

*Full Length Research Paper*

# **Influence of salicylic acid on growth and some biochemical parameters in a C<sub>4</sub> plant (*Panicum miliaceum* L.) under saline conditions**

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**Salt stress is considered as a restricting factor for plant products. Therefore, many compounds were applied to minimize the harmful effects of this stress. In this study, millet plants (Isfahan cultivar) were sprayed with 0.1, 0.3, 0.5 and 1 mM salicylic acid and then treated with 0, 150, 225 and 325 mM NaCl. Our results show that in plants not sprayed with salicylic acid but treated with NaCl, shoot length, wet weight of shoot and root, dry weight of shoot and free proline content of root significantly decreased but malondialdehyde and free proline content of leaves significantly increased. But in plants pretreated with salicylic acid, reduction in these parameters were moderate and 0.1 and 0.3 mM salicylic acid had better effect on these parameter. Therefore, we conclude that some concentration of salicylic acid could decrease harmful effects of NaCl in this plant.**

**Key words:** Millet, NaCl, salicylic acid, proline, malondialdehyde (MDA).

## **INTRODUCTION**

Salicylic acid is an endogenous growth regulator with phenolic nature (Sakhabutdinova et al., 2003). The commercially available form of salicylic acid is acetyl salicylic acid (ASA) (Canakci and Munzuroğlu, 2007). SA acts as a potential, non-enzymatic antioxidant as well as a plant growth regulator and plays an important role in regulating some plant physiological processes (Noreen et al., 2009) such as stimulating adventitious organ, development, herbicidal effect and providing resistant biotic and abiotic stress (Canakci and Munzuroğlu, 2007; Hussein et al., 2007). Salinity is one of the major environmental factors that limits plant growth and productivity (Parida and Das, 2004). It was estimated that up to 20% of irrigated lands in the world are affected by different levels of salinity and sodium content (Mostafazadeh-Fard et al., 2007). Many crop species are sensitive to high concentration of salt and negative impacts on agricultural production (Zörb et al. 2004).

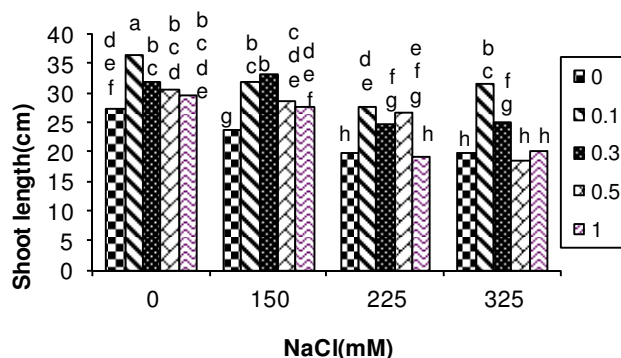
Photosynthesis and cell growth can be affected by salinity (Munnus et al., 2006). Salinity had inhibitory effect on cell division and enlargement in growth points (Maghsoudi and Maghsoudi, 2008). Plants that are exposed to salt stress accumulate damaging oxygen species, which can damage membrane lipids (Zörb et al., 2004). On the other hand, some components such as proline were produced in plants that were exposed to salt stress. Proline is free amino acids and widely occurs in higher plants that are exposed to salt stress. It is osmotically very active and contributes to membrane stability and reduces the effects of NaCl on cell membrane disruption (Mansour, 1998; Paeviz and Satyawat, 2008). The present study was initiated to investigate the effect of salicylic acid in decreasing harmful effects of NaCl in millet plant and determine the optimum concentration of salicylic acid for best growth in this plant.

## **MATERIALS AND METHODS**

Seeds of proso millet (*Panicum miliaceum* L.) were obtained from conducted in greenhouse at Isfahan Payam Noor University. The

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**Abbreviations:** MDA, Malondialdehyde; SA, salicylic acid.



**Figure 1.** Effect of salicylic acid on shoot length of *P. miliaceum* under saline condition.

Isfahan Agricultural Research Center. The present study was mean temperature was about 25°C in 12 h/8 h light/dark. Surface of seeds were sterilized in 0.5% sodium hypochlorite solution for 20 min and were sown in pots containing perlite. Plants were irrigated with distilled water and Long Ashton nutrient solution. After 30 days, plants were sprayed with 0, 0.1, 0.3, 0.5 and 1 mM salicylic acid four times with two day intervals. Control plants were sprayed with distilled water and then treated with 0, 150, 225 and 325 mM NaCl for 20 days.

