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Original Research Article

Effect of tannery soaking water on antioxidant enzymes of *Salicornia brachiata*

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A B S T R A C T

Leather processing generates wastewater containing high amounts of Total Dissolved Solids (TDS), apart from salinity. Antioxidant enzymes are involved in resisting various environmental stresses including salinity. The objective of this study was to assess the effect of salinity stress on the activities of antioxidant enzymes and polyphenol content in *S.brachiata.* The halophyte, exposed to varying concentrations of tannery soaking water (2500, 5000, 7500, 10000, 12500 and 15000 ppm of NaCl) in pots, were observed under laboratory conditions for a period of 90 days. Polyphenol content was found to increase on the 90th day in *S.brachiata* treated with 15000 ppm of soaking water. The effects of salinity stress on the activities of antioxidant enzymes such as PolyPhenol Oxidase (PPO), Catalase (CAT) and Superoxide Dismutase (SOD) showed significant differences.

Keywords

Salicornia brachiata; Halophyte; Polyphenol; Antioxidant enzymes; Soaking water.

Introduction

Salinity is a serious threat for global agriculture. Approximately 100 million hectares of the land worldwide have been adversely affected by salinity (Ghassemi *et al*., 1995). High exogenous salt concentrations cause an imbalance of the cellular ions resulting in ion toxicity, osmotic stress and production of Reactive Oxygen Species (ROS) (Cheeseman 1988; Srivastava *et al*., 2007; Mishra *et al*., 2008). ROS are highly reactive and in the absence of any protective mechanism, they can seriously disrupt normal metabolism through oxidative damage to lipids, proteins and nucleic acids. Salinity in soil poses stressed conditions for the growth of plants. Under natural conditions of growth and development, plants are inevitably exposed to different types of stresses that might cause increased production of Active Oxygen Species (Smirnoff 1993). Antioxidants are compounds that can delay or inhibit the oxidation of lipid or other molecules by inhibiting the initiation

or propagation of oxidizing chain reactions (Velioglu *et al*., 1998). In order to counteract the potential damage resulting from excess reactive oxygen and to maintain homeostasis, living organisms have evolved a delicate antioxidant mechanism consisting mainly of two systems – the enzyme system and the non-enzyme system. Plants have evolved various protective mechanisms to eliminate or reduce AOS (Foyer *et al*., 1994b). The interest on plant antioxidant defense mechanism has increased over the years. The capacity of the antioxidant defense system is often increased under stress conditions (Gressel and Galun 1994).

Salt-tolerant plants, in addition to the ability to regulate ion and water movements, often have better antioxidative systems for effective removal of AOS (Rout and Shaw 2001). These AOS are detoxified by the sequential and simultaneous action of a number of enzymes, including Glutathione Reductase (GR), Superoxide Dismutase (SOD), Polyphenol Oxidase (PPO), Catalase (CAT) and Glutathione transferase (GST). Superoxide dismutase, located in various cell compartments, is a major scavenger of superoxide and its enzymatic action results in the formation of H_2O_2 and O_2 (Smirnoff 1993; Ozden *et al*., 2009).

Catalase is present in peroxisomes, glyoxysomes and mitochondria, but is apparently absent in the chloroplast. Dismutases, mostly in the photorespiratory/respiratory chain, transform H_2O_2 into water and molecular O_2 (Willekens *et al*., 1997), whereas POD decomposes H_2O_2 by oxidation of co-substrates such as phenolic compounds and/or antioxidants. The antioxidant activity of phenolic compounds is mainly due to the redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides and have health functional properties that may protect humans from various diseases (Heinonen *et al*., 1998; Rice-Evans and Miller 1998).

Among all industries, tannery industries generate large amount of wastewater possessing high TDS, which is agronomically beneficial to plants and is readily assimilated by the soil. The continuous input of waste containing salinity over agricultural land causes an imbalance in the ecosystem. Various remediation methods are available for the treatment of salt contaminated soils. As these are expensive, alternative cost-effective in situ methods are highly desirable. The role of plants in phytoremediation of contaminated soil and wastewater has been explored (Salt et al., 1995; Sinha 1999; Lasat 2002; Sinha et al., 2002). A potentially decisive factor in determining the outcome of oxidative stress is the speed with which antioxidants can activate their antioxidant reserves, either by synthesizing antioxidants or utilizing pre-existing pools.

Saline stress could be remediated by using plant species capable of growing in highly saline conditions. It would, therefore, be beneficial to explore the potential of salt tolerance by various species of plants. *Salicornia brachiata* is highly salt-tolerant and can accumulate 30-40% NaCl in its dry weight. Hence, *S.brachiata* was selected for evaluating the polyphenolic content and changes in its antioxidant enzymes (polyphenol peroxidase, Catalase and superoxide dismutase) when exposed to salinity stress.

