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Research Paper

Significance of antioxidant enzymes in stress signaling in *Withania sominifera (L.) Dunal*

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Abstract

Plant exposed to stress undergoes changes in their metabolism in order to adapt to the changes in their environment. The present study was conducted to reveal the role of antioxidant enzymes like superoxide dismutase and peroxidase during acetyl salicylic acid and sodium chloride salt (NaCl) signaling in Withania sominifera (L.) Dunal. using enzyme activity staining on acrylamide gels. The results showed that during acetyl salicylic acid signaling, superoxide dismutase (SOD) activity increased in 2nd and 4th hrs and decreased till 6th hrs and further increased up to 10th hrs. Similarly in peroxidase, a low enzyme activity was observed in 0 and 6th hrs while in 2nd, 4th, 8th and 10th hrs the expressions of three isozymes of peroxidases were observed. Activity by 6th hrs may result in increased accumulation of H_2O_2 resulting in oxidative burst. The decrease in, superoxide dismutase activity may be due to the feedback inhibition mechanism of the enzyme. During sodium chloride salt stress only, superoxide dismutase activity was localized since Peroxidase (POD) activity was negligible. In different sodium chloride salt (concentration from 1000 to 6000 ppm, the, superoxide dismutase activity was maximum increase during 5000 ppm treatment revealed by the presence of three bands. During time dependent sodium chloride salt treatment, the superoxide dismutase activity increased during 2nd to 8th hrs of treatment revealed by the presence of two bands. The result in the present study highlight a probable occurrence of oxidative burst in Withania sominifera (L.) during 4th and 6th hrs of stress signaling. Sometimes high amount of Hydrogen Peroxide accumulated and generating PCR/HR signals against plant pathogens to protect plant.

Keywords: Antioxidant, Acetyl Salicylic Acid, Di Amino Benzedene (DAB), Peroxidase (POD), Superoxide, Dismutase (SOD), Tetra Methylene Benzedene(TMB).

Introduction

Abiotic and biotic stresses are known to act as a catalyst in producing free radicals resulting in oxidative stress in plants, where reactive oxygen species (ROS) such as superoxide radical (O_2^-), hydrogen

peroxide radical (H_2O_2), alkoxyl radical (RO^-), hypochlorous acid (HClO), and the highly reactive hydroxyl radical (OH^-) are produced [1,2,3,4,5,6,7,8,9]. These oxidants can damage cells by starting chemical chain reactions such as lipid peroxidation, or by oxidizing DNA or proteins [10,11]. Hence, plants and animals maintain complex systems of multiple types of antioxidants, such as Catalase, Superoxide dismutase (SOD) and various Peroxidases, Glutathione reductase (GR) and Glutathione-synthesizing enzymes to contain the damage caused by Reactive Oxygen Species (ROS) [12,13]. Oxidative stress is essentially a regulated process, the equilibrium between the oxidative and antioxidative capacities determining the fate of the plant. Under non-stressful conditions, the antioxidant defense system provides adequate protection against active oxygen and free radicals. In response, the level of the antioxidative defense system is increased. In plants, CuZn-Superoxide dismutase is the most abundant and has been localized in the cytosol, chloroplast, and mitochondrial matrix has also been found in peroxisomes [14] and apoplast [15,16]. Peroxidase (EC 1.11.1.7) is a ubiquitous plant enzyme that catalyzes the oxidation of cellular components by either Hydrogen Peroxide or organic hydrogen peroxides. Peroxidases are heam containing enzymes that use Hydrogen Peroxide as the electron acceptor to catalyze the number of oxidative reactions. Peroxidases are found in bacteria, fungi, plants and animals. Peroxidase is belonging from three major classes, they are class I, II and III.

Effects of Salicylic Acid at different concentrations on heat tolerance were examined in Kentucky bluegrass and activities of antioxidant enzymes, superoxide dismutase and catalase was shown increased. Treatment of seedlings with Salicylic Acid caused a transitory enhancement of O_2^- and Hydrogen Peroxide production by plants and simultaneous increase in the activity of Superoxide dismutase [17, 18].

Change in Superoxide dismutase activities and Ascorbate peroxidase (APX) [19, 20] under different stress conditions like drought, high temperature and salinity was demonstrated in wheat [21]. Soyabean [22] citrus [23], pea [24], *Foxtail millet* [25] *Suaeda salsa* [26] and *Hydrilla vercticillata*.

