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**ORIGINAL ARTICLE**

# Salt-induced changes in germination and vegetative stages of *Anethum graveolens* L.

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The aim of this experiment was to determine the response of dill (*Anethum graveolens* L.) to salt stress during germination and vegetative stages. In the first stage, response of dill seeds germination to levels of salinity (0, 25, 50, 75 and 100 mM) was investigated. In the second stage, influence of salt stress on physiological and biochemical parameters in dill seedlings were investigated. Results showed germination rate and percentage, radical, plumule length and dry weight decreased significantly with the increase of salinity levels. Effect of salt stress on amount of chlorophyll a, b and total was significant. Results indicated that, amount of proline, total soluble carbohydrates and proteins and catalase (CAT) activity in shoots and roots significantly increased with the increase of salinity. These results showed that dill maintained higher carbohydrates, proteins, proline and activity of CAT under salt stress and these traits could have partially to its salt tolerance.

*Key words: catalase, chlorophyll, dill, proline, salt stress*

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Salt stress has become one of the most damaging environmental hazards to crop productivity all over the world (Ashraf and Ali, 2008). This adverse environmental condition impairs plant growth by both water deficit and ionic toxicity (Munns and Tester, 2008). Firstly, the hyperosmolarity of the soil solution restricts water uptake by the roots and triggers transient changes in the plant water relations (Bray *et al*., 2000; Munns and Tester, 2008). Secondly, the accumulation of saline ions in the tissues leads to the salt-specific toxic effects on the plant metabolism (Maathuis and Amtmann, 1999; Apse and Blumwald, 2007). Salt stress changes the morphological, physiological and biochemical responses of plants (Amirjani, 2010). There is evidence that high salt concentrations cause an imbalance in cellular ions, resulting in ion toxicity and osmotic stress, leading to the generation of reactive oxygen species (ROS) which cause damage to DNA, lipids and proteins (Yasar *et al*., 2006). At the same time ROS causes chlorophyll degradation

and membrane lipid peroxidation, decreasing membrane fluidity and selectivity. Plants possess several anti-oxidant enzyme systems that protect their cells from the negative effects of ROS. These include non-enzymatic anti-oxidants such as ascorbic acid, glutathione and carotenoids, as well as antioxidative enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) (Zhu *et al*., 2004). Biochemical studies have shown that plants under salinity stress accumulate compatible solutes. These metabolites include carbohydrates, such as manitol, sucrose and raffinose oligosaccharides and nitrogen compounds, such as proteins, amino acids and polyamines (Bohnert *et al*., 1995). In this study effect of salinity on germination, amount of chlorophyll, proline, soluble carbohydrates and activity of catalase enzyme in Dill (*Anethum graveolens* L.) is investigated.

## MATERIALS AND METHODS

### *Germination experiment*

The seeds of dill (*Anethum graveolens* L.) were surface-sterilized for 2 min in sodium hypochlorite solution and then rinsed with distilled water. After sterilization, seeds were transferred in sterile petri dishes on a dual filter paper and then moistened with 5 ml disilled water (control) or saline water solution at 0, 25, 50, 75 and 100 mM NaCl. To prevent infection and evaporation of solution, all of the plates were closed with parafilm. The Petri dishes were incubated in a germinator at 20°C and 12h illumination. Number of germinated seeds were recorded daily and number of final germinated seeds were registered when seed germination was stopped for eleven days. Mean plumule and radicle lengths at the end of germination were measured. Dry weights of seedlings was determined after oven drying at 70°C. Germination percentage (GP) and germination rate (GR) were calculated by equation 1 and 2.

Equation 1; GP= Ni / N × 100

Where, GP= germination percentage, $N_i$=number of germinated seed till $i^{th}$ day and N are total number of seeds.

Equation 2; $$R_s = \sum_{i=1}^{n} \frac{S_i}{D_i}$$

Where, $R_s$= germination rate, $S_i$= number of germinated seeds in each numeration, $D_i$= number of days till nth numeration and n are number of numeration times.

### *Seedling experiment*

After sterilization, fifteen seeds were sowed in a plastic pot (20 × 25) contained non-saline sandy loam soil. Pots were transferred to green house under conditions of 26/18°C day/night temperature and natural light. The pots were irrigated by distilled water. Treatments in this stage was similar to germination. Treatment were applied to each pot when second leaf was completely expanded and plants were harvested after thirty days.

