I.J.S.N., VOL. 2(2) 2011: 168- 175 ISSN 2229 – 6441

INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

EFFECT OF SALT STRESS ON ION DISTRIBUTION AND PROLINE ACCUMULATION IN *FOENICULUM VULGARE* USING *IN VITRO* TECHNIQUE

[1]Khorami, R., [2]Safarnejad, A. and [1]Shourvarzi, M.
[1]Islamic Azad University of Mashhad, Iran.
[2]Agricultural Biotechnology Research Institute of Iran (ABRII)-East and North-East Branch, P.O. Box:Mashhad, Iran.

ABSTRACT

Fennel is herbaceous, aromatic plant and belongs to the Apiaceae family with scientific name of *Foeniculum vulgare* that is the medicinally most important plant. Salinity is a major environmental stress that affects almost every aspect of the physiology and biochemistry of plants and significantly reduces growth and yield of plants. Proline accumulation is one of the most frequently reported modifications induced by salt stress in plants, and it is often considered to be involved in stress resistance mechanisms. Also plant adaptations to salinity include sequestration of salt ions in vacuoles to maintain ionic homeostasis in the cells. In this research, the effects of NaCl salinity stress on proline accumulation, ions distribution include Na^+, K^+ and Ca^{2+}, ratio of K^+/Na^+ and necrosis percentage of callus, in different explants of *Foeniculum vulgare* using *in vitro* technique was investigated. In order to this, explants of root, hypocotyl and cotyledon of sterilized seedling were cultured on MS medium containing plant growth regulators of NAA (2 mg L^{-1}) and kinetin (0.5 mg L^{-1}). Then, calli were transferred to media supplemented with concentrations 0, 50, 100 and 150 mM of NaCl. Experiment was done in completely randomized design with 12 treatment and 15 replications. After four weeks of salinity treatment, the some of biochemical traits in both control and stress conditions were determined. The results showed, with increasing salt stress, Na^+ concentration increased but K^+ and Ca^{2+} concentrations were decreased. Necrosis percentage of different callus of *Foeniculum vulgare* unchanged at 50 mM NaCl but was increased at higher salinities and the K^+/Na^+ ratio was significantly reduced at 50 mM NaCl and higher salinities. Also with increasing of NaCl, proline accumulation was increased in callus of root, hypocotyl and cotyledon explants and there was significant different between proline accumulation of calli in different concentration of NaCl. Cotyledon calli had the maximum concentration of proline and K^+ at salinity treatment.

KEY WORD: Salinity, NaCl, *In vitro, Foeniculum vulgare,* proline

INTRODUCTION

Fennel (*Foeniculum vulgare*) is an annual, biennial or perennial plant depending on the variety, belonging to Apiaceae family that is one of the most important medicinal species with high economic value which is used for pharmaceutical, food, hygienic and cosmetic industries (Ashraf and Akhtar, 2004; Hunault *et al*., 1984). Although fennel occurs worldwide, it is widely grown in arid and semi-arid regions where high concentration of salts is an important characteristic of the soils (Munns, 2002; Qasim *et al*., 2003).

Several environmental factors adversely affect plant growth and development and final yield performance of a crop (Bohnert *et al*., 1995; Ashraf and Foolad, 2007). Salinity, drought and nutrient imbalances (including mineral toxicities and deficiencies) are among the major environmental constraints to crop productivity worldwide (Ashraf and Foolad, 2007). It is estimated that less than 10% of the world's arable lands may be free of major environmental stresses (Dudal, 1976), and approximately a third of the world's irrigated lands (Munns, 2002) and half of the lands in semi arid and costal regions are affected by salinization (Flowers and Yeo, 1995). Furthermore, each year there is a deterioration of 2 million ha (about 1%) of world agricultural lands to salinity, leading to reduced or no crop productivity (Tanji, 1990). Plants face two basic problems in saline environments: First, excess salt in soil lowers the osmotic potential of soil water and leads to decreased water uptake and consequently water deficit in plants. This in turn leads to perturbations in cell division and/or extension and influences the integrity of metabolic reactions in plants. Second, increased uptake and accumulation of Na^+ and Cl^- ions, decreases the absorption of essential minerals and imposes toxicity to plants (Tester and Davenport, 2003; Hopkins, 1999; Munns and Tester, 2008; Karimi *et al*., 2009). In response to salinity stress, the wide variety of physiological and biochemical changes occurs in plants (Qasim *et al.,* 2003). Among them, sequestration of salt ions in vacuoles and accumulation of low molecular weight solutes in the cytosol, such as proline commonly referred to as compatible solutes to balance the osmotic pressure (Hopkins, 1999; Jampeetong and Brix, 2009). In addition to its role as an osmolyte for osmotic adjustment (Ketchum *et al*., 1991), proline contributes to stabilizing sub-cellular structures (e.g. membranes and proteins), scavenging free radicals (Apel and Hirt, 2004), and buffering cellular redox potential under stress conditions (Ashraf and Foolad, 2007). Hossain *et al*., 2007) found that among the different parameters responding to NaCl stress, rapid accumulation of free proline within the cell is the most significant one. As increase NaCl stress, endogenous free proline content of *Chrysanthemum morifolium* increased. Also, in cells of *Distichlis spicata* treated with 200 mm NaCl, the cytosolic proline concentration was estimated to be more than 230

