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RESEARCH ARTICLE

ANTIOXIDANT ENZYMES ACTIVITY IN LEAVES OF NACL STRESSED C_4 PLANT (*ZEA MAYS* L.)

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Abstract

The present study was carried out to determine the antioxidant enzymes such as 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), Polyphenol oxidase (PPO EC, 1.10.3.1) were analyzed in *Zea mays* L. in order to explore the plant's protective mechanism against reactive oxygen species under salt stress. The experiment was factorial based on completely randomized block design with three replications. These enzymes were examined and analyzed in 45 days old plants. The first 10 days of normal growth and 10 days of salt stress was applied. After salt treatment 7th day the plants were analyzed in various concentrations of 0mM (control), 25mM, 50mM, 75mM and 100mM NaCl. The NaCl stress enhanced all the antioxidant enzymes were augmented with increasing salinity compared with respective control. The results suggest that maize plants may increase the activity of antioxidant enzymes to have superior protection system against oxidative damage under NaCl stress.

Key words: Antioxidant enzyme, ROS, NaCl, Maize.

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Introduction

High salinity is one of the very noxious environmental issues that hostility affects enormous areas of cultivated land. Plant growth, physiological and metabolic processes are affected, leading to vital reductions in world-wide crop productivity (Zhang et al. 2009). Abiotic stresses have an effect on plant metabolism, disrupt cellular physiological state and disconnect major physiological and biochemical processes (Siringam et al. 2011). Plant growth is

always under the menace of excess of abiotic stress factors such as drought, heat, salinity and serious metal toxicity (Siringam et al. 2012). Misra et al. (1997) reported that NaCl stress causes loss in turgidity by osmotic stress resulting in stomata closure, which eventually lowers CO_2 supply to leaves and photosynthetic activity is reduced. Furthermore, salinity can even upset non stomatal attributes like loss of green pigments and dropping of photosynthetic enzyme's activity. McCord (2000) revealed that photosynthetic activity is coupled with an accumulation of reactive oxygen species (ROS) that ultimately leads to oxidative stress and stimulate toxic reactions like DNA mutation, lipid peroxidation and protein degradation. For example, Hydrogen peroxide is considered as one of the potential ROS which represses the Calvin cycle (Ashraf 2009; Abogadallah 2010). Salt-induced damage to essential cell membrane has been observed in the form of solute leakage, which has been reported in mungbean (Saha et al. 2010).

To mitigate the ROS induced oxidative effects, plants have an antioxidant defense system that involves generation of different sorts of antioxidants like catalase, peroxidase, ascorbate peroxidase, polyphenol oxidase and superoxide dismutase (Shahid et al. 2011). Many abiotic stresses are shown to secondarily cause oxidative stress in plants through the improved production of ROS like OH, O_2- and H_2O_2 (Ozgur et al. 2013). These ROS can damage nucleic acids of cells as well as proteins and essential membrane lipids. Levels of ROS in plant cells are normally mediated by protective antioxidant activities (Foyer & Noctor, 2013; Ozgur et al. 2013).

Under stress conditions, SOD enzyme converts superoxide ion to H_2O_2 and molecular oxygen thus playing pivotal role in defense mechanisms (Harinasut et al. 2003). Peroxidase catalyst deteriorates H2O2 by oxidation of co-substrates, for example, phenolic mixes and cell reinforcements (Gaspar et al. 1991). Catalase enzyme plays an important role in the acquisition of tolerance to oxidative stress in the adaptive response of plant cells (Abassi et al. 1998). Foyer and Lelandais (1996) observed that ascorbate peroxidase is the most important peroxidase in detoxifying H_2O_2 catalyzing the reduction of H_2O_2 to water. APX along with monodehydro Ascorbate reductase, dehydro Ascorbate reductase and GR eliminate H2O2 through the Foyer–Halliwell–Asada pathway (Halliwell 2006). The purpose of the present investigation was to evaluate anti-oxidative defense system stimulation against reactive oxygen species under salt stress.