Materials and Methods

Plant Material and Growth Conditions

S. brachiata is a halophytic plant, commonly known as sea purslane. The thick, fleshly plants are borne on succulent, pinkish green stem and are leafless. The plants used in this work were obtained by cutting propagation method. *S. brachiata* cuttings were grown in pots containing sandy soil and organic manure in the ratio of 1:1 (w/w), irrigated with tap water for a period of one month. From the soaking water of the tannery, solutions possessing varying concentrations (2500, 5000, 7500, 10000, 12500 and 15000 ppm of NaCl) were prepared. Every day, the plants were irrigated with 100 mL of Hoagland's solution for 90 days, under laboratory conditions. The plants irrigated only with Hoagland's solution were treated as control.

Determination of Polyphenol Content

Total polyphenolic content of the plant extract was determined, as described by Dewanto (2002). Three hundred microlitre of the above sample was taken into test tubes followed by the addition of 1.5 mL of a Folin–Ciocalteau's reagent (10 x dilutions) and 1.2 mL of sodium carbonate (7.5% w/v). This solution was vortexed for 15 sec and allowed to stand for 30 min at 40°C for color development. The absorbance was measured at 760 nm against the blank. The total polyphenolic content was expressed in mg tannic acid equivalent per gram of dry weight (mg TAE/g dry weight) through a standard graph.

Enzyme Extraction Assay

Samples of *S. brachiata* treated with varying concentrations of NaCl were ground to fine powder with liquid nitrogen and extracted with ice cold 0.1 mM phosphate buffer (pH 7.8), containing 1 mM EDTA, 1 mM PMSF and 0.5% PVP. The supernatant obtained upon centrifuging the homogenate at 14000 × g for 20 min and 4°C was used for the enzyme activity and protein estimation (Bradford 1976).

Determination of Polyphenol Oxidase

PPO activity was assayed spectrophotometrically as described by Kumar and Khan (1982). The assay mixture contained 2 mL of 0.1 M phosphate buffer (pH 6.0), 1 mL of 0.1 M Catechol and 0.5 mL of enzyme extract. The sample was incubated for 5 min at 25°C and the reaction was stopped by adding 1 mL of 2.5 N H_2SO_4. The absorbance of the purpurogallin formed was measured at 495 nm. PPO activity was expressed in U mg^{-1} protein (U = Change in 0.1 absorbance min^{-1} mg^{-1} protein).

Determination of Catalase Activity

The procedure of Chandlee and Scandalios (1984) was employed for the assay of CAT. The assay mixture contained 2.6 mL of 50 mM potassium phosphate buffer (pH 7.0), 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 in absorbance at 240 nm. The enzyme activity was expressed in U mg^{-1} protein (U = 1 mM of H_2O_2 reduction min^{-1} mg^{-1} protein).

Determination of superoxide dismutase activity

The activity of SOD was determined from its ability to inhibit the photochemical reduction of nitro-blue tetrazolium (NBT)

using the method of Beauchamp and Fridovich (1971). Three mL of reaction mixture contained 33 mM NBT, 10 mM methionine, 0.66 mM EDTA and 3.3 mM riboflavin in 50 mM phosphate buffer, pH 7.8. This reaction mixture was added to 1 mL of enzyme extract. Illumination, in glass test tubes by 2 sets of Philips 40 W fluorescent tubes in a single row, was done to initiate the reaction at 30°C for 1 h. Identical solutions kept under dark served as blank. The absorbance was measured at 560 nm in the spectrophotometer against the blank. SOD activity was expressed in U mg $^{-1}$ protein. One unit (U) is defined as the amount of change in the absorbance by 0.1 h^{-1} mg^{-1} protein.

Results and Discussion

Salinity of tannery waste water makes it complex to be treated by conventional biological treatment. Salinity exposure can lead to various physiological and biochemical changes within plant cells causing numerous changes in the structure and function (Deepa *et al., 2013*; Ramesh Kannan *et al., 2013*). Salt tolerant plants can adapt to these saline stresses and degrade the organics in saline wastewater. This report deals with the changes induced in the antioxidative enzyme profile of *S.brachiata* by salinity.