mM (Ketchum *et al*., 1991). Proline accumulation in plant tissues under salt stress as a mechanism to maintain cell turgor, water uptake and salinity tolerance, has been reported for many crops (Hsu *et al*., 2003). Furthermore, salinity stress causes an imbalance in the uptake of mineral nutrients and their distribution within the plants. Nutrient uptake by plants can be reduced by excessive salts in the soil solution, either by direct competition between ions or by increased osmotic potential of the solution reducing the mass flow of mineral nutrients to the root surface (Shannon and Grieve, 1999; Zhu, 2001). Generally, plants grown under NaCl salinity show an increase in sodium concentration, and a decrease in potassium and calcium (Tattini *et al*., 1992; Chartzoulakis *et al*., 2002; Khan, 1993). When the plants are not able to control Na^+ uptake over K^+ uptake, an accumulation of Na^+ in plant tissues at excessive levels is expected, causing cell damage. To minimize the toxic effects of salts in plant cells, plants should develop mechanisms to maintain low concentrations of Na^+ in cytosol by compartmentation of Na^+ into vacuoles (Zhu, 2003; Munns, 2005). Results indicate that both the absolute concentrations of K^+, Na^+, and Ca^{2+} in plants and the magnitude of the imbalance between these ions in the critical cell organelles such as cytosol and vacuoles are important in the differential expression of salt tolerance. Therefore, in many research groups, investigations dealing with the development of salt-tolerant varieties have concentrated on the uptake, transport, and accumulation of K^+, Na^+, and Ca^{2+} in plants (Colmer *et al*., 2006; Munns *et al*., 2006). The concentrations of these nutrients and their ratios are widely used as screening parameters in ranking varieties for their tolerance to salt toxicity. These parameters are reliable and useful in screening varieties for salt stress tolerance (Eker *et al*., 2006). Different strategies are in progress for the development of NaCl tolerant plants (Hossain *et al*., 2007). For instance, *in vitro* culture, use as a tool to assist in breeding improved cultivars by increasing the genetic variability. This method is based on the induction of genetic variation among cells, tissues and/or organs in cultured and regenerated plants (Mohamed *et al*., 2000). Although there are genetic, biochemical and physiological constraints in obtaining stress tolerant plants through *in vitro* culture, Nabors (1990) pointed out that this technique has been successfully used to produce stress tolerant plants from several species. Number of papers have been published on development and isolation of NaCl-tolerant cell/callus lines using *in vitro* technique (Olmos *et al*., 1994; Tal, 1994; Patnaik and Debata, 1997).

Keeping in mind, *in vitro* selection importance in characteristics of salt tolerance lines and since maintaining a better nutrition with K^+ and Ca^{2+}, limiting Na^+ uptake and consequently higher K^+/Na^+ ratio and proline content are highly important traits contributing to high salt stress tolerance in plants, that are often used as the screening parameters for identification of salt stress-tolerant varieties (Song *et al*., 2006). In the present study an attempt has been made to characterize NaCl tolerant callus lines and investigated the effect of salinity on proline accumulation and ions contents in root, hypocotyls and cotyledon explants of *Foeniculum vulgare* by *in vitro* technique.

MATERIALS AND METHODS

Seeds of Esfahan populations of *Foeniculum vulgare* were used in the present experiments. The seeds were surface sterilized using 20% sodium hypochlorite solution for 10 minutes, followed by thorough washing in sterile distilled water three times. Then the seeds were cultured on the surface of a germination medium containing MS (Murashing and Skoog, 1962) basal media, with 30 g L^{-1} sucrose, 8 g L^{-1} agar but without growth regulators. The medium was adjusted to pH 5.7 before autoclaving at 121°C for 20 min at 1.16 kg cm^{-1} pressure. All the sample were incubated in growth chamber at 25 ± 2 °C temperature under photoperiod of 16 h of light and 8 h of darkness. Explants of root, hypocotyl and cotyledon were taken from 9 days old seedling of *Foeniculum vulgare.* The explants were placed on the surface MS medium supplemented with NAA (2 mg L^{-1}) and kinetin (0.5 mg L^{-1}) for callus induction. After 28 days, each complete explant with its attached callus was transferred to the same medium but supplemented with 0 (control), 50, 100 and 150 mM sodium chloride, and incubated as before. Since sodium chloride represents the major source of salt in irrigation water and soil solutions (Chelli-Chaabouni *et al*., 2010; Turkan and Demiral, 2009), it was used as the source of salt throughout the experimental assays. At the third and fourth week of NaCl treatment, necrosis callus pieces were rejected and necrosis percentage of callus was investigated. In fact only healthy growing callus were selected and after four weeks of salinity treatment, content of ions include Na^+, K^+ and Ca^{2+} were measured in the different callus. At the same time, concentration of proline of the callus under saline conditions was also assessed. For proline analysis, at first, 0.2 g of different calli were frozen in liquid nitrogen and were homogenized in a methanol: chloroform: water (MCW 12: 5: 1 /V) solution. The homogenate was centrifuged at 5000 × g for 10 min. Then 2.5 ml of acid-ninhydrin and 2.5 ml of acetic acid were added into 2 ml of the homogenate in a test tube. The tubes were incubated in a boiling water bath for 60 minutes. After cooling of the tubes in ice, five milliliters of toluene were added to each test tube and vortexes for 15–20 s. The upper (toluene) phase decanted into a glass curette and absorbance read at 515 nm (Safarnejad *et al.,* 1996). The concentrations of sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}) were analysed on samples (0.1-0.01 g DW) of dried plant materials which were ground finely in a mill grinder. The samples were digested in 5 ml concentrated HNO_3. A flame photometer (model JEN WAY, PFP-7) was employed to determine ions concentrations.

