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REGULAR ARTICLE

RESPONSE OF ANTIOXIDANT METABOLISM TO NaCl STRESS IN THE HALOPHYTE *SALICORNIA BRACHIATA* ROXB.

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SUMMARY

The present study was conducted to determine how salinity affects growth and enzymatic activity in shoot and root organs of *Salicornia brachiata* Roxb. The seedlings were treated with various concentration of exogenous addition of NaCl ranging from 0 to 700mM was added. The 700mM NaCl treatment significantly reduced the growth rate. The shoot and root length, fresh and dry weight increased significantly upto 400mM NaCl and thereafter drastically reduced. The antioxidant enzyme such as catalase (CAT), peroxidase (PO), polyphenoloxidase (PPO), superoxide dismutase (SOD) activity increased upto 400mM NaCl. Beyond this level all the enzyme activity gradually reduced. The increase in the enzyme activity was probably due to the direct influence of salt stress on enzyme synthesis and the induction of enzyme synthesis by salt stress may play a role in the salt tolerance.

Keywords: Growth, catalses, peroxidase, polyphenoloxidase, Superoxide dismutase

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1. Introduction

Soil salinity problems in agriculture are world-wide being a major limiting factor on crop productivity. The restriction to plant growth and productivity originated by salinity is especially acute in arid and semi-arid region [1]. Even under normal condition, relative oxygen species such as superoxide radical (O_2-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH) and singlet oxygen (O_2) are metabolic by products of plant cells. Much of the injury to plants imposed by stress exposure is associated with oxidative damage at the cellular level [2]. There is increasing evidence that NaCl salinity is one factor leading to oxidative stress in plant cells [3].

CAT catalyses to H_2O_2 to water and O_2 which does not need a reducing substrate for its activity [4]. CAT has been found predominantly in leaf provision where it functions chiefly to remove H_2O_2 formed in photorespiration. Several studies have demonstrated that salt tolerant species increased their antioxidant enzyme activities and antioxidant contents in response to salt treatment where as salt-sensitive species failed to do so [5]. On the other hand little is known about NaCl–dependent changes of antioxidant

system components in halophytes. A correlation between the antioxidant capacity and NaCl tolerance has been demonstrated innumerous plant species such as Cakile maritima [6] *Cassia angustifolia* [7] and *Suaeda salsa* [8].

Superoxide dismutase (SOD) can convert O_2- to H_2O_2. Other enzymatic and non-enzymatic reaction can contribute to H_2O_2 generation. H_2O_2 acts as precursor of more cytotoxic or highly reactive oxygen, derivatives, such as peroxynitrite or OH. So, it is very important to scavenger excess H_2O_2. Excess H_2O_2 can be removed using catalase (CAT) and ascorbate-glutathione [9]. The objectives of the present study are to examine the NaCl induced effect on certain antioxidant enzymes in the leaf tissue of a halophytic herb.

2. Materials and Methods

One month old healthy seedlings of *Salicornia brachiata*, 2 to 3 cm height were uprooted from the mangrove belt at Pichavaram, south east coast of India without damaging the root system. Seedlings were washed thoroughly and brought to the botanical garden of Annamalai University. Polythene sleeves (7″ × 5″) were filled with homogenous mixture of garden soil comprising of red earth, sand and farmyard manure in the ratio of 1:2:1. The plants were irrigated with tapwater and allowed to establish well, after well established plants were selected and kept in 8 treatments (0-700 mM). The treatment was continued for 10 days, the required millimolar NaCl concentration reached and then irrigated with tap water. The above 700mM NaCl could not survive a weak after salts treatment. So the experimental site maintains only upto 700mM. The samples were collected randomly at 90th and 180th sampling and the data were analysed by the method of complete randomized block design [10]. Growth was determined as fresh and dry masses. Dry weight was determined after drying the plant parts at 800C for 48 hours to a constant weight.

