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Antioxidant enzymes activity in leaves of salt stressed *Excoecaria agallocha* L.

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ABSTRACT

Objective: To identify the effect of different concentrations of NaCl on some antioxidant enzyme activities of leaves of salt-stressed true mangrove. **Methods:** *Excoecaria agallocha* (*E. agallocha*) subjected to varying levels of NaCl. Salt stress was imposed on 60 days old plants with ten different concentrations of NaCl (0, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1 000 mM). **Results:** *E. ahallocha* seedlings survived up to 500 mM. Above 500 mM the seedlings did not survived. The leaves of 60 day old plants were used for the analysis of enzyme activities. NaCl stress enhanced the enzymatic antioxidants such as catalase, peroxidase, polyphenoloxidase and superoxide dismutase. The highest CAT, PPO activities in the leaves enhanced gradually up to 300 mM NaCl concentrations, the highest SOD, POX activities in the leaves were observed at NaCl concentrations of 400 mM (SOD), and 500 mM (POX) respectively. **Conclusion:** Increase in anti-oxidant enzyme activity could be a response to cellular damage induced by NaCl.

1. Introduction

Mangrove forests are widely distributed in the inter-tidal zones of the tropical and subtropical areas of the globe[1]. Soil salinity is one of the major abiotic stresses that adversely affect plant productivity and quality[2]. It was estimated that up to 20% of irrigated lands in the world are affected by different levels of salinity and sodium content[3]. Salinity stress limits plant growth by adversely affecting various physiological and biochemical processes like photosynthesis, antioxidant phenomena, nitrogen metabolism and ion homeostasis[4, 5]. The primary effects of salt problem are the changes in membrane permeability[6]. Salt stress in soil or water is one of the major abiotic stresses especially in arid and semi-arid regions and can severely limit plant growth and yield. Salt stress can lead to stomatal closure, which reduces CO_2 availability in the leaves and inhibits carbon fixation, exposing chloroplasts to excessive excitation energy, which in turn could increase the generation of reactive oxygen species (ROS) and induce oxidative stress[7, 8]. Much of the injury to plants exposed to stress is connected with oxidative damage at the cellular level [9]. If there is a serious imbalance in any cell compartment between the production of reactive oxygen species (ROS) and antioxidant defence, oxidative stress and damage occurs [10]. ROS can seriously disrupt normal metabolism through oxidative damage to lipids, protein and nucleic acids [11].

Plants have developed the scavenging mechanism of ROS categorized as enzymatic and non-enzymatic [12, 13]. Several studies have pointed out that salt-tolerant species increased their antioxidant enzyme activities and antioxidant contents in response to salt treatment, whereas salt-sensitive species failed to do so [13, 14]. Increased SOD, POD and CAT activities are closely related to salt tolerance of many plants as reported in various researches [15-17]. Increase in the production of ROS are scavenged by antioxidant system which includes superoxide dismutase (SOD EC, 1.15.1.1), peroxidase (POX EC, 1.11.1.7), catalase (CAT EC, 1.11.1.6), Ascorbate peroxidase (APX EC, 1.11.1.11), polyphenoloxidase (EC, 1.10.3.1) enzymes in plants. There are many studies that there is a correlation with increasing antioxidant enzyme activities with coping oxidative stress in plants. Similarly, it is well known that salt stress can also enhance the anti-oxidant enzyme activities in plants for alleviating the oxidative damage [16]. In the present investigation anti-oxidative enzymes of *Excoecaria agallocha* (*E. agallocha*) under NaCl salinity were assayed.

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2. Materials and methods

E. agallocha L., an evergreen mangrove species belonging to the family Euphorbiaceae was used for the present investigation. This species is naturally growing in abundance in the salt marshes of Pichavaram on the east coast of Tamil Nadu, India about 10 km east of Annamalai University campus. The mature seedlings were collected from Pichavaram. Healthy seedlings with uniform size were planted individually in polythene bags (7 cm × 5 cm) filled with homogenous mixture of garden soil containing red earth, sand and farmyard manure mixed in the ratio of 1:2:1 and polythene bags were irrigated regularly. One month old seedlings were subjected to salt stress with different NaCl concentrations. The treatment constituted (control), 100, 200, 300, 400, 500, 600, 700, 800, 900, 1 000 mM NaCl. Fifty plants were treated with each of the NaCl concentrations. A control was maintained without any exogenous addition of salts. First samples for these studies were collected on the 60th day after salt treatment.