#### Shoot length, dry and fresh weight measurement

The shoot and root fresh weight were weighted and shoot lengths were measured with ruler. The samples were dried in oven at 70°C for 42 h, and the dry weights were determined.

#### Proline measurement

To determine free proline level, wet leaf samples from each treatment were homogenized in 3% sulpho salicylic acid and centrifuged in 4°C for 10 min at 5000 rpm/min with 2 ml upper aqueous phase, 2 ml acid ninhydrin and 2 ml of glacial acid added, and the reaction mixture was incubated at 100°C for 1 h. The reaction mixture was placed on ice and extracted with 4 ml toluene. Proline content was measured by spectrophotometer and absorbance was read at 520 nm (Bates et al., 1973).

#### Lipid peroxidation

Lipid peroxidation was determined by estimating the MDA content in 1 g leaf fresh weight according to Heath and Packer (1969). The concentration of MDA was calculated from the absorbance at 532 nm (correction was done by subtracting the absorbance at 600 nm for non-specific turbidity) by using extinction coefficient of 155 m<sup>1</sup>cm<sup>-1</sup>.

#### Statistical analysis

This research was performed on randomized factorial design and data analyses were done by SAS and MSTATC computer programs and comparison of the means was performed by Duncan test. The result of the experiment was reported with means of three repeat

from each group (n = 3) and comparisons with P≤0.05 were considered significantly different and figures were drawn with Microsoft Excel.

## RESULTS

### Shoot length

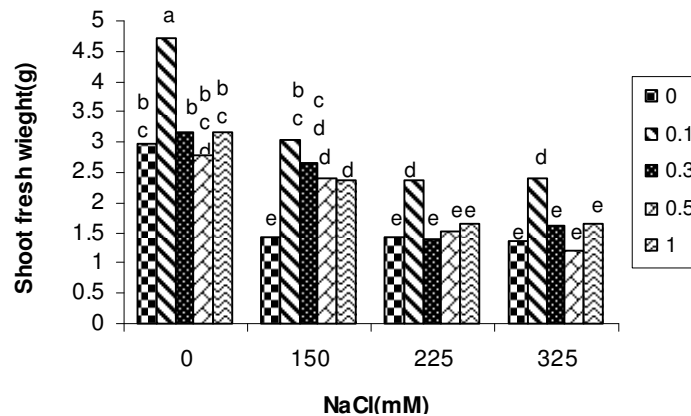
Shoot length was increased in control plants that were pretreated with SA. Shoot length significantly (P≤0.05) decreased in all levels of salinity as compared to the control plants. Shoot length was significantly increased when plants were sprayed with 0.1 and 0.3 mM salicylic acid when treated with NaCl. But minimum shoot length was observed when plant was pretreated with 1 mM SA and then treated with 225 and 325 mM NaCl (Figure 1).

### Shoot fresh weight

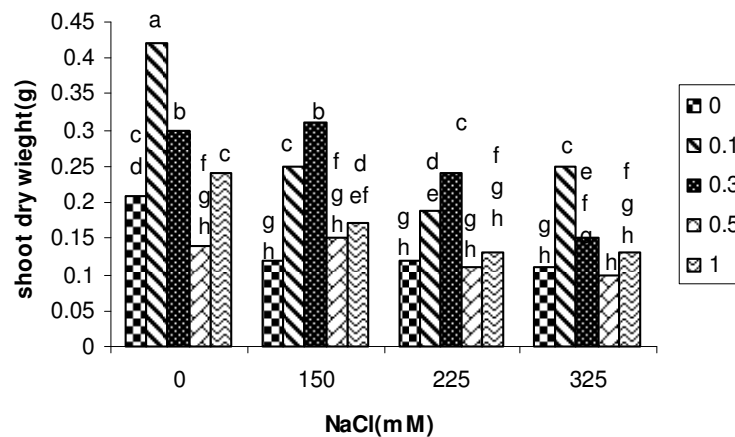
Shoot fresh weight increased in the control plants pretreated with 0.1 mM SA. The result shows that in all salinity treatments, shoot fresh weight decreased. The best concentration of SA was seen in 0.1 mM but significantly (P≤0.05) increased shoot fresh weight in three levels of salinity. Application of 0.3, 0.5 and 1 mM SA significantly increased shoot fresh weight in plants treated with 150 mM NaCl (Figure 2).