Enzyme assay

Catalase (H_2O_2:H_2O_2 oxidoreductase (EC 1.11.1.6)

Catalase activity was measured by the methods of [11]. One gram of shoot samples was homogenized in 10ml of 0.1M sodium phosphate buffer pH 7 and centrifuged 40C for 10 min. at 10,000 rpm. An aliquot of 1ml of the supernatant of the enzyme extract was added to the reaction mixture containing 1ml of 0.01M H_2O_2 and 3ml of 0.1 M sodium phosphate buffer. The reaction was stopped after an incubation of 5 min. at 200C by adding 10 ml of 1% H_2O_2. The acidified medium without or with the enzyme extract was titrated against 0.005 N KMNO4 and catalase activity was expressed as n moles of H_2O_2 destroyed per min per ml per gram fresh weight. A blank was prepared by adding the enzyme extract at zero time.

Peroxidase (Donor: Hydrogen peroxidant oxireductase (EC1.11.17)

Extraction:

One gram of fresh plant material was homogenized with 20ml of ice cold extraction medium containing 2 mM $MgCl_2$, 1 mM EDTA, 10 mM β-mercaptoethanol 7 per cent PVP and 10mM sodium metabisulphate. The homogenate was strained through two layers of cheese cloth and centrifuged at 10,000 rpm for 15 min. The supernatant was made upto 20ml with the same buffer and it was used as the source enzyme.

Assay

Peroxidase activity was assayed by the method of [12]. Assay mixture of peroxidase

contained 2ml of 0.1M phosphate buffer (pH 6.8). 1ml of 0.001 M pyrogallol, 1ml of 0.005 M hydrogen peroxide and 0.5 ml of enzyme extract. The solution was incubated for 5 min. at 250C, after which the reaction was terminated by adding 1 ml of 2.5N sulphuric acid. 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 acid. The activity was expressed in unit = 0.1 absorbance min-1 mg-1 protein.

Polyphenoloxidase: (O-diphenol: O_2 oxido-reductase, EC 1.10.3.1)

Polyphenoloxidase activity was assayed by the method of [12]. Assay mixture for polyphenoloxidase contained 2 ml of 0.1 M phosphate buffer (pH 6.0). 1ml of 0.1M catechol and 0.5 ml enzyme extract. This was incubated for 5 min. at 250C, after which reaction was stopped by adding 1 ml of 2.5 N 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 absorbency by 0.1 min-1 under the assay condition.

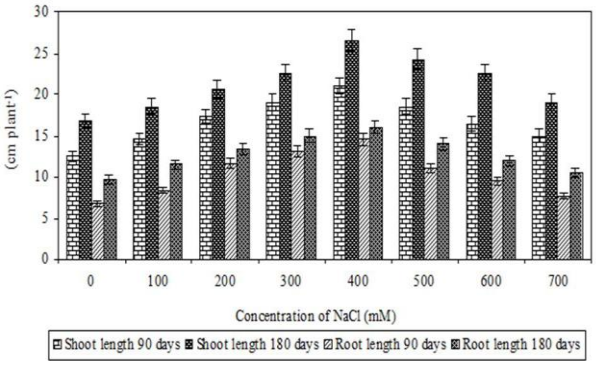
Superoxide dismutase: (EC.1.15.1.1)

SOD activity was determined as described by [13] with some modifications. Shoot tissue (1g) was ground to a fine powder in liquid nitrogen and then homogenized with 4ml 100 mM potassium phosphate (pH 7.8) containing 2mM ethylenediaminetetra acetic acid (EDTA) and 1% polyvinyl pyrrolidone (PVP) at 40C. The homogenate was filtered through four cheese cloth layers and centrifuged at 15, 000g for 10 min. The assays were performed at 250C in 3ml cuvette containing 100mM phosphate buffer (pH 7.8), 2mM EDTA, 0.015 mM ferricytochrome c and 0.5 mM xanthine. One unit of SOD activity was defined as the amount of enzyme that inhibited the ferricytochrome c reduction rate by 50%. It was measured by recording the absorbance at 550 nm.