2.1. Extraction of enzymes and assays

2.1.1. Enzymatic antioxidants

Two grams of young leaves were macerated to powder with liquid nitrogen by a mortar-pestle; then 0.1 g PVP and 5 mL of extraction buffer (consisting of 1 M Sucrose, 0.2 M Tris-Hcl and 0.056 M β-Marcaptoethanol; pH adjusted at 8.5) was added and homogenized. The extracts were centrifuged at 10 000 rpm for 20 min at 48 ℃; supernatants were used as samples for enzyme assay.

2.1.2. Estimation of catalase: (CAT: E.C.1.11. 1.6)

Catalase (CAT:EC.1.11. 1.6) was measured [18] by change in absorbance at 240 nm. An assay mixture contained 2.6 mL of 50 mM potassium phosphate buffer (pH7.0), 0.4 mL of 15 mM H_2O_2 and 0.04 mL of enzyme extract. The decomposition of H_2O_2 was followed by the decline absorbance at 240 nm. The enzyme activity is expressed in units per min per mg protein.

2.1.3. Estimation of peroxidase: (POX: E.C.1.11.1.7)

Peroxidase (POX : E.C.1.11.1.7) activity was measured[19] following the change in absorbance at 470 nm due to 2 mL of 0.1 M phosphate buffer (pH 6.8), 1 mL of 0.001 M pyrogallol, and 1 mL of 0.005 4 M hydrogen peroxide and 0.5 mL of enzyme extract. The reaction mixture was incubated for 5 minutes at 25 ℃, after which the reaction was terminated by adding 1ml of 2.5N sulphuric acid. The activity is expressed in unit per minute per mg protein.

2.1.4. Estimation of superoxide dismutase: (SOD: E. C. 1. 15.1.1)

Superoxide dismutase (SOD: E. C. 1. 15.1.1) was assayed as described by Beauchamp and Fridovich[20]. The reaction mixture contained 1.17 μM × 10^{-6} M riboflavin, 0.1 M methionine, 2 μM × 10^{-5} M potassium cyanide and 5.6 μM × 10^{-6} M Nitroblue tetra-zolium salt (NBT) dissolved in 3 mL of 0.05 M sodium phosphate buffer (pH 7.8). Three mL of the reaction medium was added to 1 mL 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 ℃ for an hour. Identical solutions were kept under dark served as blanks. The absorbance was read at 560 nm in the spectrophotometer against the blank. Superoxide dismutase activity is expressed in units. One unit is defined as the amount of change in the absorbance by 0.1 per hour per mg protein under assay condition.

2.1.5. Polyphenoloxidase (E.C.1.10.3.1)

Polyphenoloxidase activity was assayed [19]. Assay mixture for polyphenoloxidase contained 2 mL of 0.1 M phosphate buffer (pH 6.0), 1 mL of 0.1M catechol and 0.5 mL of enzyme extract. This was incubated for 5 minutes at 25 ℃, after which the reaction was stopped by adding 1 mL of 2.5N sulphuric acid. The absorbance of the purpurogallin formed was recorded at 495 nm. The enzyme activity is 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.

3. Results

3.1. Effect of salinity on catalase activity

The effect of NaCl on the catalase activity in the leaves at various NaCl concentrations is presented in Figure 1. There was a steady increase in the catalase activity up to 300 mM NaCl.