### *Chlorophyll measurement*

Amount of chlorophyll a, b and total, measure by Arnon (1967) method. Therefore, 0.03 gr leaf samples was prepared and were beaten by acetone 80% in the porcelain mortar. Remained leftovers in the mortar was completely washed and Final volume was reached to 25 ml by acetone 80%. After centrifugation at 4000rpm, absorption of samples were read in wavelength 663, 645 and 652 nm. Clorophyll a, b and total for each sample were determined by using the following formulas:

Chl.a = {2.69 (A663) - 12.7 (A 645)}×V/W× 1000

Chl.b = {22.9 (A645) - 4.68 (A 663)}×V/W× 1000

Chl.T = {20.2 (A645) - 8.02 (A 663)} 1000×V/W×

Where, V= volume of purified solution, W= wet weight and A are optical absorption in wavelengths 663, 652 and 645 nm.

***Proline***

Proline content was determined based on the method of Bates *et al*. (1973). 0.5gr plant tissue (shoot and root) was homogenized with 10 ml of 3% aqueous sulfosalicylic acid and centrifuged for 10 min, 2ml of supernatant were mixed with 2ml of glacial acetic acid and 2ml of acid ninhydrin for 1 h at 100 °C. The developed colour was extracted in 4ml toluene and measured colourimetrically at 520nm. Content of proline was expressed as µmol/g FW.

***Soluble carbohydrates***

For measurement of soluble carbohydrates, a phenol-sulfuric acid assay was used (Dubois *et al*., 1956). A volume of 0.5 mL of 5% (v/v) phenol solution and 2.5 mL of concentrated sulfuric acid were added to 0.5 mL aliquots. The mixture was shaken, heated in a boiling water-bath for 20 min and cooled to room temperature. The absorption was then determined spectrophotometry at 490 nm.

***Total soluble proteins***

Samples were homogenized in ice-cold extraction buffer (50 mM potassium phosphate, pH 7.4, 1 mM EDTA). The extracts were centrifuged at 15,000×g for 20 min, and the resulting supernatants were used for estimation of soluble protein contents. Protein contents were assayed following Bradford's method (1976) with a standard curve prepared using bovine serum albumin.

***Catalase activity***

Shoot and root tissue (1gr fw) were homogenized in 3 ml 50 mM potassium phosphate buffer, pH 7.0, including 1 mM EDTA, 1 mM PMSF and 1% PVP. The homogenate was centrifuged at 15000 rpm, at 4 °C for 20 min. CAT activity was determined by measuring $H_2O_2$ consumption in reaction mixture containing 50 mM sodium phosphate buffer (pH 7.0), 15 mM $H_2O_2$ and extract. at 240 nm against a calibration curve. The enzyme activity was determined by measuring the decrease in absorbance at 240 nm and 30°after 60s. Activity was expressed as µM decomposed $H_2O_2$ per mg protein (Dhindsa *et al*., 1981).

***Statistical analysis***

The experiment was as factorial based on a randomized complete block design with three replications. Data were analyzed using Mstat C and JMP. Means were compared by Duncan Multiple Rang Test ($P \leq 0.05$).

## RESULTS AND DISCUSSION

The results showed that the germination percentage and rate of dill seeds were strongly affected by all salt treatments. Strong reduction was observed mainly at higher level of salt concentration compared to control (Table 1). Several studies have reported the reduction of seed germination by salinity (Dogan *et al*., 2008; Ayaz *et al*., 2000). For example, Amzallag (2000) reported that in comparison with the control, germination decreased markedly under NaCl. According to Ayaz *et al*., (2000), decrease of seed germination under salinity stress conditions is due to occur of some metabolically disorders. It seems that, decrease of germination percentage and germination rate is related to reduction in water absorption into the seeds at imbibition and seed turgescence stages (Mostafavi, 2011).

Plumule and radical length of dill seedlings declined significantly in all the salt treatments

compared to the control (Table 1).

High salinity may inhibit root and shoot elongation due to slowing down the water uptake by the plant (Jamil *et al*., 2006). Neumann (1995) indicated that salinity can rapidly inhibit root growth and hence capacity of water uptake and essential mineral nutrition from soil.

Differences in dry weight of plumule and radicle were significant in all salinity levels. These traits decreased with increasing in salinity level (Table 1). Reduced seedling weight has also been reported (Jeannette *et al*., 2002 ; Keshavarzi, 2011). Amiri *et al*., (2010) reported seedling dry weight of *Cynara scoolymus* and *Echinacea purpurea* was decreased by increasing salinity stress.

Results showed that amount of chlorophyll a, b and total decreased by all of NaCl concentrations compared to control. Maximum decrease was shown at 100 mM NaCl concentration (Table 2). NaCl stress decreased chlorophyll content of the plant by increasing the activity of cholorophyllase enzyme (Noreen and Ashraf, 2009). The decrease in chlorophyll content under salinity conditions is reported by Yasar *et al*., (2008), Kusvuran (2010) and Nazarbeygi *et al*., (2011).