The experimental design was a completely randomized design with 12 treatment and 15 replications per treatment. Analysis of variance (ANOVA) technique was employed for carrying out statistical analysis of data collected (Steel and Torie, 1980) using SAS statistical software (SAS Institute Inc., 2001).

RESULTS

The proline accumulation in different explants of cotyledon, hypocotyl and root was significantly ($p<0.01$) increased with the enhancement of salinity levels in the media (Figure 1). The proline content was 0.0033 µmol g^{-1} FW at salinity level of 0 mM (control) but it increased

significantly up to 0.011 µmol g^{-1} FW at the presence 150 mM of NaCl. Therefore, proline concentration was increased 70% at 150 mM NaCl in comparison with the control (Table 1). In addition, a highly significantly increase was observed in 50 mM NaCl as compared to others treatments. Hence, the highest proline accumulation was in 50 mM NaCl and the lowest was in the non-stressed conditions (Table 1). Results showed that, the free proline content was higher in the explants of cotyledon and hypocotyl than the root at different salinity levels and cotyledon maintained higher level of free proline content than the other explants (Figure 1). According to results of analysis of variance, proline accumulation in different levels of salinity stress and different explants were significant. Also there was significant difference between stress levels and explants interaction for proline accumulation (Table 2). In general, the results indicated that the free proline contents were significantly increased in all the explants under salt stress.

The salinity treatments significantly affected ion concentrations in the different explants (Figure 2). Increasing the concentration of NaCl in medium led to increased accumulation of Na^+ in the different explants tested, but not proportionally (Table 1). The amount of Na^+ was significantly ($p<0.01$) increased at 50 mM of NaCl but reduced with increasing salinity, especially in the roots (Figure 2). Therefore, after an initial increase in accumulation of Na^+, with increasing salinity, it declined at 100 and 150 mM of NaCl. Hence the highest amount of Na^+ was observed at 50 mM of NaCl, whereas the lowest value was observed at the control (Table 1). The concentration of Na^+ in the different explants was especially affected as concentrations were more than 42% higher in the highest salinity treatment (150 mM NaCl), compared with the control treatment (Table 1). The Na^+ content was higher in cotyledons than in roots. Indeed the content of Na^+ in cotyledons increased from 8.8 ppm in the control treatment to 21.49 ppm at the high salinity treatments, but the contents in the roots were less affected by the salinity treatments (Figure 2). Salinity had highly significant effect ($p<0.01$) on K^+ accumulation and K^+/Na^+ ratio in all explants (Figure 2). The amount of both K^+ content and K^+/Na^+ ratio decreased with the increase in NaCl level in the medium (Table 1). However, K^+ concentrations in cotyledon were higher at 50 mM salinity compared with the control, but at 150 mM salinity the concentrations were again lower (Figure 2). Content of K^+ was 21 ppm in control while at 100 and 150 mM of NaCl, it decreased to10.15 and 5.95 ppm respectively. Therefore K^+ accumulation decreased to 71.6% at 150 mM NaCl as compared to the control (Table 1). Also, K^+/Na^+ ratio was reduced by 85.3% at 150 mM salt level (Table 1). According to results of analysis of variance, K^+ concentrations and K^+/Na^+ ratio in different levels of salt stress and different explants were significant (Table 2). Similarly, In the presence of NaCl in the medium, the concentrations of Ca^{2+} decreased in cotyledon, hypocotyl and root (Figure 2). Data regarding Ca^{2+} concentration shows that increasing salinity of the medium had a significant decreasing effect ($p<0.01$) in the different explants (Table 1). The Ca^{2+} concentration in the hypocotyl was higher than in the other explants at different salinity treatments (Figure 2). Ca^{2+} concentration decreased under both levels of salinity (50 and 100 mM NaCl) that was 6.2 and 5.6 ppm, respectively. Also, treatment of 150 mM NaCl caused a 37.6% decrease of Ca^{2+} accumulation as compared to control (Table 1). It was concluded that with increasing salinity, there was a significant reduction in K^+ and Ca^{2+} concentrations in the callus of different explants. Pattern of accumulation of ions varied significantly in different treatment of salinity. Also, the interaction between explants and stress levels for ions contents was significant at $P<0.01$ (Table 2).

The increased NaCl concentration in culture medium resulted in increased necrosis percentage of callus (Table 1). Although, the salinity increased necrosis percentages of callus, but with increasing concentration of NaCl from 0 to 50 mM, no increased in necrosis percentages was observed and at 100 mM and 150 mM salinity was significantly higher (Figure 1). The results indicated that, more than 70% of the calli were necrotic in 150 mM NaCl compared to the control (Table 1). However, the necrosis percentage of callus was significantly ($p<0.01$) different among the various treatments of salt stress. Explants of cotyledon growing in 150 mM NaCl had less than 35% of the necrosis level, whereas more than 60% of the explants of root were necrotic in 150 mM NaCl (Figure 1).

TABLE 1. Means comparison of different treatments of salinity in *Foeniculum vulgare*.

Salinity (mM)	Proline (µmol g^{-1} FW)	Na^+ (ppm)	K^+ (ppm)	Ca^{2+} (ppm)	K^+/Na^+	Necrosis (%)
Control	0.0033d	8.47d	21a	8a	2.87a	0c
50	0.021a	16.84a	19.15b	6.27b	1.17b	0c
100	0.014b	14.65b	10.15c	5.60c	0.77c	53.76b
150	0.011c	12.05c	5.95d	4.99d	0.42d	72.72a

Different letters indicate significant difference between means at $P<0.01$.