Polyphenol Content

Plants can synthesize and accumulate phenolic compounds in response to stress (Dixon and Paiva 1995; Mamdouh *et al.*, 2002). Phenolic compounds are some of the most effective antioxidative constituents in plants materials. Phenol accumulation could be cellular adaptive mechanisms for scavenging oxygen radicals during stress, and this free radical scavenger could be oxidized in the system of this tissue preventing sub-cellular damage. The antioxidant activity of phenolic compounds can play a vital role in neutralizing ROS (Zheng and Wang 2001). ROS contribute to various environmental stresses including salinity. Generally, it is known that total polyphenols are highly correlated with antioxidant activity (Manach *et al*., 2005). The polyphenol content of *S.brachiata* extracts varied significantly as shown in the Table 1. The maximum polyphenolic content was 75.66 mg/ g at 15000 ppm of saline stress while it was 19.37 mg/g in that of the control.

Effect of Soaking water on PPO Activity

The changes in the PPO activity upon exposure of varying concentrations of soaking waste water of tannery are represented in Fig. 1. PPO activity of *S.brachiata* was at the maximum of 4.2 U mg^{-1} protein at 12500 ppm as observed on the 60th day. On the 90th day, the PPO activity significantly reduced when compared to the control. Increased PPO activity under stress indicates the ability to oxidize and degrade the toxic substances such as phenolic compounds which are generally accumulated during salt stress. It is believed that the action of NaCl is due to the formation of complex between the halide ion and copper in the enzyme (Zawistowski *et al.,* 1991).

Changes in CAT Activity under Soaking water of Tannery

The level of antioxidative response depends on the species, development and the metabolic state of the plant, as well as the duration and intensity of stress (Reddy *et al.,* 2004). From Fig. 2 that depicts the CAT activity of the control and the

Table.1 Polyphenol content of *S.brachiata* under soaking water of tannery

Sl.No	Salinity levels in ppm	Polyphenolic content mg/g TAE
1	0	19.37 ± 0.64
2	2500	24.78 ± 0.56
3	5000	36.45 ± 0.67
4	7500	48.89 ± 0.45
5	10000	59.75 ± 0.56
6	12500	67.34 ± 0.35
7	15000	75.65± 0.56

Fig.1 Effect of soaking water in tannery wastewater on polyphenol oxidase (PPO) activities in *S.brachiata* at different days interval. Values are given as mean±SD of three replicates.

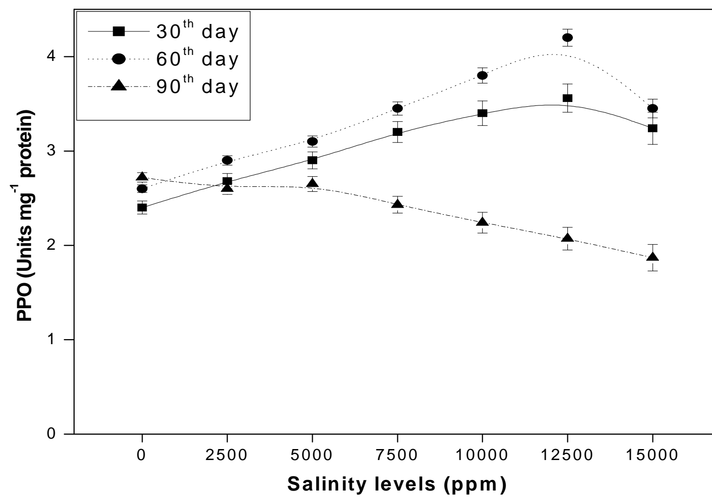


Fig.2 Effect of soaking water in tannery wastewater on catalase (CAT) activities in *S.brachiata* at different days interval. Values are given as mean±SD of three replicates.

