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Original Article

Effects of Salinity Stress on Growth and antioxidant enzymes of the Halophyte Sesuvium portulacastrum

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Abstract

The present investigation was made to study the effect of different concentrations of sodium chloride on growth, and antioxidant enzymes of the seedlings of Sesuvium portulacastrum. The plant could survive a wide range of 100-1000 mM NaCl concentrations. The upper limits for the survival of the seedlings were up to 600 mM NaCl. The highest numbers of leaves, leaf area, shoot length, root length, fresh and dry weight were recorded at 600 mM NaCl concentration. Beyond 600 mM NaCl, the growth parameters reduced drastically. The antioxidant enzymes such as catalase (CAT), peroxidase (POX) polyphenol oxidase (PPO) and superoxide dismutase (SOD) increased up to the optimum level of 600mM NaCl concentration and beyond these levels the contents decreased marginally.

Key words: NaCl, salinity, halophytes, antioxidant enzymes, Sesuvium portulacastrum.

INTRODUCTION

Salinity is a major adverse environmental constraint to plant productivity, limiting the utilization of about 800 million ha of agricultural land globally (Yang et al., 2011; Zhang et al., 2011a). As estimated, 80,000,000 ha of cultivated land are affected by soil salinity, which corresponds to 5% of all cultivated land (Askaril et al., 2006). High levels of soil salinity can cause water deficit, ion toxicity, and nutrient deficiency leading to molecular damage and even plant death (Maggio et al., 2010).

Salt induces osmotic stress by limiting absorption of water from soil, and ionic stress resulting from high concentrations of potentially toxic salt ions within plant cells. Plants have evolved a variety of protective mechanisms to allow with these unfavorable environmental conditions for survival and growth including the accumulation of ions and osmolytes such as proline. The accumulation of these compounds prevents water loss and ion toxicity. The alleviation of oxidative damage and increased resistance to salinity and other environmental stresses are often correlated with an efficient antioxidative system (Manivannan et al., 2007).

Salinization of agricultural soils is a worldwide concern, especially in irrigated lands. Saline soil is characterized by the presence of toxic levels of sodium and its chlorides and sulphates.

Salinity tolerance is defined as the ability of plants to continuously grow under salt stress conditions (Munns, 2002). Another major factor of salt tolerance mechanisms is the ability of plant cells to adjust osmotically and to accumulate organic solutes (proteins, sugar, amino acids, etc.). The accumulation of these compounds is not only important for cell osmoregulation but also for the protection of subcellular structure (Munns, 2002) and maintenance of protein structures. Many of the physiological adaptations of plants to saline conditions are similar to the adaptations shown by plants to desiccation stress and it has been suggested that plants showing drought resistance would also exhibit salinity tolerance (Munns, 2002). However, in some halophytes, the salt tolerance mechanisms are not sufficient for tolerance of drought or frost (Ueda et al., 2003). Halophytes and other salt-tolerant plants may provide sensible alternatives for many developing countries (Squires and Ayoub, 1994).
The present study was made to investigate the effect of NaCl on growth and enzymes of *Sesuvium portulacastrum* and optimum salt tolerance of this species.

**MATERIALS AND METHODS**

*Sesuvium portulacastrum* L. a fast growing herbaceous, perennial dicotomous, halophyte species belonging to the family Aizoaceae was used for the present investigation. This species was naturally growing in abundance in salt marshes in the mangrove area of Pichavaram, on the east coast of Tamil Nadu, India about 10 km east of Annamalai University campus. *Sesuvium portulacastrum* was propagated by cutting, three centimeter long stem cuttings with one node and two opposite leaves were taken from mother plants. Healthy cuttings with uniform size were planted individually in polythene bags (7" x 5"). The polythene bags filled with homogenous mixture of garden soil containing red earth, sand and farmyard manure (1:2:1). The cuttings were irrigated with tap water and allowed to establish well. After establishment in polythene bags for 30 days about 300 healthy plants were subjected to saline treatment. The treatment constituted control (0), 200, 400, 600, 800 and 1000 mM NaCl concentration. Salt solutions were prepared with NaCl (Laboratory Grade, Glaxo Laboratories, India). The treatments were continued until the plants received the required concentrations of the salt, after this all the plants were irrigated with tap water. The experimental yard was roofed with transparent polythene sheet at the height of 3 m from the ground in order to protect the plants from rain. Sampling for various studies was taken on the 60th day after NaCl treatment.