Materials and Methods

A pot experiment was conducted in the Botanical garden of the Thirumahal Mills College, Tamil Nadu under natural environmental condition. The seeds of maize were procured from Syngenta Ltd. The equal sizes of seeds were sown in plastic pots. The pots with 15cm height and 18cm wide were chosen. The pots were filled with homogenous mixture of the garden soil containing red soil, sand along with farmyard manure in the ratio of 1:2:1. In each pot, eight seeds were sown and one plant was maintained finally. The pots were masterminded in Completely Randomized Block Design (CRBD) and were flooded with faucet water. After 10days, the well-established plants were selected for saline treatment. The saline treatment consisted of (0, 25, 50, 75 and 100) seven different concentrations of NaCl. Fifty plants were treated with every one of the NaCl focus. A control was maintained without any exogenous addition of salt. The sampling of these studies was collected on the 7^{th} day after the salt treatment.

Extraction of enzymes and assays

Enzymatic antioxidants

Two grams of youthful leaves were macerated to powder with fluid nitrogen with a mortar-pestle; at that point 0.1 g PVP and 5 ml of extraction cradle (comprising of 1 M Sucrose, 0.2 M Tris-HCl and 0.056 M β-Marcaptoethanol; pH changed at 8.5) was added and homogenized. The extracts were centrifuged at 10,000 rpm for 20 min at 48°C; supernatants were used as samples for enzyme assay.

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 & Fridovich, (1971). The reaction mixture contained 1.17 µM × 10^{-6}M riboflavin, 0.1 M methionine, 2µM × 10^{-5}M

potassium cyanide and 5.6 µM × 10^{-6}M Nitroblue tetra-zolium salt (NBT) dissolved in 3ml of 0.05 M sodium phosphate buffer (pH7.8). Three ml of the response medium was added to 1 ml of protein separate. The combinations were enlightened in glass test containers of chose uniform thickness. The light was performed by two arrangements of Philips 40W fluorescent cylinders. The test tubes were organized in a solitary column, with a bunch of cylinder lights fixed on one or the other side. Illumination was started to initiate the reaction at 30˚C for an hour. Indistinguishable arrangements were held under dull filled in as spaces. The absorbance was read at 560nm in the spectrophotometer against the blank. Superoxide dismutase activity is expressed in units. One unit is characterized as the measure of progress in the absorbance by 0.1 every hour per mg protein under test condition.

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

Catalase (CAT: EC.1.11. 1.6) was measured according to Chandlee & Scandalios, (1984) by change in absorbance at 240nm. An assay mixture contained 2.6ml of 50mM potassium phosphate buffer (pH7.0), 0.4ml of 15mM H_2O_2 and 0.04ml of enzyme extract. The decomposition of H_2O_2 was followed by the decline absorbance at 240nm. The compound movement is communicated in units per min per mg protein.

Estimation of Ascorbate peroxidase (APX E.C. 1.11.1.11)

APX (EC: 1.11.1.11) activity was assayed following the method of Nakano & Asada, (1981). The response cushion arrangement contained 50 mM K-phosphate support (pH 7.0), 0.5 mM AsA, 0.1 mM H2O2, 0.1 mM EDTA and compound concentrate in a last volume of 0.7 ml. The reaction was initiated by the addition of H_2O_2 and activity was measured by observing the decrease in absorbance at 290 nm for 1 min using an extinction coefficient of 2.8 mM^{-1} cm^{-1}.

Estimation of polyphenoloxidase (PPO E.C.1.10.3.1)

Polyphenoloxidase activity was assayed by the method of Kumar & khan, (1982). Assay mixture for polyphenoloxidase contained 2ml of 0.1 M phosphate buffer (pH 6.0), 1ml of 0.1M catechol and 0.5ml 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 framed was recorded at 495nm. The compound movement is communicated in units. One unit is characterized as the measure of purpurogallin shaped, which raised the absorbance by 0.1 every moment under the examine condition.