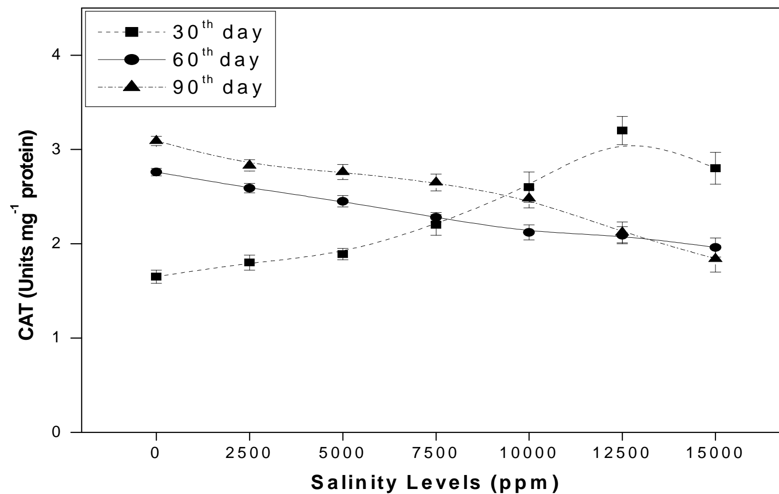
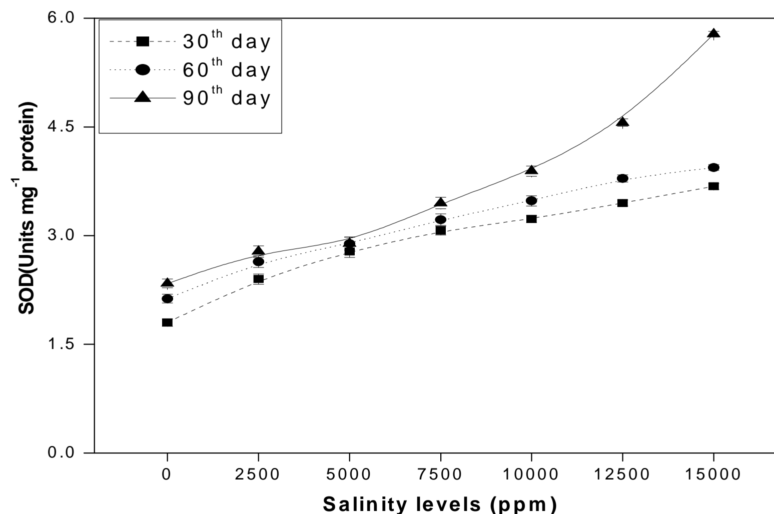


Fig.3 Effect of soaking water in tannery wastewater on superoxide dismutase (SOD) activities in *S.brachiata* at different days interval. Values are given as mean±SD of three replicates.



experimental plants of *S.brachiata*, it is noted that there was an increase till the 30th day of the experiment, which was the maximum recorded (3.2 U mg^{-1} protein at 12500 ppm). On the 60th day and 90th day, the activity in treated *S.brachiata* was drastically reduced when compared to the control. The results indicate a decline in CAT activity under extreme salinity, which suggests that CAT appears not to be an efficient scavenger of H_2O_2 in *S. brachiata*. This enzyme has a relatively poor affinity for H_2O_2 and in the presence of light, undergoes photoinactivation with subsequent degradation (Shang and Feierabend 1999). The changes in CAT may vary according to the intensity of stress, time of assay after the stress and induction of new isozyme (Shim et al. 2003).

SOD Activity of *S.brachiata* under Saline Stress

The activity of SOD increased with increasing concentrations of NaCl during the growth period as shown in Fig. 3. On the 30th day, the activity of superoxide dismutase recorded at 15000 ppm was more than 220% when compared to the control. SOD activities of 5.78, 4.56 and 3.89 U mg^{-1} protein were observed at 15000 ppm, 12500 ppm and 10000 ppm NaCl, respectively, on the 60th day. By the end of the 60th day, the activity was the highest (3.94 U mg^{-1} protein) at 15000 ppm NaCl. Many stress situations cause an increase in the total foliar antioxidant activity (Pastori *et al*,. 2000). SOD represents an important protective mechanism against possible NaCl – induced ROS production. Adaptation to high NaCl levels involves an increase in the antioxidant capacity of the cell in order to detoxify ROS. It has been reported that salt stress enhance superoxide anion (Bellaire *et al.,* 2000; Xu *et al.,* 2011) which can be converted to H_2O_2 through both enzymatic and non-enzymatic reactions. In the present study, the

elevated levels of SOD activities under salt stress may directly modulate the amount of ROS (Reactive Oxygen Species). The H_2O_2 formed as a product of SOD activity should be a potential damaging agent. The plant showed increased activities of PPO and CAT under low salinity and increased SOD activity at high salinity, suggesting the existence of an effective scavenging mechanism to remove ROS, because salinity is thought to play a critical role in plant salt tolerance.

The results of the study clearly shows that saline stress results in the increase of Polyphenol content, changes in antioxidant enzymes such as PPO, CAT and SOD. The PPO activity, maximum at 12500 ppm was observed on the 60th day of experimental plants. CAT activity increased till the 30th day at 12500 ppm of NaCl. SOD acts as the best scavenger when compared to the activity of other enzyme. The increase in the enzyme activity might be due to the direct influence of salt stress on enzyme synthesis and its induction by salt stress may play a role in salt tolerance. This proves that *S.brachiata* can be used for phytoremediation of salinity contaminated lands. *S.brachiata* which is considered to be salt tolerant, might have limited ROS scavenging system, apart from tolerance mechanisms, to cope with salt stress.

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