**Shoot length** (cm plant⁻¹).

Plant height was recorded by measuring the height of the plant from the surface of the soil to the tip of the top most leaf. This was recorded on 60th days after treatment and expressed in cm plant⁻¹.

**Root length** (cm plant⁻¹)

The root length was measured from the point of first cotyledonary node to the tip of longest root and expressed in cm plant⁻¹.

**Total leaf area**

The total leaf area was calculated by measuring the length and width and number of leaves and multiplied by a correlation factor (0.66) derived from the method of Yoshida *et al.* (1972). Leaf area (cm²) = L x W x 0.66.

**Fresh weight and dry weight**

For the Estimation of fresh weight leaf, stem and root portions were separated and weighted. They were dried in a hot air oven at 80°C for 24 hours. Then, the dry weight was taken by using an electronic balance.

**Estimation of catalase** (EC 1.1.1.6)

Catalase activity was assayed as described by Chandlee and scandalios (1984). 500 mg of frozen material was homogenized in 5ml of ice cold 50 mM PMSF (Phenyl methyl sulfonyl fluoride). The extract was centrifuged at 4°C for 20 min at 12500 rpm. The supernatant was used for enzyme assay. The activity of enzyme catalase was measured using the method of Chandlee *et al.* (1984) with modification. The assay mixture contained 2.6 ml of 50 mM potassium phosphate buffer (pH 7.0), 0.4 ml of 15 mM H₂O₂ and 0.04 ml of enzyme extract. The decomposition of H₂O₂ was followed by the decline absorbance at 240 nm. The enzyme activity was expressed in units per min per mg protein.

**Estimation of peroxidase** (donor: hydrogen peroxide oxidoreductase; (EC. 1.11.17)

Peroxidase activity was assayed by the method of kumar and khan (1982). Assay mixture of peroxidase contained 2ml of 0.1 M phosphate buffer (pH 6.8), 1ml of 0.001M pyrogallol, and 1ml of 0.005M hydrogen peroxide and 0.5ml of enzyme extract. The reaction mixture was incubated for 5 minutes at 25°C, after which the reaction was terminated by adding 1ml of 2.5 N sulphuric acids. The amount of purpurogallin formed was determined by reading the absorbance at 420 nm against a blank prepared by adding the extract after the addition of 2.5 N sulphuric acids at zero time. The activity was expressed in unit per minute per mg protein.

**Estimation of polyphenoloxidase** (O-Diphenol: O₂ oxido-reductase, EC. 1.10.3.1)

Polyphenol oxidase activity was assayed by kumar and khan (1982). Assay mixture for polyphenol oxidase contained 2 ml of 0.1M phosphate buffer pH (6.0), 1ml of 0.1M catechol and 0.5 ml of enzyme extract. This was incubated for 5 minutes at 25°C, after which the reaction was stopped by adding 1ml of 2.5N sulphuric acid. The absorbance of the purpurogallin formed was recorded at 495 nm. The enzyme activity was expressed in units. One unit is defined as the amount of purpurogallin formed, which raised the absorbance by 0.1 per minute under the assay condition.