3. Results and Disscussion

The maximum percentage was observed of value at 400mM the shoot length (68%), root length (113.23%) and in fresh weight of shoot and root (65.85% ; 57.89%) and in dry weight of shoot and root (170.49% ; 138.81%) higher when compared to control on 90th day respectively. The F values were significant at 1% and 5% level. In 700mM NaCl the growth rate was slightly higher when compared to control and the percentage of increases was observed in shoot and root length (20% and 11.76%); fresh weight of shoot and root (39.02 and 3.15%) and in dry weight of shoot and root (88.69 and 77.91%) on 90th day after salt treatment. (Fig 1a,1b,1c).

Fig. 1a. Effect of NaCl on shoot and root length (cm plant-1) of *Saclicornia brachiata* on 90th and 180th day after salt treatment (Mean ± SE, n=5) F values Shoot=9.16: 36.62 Root=73.38: 599.05 Significant at 5% level



The highest activity of catalase, peroxidase, polyphenoloxidase, acid phosphates and superoxide dismutase increased considerably upto 400mM NaCl and thereafter all the activites drastically reduced. The maximum percentage of the value recorded at 400mM

and this was CAT (63.33%); PO (28.21%); PPO (78.02%), Alkalin phosphatase (83.61%) and SOD (23.83%) higher when compared to that of control on 90th day respectively. The F values were significant at 5% level. The highest ATPase and NRase activity increased upto extreme level of 700mM NaCl and this was 83.09% and 76.92 % higher when compared to control on 90th day after salt treatment (Fig. 2a,2b,2c).

Fig. 1b. Effect of NaCl on fresh weight of shoot and root (g plant-1) of *Saclicornia brachiata* on 90th and 180th day after salt treatment (Mean ± SE, n=5) F values of Shoot = 66.94: 110.94 Root = 76.68:148.09 Significant at 5% level

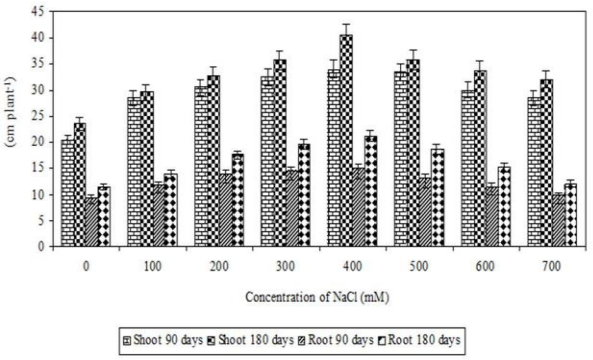
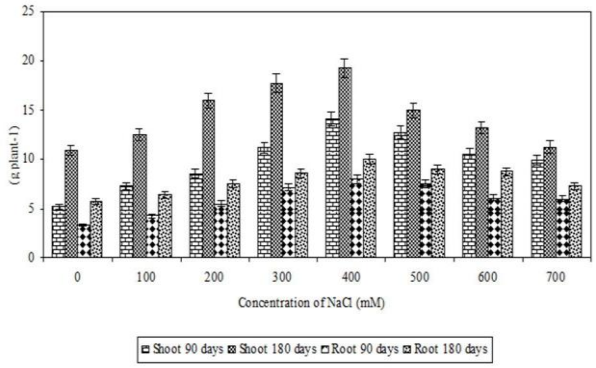


Fig. 1c. Effect of NaCl on dry weight of shoot and root (g plant-1) of *Saclicornia brachiata* on 90th and 180th day after salt treatment (Mean ± SE, n=5) F values Shoot = 20.29:73.29 Root = 64.47: 258.00 Significant at 5% level



The data also show that there is depressed in growth at high salinities. A stimulation of growth in response to moderate levels of NaCl salinity has been reported for several halophytes such as *Kandelia candel* [14], *Chenopodium quinoa* [15] and *Rhizophora apiculata* survives double the strength of sea water [16]. In relation to seedling growth, NaCl suppresses the cotyledons and the embryonic axis. They are smaller than in distilled water because of reduced fresh weight resulting from reduced water absorption. The fresh weight increase is largely attributed to cell enlargement by water absorption, cell vacuolation and turgor-driven wall expansion[17].