Materials and Methods

Leaves from *Withania somnifera* (L). Dunal (Ashwagandha), were collected from the Vegetative Propagation Complex, Institute of Forest Genetics and Tree Breeding, Coimbatore, Tamil Nadu. The leaf discs of *Withania sominifera* were incubated in 1000 ppm solution of acetyl salicylic acid (Qualigens Fine Chemical Ltd., India), sodium chloride salt stress (1000 ppm). The leaves discs were taken out at regular interval of 2 hrs from the solution including the 0^{th} has till 10^{th} and 12^{th} hrs ground into a fine powder with liquid nitrogen and was suspended in 1:3 (w/v) of extraction buffer (0.2 M phosphate buffer, pH-7.8 and 7.0). The homogenate was centrifuged at 10,000 rpm for 20 minutes at 4ºC. The pellet was discarded and the supernatant used for Superoxide dismutase and Peroxidase activity determination. Determined the protein conc. spectrophotometrically (SPECGENE, U.K.) at the wavelength of λ_{280} and λ_{260} in µg/ml and Non-denaturing PAGE was performed and Superoxide dismutase was localized in the gel by method of Beauchamp and Fridovich [27] with few modifications. Superoxide dismutase localization on gel was observed as negative staining with colourless bands against background of formazan blue. Peroxidases activity were localized by two protocols viz. diamino benzedene (DAB) method [28] and (Tetramethylene benzedene and Hydrogen Peroxide) (Bangalore Genei Ltd, India) method. Observations were made for appearance of yellowish brown color bands revealing presence of Peroxidase.

The leaf discs were subjected to various sodium chloride salt concentrations from 1000 ppm to 6000 ppm at three hrs and extracted protein samples were used for the enzyme assay.

Results and Discussion

Effect of acetyl salicylic acid on Superoxide dismutase and Peroxidase

The present study was undertaken to elucidate the role of superoxide dismutase and peroxidase during Acetyl salicylic acid signaling and sodium chloride salt signaling in *Withania sominifera* (L.) Dunal using enzyme activity staining on acrylamide gels. The results showed that during acetyl salicylic acid signaling, Superoxide dismutase activity increased in 2nd and 4th hrs and decreased till 6th hrs and further increased up to 10th hrs in (Figure 1). Similarly in Peroxidase, a low enzyme activity was observed in 0 and 6th hrs while in 2nd, 4th, 8th and 10th hrs the expressions of three isozymes of Peroxidases were observed (Figure 2).

The decrease in Peroxidase activity by 6th hrs may result in increased accumulation of Hydrogen Peroxide resulting in oxidative burst. The decrease in Superoxide dismutase activity may be due to the feedback inhibition mechanism of the enzyme. The results showed that, high concentration of Hydrogen Peroxide resulted in a linear decrease in Superoxide dismutase activity in *Staphylococcus aureus*.

During sodium chloride salt stress only Superoxide dismutase activity was localized since Peroxidases activity was negligible. In different sodium chloride salt concentration from 1000 to 6000 ppm, the Superoxide dismutase activity was maximum increase during 5000 ppm treatment revealed by the presence of three bands in (Figure 3), similar result studied in chloroplasts of *S. salsa* [29]. During time dependent sodium chloride salt treatment, the Superoxide dismutase activity increased during 2nd to 8th hrs of treatment revealed by the presence of two bands (Figure 4).

Superoxide dismutase activity is increased initially with increasing sodium chloride salt concentration, but due to high salinity (6000 ppm sodium chloride salt concentration) increased in Hydrogen Peroxide concentration which causes oxidative brust, similar result is also studied in *C. maritima* [30].

Leaf morphology during ASA signaling

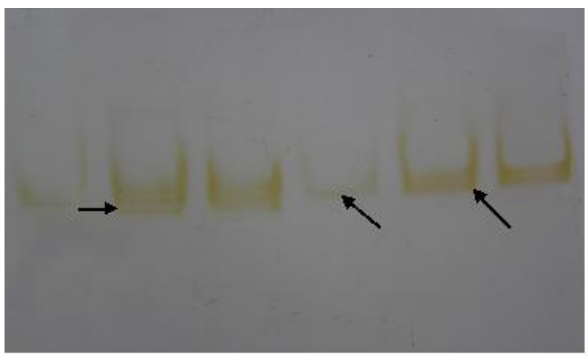

Figure 1: Effect of ASA stress on total Peroxidase activity

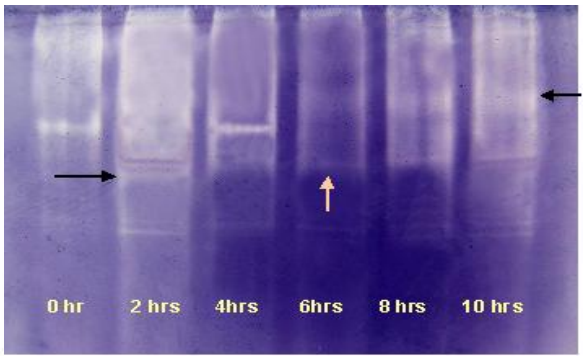

Figure 2: Effect of ASA stress on Superoxide Dismutase activity

Figure 1-2: Effect of ASA on Superoxide Dismutase and Peroxidase activity in *Withania somnifera*