**Estimation of Superoxide dismutase** (SOD) (EC 1.5.1.1)

Superoxide dismutase activity was assayed as described by Beauchamp and Fridovich (1971). The reaction mixture contained 1.17M X 10-6M riboflavin, 0.1M methionine, 2H M x 10-5m potassium cyanide and 5.6H M x 10-5M nitroblue tetra-zolium salt (NBT) dissolved in 3ml of 0.05M sodium phosphate buffer (pH 7.8). Three ml of the reaction medium was added to 1ml of enzyme extract. The mixtures were illuminated in glass test tubes of selected uniform thickness. The illumination was performed by two sets of Philips 40W fluorescent tubes. The test tubes were arranged in a single row, with a set of tube lights fixed on either side. Illumination was started to initiate the reaction at 30°C for an hour. Identical solutions were kept under dark served as blanks. The absorbance was read at 560nm in the Spectrophotometer against the blank. Superoxide dismutase activity was expressed in units. One unit is defined as the amount of change in the absorbance by 0.1 per hour per mg protein.
Table 1: Effect of NaCl on shoot length, root length (cm plant⁻¹), number of leaves (plant⁻¹), leaf area (cm² plant⁻¹) and whole plant fresh weight and dry weight (g plant⁻¹) of Sesuvium portulacastrum on 60th day after treatment.

<table>
<thead>
<tr>
<th>Concentration (mM)</th>
<th>Shoot length</th>
<th>Root length</th>
<th>Total no. of leaf</th>
<th>Leaf area</th>
<th>Fresh weight</th>
<th>Dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>23.4</td>
<td>19.6</td>
<td>72</td>
<td>86.46</td>
<td>41.45</td>
<td>1.836</td>
</tr>
<tr>
<td>200</td>
<td>30.7±2.14</td>
<td>27.4±1.64</td>
<td>91±5.46</td>
<td>1.26±6.15</td>
<td>52.46±3.4</td>
<td>2.549±0.15</td>
</tr>
<tr>
<td>400</td>
<td>42.4±2.96</td>
<td>39.7±2.77</td>
<td>105±7.35</td>
<td>127.8±8.94</td>
<td>63.41±3.80</td>
<td>2.935±0.20</td>
</tr>
<tr>
<td>600</td>
<td>61.5±4.30</td>
<td>53.7±3.75</td>
<td>132±9.27</td>
<td>165.3±11.57</td>
<td>74.43±5.21</td>
<td>3.134±0.25</td>
</tr>
<tr>
<td>800</td>
<td>45.3±3.62</td>
<td>38.4±3.07</td>
<td>110±8.80</td>
<td>133.9±10.71</td>
<td>59.45±4.75</td>
<td>2.565±0.20</td>
</tr>
<tr>
<td>1000</td>
<td>22.4±1.79</td>
<td>18.4±1.42</td>
<td>86±6.88</td>
<td>89.47±7.15</td>
<td>38.39±3.07</td>
<td>1.106±0.08</td>
</tr>
</tbody>
</table>

+ Denote Standard deviation
Figures in parentheses are percentage increase\ decrease over control.

Table 2: Effect of NaCl on Catalase, peroxidase, polyphenol oxidase and superoxide dismutase in the leaves of Sesuvium potulacastrum on 60th day after treatment.

<table>
<thead>
<tr>
<th>Concentration (mm)</th>
<th>Catalase</th>
<th>Peroxidase</th>
<th>Polyphenol oxidase</th>
<th>Superoxide dismutase</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>2.426</td>
<td>1.116</td>
<td>1.626</td>
<td>1.826</td>
</tr>
<tr>
<td>200</td>
<td>2.939±0.17</td>
<td>1.726±0.10</td>
<td>2.015±0.12</td>
<td>2.679±46.17</td>
</tr>
<tr>
<td>400</td>
<td>3.260±0.22</td>
<td>2.226±0.13</td>
<td>2.765±0.19</td>
<td>2.115±15.82</td>
</tr>
<tr>
<td>600</td>
<td>4.016±0.32</td>
<td>2.996±0.20</td>
<td>2.997±0.26</td>
<td>3.127±71.24</td>
</tr>
<tr>
<td>800</td>
<td>3.105±0.24</td>
<td>1.711±0.13</td>
<td>1.427±0.11</td>
<td>1.786±52.19</td>
</tr>
<tr>
<td>1000</td>
<td>2.162±0.17</td>
<td>1.015±0.08</td>
<td>1.015±0.08</td>
<td>1.324±27.49</td>
</tr>
</tbody>
</table>

+ Denote Standard deviation
Figures in parentheses are percentage increase\ decrease over control.