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

Peroxidase (POX : E.C.1.11.1.7) activity was measured by Kumar & khan, (1982) following the change in absorbance at 470nm due to 2ml of 0.1 M phosphate buffer (pH 6.8), 1ml of 0.001 M pyrogallol, and 1ml of 0.0054 M 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 2.5N sulphuric acid. The movement is communicated in unit every moment per mg protein.

Statistical analysis

The experiment was placed in a completely randomized black design (CRBD) with three replicates of the each treatment. The results were analyzed by one-way ANOVA with the help of SPSS 16.0 software package. Means and standard deviation were determined from three replications.

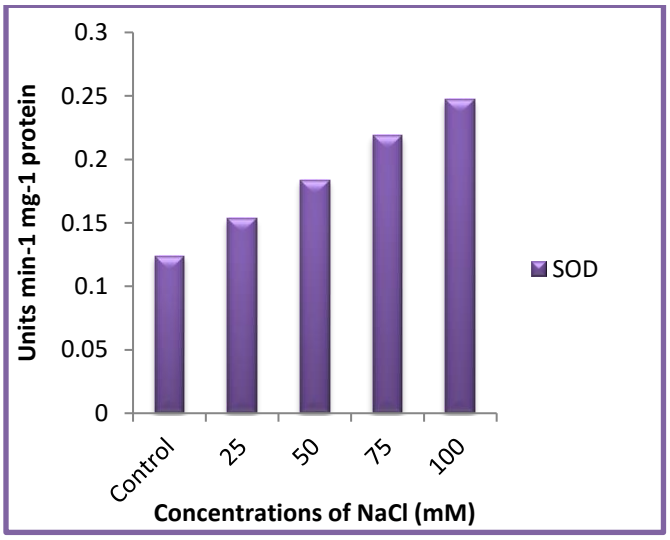
Results and Discussion

The activities of antioxidant enzymes such as SOD, CAT, APX, PPO and POX in maize cultivar under different levels of salinity treatments were assayed.

Salt stress exaggerated SOD activity at the 7th day after salt treatment with increasing salinity up to 100mM NaCl compared with the control plants and the data are given in Figure 1. Sajjad and Pakniyat (2012) reported that SOD is one of the major anti-oxidative enzymes present in all aerobic organisms and largely in sub cellular elements that generate activated oxygen and this enzyme is concerned in dismutation of superoxide radicals to hydrogen peroxide and oxygen. The primary line of defense in oxidative stress is that the action of SOD that converts

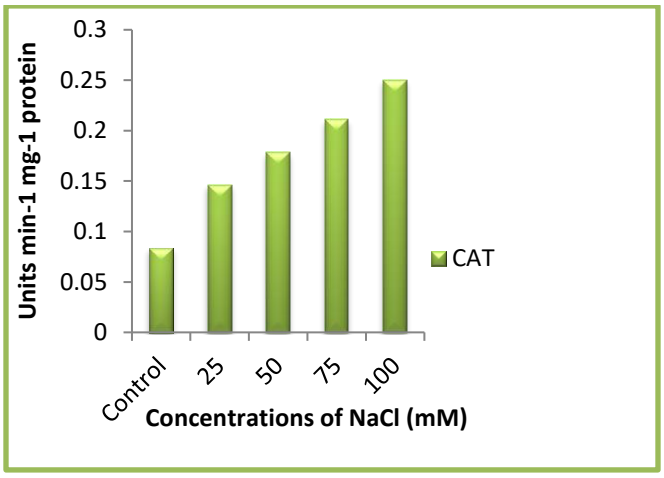
O_2- to H_2O_2 (Ashraf 2009). The increase in Hydrogen peroxide is extremely detrimental for the cells; therefore it gets dismutated to water and oxygen (Ashraf 2009). Ahmad and Umar (2011) reveled that increased SOD activity enables plants to protect the oxidative damage caused by exposure to NaCl salinity stress. Printed reports additionally showed that over expression of SOD resulted in efficient stress protection against NaCl stress in *B. vulgaris* (Bor et al. 2003), cotton (Meloni et al. 2003) and rice (Satpal Turan & BaishnabTripathy 2012).

