. The salinity concentrations in the irrigation water were 0, 1000, 2000 and 3000 ppm. Thirty days after treatment, seedling length, water status and cell membrane stability were measured. Then, the plants were harvested and dried at 80°C for 2 h. The samples were grinded and kept for chemical analysis.

The seedling length was measured using a ruler. The relative values were used to avoid the differences among species under control. The relative value was calculated by dividing the value on the highest value among treatments. Water status of the seedlings was determined by measuring relative water content (RWC) according to Loutfy et al. (2012).

$$RWC(\%) = \frac{FW - DW}{TW - DW} \times 100$$

Where FW is the fresh weight, DW is the dried weight and TW is the turgid weight of tissue after being soaked in water for 12 h at room temperature.

Cell membrane stability was measured by ion leakage (IL) from seedlings using the method described by Soliman et al. (2011). The sampled seedlings were cut into discs 2 mm in diameter. The discs were rinsed three times with distilled water and 10-15 discs were put in a test tube containing 6 ml distilled water. The test tubes were agitated on a shaker for about 1h and conductivity (C1) of the solution was measured with a conductivity meter (Cyberscan100; Iuchi, Tokyo, Japan). Seedling discs then were heated in an oven at 70 to 80°C for 1h, and the conductivity of the solution containing the dead tissue (C2) was measured after the tubes had cooled down to room temperature and had been agitated on a shaker for 1h. The relative ion leakage was calculated as (C1/C2) × 100.

Chemical analysis

Samples (0.2 g) were digested with 10 ml sulfuric acid (H_2SO_4) at 200°C for 2 h. After cooling, 5 ml 30% hydrogen peroxide (H_2O_2) was added and heated at 200°C for another 2 h. The digested samples were completed to 50 ml using distilled water. Na^+ and K^+ contents were measured using Flame spectrophotometer (Kalra, 1998).

Free proline was determined according to Bates et al. (1973). Briefly, samples (100 mg) were homogenized in 10 ml of 3% aqueous sulfosalicylic acid for 10 m, followed by filtration. Two millilitres of the filtrate were mixed with 2 ml of glacial acetic acid and 2 ml of acid ninhydrin and then warmed in water bath for 1h at 90°C. After cooling, the developed colour was extracted in 4 ml toluene and measured by photometric method using T80 UV-VIS Spectrophotometer at 520 nm against toluene. A standard curve with proline was used for the final calculations.

Statistical analysis

The statistical difference among salinity treatments was tested by analysis of variance (ANOVA) for each species. The analysis was carried out using JMP (version 4; SAS Institute, Cary, NC, USA). The experiment was set up in a randomized block layout incorporating five replications (pots).

RESULTS

One-way ANOVA showed significant decreases in germination rate (GR), seedling length and relative water content (RWC) under salinity stress conditions (Table 1). On the contrary, cell membrane stability as expressed by ion leakage (IL) showed no significant changes under stress. Germination rate showed no significant difference

Table 1. Effect (*F* value) of salinity stress on germination rate (GR), Seedling length, relative water content (RWC) and Ion leakage (IL) of different species.

Species	GR (%)	Length (cm)	RWC (%)	IL (%)
Caraway	9.80***	10.43***	10.20***	1.30
Celery	13.32***	23.64***	5.68**	2.13
Dill	1.08	34.24***	5.99**	5.05*
Fennel	1.73	19.85***	2.46	3.11
Parsley	6.48**	7.31**	9.23***	2.19

*, **, and *** represent significance at probability levels of 5, 1, and 0.1%, respectively.

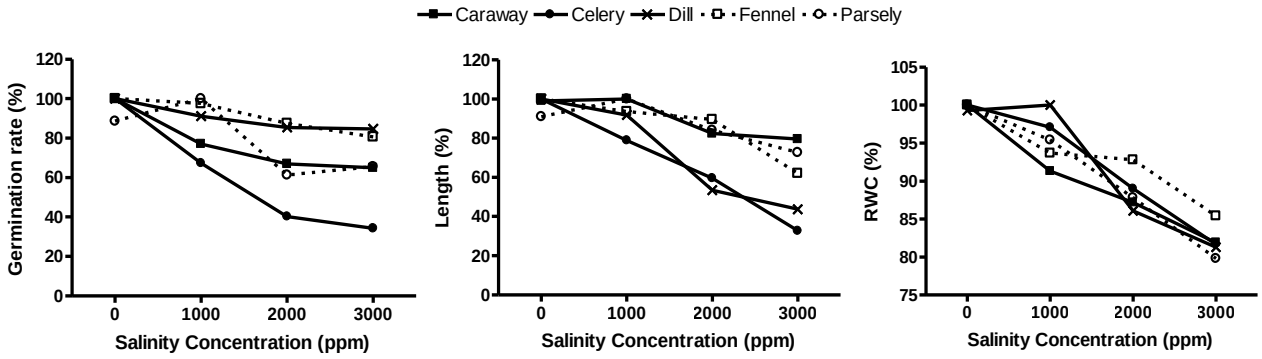

Figure 1. The relative values of germination rate (%), seedlings length and relative water content under different salinity stress treatments of different species.