TABLE 2. Analysis of variance for studied traits of *Foeniculum vulgare* under salinity stress.

Source of variation	Degrees of freedom	Proline (µmol g^{-1} FW)	Na^+ (ppm)	K^+ (ppm)	Ca^{2+} (ppm)	K^+/Na^+	Necrosis (%)
Treatment	23	0.00042**	734.40**	1348.28**	36.21**	28.82**	2951.29**
Salinity	3	0.00096**	1165.75**	4651.86**	152.24**	105.97**	18608.87**
Explant	2	0.0021**	4460.27**	2358.14**	109.57**	4.03**	574.97**
Salinity× Explant	6	0.00023**	622.71**	236.38**	17.77**	32.96**	206.86**
Error	336	0.00000003	6.52	9.80	0.47	0.20	0.102

*, **: Significant difference at 0.05 and 0.0.1 probability level, respectively.

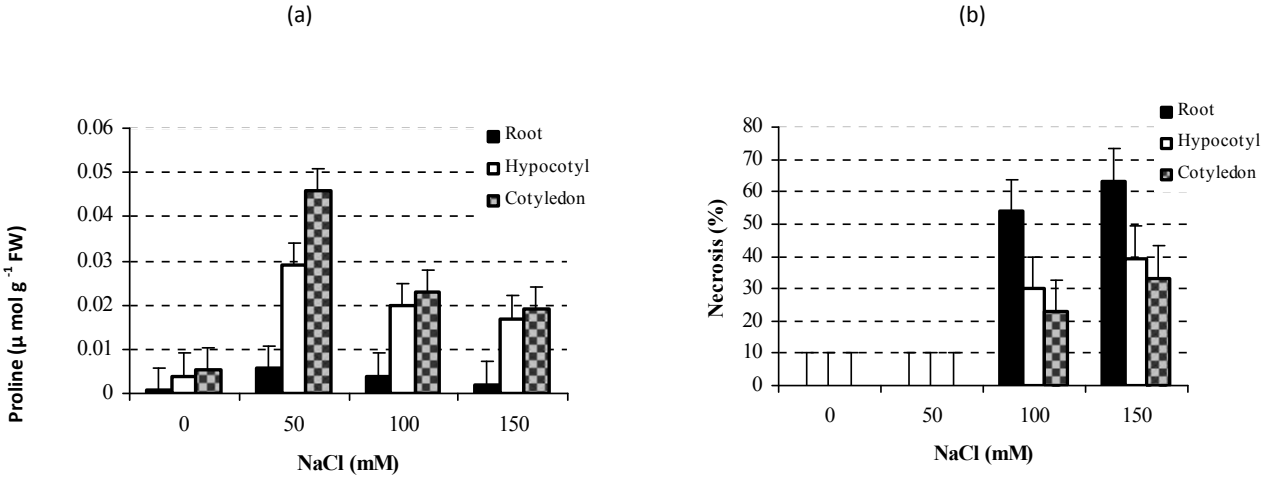

FIGURE 1. Interaction effect of salinity and explants on proline level (a) and necrosis level (b) in root, hypocotyl and cotyledon of *Foeniculum vulgare*

DISCUSSION

Since the field evaluation of salt effects is highly correlated with environmental conditions, *in vitro* screening techniques would allow for a better control of culture conditions (Vijayan *et al*., 2003; Chelli-Chaabouni *et al*., 2010). On the other hand, plant response to abiotic stress is a complex phenomenon, and *in vitro* culture could be used to enhance selection process for these stresses. Thus, the main objective of this study was to evaluate the *in vitro* selection for salt tolerance of *Foeniculum vulgare*, based on the effects of salinity on the concentrations of proline, ions contents and necrosis percentage of the different explants. Because, most of the works so far done in relation to *in vitro* selection is based on ion homeostasis and proline pool, too (Olmos *et al*., 1994; Patnaik and Debata, 1997). To cope with salt stress, plants employ different biochemical and physiological processes. One of the vital processes is production of low molecular weight solutes which called compatible solutes or osmolytes. The accumulation of compatible solutes may help to maintain the relatively high water content necessary for plant growth and cellular function (Kumar *et al*., 2003; Jampeetong and Brix, 2009). Proline is a main osmolyte which accumulate under saline conditions in many plants and it is one of the adaptation mechanism to salinity and water deficit (Kumar, 2003). In the present study, after 28 days of *in vitro* culture, salinity levels ranging between 0 and 150 mM NaCl were noted to induce a significant increase in the free proline content of *Foeniculum vulgare*. These results were in agreement with those previously reported by Ashraf and Akhtar (2004) for sweet fennel in greenhouse conditions. Similar results were also obtained on lentil (Misra and Saxena, 2009), *Salvinia natans* (Jampeetong and Brix, 2009), *Pistacia* (Chelli-Chaabouni *et al*., 2010) and *Citrus* (Ferreira and Lima-Costa, 2006). The accumulation of proline as an osmotic tolerance mechanism has been widely observed in many plants including some medicinal plants such as ajwain (Ashraf and orooj, 2006), anise and coriander (Zidan and Elewa, 1995). It had been reported that the accumulation of proline under conditions such as high salinity, in many plants, has been correlated with stress tolerance (Chelli-Chaabouni *et al*., 2010; Misra and Gupta, 2005). Accumulation of proline with increasing NaCl, which might be attributed to the strategies adapted by plants to cope up with stress conditions. In this investigation, higher accumulation of proline was found in cotyledon and hypocotyl than in root at presence of salt stress, which was