### Shoot dry weight

Application of 0.1, 0.3 mM SA increased shoot dry weight in control plants. In plants treated with NaCl, shoot dry weight significantly reduced. In plants pretreated with 0.1 mM SA and treated with NaCl, shoot dry weight significantly increased. Maximum shoot dry weight was observed in plants sprayed with 0.3 mM SA and treated with 150 mM NaCl (Figure 3).



**Figure 2.** Effect of salicylic acid on shoot fresh weight of *P. miliaceum* under saline condition.



**Figure 3.** Effect of salicylic acid on shoot dry weight of *P. miliaceum* under saline condition.

### Root fresh weight

Application of 0.1, 0.3 and 1 mM SA increased root fresh weight in control plants. Root fresh weight significantly decreased when plants were treated with 150 mM NaCl. When plants were sprayed with 0.1 mM SA and then treated with different concentration of NaCl, root fresh weight significantly increased and 0.3 mM SA significantly increased root fresh weight in plants which were treated with 150 and 225 mM NaCl (Figure 4).

### Root dry weight

Application of SA increased root dry weight in the control plants. Different concentrations of salt stress did not have significant effect on root dry weight. In plants pretreated with 0.1 and 0.3 mM SA and treated with NaCl, root dry

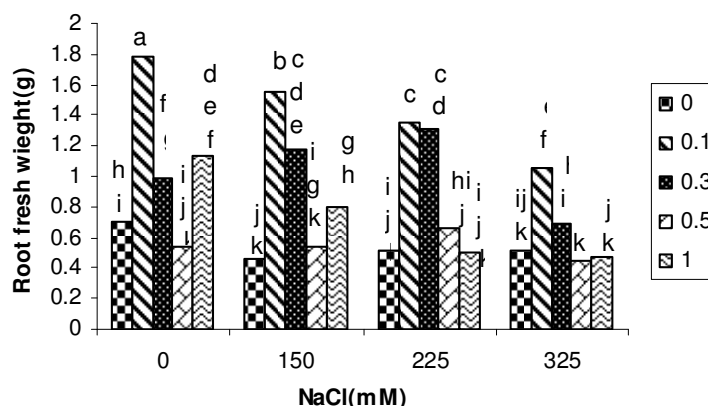
weight significantly increased (Figure 5).

### Shoot proline content

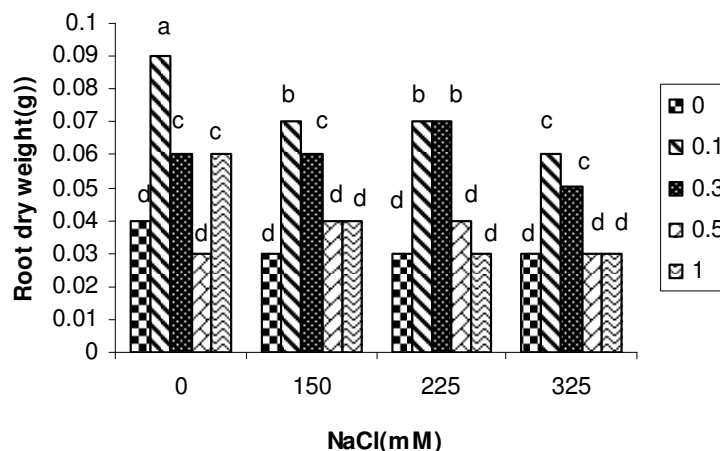
Proline content was measured in leaf and root tissue. In the control plants pretreated with SA, shoot proline content significantly increased. In plants treated with NaCl, leaf proline content significantly increased, also in plants pretreated with 0.1 and 0.3 mM SA and treated with NaCl, leaf proline content significantly increased. Minimum content of leaf proline was recorded in plants sprayed with 0.5 mM SA and treated with NaCl (Figure 6).