- **protein under assay condition.**

**Statistical analysis**

Each treatment was analyzed with at least five replicates and a standard deviation (SD) was calculated; data are expressed in mean ± SD of five replicates.

- **RESULTS**

**Number of leaves**

The result on the effect of NaCl on the number of leaves per plant is given in Table (1). NaCl had increased the number of leaves with the increasing concentrations up to 600mM. At higher concentrations, a gradual reduction in number of leaves was noticed.

**Leaf area**

The effect of different concentrations of NaCl on the leaf area per plant is presented in Table (1). The maximum increase in leaf area was observed on the 600mM NaCl. In the optimum concentration, the calculated leaf area was high when compared to that of control. Beyond this optimum concentration, there was a gradual reduction in leaf area was noticed.

**Fresh and dry weight**

The results on the effect of NaCl on the fresh and dry weight are presented in Table (1). The fresh weight of leaf, stem and root increased with increasing salinity upto 600 mM NaCl and there after it had decreased. The highest increase in fresh and dry weight was recorded at 600 mM NaCl on 60th day samples.

**Effect of salinity on catalase activity**

The effect of NaCl on the catalase activity in the leaves at various NaCl concentrations is presented in Table (2). There was a steady increase in the enzyme activity with increasing salinity upto 600mM in Sesuvium portulacastrum. At higher concentrations, the enzyme activity decreased.

**Effect of salinity on peroxidase activity**

The peroxidase activity showed a similar increasing trend as that of catalases upto the optimum level of NaCl salinity and data are given in Table (2). The highest activity was recorded in Sesuvium portulacastrum at the optimum level (600mM NaCl). Even at the extreme salinity the peroxidase activity was equal to that of control.
Effect of salinity on polyphenol oxidase activity

The sodium chloride salinity enhanced the PPO activity upto the optimum level of 600mM in Sesuvium portulacastrum and the data are given in Table (2). Beyond, the optimum level, the PPO activity reduced gradually.

Effect of Salinity on Superoxide dismutase

The effect of NaCl on the superoxide dismutase activity in the leaves at various NaCl concentrations is given in Table (2). There was a steady increase in the SOD activity up to 600mM NaCl. At higher concentrations the enzyme activity declined gradually. The maximum SOD activity was observed at 600mM NaCl on 60th day.

DISCUSSION

The NaCl had stimulatory effect on leaf production up to the optimum concentration. However, the leaf number decreased at higher concentrations. The decline in leaf number at high concentrations was due to the leaf fall because of ageing. Salinity has been shown to be one of the external factors that influence the process of senescence and the consequent shedding of leaves (Pool et al., 1975). The number of leaves reduced only at the highest salt concentration but the dead leaves were increased with salinity as a mean of protecting the young growing leaves to toxic levels of the salts as well as off-loading the plants of excess salts (Wahome, 2001).

In the present study, NaCl increased the leaf area considerable at the optimum concentration. The increase in the leaf area could be due to the increase in the volume of mesophyll cells due to increase in water content of the leaves and increase in succulence. The external NaCl stimulated the leaf area at optimum level of seawater in Rhizophora mangle (Hwang and Chen, 1995). Salinity has been reported to promote succulence in several plant species viz., Plantago maritima (Flanagan and Jefferies, 1988) and Sesuvium portulacastrum (Venkatesalu and Chellappan, 1993). Succulence enables the dilution of internal ion content. It has been related with the performance of eu-halophytes like Suaeda fruticosa (Khan et al., 2000) and Salvadoria persica (Maggio et al., 2003).