more prominent in explants of cotyledon. Ashraf and Akhtar (2004) also noticed that rise in proline content in the shoots of *Foeniculum vulgare* shows the positive role of proline in the salt tolerance of this crop. As our findings, increase of proline in cotyledon of *Foeniculum vulgare* under salinity treatment can reduce the harmful effects of stress conditions and thus prevents the necrosis of these calli, while a small increase in proline content was observed in root. Also, root has been found to be more affected than both cotyledon and hypocotyl by an increasing supply of NaCl in the medium, although all the explants were inhibited by salt, the effects were more pronounced on root. It is suggested that proline can be utilized both as a carbon and/or nitrogen source for rapid recovery from the salinity stress and post stress growth (Hossain *et al.*, 2007). This may be the reason that when callus transferred to NaCl medium, high level of proline was observed under stress condition, in this experiment. In addition, proline serves as a sink for energy to regulate redox potentials, as a hydroxy radical scavenger, as a solute that protects macromolecules against denaturation, as means of reducing the acidity in the cell, and proline protect plants against free-radical induced damage by quenching of singlet oxygen (Kumar *et al*., 2003; Misra and Saxena, 2009).

In this study, increasing the concentration of NaCl in the medium led to a higher accumulation of sodium in the callus of the tested explants and the effect of salinity was significant on Na^+ concentrations. Similar results have been found in a number of studies as in wheat (Begum *et al*., 1992) and *Nigella sativa* (Hussain *et al*., 2009). High NaCl concentrations in the growth medium of plants generate primary and secondary effects that negatively affect plant growth and development. Primary effects are ionic toxicity and osmotic stress. Ionic toxicity occurs because high concentrations of Na^+ and Cl^- in the cytoplasm of cells disturb several biochemical and physiological processes, and osmotic stress is induced by the lowering of the water potential causing turgor reduction and cellular water loss. Secondary effects of NaCl stress include inhibition of K^+ uptake, membrane dysfunction and generation of reactive oxygen species in the cells (Rout and Shaw, 2001; Jampeetong and Brix, 2009). In this experiment, the level of necrotic callus was not significantly increased at 50 mM NaCl, while high level of necrotic was observed in 100 and 150 mM of NaCl, it suggested that, these changes were probably caused by the ionic toxicity of Na^+ and Cl^- at concentrations higher than 100 mM NaCl. Munns (2002) found that ionic toxicity of Na^+ and Cl^- generally occurs at concentrations in the cytoplasm exceeding 100 mM where most enzymes start to become inhibited. Jampeetong and Brix (2009) indicated that Na^+ competes with K^+ for uptake into cells, particularly when the external concentrations of Na^+ are substantially higher than that of K^+, and the ability to maintain Na^+/K^+ homeostasis in the cells is crucial for the salt tolerance of plants. Our results showed with increasing of salt stress, potassium concentrations decreased in *Foeniculum vulgare* cotyledon, hypocotyl and root and maximum reduction was observed at 150 mM NaCl. This may be due to a direct effect of sodium, displacing potassium, and/or causing loss of potassium from the root tissue that might have increased the stress severity. It has been reported that K^+ concentration is declined by increasing NaCl stress in the nutrient solution or soil in plants as diverse as maize and barley (Benes *et al*., 1996) and olive (Tattini *et al*., 1992; Chartzoulakis *et al*., 2002). Similar results were also obtained in *Sorghum bicolor* (Yang *et al*., 1990) and *Salvinia natans* (Jampeetong and Brix, 2009). Therefore numerous studies with a wide variety of horticultural crops have shown that potassium concentration in plant tissue declines as the salinity in the root media increases. In the present investigation, K^+/Na^+ ratio of callus grown in high saline medium showed a decline with increasing external NaCl concentrations. This observation is in agreement with earlier reports that in some callus lines of *Cymbopogon martini* (Roxb.) that the Na^+ uptake increased with the corresponding increase of NaCl level in medium while that of K^+ and K^+/Na^+ ratio showed a decline (Patnaik and Debata, 1997). Also, we observed that NaCl had a highly significant effect on Ca^{2+} concentration in *Foeniculum vulgare*. Reduction in Ca^{2+} with the increase in salinity levels was observed in this plant, these results are similar with Ashraf and Khanum (1997) in wheat. Also similar results have been found in *Nigella sativa* (Hussain *et al*., 2009). It seems that high Na^+ concentration in the media inhibits uptake and transport of Ca^{2+} and therefore induce calcium deficiency in plant. The Ca^{2+} plays an important role in regulating ion transfer into plant cells growing in saline medium. As calcium can affect membrane stability and ion translocations. In addition, calcium is a non-toxic inorganic nutrient that is very effective in detoxifying high concentrations of other elements in plants under saline medium (Greenway and Munns, 1980). Thus Ca^{2+} may be an important factor in controlling salinity response of plants. Similarly, Ashraf and Akhtar (2004) for *Foeniculum vulgare* grown *in vivo* reported that salt stress significantly increased Na^+ and Cl^- concentrations of both shoots and roots. The concentration of both ions in shoots and roots increased with increasing NaCl concentration and concentration of Na^+ was higher in the shoots than that in the roots. A consistent decrease in concentrations of K^+ and Ca^{2+} in shoots and K^+ in roots was found with increasing concentration of NaCl in the rooting medium. Results obtained from the present experiment indicated that levels of 50 mM NaCl did not affect *Foeniculum vulgare* callus, but high levels of NaCl in the medium led to increased necrosis in all explants. It could therefore be attributed to the increase levels of Na^+ and reduction of K^+ and Ca^{2+} in callus of different explants. Under 150 mM NaCl, accumulation of Na^+ in the cotyledon calli was higher than that of root and hypocotyl, however a lower degree of necrosis of cotyledon was also observed in the 100 and 150 mM NaCl range. This explant also exhibited a higher tolerance to the NaCl treatment *in vitro* than the other explants. Since proline, potassium and calcium accumulated in cotyledon calli rather than in root by salinity effect, this indicated the importance role of proline, K^+ and Ca^{2+} in salt tolerance.