Figure: 1. Effect of different concentrations of NaCl treatment on SOD activity (units $min^{-1}mg^{-1}$ protein) in leaves of *Zea mays* 7th day after salt treatment.



(Values are mean ± S.D. of 3 samples, n-3 and expressed in units min^{-1} mg^{-1} protein)

Figure: 2. Effects of NaCl on catalase activity (units min^{-1} mg^{-1} protein) of 7th day old maize plants grown with (25-100mM) and without (control) salt stress.



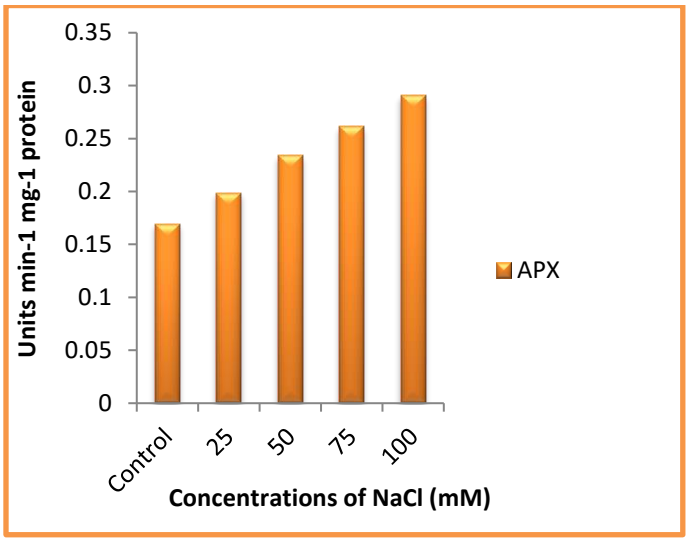
(Values are mean ± S.D. of 3 samples, n-3 and expressed in units min^{-1} mg^{-1} protein)

Catalase activity was influenced significantly in leaf tissues at 100mM NaCl concentrations are presented in Figure 2. Noreen et al. (2010) estimated that under saline stress increase in catalase activity which reduces the toxic levels of H_2O_2 and protects the cell from oxidative damage. Catalase is essential for ROS detoxification during stress. Catalase is one of the H_2O_2 detoxifying enzymes, where it removes H_2O_2 fashioned throughout photorespiration. Increase in CAT activity is supposed to be an adaptive trait probably helping to overcome the destruction to tissue metabolism by reducing venomous levels of H_2O_2 (Sekmen et al. 2007). CAT activity increases with the increase in NaCl concentration in maize plants (Arora et al. 2008). Similar results were reported by Azevedo-Neto et al., (2006) in maize, Koca et al. (2007) in sesame and Sudhakar et al. (2001) in mulberry cultivars.

Figure 3 showed APX activity was enhanced in *Z. mays* plant leaves at 100mM under salt treatment alone as compared to non-saline plants. Another versatile antioxidant enzyme is ascorbate peroxidase which utilizes ascorbate (AsA) as electron donor and scavenges H_2O_2 in water-water and ascorbate glutathione cycles. H_2O_2 is reduced to water by APX and plays a vital role in cell defense mechanism (Kangasjarvi et al. 2008; Ashraf 2009). Ascorbate peroxidase could be a central component of AsA-GSH cycle, and plays a pivotal role in the control of intracellular ROS levels. APX utilizes two molecules of AsA to reduce H_2O_2 with a

concomitant generation of two molecules of MDHA. In the present study, upon imposition of salt stress APX activity accumulated considerably. Increase in APX activity in response to salt stress was also reported in *Brassica napus* (Hasanuzzaman et al. 2011) and *Brasssia juncea* (Mittal et al. 2012).