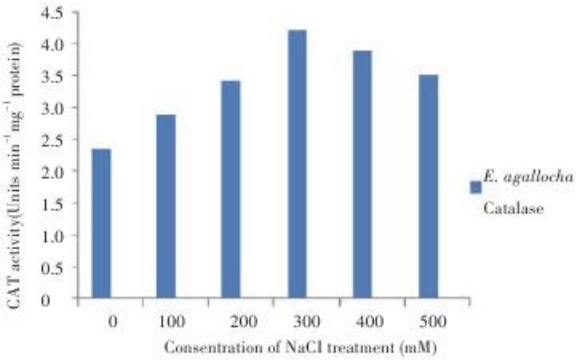

Figure 1. Effect of different concentrtions of NaCl on CAT activity. (units min^{-1} mg^{-1} protein) in leaves of *E. agallocha* 60th day after salt treatment.

3.2. Effect of salinity on peroxidase activity

The effect of NaCl on the peroxidase activity in the leaves at various NaCl concentrations is presented in Figure 2. The peroxidase activity showed a similar increasing trend as that of catalyses up to the optimum level of NaCl salinity. The maximum POX activity was observed up to 500 mM on 60th day.

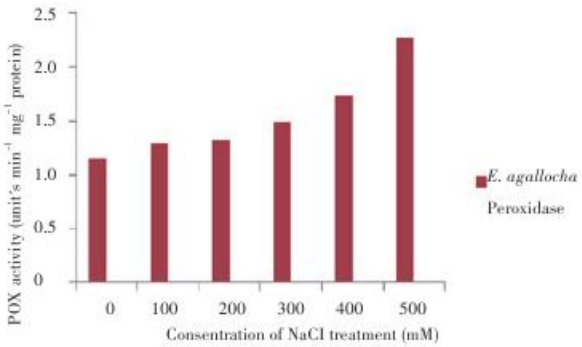

Figure 2. Effect of different concentrtions of NaCl on POX activity. (units min^{-1} mg^{-1} protein) in leaves of *E. agallocha* 60th day after salt treatment.

3.3. Effect of salinity on superoxide dismutase

The effect of NaCl salinity stress on the superoxide dismutase activity in the leaves at various NaCl concentrations is given in Figure 3. There was a steady increase in the superoxide dismutase activity up to 400 mM NaCl. The higher value of superoxide dismutase activity was observed at 400 mM.

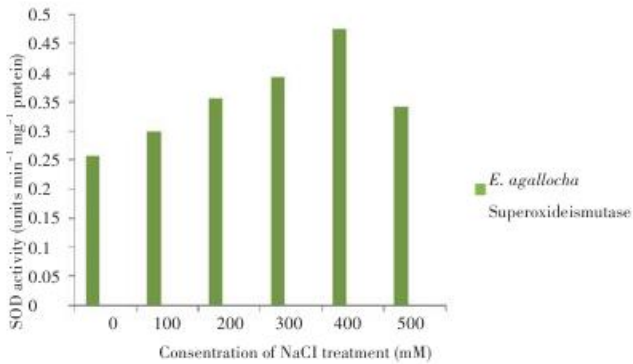

Figure 3. Effect of different concentrtions of NaCl on SOD activity. (units min^{-1} mg^{-1} protein) in leaves of *E. agallocha* 60th day after salt treatment.

3.4. Effect of salinity on polyphenoloxidase

The effect of NaCl salinity enhanced the polyphenoloxidase activity up to the optimum level of 300 mM in *E. agallocha* and the data are given in Figure 4. There was a steady increase in the polyphenoloxidase activity up to 300 mM NaCl.

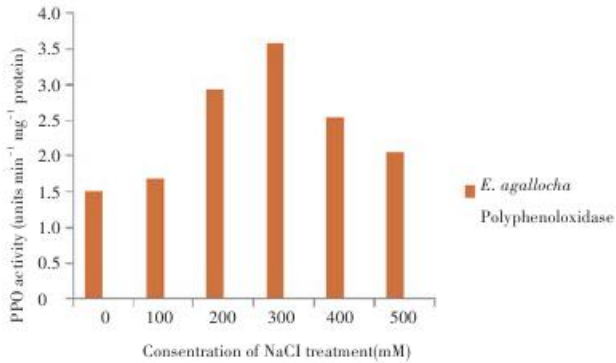

Figure 4. Effect of different concentrtions of NaCl on PPO activity. (units min^{-1} mg^{-1} protein) in leaves of *E. agallocha* 60th day after salt treatment.