In conclusion, the findings presented in the current paper demonstrate that, *Foeniculum vulgare* is a salt-sensitive species lacking mechanisms to maintain ionic homeostasis in the cells and it is lacking efficient measures to cope with exposure to high salinity. The obtained results are in agreement with those reported by Ashraf and Akhtar (2004) who showed that *Feniculum vulgare* is a

moderately salt sensitive crop. The contents of proline in the different explants increased at high salinity, indicating a capacity of *Foeniculum vulgare* to synthesize proline as a compatible compound. It seems that *In vitro* selection of *Foeniculum vulgare* respond to salinity in a similar way as the whole plant under *in vivo* conditions. Therefore, *in vitro* culture is an efficient and fast technique for understanding and knowledge the mechanisms of salt tolerance in plants.

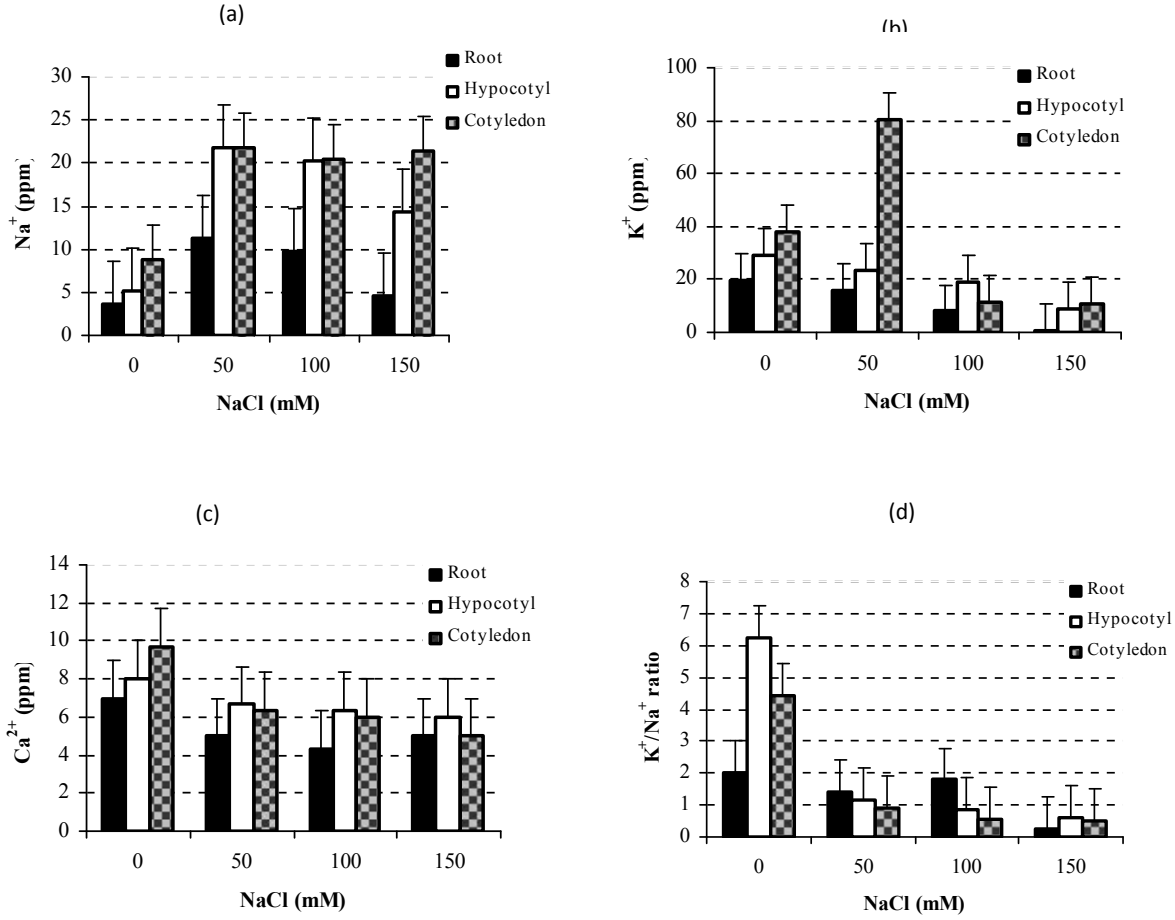

FIGURE 2. Interaction effect of salinity and explants on Na^+, K^+ and Ca^{2+} concentration (a,b,c) and K^+/Na^+ ratio (d) in root, hypocotyl and cotyledon of *Foeniculum vulgare*

ACKNOWLEDGEMENTS

The authors wish to thank Agriculture and Natural Resources Research Center of Razavi Khorasan and Islamic Azad University of Mashhad, Iran, for funding this research.