Leaf morphology during NaCl stress

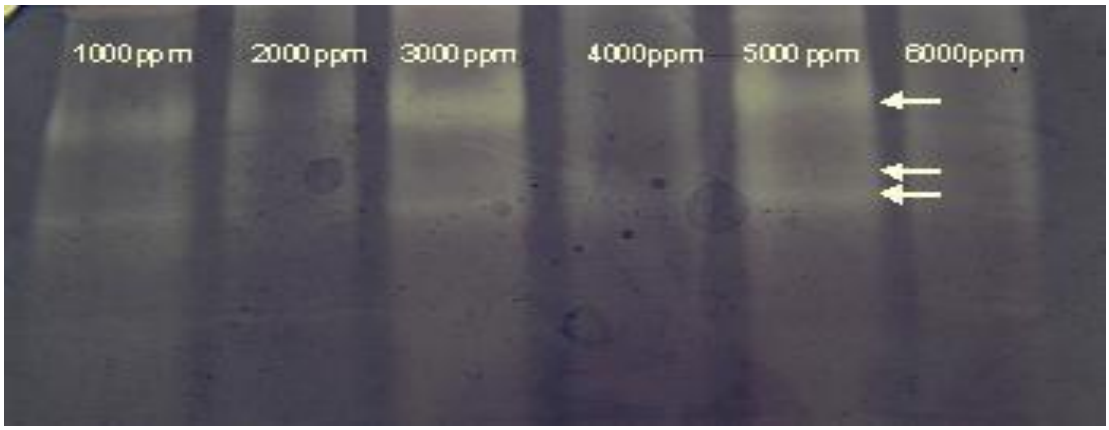

Figure 3: Effect of NaCl concentration on Superoxide Dismutase

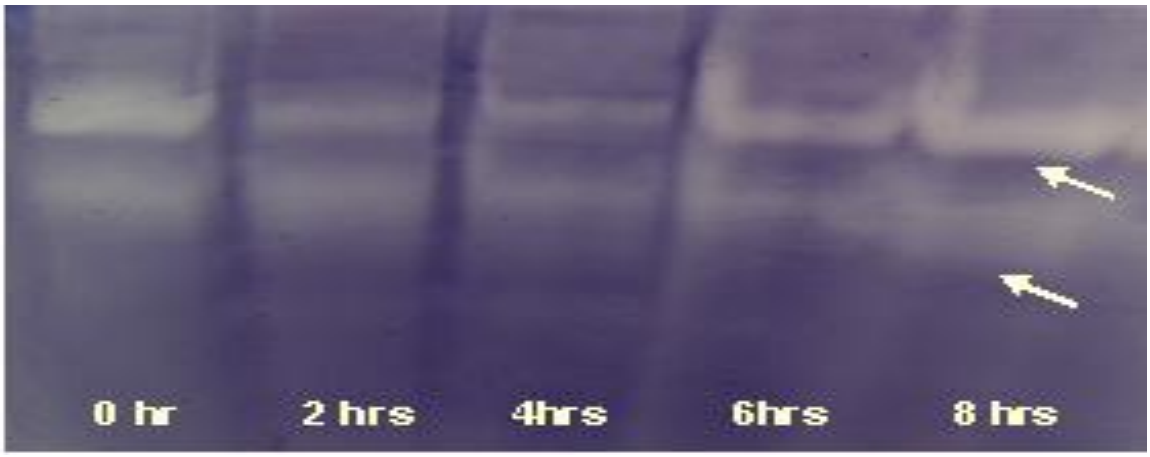

Figure 4: Effect of NaCl stress on Superoxide Dismutase

Figure 3-4: Effect of NaCl stress on Superoxide Dismutase activity in *Withania somnifera*

Conclusion

Cells are protected against oxidative stress by an interacting network of antioxidant enzymes. Present study was undertaken to elucidate the role of Superoxide dismutase and Peroxidase during Acetyl salicylic acid signaling and sodium chloride salt signaling in *Withania sominifera* (L.) Dunal using enzyme activity staining on acrylamide gels with regular intervals was documented. During Acetyl Salicylic Acid stress the Superoxide dismutase activity increased significantly by the 4th hrs of treatment, and decreased till 6th hrs and same in case of Peroxidase The decrease in Peroxidase

activity by 6th hrs may result in increased accumulation of Hydrogen Peroxide resulting in oxidative burst. The decrease in Superoxide dismutase activity may be due to the feedback inhibition mechanism of the enzyme. Superoxide dismutase activity is increased initially with increasing sodium chloride salt concentration, but due to high salinity (6000 ppm sodium chloride salt concentration) may resulted increase in Hydrogen Peroxide concentration which cause oxidative brust. Sometimes high amount of Hydrogen Peroxide accumulated and generating PCR/HR signals against plant pathogens to protect plant.

The importance of both antioxidant enzymes in stress defense has been demonstrated in transgenic plants over expressing Ascorbate peroxidase (APX) and Superoxide dismutase, which showed enhanced oxidative stress tolerance. While the role of the Superoxide dismutases in maintaining its potential therapeutic application, cosmetic products to reduce free radical damage to skin, for example to reduce fibrosis following radiation for breast cancer. Several studies over the past few years have looked into the role of *Withania somnifera* in having anti-inflammatory, anti-tumor, anti-stress, and antioxidant, mind-boosting, immune-enhancing, and rejuvenating properties.

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