Figure 1 shows NaCl induced proline accumulation in shoot and root. The proline content increased with the increasing concentration of the salt. The highest concentration of NaCl (100 mM) increased proline content approximately 72% and 89% in shoot and root, respectively compared to the control. Many authors were reported the increase of proline accumulation under salt stress in different plants (Mohamed *et al*., 2007; Misra and Gupta, 2005; Fayek *et al*., 2010).

Total soluble carbohydrates concentration was significantly increased in shoot and root under salinity (Figure 2). Carbohydrate concentration, beside its role in decreasing water potential contributes in preventing oxidative damage and maintaining the structure of proteins and membranes under moderate dehydration during drought period. Carbohydrate also serve as signaling molecules for sugar-responsive genes which leading to different physiological responses like defence responses and turgor-driven cell expansion (Hoekstra *et al*., 2001).

The effect of NaCl on total proteins is shown in Figure 3. Total proteins content in 100 mM NaCl-treated plants was 21% and 19% higher than control plants in shoot and root, respectively. Venkatesalu *et al*., (1994) reported an increase in protein content with increasing NaCl salinity in *Sesuvium portulacastrum*. Muthuchelian *et al*., (1995) also reported that salt stress increased the protein content in *Erythrina variegate* seedlings. Proteins may provide a storage form of nitrogen that is re-utilized later and may play a role in osmotic adjustment (Muthuchelian *et al*., 1995).

The activity of CAT in treated plants progressively increased with increasing concentrations as compared to control (Figure 4). By increasing of salt concentration from 0 to 100 mM, activity of CAT was increased by 57% and 62% in shoot and root, respectively. CAT activity is supposed to be an adaptive trait possibly helping to overcome the damage to the tissue metabolism by reducing toxic levels of $H_2O_2$ produced during cell metabolism (Del Rio *et al*., 2012). CAT activity changes have been studied in many other plants. CAT activity has been found to increase under salt stress in mulberry (Sudhaker *et al*., 2001), rice (Swapna, 2003) and pistachio (Abbaspour, 2012). In our study, we found that CAT activity was higher in root compared to shoot under all conditions. This

may be due to root are directly exposed to salt and bearing high $H_2O_2$ content.

**Table 1:** Effect of salt stress on germination characteristics of dill seeds

| NaCl Treatments (mM) | Germination percentage | Germination rate | Plumule length (mm) | Radicle length (mm) | Plumule dry weight (mg) | Radicle dry weight (mg) |
|---|---|---|---|---|---|---|
| 0 | 71.00 a | 15.68 a | 39.67 a | 83.33 a | 7.36 a | 9.07 a |
| 25 | 56.67 b | 11.00 ab | 30.67 b | 65.33 b | 6.37 b | 8.30 a |
| 50 | 47.67 bc | 8.20 b | 25.00 c | 53.00 c | 4.86 c | 7.17 b |
| 75 | 39.67 cd | 7.68 b | 19.34 d | 43.00 d | 2.86 d | 5.30 c |
| 100 | 27.67 d | 6.05 b | 12.67 e | 28.33 e | 1.90 e | 3.43 d |

+ Duncan test (0.05) is to compare mean performances among salinity levels. Different letters represent a significant difference P≤ 0.05 between treatments.

**Table 2:** The effect of salt stress on chlorophyll a, b and total in leaves of dill

| NaCl Treatments (mM) | Chlorophyll a ( mg/g FW) | Chlorophyll b (mg/g FW) | Total Chlorophyll mg/g FW)) |
|---|---|---|---|
| 0 | 1.57 a | 1.07 a | 2.71 a |
| 25 | 1.45 b | 0.96 a | 2.23 b |
| 50 | 1.26 c | 0.79 b | 2.15 b |
| 75 | 1.11 d | 0.68 b | 1.79 c |
| 100 | 0.98 e | 0.56 c | 1.58 d |

+ Duncan test (0.05) is to compare mean performances among salinity levels. Different letters represent a significant difference P≤ 0.05 between treatments.

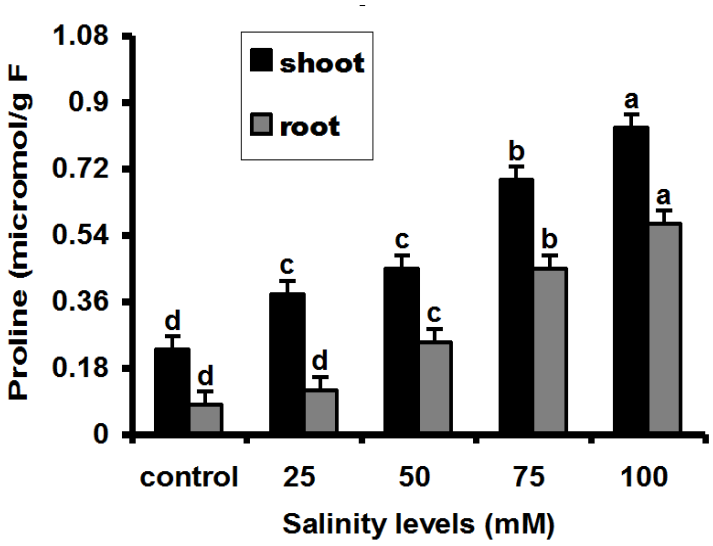


**Figure 1.** Effect of salt stress on proline content in shoots and roots of dill plantlets, Values are mean of three replicates + SD.