Table 2. Effect (*F* value) of salinity stress on sodium (Na^+), potassium (K^+), K^+/Na^+ ratio and praline content (mg/g^{-1} DW) of different species.

Species	Na^+	K^+	K^+/Na^+	Proline
Caraway	69.5***	613.3***	185.3***	283.4***
Celery	38.6**	67.6***	4.07	79.3***
Dill	15.5*	361.2***	189.9***	143.5***
Fennel	31.6**	2.3	18.8**	106.7***
Parsley	49.5**	25.7**	23.92**	52.9**

*, **, and *** represent significance at probability levels of 5, 1, and 0.1%, respectively.

under control condition, while the differences appeared clearly under salinity stress conditions.

On the other hand, significant differences were shown among species in regard of seedling length even under control conditions as a result of different genetic background and growth behavior. The species showed clear differences in their response to stress especially in the high level of salinity (3000 ppm) in regard of GR, relative seedling length and RWC (Figure 1). There was no significant difference among species in regard of IL. Table 2 showed that salinity stress had significant effects on sodium (Na^+), potassium (K^+), K^+/Na^+ ratio and proline content. The concentration of Na^+ increased significantly in all species under stress conditions. However, the species showed different responses regarding to K^+ concentration. The species showed significant decreases in K^+/Na^+ ratio under salinity stress except for Celery (Figure 2). Significant differences were shown among species under control and salinity stress conditions. Proline content differed significantly among species under control and salinity stress conditions (Figure 3). The highest value of proline was shown at 3000 ppm treatment for all species except Fennel which showed highest value of proline at 2000 ppm, followed by significant decrease at 3000 ppm.

DISCUSSION

Salinity stress has a critical impact on the growth and

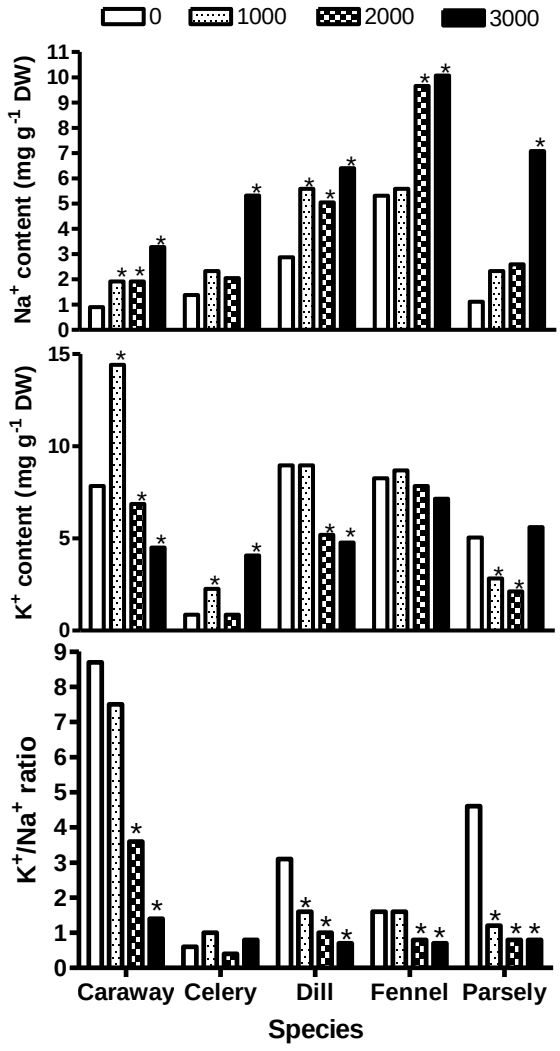

Figure 2. Changes in sodium (Na^+), potassium (K^+) and K^+/Na^+ ratio under salinity stress treatments of different species.