REFERENCES

Apel, K. and Hirt, H. (2004) Reactive oxygen species: metabolism, oxidative stress and signal transduction. Annual Review of Plant Biology, 55: 1331-1341.

Ashraf, M. and Akhtar, N. (2004) Influence of salt stress on growth, ion accumulation and seed oil content in sweet Fennel. Biologia Plantarum, 48 (3): 461- 464.

Ashraf, M. and Foolad, M. (2007) Roles of glycin- betaien and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany, 59: 206 - 216.

Ashraf, M. and A. Khanum (1997) Relationship between ion accumulation and growth in two spring wheat lines

differing in salt tolerance at different growth stages. J. Agron. Crop Sci., 178(1): 39-51.

Ashraf, M. and Orooj, A. (2006) Salt stress effects on growth, ion accumulation and seed oil concentration in an arid zone traditional medicinal plant ajwain (*Trachyspermum ammi* L. Sprague). J. of Arid Environments, 64: 209-20.

Begum, F. J. L., Karmoker, Q. A. F. and Moniruzzaman, A. F. M. (1992) The effect of salinity on germination and its correlation with K^+, Na^+, Cl^- accumulation in germinating seeds of *Triticum aestivum* L. cv. Akbar. Plant Cell Physiol., 33: 1009-14.

Benes, S.E., Aragüés, R., Austin, R.B. and Grattan, S.R. (1996) Brief pre- and post-irrigation sprinkling with fresh water reduces foliar salt uptake in maize and barley sprinkler irrigated with saline water. Plant and Soil, 80: 87-95.

Bohnert, H. J., Nelson, D. E. and Jensen, R. G. (1995) Adaptations to environmental stresses. The Plant Cell, 7: 1099-1111.

Chartzoulakis, K., Loupassaki, M., Bertaki, M. and Androulakis, I. (2002) Effects of NaCl salinity on growth,

ion content and CO2 assimilation rate of six olive cultivars. Sci. Hortic. 96: 235-247.

Chelli-Chaabounia, A., Mosbahb, A.B., Maalejb, M., Gargouric, K., Gargouri-Bouzidd, R. and Drirab, N. (2010) *In vitro* salinity tolerance of two pistachio rootstocks: Pistacia vera L. and P. atlantica Desf. Environmental and Experimental Botany, 69: 302-312.

Christian, J.H. (1955) The influence of nutrition on the water relation of *Salmonella oranienburg*, Aust. J. Biol. Sci., 8: 75-82.

Colmer, T.D., Flowers, T.J. and Munns, R. (2006) Use of wild relatives to improve salt tolerance in wheat. J. Exp. Bot., 57: 1059-1078.

Dudal, R. (1976) Inventory of Major Soils of the World with Special Reference to Mineral Stress. Cornell Univ. Agric. Exp. Stn, Ithaca, pp: 3.

Eker, S., Comertpay, G., Konuskan, O., Ulger, A. C., Oztrk, L. and Cakmak, I. (2006) Effect of salinity stress on dry matter production and ion accumulation in hybrid maize varieties. Turkish Journal of Agriculture and Forestry, 30: 365-373.

Ferreira, A.L., Lima-Costa, M.E. (2006) Metabolic responses to salt stress in cell suspension cultures of sensitive and resistant Citrus. J. Hort. Sci. Biotech., 81 (6): 983-988.

Flowers, T.J., Yeo, A.R. (1995) Breeding for salinity resistance in crop plants. Aust. J. Plant Physiol., 22: 857-884.

Greenwey, H. and Munns, R. (1980) Mechanism of salt tolerance in nonhalophyte. Ann. Rev. Plant Physiol., 31: 149-190.

Hopkins, W.G. (1999) Introduction to Plant Physiology. John Wiley & Sons, Inc., New York, USA.

Hossain, Z., Mandal, A.K. A., Datta, S. K. and Biswas, A. K., (2007) Development of NaCl-tolerant line in *Chrysanthemum morifolium* Ramat. Through shoot organogenesis of selected callus line. Journal of Biotechnology, 129: 658-667.

Hsu, S.Y., Hsu, Y.T. and Kao, C.H. (2003) The effect of polyethylene glycol on proline accumulation in rice leaves. Biol. Plant., 46: 73-78.

Hunault, G. (1984) *In vitro* culture of Fennel tissue (*Foeniculum vulgare* Miller) from cell suspension to mature plant. Scientia Horticulturae, 22 (1-2): 55-65.

Hussain, K., Majeed, A., Nawaz, K., Hayat, B. K. and Nisar, M. F. (2009) Effect of Different Levels of Salinity on Growth and Ion Contents of Black Seeds (*Nigella sativa* L.). Current Research Journal of Biological Sciences, 1(3): 135-138.

Jain, M., Mathur, G., Koul, S., and Sarin, N.B. (2001) Ameliorative effects of proline on salt stressinduced lipid peroxidation in cell lines of groundnut (*Arachis hypogaea* L.). Plant Cell Reports, 20: 463-468.

Jampeetong, A. & Brix, H. (2009) Effects of NaCl salinity on growth, morphology, photosynthesis and proline accumulation of *Salvinia natans*. Aquatic Botany, 91: 181-186.

Karimia, E., Abdolzadeha, A. and Sadeghipour, H. R. (2009) Increasing salt tolerance in Olive, *Olea europaea* L. plants by supplemental potassium nutrition involves changes in ion accumulation and anatomical attributes. International Journal of Plant Production, 3 (4): 49-60.