### Root proline content

In the control plants pretreated with 0.1, 0.3 and 0.5 mM SA, root proline content significantly increased. Root



**Figure 4.** Effect of salicylic acid on root fresh weight of *P. miliaceum* under saline condition.



**Figure 5.** Effect of salicylic acid on root dry weight of *P. miliaceum* under saline condition.

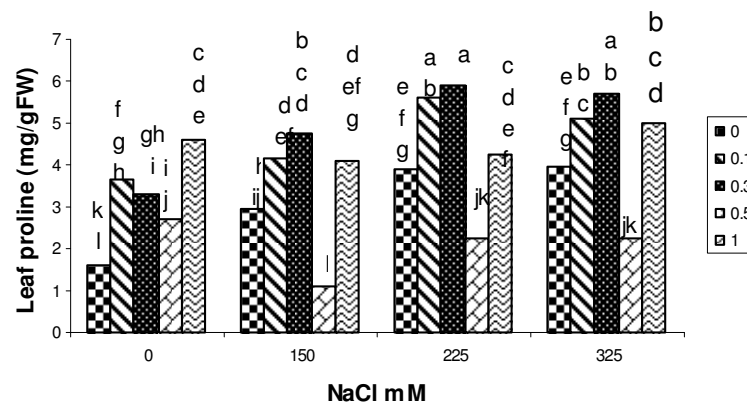
proline content significantly reduced in plants treated with 150 and 225 mM NaCl. In plants pretreated with 0.1, 0.3, 0.5 and 1 mM SA and then treated with NaCl, proline content significantly increased but in plants pretreated with 1 mM SA and then treated with 325 mM NaCl, root proline content significantly decreased (Figure 7).

### Lipid peroxidation

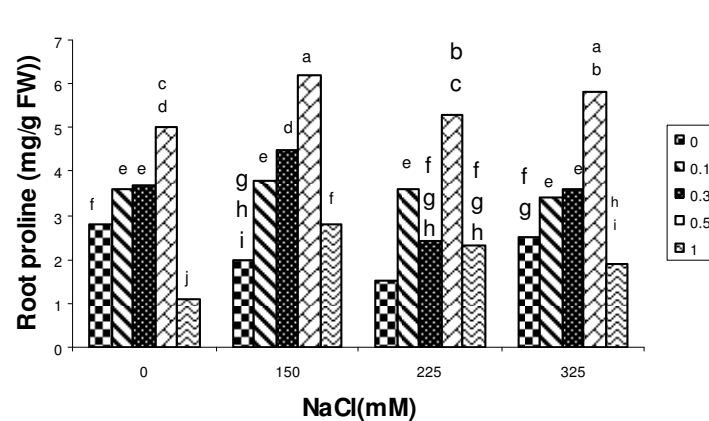
Salt stress did not have any effect on MDA in the control plants but MDA content significantly increased in plant treated with 325 mM NaCl. In plants sprayed with 0.1 mM SA and treated with different concentration of NaCl, MDA content significantly decreased and plants pretreated with 0.3, 0.5 and 1 mM SA and treated with 325 mM NaCl, MDA content significantly decreased (Figure 8).

### DISCUSSION

Shoot and root length are the most important parameters for salt stress because roots have direct contact with soil and absorb water from soil and supply to leaves in plant. Therefore, shoot and root length play important role in response to salt stress (Jamil and Rha, 2004). This study shows that shoot length, fresh and dry weight of shoot and fresh weight of root significantly decreased by salinity. It is similar to the results reported by Jamil et al. (2007). They observed that the root and shoot length, dry and fresh weight was significantly inhibited in cabbage (*Brassica oleracea capitata* L.) treated with salinity, also Tavali et al. (2009) indicated that in *Hordeum vulgare* and *Hordeum bulbosum*, shoot and root length was reduced by increasing salinity levels. But our results show that different concentration of salt stress did not have any