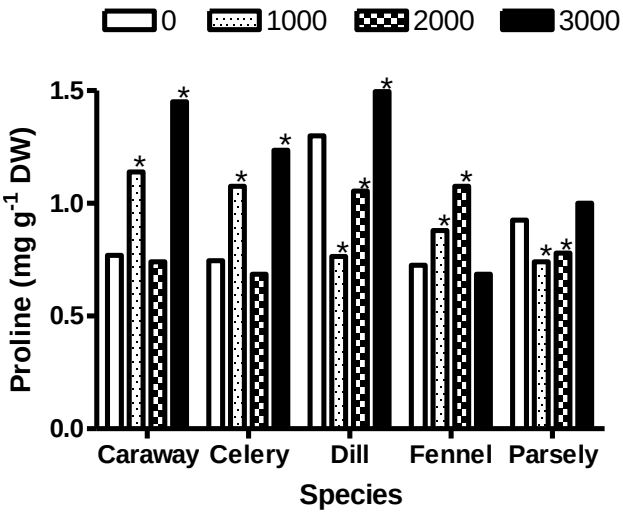

Figure 3. Changes in Proline content under salinity stress treatments of different species.

productivity of plants (Flowers, 2004; Munns and Tester, 2008; Rasool et al., 2013a). Salinity stress inhibits the growth of plants at early seedling and developed seedling stages (Ferdose et al., 2009; Silva et al., 2014). In this study, the impact of saline irrigation water on germination and early growth stages were studied. Five species belonging to the family *Apiaceae* (*Umbelliferae*) were used. The saline water had negative impacts on germination rate and growth measured by seedling length (Figure 1). There were significant differences among species responding to stress. The decreases in GR under stress on the control group, were 25, 35 and 65% for Parsley, Caraway and Celery, respectively.

The decreases in GR for Fennel and Dill were not significant. Consequently, the decreases in leaf length under stress relative to control were varied within a range of 20~67%. Celery showed the highest decreasing rate, while Caraway showed the lowest decreasing rate. These results show the differences among species in response to saline water stress.

The tolerance of plants to unfavorable environmental conditions is a complex trait which is associated with different mechanisms. In earlier studies, it was suggested that the functional damage due to high temperature is caused mainly by oxidative stress (Rasool et al., 2013b) which caused severe damage to cell membrane stability (Radi et al., 2013). On the other hand, salinity stress is associated mainly with two mechanisms: osmotic stress and ions toxicity. The high Na+ concentration in saline water lowers the water potential in the rooting zone which in turn decreases water permeability into plant tissue (Taiz and Zeiger, 2002; Munns, 2005; Koyro et al., 2011).

In this study, the association of damage caused by salinity stress with oxidative stress, osmotic stress and ion toxicity was examined. The cell membrane stability expressed by ion leakage (IL) was used as an indicator of oxidative stress. Osmotic stress was measured by relative water content (RWC) which is commonly used as an indicator of water status. Also, the accumulation of Na^+ ion was measured to examine ion toxicity. In this study, IL did not show significant changes under stress conditions compared to control. ANOVA analysis shown in Table 3, suggests that the declines in GR and seedling length were greatly associated with RWC and less associated with relative Na^+ content.

RWC associated positively with GR and seedling length, while relative Na^+ content associated negatively with both (Figure 4). Also, significant correlation was observed between RWC and relative Na^+ content (Figure 5).

Table 3. Effect (*F*-value) of species as well as relative water content (RWC) and relative Na+ on germination rate (GR) and relative seedling length (r.Length), with overall coefficient of determination (R^2).

Parameter	d.f.	GR %	r. length
F-value			
Species	4	4.5*	5.2**
RWC	1	23.1***	52.9***
R^2 (%)		75.0	83.3
Species	4	2.4	1.9
Relative Na^+		1	8.1*
R^2 (%)		58.2	49.9

*, ** and *** P < 0.05, 0.01 and 0.001, respectively.

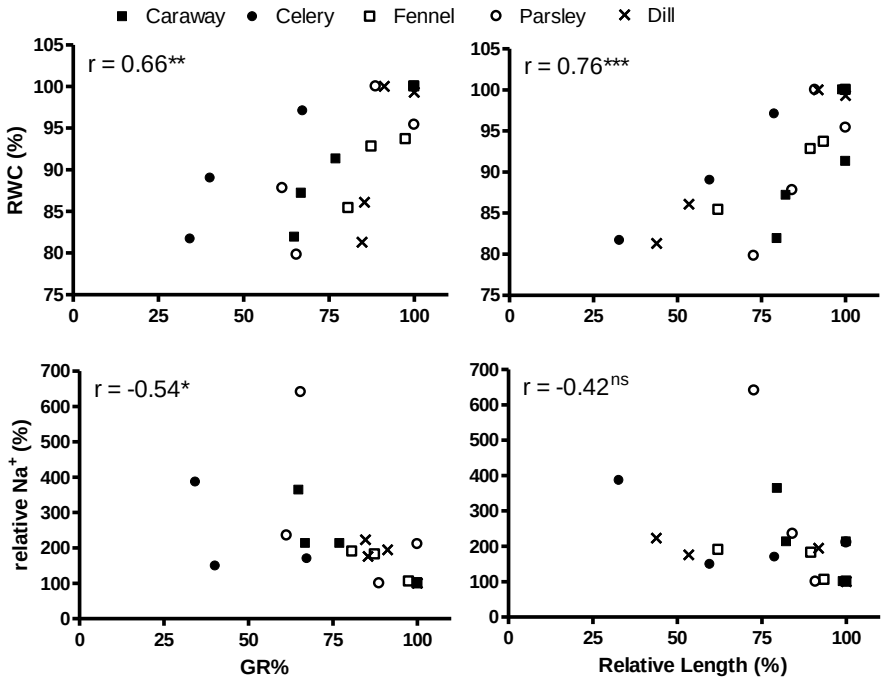

Figure 4. Correlation of germination rate (GR%) and seedling length with relative water content (RWC) and relative Na^+ content.