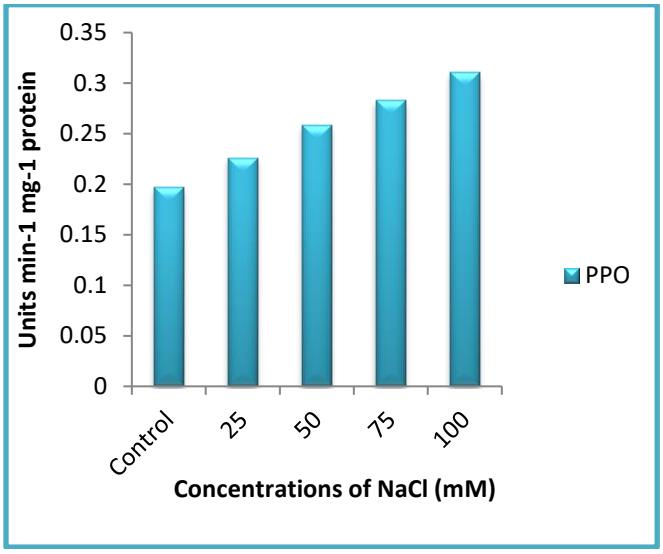
Figure: 3. The effect of various concentrations of NaCl treatment on ascorbate peroxidase activity (units min^{-1} mg^{-1} protein) in leaves of maize plants 7th day after salt treatment.



(Values are mean ± S.D. of 3 samples, n-3 and expressed in units min^{-1} mg^{-1} protein)

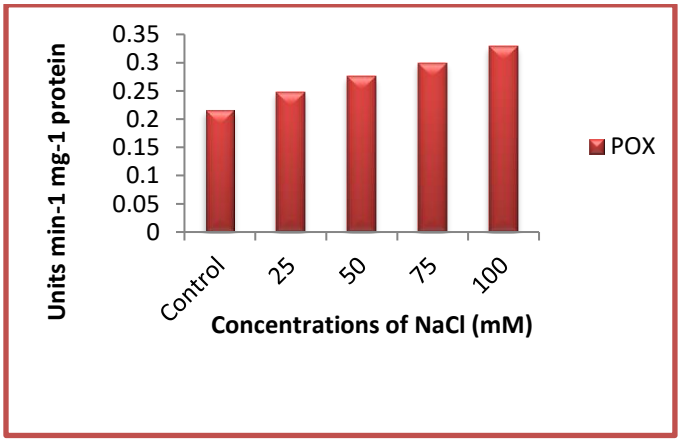
The sodium chloride salinity promoted the PPO activity up to the 100mM in *Z.mays*. The data are given in Figure 4. POX and PPO are two significant chemicals which effectively take an interest inside the oxidation of phenolic builds (Dolatabadian et al. 2009). The redoubled PPO activity in maize plants might reduce the phenol accumulation in plants under stress. PPO activity gradually increased with increasing NaCl concentrations in bean plants (Demir & Kocaliskan 2001). PPO activity increases during the progression of stress and is a possible tolerance mechanism as demonstrated in *C. angustifolia* seedlings subjected to salt stress (Agarwal & Pandey 2004).

Figure: 4. Effect of NaCl on PPO activity (units min^{-1}mg^{-1} protein) in leaves of *Zea mays* 7th day after salt treatment.



(Values are mean ± S.D. of 3 samples, n-3 and expressed in units min^{-1} mg^{-1} protein)

Figure: 5. Effect of different concentrations of NaCl treatment on Peroxidase activity (units min^{-1}mg^{-1} protein) in leaves of *Zea mays* 7th day after salt treatment.



(Values are mean ± S.D. of 3 samples, n-3 and expressed in units min^{-1} mg^{-1} protein)

Conclusion

In summary, the result of this study indicates that increased activity of antioxidant defense system may be having a better protection against reactive oxygen species under salinity stress.

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