Fig. 2a. Effect of NaCl on catalase, peroxidase and polyphenol-oxidase in shoot of *Salicornia brachiata* (Mean ± SE, n=5) F values CAT = 11.014:55.436 PO= 42.43:386.88 PPO = 7.928: 53.51 Significant at 5% level

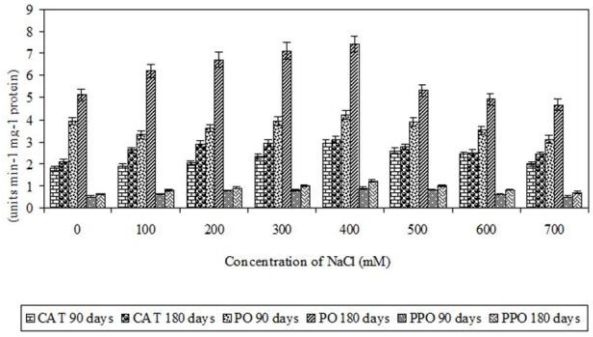
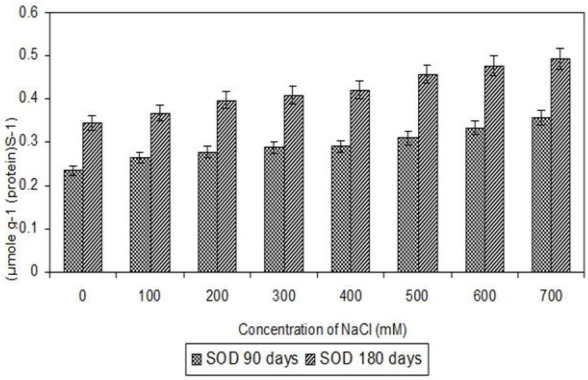


Fig. 2b. Effect of NaCl on SOD in shoot of *Salicornia brachiata* (Mean ± SE, n=5) F values of SOD = 57.68:544.00 Significant at 5% level



Many salt tolerant species are reported to reduced membrane damage by increasing enzymatic defenses against oxygen radicals [18]. Enhanced activity of catalase has been

reported to be essential for the survival of the halophytes, *Halimione portulacoides* in natural saline habitats [19]. Catalase reacts with H_2O_2 directly to form water and oxygen [18]. Decline in catalase activity with the progress of water stress has been reported in *Oryza sativa* [20] and in certain halophytes such as *Avicennia marina* [21] and in *Bruguiera gymnorrhiza* [22].

Increase in peroxidase activity indicates the formation of large amount of H_2O_2 and which can release enzyme from membrane structure [23]. Water stress can increase the accumulation of enzyme substrates such as ascorbate and glutathione which in turn are scavengers of activated oxygen species. High soluble peroxidase activity has been reported in plants and water stress condition [18]. Significant increase in the peroxidase activity in the halophytes such as *Halimione portulacoides [19], Suaeda nudiflora [24] and Aegiceras corniculatum* [25] in the salt tolerant varieties of *Pisum sativum* [26], salt tolerance of spinach leaves [27]. Increase in polyphenol oxidase activity along with peroxidase is generally reported in species under various environmental stresses [28]. Increased poly phenoloxidase activity under stress indicates the ability to oxidize and degrade the toxic substance such as phenolic compounds which are generally accumulated during salt stress.

An increases in SOD and a significant enhancement of APX activities, both of which occur at and/below 200mM NaCl, the activity of the other antioxidative enzymes diminished (CAT) or remain unchanged and then decreased at the highest NaCl concentration (400mM). SOD represents and important protective mechanism against possible NaCl – induced ROS production [3]. An interesting aspect is that the induction of SOD activity coincided with changes in the specific activity of POD and of the ASC-GSH cycle enzymes. Thus the expected increase in H_2O_2 as result of the SOD reaction was accompanied by an increased enzymatic capacity to decompose it. This was particularly clear in jerba plants, in which a greater and parallel increase in SOD, POD activities occurred under low or high salinity.

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