Khan, A.A. (1993) Preplant physiological seed conditioning, Hortic. Rev., 13:131-181.

Ketchum, R.E.B.,Warren, R.C., Klima, L.J., Lopez-Gutierrez, F. and Nabors, M.W. (1991) The mechanism and regulation of proline accumulation in suspension cultures of the halophytic grass *Distichlis spicata* L., J. Plant Physiol., 137: 368-374.

Kumar, S. G, Matta Reddy, A. and Sudhakar, C. (2003) NaCl effects on proline metabolism in two high yielding genotypes of mulberry (*Morus alba* L.) with contrasting salt tolerance. Plant Science, 165:1245-1251.

Mohamed, M.A.H., Harris, P.J.C. and Henderson, J., 2000. *In vitro* selection and characterization of a druogh tolerant clone of *Tagetes minuta*. Plant Science, 159: 213-222.

Misra, N. and Gupta, A.K. (2005) Effect of salt stress on proline metabolism in two high yielding genotypes of green gram. Plant Sci., 169: 331-339.

Misra, N. and Saxena, P. (2009) Effect of salicylic acid on proline metabolism in lentil grown under salinity stress. Plant Science, 177: 181-189.

Munns, R. (2002) Comparative physiology of salt and water stress. Plant Cell Environ., 25: 239-250.

Munns, R. (2005) Genes and salt tolerance: bringing them together. New Phytol., 167: 645-663.

Munns, R., James, R.A. and Lauchli, A. (2006) Approaches to increasing the salt tolerance of wheat and other cereals. J. Exp. Bot., 57: 1025-1043.

Munns, R. and Tester, M. (2008) Mechanisms of salinity tolerance. Ann. Rev. Plant Biol., 59: 651-681.

Murashing, T. & Skoog F. (1962) A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiologia Plantarum. 15: 473- 497.

Nabors, M.W. (1990) Environmental stress resistance procedure and applications, in: J.D. Philip (Ed.), Plant Cell Line Selection, VCH, Weinheim, pp. 167-185.

Olmos, E., Hernandez, J.A., Sevilla, F. and Hellin, E. (1994) Induction of several antioxidant enzymes in the

selection of a salt tolerant cell line of *Pisum sativum*. J. Plant Physiol., 144: 594-598.

Patnaik, J. and Debata, B.K. (1997) *In vitro* selection of NaCl tolerant callus ines of *Cymbopogon martini* (Roxb.) Wats. Plant Sci., 124: 203-210.

Qasim, M., Ashraf, M., Ashraf, M.Y., Rehman, S.U. and Rha, E.S. (2003) Salt-induced changes in two canola cultivars differing in salt tolerance. Biol. Plant., 46: 629-632.

Rashid, P., Karmoker, J.L., Chakrabortty, S. and Sarker, B. C. (2004) The Effect of Salinity on Ion Accumulation and Anatomical Attributes in Mungbean (*Phaseolus radiatus* L. cv. BARI-3) Seedlings. International Journal of Agriculture & Biology, 6(3): 495-498.

Rout, N.P. and Shaw, B.P. (2001) Salt tolerance in aquatic macrophytes: ionic relation and interaction. Biol. Plant, 44: 95-99.

Safarnejad, A., Collin, H., Bruce, K.D. & McNeilly, T. (1996) Characterization of alfalfa following *in vitro* selection for salt tolerance. Euphytica, 92:55-61.

Shannon, M.C. and Grieve, C.M. (1999) Tolerance of vegetable crops to salinity, Sci. Hortic., 78: 5-38.

Song, J.Q., Mei, X.R. and Fujiyama, H. (2006) Adequate internal water status of NaCl-salinized rice shoots enhanced selective calcium and potassium absorption. Soil Sci. Plant Nutr., 52: 300-304.

Tal, M. (1994) *In vitro* selection for salt tolerance in crop plants: theoretical and practical considerations. *In vitro* Cell. Dev. Biol., 30: 175-180.

Tanji, K.K. (1990) Nature and extent of agricultural salinity. In: Tangi, K.K. (Ed.), Agricultural Salinity Assessment and Management. American Society of Civil Engineers, New York, pp. 1-13.

Tattini, M., Bertoni, P. and Caselli, S. (1992) Genotipic responses of olive plants to sodium chloride .J. Plant Nutr., 15: 1467-1485.

Tester, M. and Devenport, R., (2003) Na^+ tolerance Na^+ transport in higher plants. Annal Bot., 91: 503-527.

Vijayan, K., Chakraborti, S.P. and Ghosh, P.D. (2003) *In vitro* screening of mulberry (Morus spp.) for salinity tolerance. Plant Cell Rep., 22: 350-357.

Yang, Y.W., Newton, R.J. and Miller, F.R. (1990) Salinity tolerance in *S. bicolor*. I. Whole plant response in sodium chloride in *S. bicolor* and *S. halepense*. Crop Sci., 30: 775-81.

Zidan, M.A. and Elewa, M.A. (1995) Effect of salinity on germination, seeding growth and some metabolic changes in four plant species (Umbelliferae). Indian J. Plant Physiol., 38: 57-61.

Zhu, J.K. (2001) Plant salt tolerance trends. Plant Sci., 6: 66-72.

Zhu, J.K. (2003) Regulation of ion homeostasis under salt stress. Curr. Op. Plant Biol., 6: 441-445.