Sodium chloride salinity increased the fresh weight of leaf, stem and root with increasing salinity upto the optimum concentrations of 600 mM in S. portulacastrum. At higher concentrations, the fresh weight of leaf, stem and root was reduced. The increase in fresh weight of the leaf tissues can be attributed to the increase in leaf thickness and the accumulation of ions and water in the tissues (Khan et al., 2005). The NaCl salinity had also increased the dry weight of the three tissues with increasing concentrations of salts upto optimum level. In the dicotyledonous halophytes, it has been reported that Na+ and Cl- ions were 30-50% of the dry weight (Flowers et al., 1986). The dry weight increase could be attributed to the accumulation of inorganic salts and organic matter in the plant tissues. The gradual decrease in fresh weight and dry weight at higher salinity levels, was evident in the present study has also been reported (Kingsbury et al 1976).

Catalase activity increased with increasing salinity up to optimum concentration of 600 mM in S. portulacastrum. Enhanced activity of catalase was reported to be essential for the survival of the halophytes, Halimions portulacoides in natural saline habitats (Kalir and Poljak off-Mayber, 1981). The catalase activity increased with increasing concentration of NaCl upto optimum level in Ipomoea pes-caprae (Venkatesan and Chellappan, 1999). The catalase activity decreased with increasing concentration in Phaseolus radiatus (Saha and Gupta, 1999).

Peroxidase activity increased with increasing salinity up to optimum concentration of 600 mM in S. portulacastrum. Increase in peroxidase activity indicated the formation of large amount of H2O2 and which could release enzyme from membrane structure (Zhang and Kirkham, 1994). Peroxidase is a scavenging enzyme which removes the toxic oxygen radicles from the cells. Significant increase in the peroxidase activity in the halophytes such as Aegiceras corniculatum (Manikandan and Venkatesan, 2004), in the salt tolerant varieties of spinach leaves (Oztirik and Demir, 2003) and Xanthosoma sagittifolium (Kamemge and Omokoto, 2003). Peroxidase activity was inhibited under NaCl and CaCl2 stress in Arachis hypogea (Satakopan et al., 1990). The increased peroxidase activity was mainly due to increased enzyme synthesis and might be useful for adaptation under conditions requiring prevention of peroxidation of membrane lipids (Kalir et al., 1984).

Polyphenol oxidase activity increased with increasing salinity up to optimum concentration of 600 mM in S. portulacastrum. Increased polyphenol oxidase activity has been reported in halophytes such as Aegiceras corniculatum (Manikandan and Venkatesan, 2004). High polyphenol oxidase activity under stress indicates its ability to oxidize and to degrade the toxic substances such as phenolic compounds which are generally reported to be accumulated during salt stress (Subhashini and Reddy, 1990). Sharp increase in polyphenol oxidase activity under salinity stress was associated with enhanced rooting in Excoecaria agallocha, Cynometra iripa and Heritiera fomes (Basak et al., 2000).

NaCl salinity enhanced the superoxide dismutase activity up to 600mM in S. portulacastrum and at higher concentrations the enzyme activity was reduced. Takemura et al. (2000) showed that this enzyme (SOD) retained full activity at least up to seawater salt levels. These enzymes differ in their response from the leaves of the secretor mangrove. Superoxide dismutase is controlled by the salt, while catalase seems to respond to the osmoticum regard less of its chemical nature. There is no doubt that exposure to high salinity incurs water stress, which has been demonstrated to elicit different antioxidative defenses in plants, invariably.
including superoxide dismutase, ascorbate peroxidase and catalase (Larson, 1995; Gosset et al., 1996). Plants with high antioxidant enzyme activities are generally more tolerant to various environmental stresses than those with low enzyme activates.

**REFERENCE**


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