These results suggest that salinity stress at germination and early growth stages is associated with osmotic stress and ion toxicity rather than oxidative stress. Osmotic stress had greater impact on germination rate and seedling growth than that of ion toxicity which had indirect effect on plant by affecting the water potential between the soil and the leaves. This is consistent with Gholamin and Khayatnezhad (2011) who concluded that the adverse effect on germination and early seedling growth under salinity stress was due to the osmotic effect than the salt toxicity.

High tolerance to salinity stress is associated with the maintenance of higher K^+, lower Na^+ and higher K^+/Na^+ ratio in the cytoplasm of mesophyll cells (James et al., 2006). The abnormal ratio of K^+/Na^+ and high concentration of total salts inhibit enzymes activity and protein synthesis (Taiz and Zeiger, 2002). Concentration of Na^+ ions at 3000 ppm treatment increased to about six times for Parsley, four times for Celery and Caraway, and twice for Dill and Fennel compared to control. These were associated with great decreases in K^+/Na^+ ratio especially for Parsley, Caraway (one-fifth) and Dill (one-fourth). There was significant difference among species in their response to salinity stress which due to their different genetic background (Figures 1 and 2).

Celery had the lowest GR and relative length at 3000

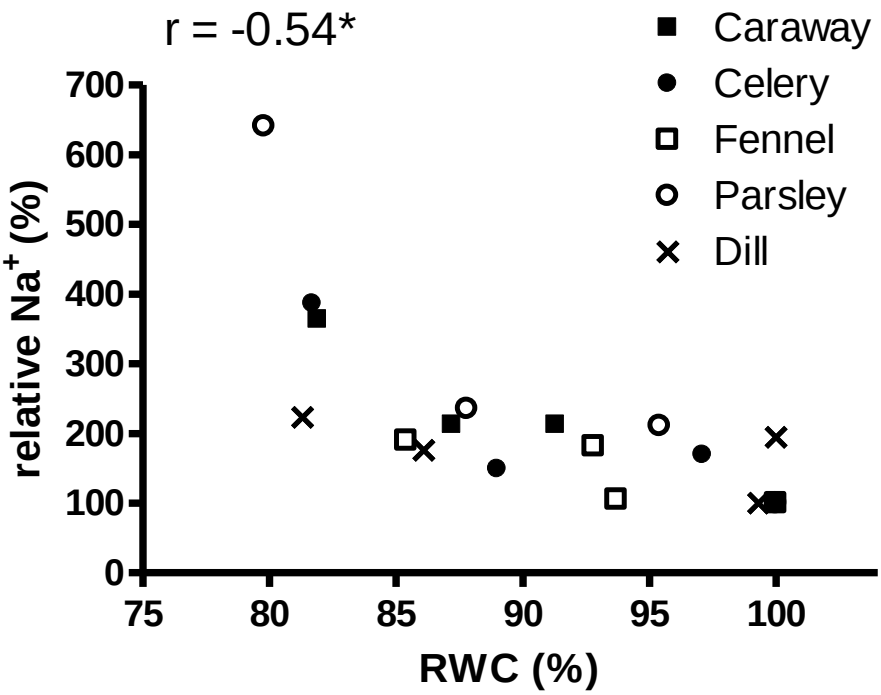

Figure 5. Correlation between relative water content (RWC) and relative Na^+ content of five species.

ppm compared to others; this shows that Celery species was the most sensitive among all in response to salinity stress. Although Dill species showed the highest GR at 3000 ppm treatment, relative length was low. This result suggested that Dill species was tolerant to salinity stress at germination stage, but seedlings growth at early stage was sensitive. Caraway species had mediate tolerance to salinity stress at germination stage and high tolerant at seedling growth stage.

The relative proline content at 3000 ppm compared to control was high for Caraway and Celery species compared to others. Both species also showed high relative Na^+ content (more than 3.5 times) compared to control. The changes in proline content did not show clear association with GR, seedling length or ion accumulations. This does not reflect the lack of role of proline in salinity stress tolerance, but this may be due to the fact that the species used in this study had wide genetic background, and roles of proline in stress response differed with each other.

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