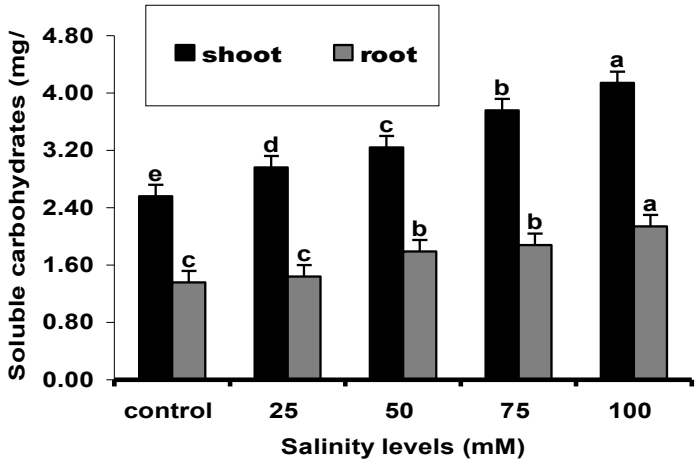


**Figure 2.** Effect of salt stress on soluble carbohydrates content in in shoots and roots of dill plantlets, Values are mean of three replicates + SD.

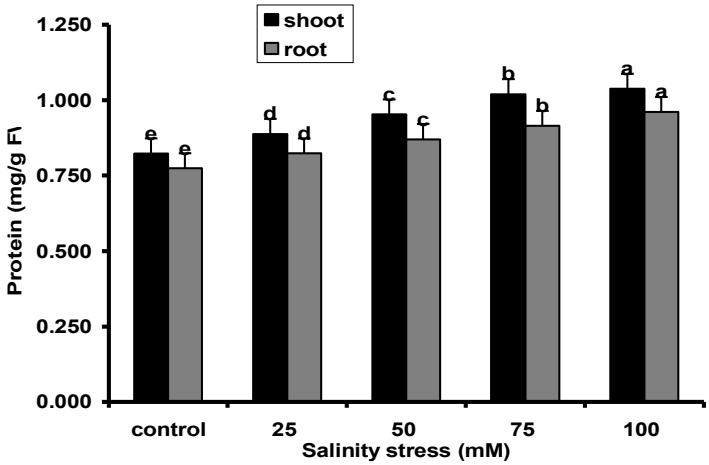


**Figure 3.** Effect of salt stress on soluble proteins content in in shoots and roots of dill plantlets, Values are mean of three replicates + SD.

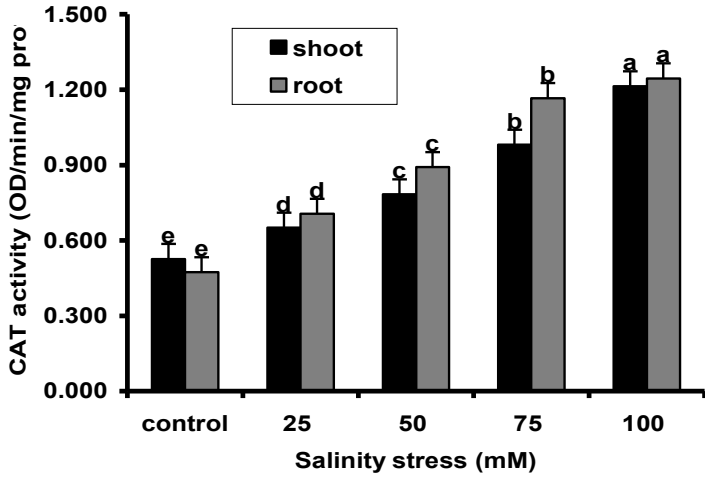


**Figure 4.** Effect of salt stress on catalase activity in in shoots and roots of dill plantlets, Values are mean of three replicates + SD.

## CONCLUSION

Our results indicated that dill seedlings can tolerate in saline condition. High proline, carbohydrates and proteins concentration in tissues is probably for adjusting osmotic potential and better water uptake under salinity. In addition to role of compatible solutes in osmotic adjustments, antioxidant enzyme of CAT also play a role in salt tolerance. These mechanisms help plant to avoid tissue death and enable to continue its growth and development under saline conditions.

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