4. Discussion

Plants resort to a range of distinct acclimation strategies in response to abiotic environmental stresses such as high salt, dehydration, cold, heat, and excessive osmotic pressure [21]. Salinity stress is an intricate phenomenon which includes osmotic stress, specific ion effect, nutrient deficiency thereby affecting various physiological and biochemical mechanisms associated with plant growth and development[22]. Soil salinity is a prevalent abiotic stress for plants. Growth inhibition is a common response to salinity and plant growth is one of the most important agricultural indices of salt stress tolerance as indicated by different studies[7].

CAT, which is involved in the degradation of hydrogen peroxide into water and oxygen, is the most effective antioxidant enzymes in preventing oxidative damage [10, 23]. Increase in 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 and protection against oxidative stress [24-26]. The catalase activity increased with increasing concentration of NaCl up to optimum level in *Ipomoea pes-caprae* [27]. Takemura *et al.*, reported an inductive response in CAT activity in the mangrove *Bruguiera gymnorrhiza* under salt stress [28]. The catalase activity decreased with increasing concentration in *Phaseolus radiatus* [29]. Increased CAT activity in *Cassia angustifolia* [30], maize [16] and *Sesamum indicum* [17] differing in salt tolerance were found. The stimulation of total CAT activity by salt stress has been observed in many plant species [31, 32]. Similarly in the present study, the salt induced enhancement of CAT activity in *E. agallocha* may suggest its effective scavenging mechanism to remove H_2O_2 and imparting tolerance against salinity induced oxidative stress.

POX activity of *E. agallocha* was increased with increasing salt concentrations. Significant increase in the peroxidase activity in the mangrove such as *Aegiceras corniculatum*[33]. Increase in peroxidase activity indicated the formation of large amount of H_2O_2 and which could release enzyme from membrane structure [34]. The enzyme POX involves in the decomposition of co-substrates such as phenolic compounds and/or antioxidants. Certain POX isomers utilize phenolic compounds and H_2O_2 to initiate biosynthesis of secondary metabolites required for the plant growth, development and differentiation[35]. POX activity increased in comparison with control under stress condition in different cultivars. Other researchers have reported the increase in POX activity in stress condition[36]. Increase in POX activity demonstrates the accumulation of H_2O_2 in salt stress condition [37].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[38].

SOD is one of several important antioxidant enzymes with the ability to repair oxidation damage caused by ROS. Thus, SOD is considered as a key enzyme for maintaining normal physiological conditions and coping with oxidative stress in the regulation of intracellular levels of ROS [10]. Sodium chloride stress caused increase in SOD activity with increasing salt concentrations in *E. agallocha*. Takemura *et al*., showed that this enzyme (SOD) retained full activity at least up to seawater salt levels [28]. 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 anti-oxidative defences in plants, invariably including superoxide dismutase, Ascorbate peroxidase and catalase [39, 40]. Plants with high antioxidant enzyme activities are generally more tolerant to various environmental stresses than those with low enzyme activates. SOD activity increased in comparison with control under stress condition in different cultivars. Others showed that salt treatment significantly increases the SOD activity. Increase in Reactive Oxygen Species (ROS), especially O_2^-, causes to increase in SOD, because this enzyme scavenges O_2^- [41]. Results in published reports also indicate that SOD over expression may be involved in the increase of stress protection observed in some transgenic plants [42, 43]. Increased polyphenoloxidase activity has been reported in halophytes such as *Aegiceras corniculatum* [33]. High polyphenoloxidase 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 [44]. Sharp increase in polyphenoloxidase activity under salinity stress was associated with enhanced rooting in *E. agalloha*, *Cynometra iripa* and *Heritiera fomes* [45].

Declare of interest statement

We declare that we have no conflict of interest.

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