**Figure 6.** Effect of salicylic acid on leaf proline content of *P. miliaceum* under saline condition.

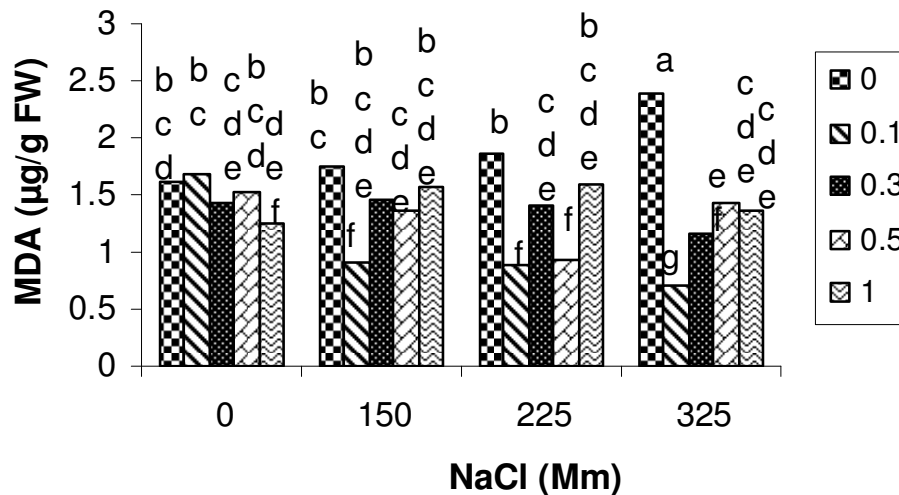


**Figure 7.** Effect of salicylic acid on root proline content of *P. miliaceum* under saline condition.

effect on root dry weight. These results are similar to reports about *Halopyrum mucronatum* (Prida and DAS, 2004). Expressed high salinity may inhibit root and shoot elongation and decrease dry and fresh weight in plant due to slowing down of the water uptake and decrease of osmotic potential by stress (Jamil et al., 2007). The thickening of cell wall and inhabitation of cell elongation are the most common effects which are important reasons for reduction in growth and development of shoot and root in salt stress. Reports have shown that salt stress have inhibitory effects on cell wall architecture (Shingh and Prasad, 2009). Cell wall associated with hydrolyses ( $\alpha$ -galactosidase,  $\beta$ -galactosidase,  $\alpha$ -glucosidase and acid phosphatase) has important role in cell elongation and growth. Salt stress inhibited the effects of some hydrolyses and decreased their activities. The growth of plants depends on the continuous growth and development of cell which require degradation of cell wall polysaccharides by the action of cell wall hydrolases

(Shingh and Prasad, 2009). Another response is due to reduced cell division and decreased elongation and growth in root and shoot under salinity, therefore, the root and shoot length, dry and fresh weight can decrease in salt stress. Our findings showed that morphological parameters decreased due to salinity stress but plants pretreated with SA improved in some parameters (Ashraf and Waheed, 1993).

Proline accumulation in response to environmental stresses had been considered by a number of researchers as an adaptive trait concerned with stress tolerance. It is generally assumed that proline is acting as a compatible solute in osmotic adjustment (Larher et al., 1993). It may act as an enzyme protectant, stabilize membranes and cellular structures during hostile conditions and detoxify free radicals (Larher et al., 1993). Proline is generally assumed to serve as a physiologically compatible solute that is increased to maintain osmotic potential in cell (Pollard and Wyn, 1979). This study



**Figure 8.** Effect of salicylic acid on MDA of *P. miliaceum* under saline condition.

showed that free proline content was significantly increased in leaves; our result are similar to the findings of Hoseini et al. (2010) with Cyprus.cv (*Carthamus tinctorius* L.) and Najafi et al. (2006) with Pea (*Pisum sativum* L.). Also, our results show that free proline content decreased in roots of plants exposed to 150 and 225 mM NaCl. This is similar to the result reported by Amini et al. (2005) which indicated that proline content in stem and leaf was higher than that in root in the two tomato cultivars under salt stress.

Higher level of proline content in stem and leaf maybe due to expression of genes encoding enzymes of proline synthesis such as pyrroline-5-carboxylate or decrease in enzymes of proline oxidative such as proline dehydrogenase which is controlled by osmotic and salinity stress (Amini and Ehsanpour, 2005). They indicated that in the two tomato cultivars that were exposed to salt stress, proline content in stem or leaf was higher than that in root.

Peroxidation of membrane lipids is an indication of membrane damage and leakage in stress conditions. This experiment showed that MDA level increased in salt stress Koca et al. (2007). Our result is similar to the findings of Tajdost et al. (2007) in maize. Other results showed that in four varieties of rice, MDA was increased in salt-sensitive varieties. MDA produced by lipid peroxidation of cell membrane is often used as an indicator of salt and oxidative damages and the levels of free radicals in living cells (Chutipaijit et al., 2009).

In this experiment when plants were sprayed with SA, there was significant decreased level of lipid peroxidation which is comparable to plants that were not sprayed with SA and treated with NaCl. SA increased amount of antioxidative enzymes activities by reducing  $H_2O_2$  level and decreasing membrane damages (Tajdost et al.,

2007). This experiment showed that proso millet ( $C_4$ ) was salt-sensitive and some morphological and biochemical parameters were decreased in salt stress.

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