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Biochemical Response of *Solanum Melongena* to Salinity Stress In Relation To Stress Factors

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Abstract: Salinity due to over accumulation of NaCl is usually of great concern and is injurious especially for plants growing in arid and semiarid regions. Considering this agricultural problem, an experiment was performed to evaluate the salinity stress response of Solanum melongena. The experiment was conducted on 60 days old plants. Four replicates were taken wherein two of the replicates were subjected to 25 mM NaCl and other two to 50 mM NaCl on every third day for duration of 10 days. The stress was found to reduce the dry and fresh weight, RWC of the leaf tissue respectively. Plants have multiple strategies to prevent oxidative damage to cells employing enzymatic antioxidants such as Peroxidase, Catalase and Polyphenol oxidase .These enzymes were estimated along with Carotenoids which help in the deactivation of ROS. Salinity induces generation of Reactive oxygen species (ROS) which occurs via electron transport reactions in the mitochondria and chloroplasts**.**

Keywords: *Solanum melongena, Brinjal, Salinity, Stress, Biomarker.*

INTRODUCTION

Brinjal is an important crop of the tropics and sub-tropics. The brinjal crop is of importance in the warm areas of east, being grown extensively in India. In India it is one of the most common, popular and principle vegetable crops grown throughout the country except high altitudes. It is a versatile crop adapted to different agro-climatic regions and can be grown throughout the year. It is a perennial crop but grown

commercially as an annual crop. A number of cultivars are grown in India, consumer's preference are dependent upon fruit, color, size and shape.

Brinjal or eggplant (Solanum melongena Linn.), is a very important common vegetable in India. It is often described as a poor person's vegetable because it is popular amongst small-scale farmers and low income consumers. It is featured in the dishes of virtually every household in India, regardless of food preferences, income levels and social status[1]. It is also used in ayurvedic medicine for curing diabetes, hyper-tension and obesity[1]. In addition, dried brinjal shoots are used as fuel in rural areas[1]. Contradictory literature exists on eggplant tolerance to soil salinity; some classified the eggplant as a moderately sensitive vegetable crop[2, 3,] whereas[4] others reported that the eggplant is sensitive to water stress caused by salinity. Studies on the eggplant is affected negatively by increasing salt at the germination and seedling stages[5]. The salt tolerance of the different varieties of the eggplants is varied from variety to variety[5]. Unlike other important Solanaceae crops such as tomato, potato, chili pepper, and tobacco, all of which originated in South America and are cultivated worldwide, eggplant (Solanum melongena L.) is indigenous to the Old World and in this respect it is phylogenetically unique[6].

In tropical and subtropical climates, eggplant can be sown directly into the garden. Eggplant grown in temperate climate fares better when transplanted into the garden after all danger of frost is passed. Seeds are typically sown eight to ten weeks prior to the anticipated frost-free date. However, from the botanical and agronomical points of view, compared with the two Solanum model species, eggplant has many unique aspects including extra-large fruit size, high tolerance to biotic and abiotic stresses, and parthenocarpy without any negative pleiotropic effects .While its nutritional value, such as content of vitamins, has not been considered remarkable, eggplant has recently begun to be reconsidered as a good source of free radical scavengers, such as anthocyanins and phenolics from the viewpoint of functional food research .This unique species, a valuable member of the Solanaceae for comparative biological studies of genetics, physiology, development and evolution of this taxon.

Abiotic stresses trigger a wide range of plant responses, from altered gene expression and cellular metabolism to changes in growth rates and crop yields. The duration, severity, and the rate at which the stress is imposed influence how a plant responds. Several adverse conditions in combination may elicit a response different from that of a single type of stress. Features of the plant, including organ or tissue identity, development age, and genotype, too influence the plants response to stress. Some responses clearly enable a plant to acclimatize to stress, whereas the functional role of other responses is not apparent. Therefore, identifying which responses promote or maintain plant growth and development during stress is important for understanding the stress response process. The ability to withstand stresses frequently becomes the limiting factor for plant growth, survival and geographical distribution. The study of the behavior of plants under stress is of practical importance from the point of view of agricultural yield.The current study aims at identifying the morphological changes in the plant growth and development caused due to salinity stress and also to check for the varied levels of enzyme biomarkers during stress.

MATERIAL AND METHODS

Growth and stress conditions: The experiment was conducted on 60 days old plants growing under natural greenhouse conditions; day/night temperature and relative humidity were: 30/25 °C and 75/70 % respectively. The average photoperiod was 12 h light/12 h dark. Four replicates were taken wherein two

of the replicates were subjected to 25 mM NaCl and other two to 50 mM NaCl on every third day for duration of 10 days. Samples used for determination of RWC, fresh and dry weight were used immediately after collection.

Carotenoids: 100 mg of the leaf tissue of each sample was taken and homogenized in 5 mL acetone using a chilled mortar and a pestle. The mixture was then filtered using a funnel and Whatman No. 2 filter paper. The filtrate was collected and to this equal volume of peroxide-free ether was added. A little bit of water was added to aid in separation. The upper ether phase containing carotenoids was taken and evaporated to dryness in a rotary evaporator under pressure at 35 ºC. The residue was then dissolved in minimum quantity of ethanol. To this 0.2 mL of 60% KOH was added to saponify the mixture. This is done in order to remove the interfering lipids and chlorophylls and also to cleave the esterified carotenoids. This mixture is boiled in a water bath for 5-10 min. To this, equal volumes of peroxide free ether is added and partitioned. The ether layer is taken and evaporated to dryness under reduced pressure in a rotary evaporator. The residue was dissolved in minimum quantity of ethanol. The absorbance was read at 450 nm and the concentration of carotenoids was calculated from the standard graph[7].

Extraction of enzymes: 100 mg of fresh leaf tissue was homogenized with 2 mL of pre-chilled 100 mM sodium phosphate buffer (pH 7.0) using chilled pestle and mortar. The homogenate was then centrifuged at 6000 rpm for 20 min at 4 °C. This supernatant was used as a source of enzymes. This set up was stored in an ice bath till the completion of the experiment. Soluble protein content was determined according to the method of Lowry[8] with BSA as the standard[8].

Catalase (CAT, EC 1.11.1.6): Catalase activity was assayed by following the decline in optical density at 240 nm ($\varepsilon = 39.4\ M^{-1}\ cm^{-1}$) according to the method of Aebi[9]. The reaction mixture consisted of 100 µl of enzyme extract and 100 mM sodium phosphate buffer (pH 7.0). The reaction was started by addition of H_2O_2 to a final concentration of 10 mM, and its consumption was measured for 2 min at an interval of 15 s. One unit of activity was defined as the amount of enzyme that catalyzes the oxidation of 1 µmol H_2O_2 min–1 under the assay conditions[9].

Guaiacol peroxidase (POX, EC 1.11.1.7): Guaiacol peroxidase activity was measured in a reaction mixture of 3.0 mL consisting of 100 mM phosphate buffer (pH 7.0) containing 20 mM guaiacol, 10 mM H_2O_2 and 100 µl enzyme extract[10]. The formation of tetraguaiacol was followed by an increase in absorbance at 470 nm (ε = 26.6 mm–1 cm–1). One unit of peroxidase is defined as the amount of enzyme needed to convert 1 µmol of H_2O_2 min^{-1} at 25 °C [10].

Polyphenoloxidase (PPO, EC 1.14.18.1): PPO was assayed spectrophotometrically at 400 nm using tertiary butyl catechol as substrate according to Kanade [11]. The assay mixture consisted of 3.0 mL sodium acetate buffer (pH 7.0), 1 mL of catechol and 0.5 mL enzyme extract. The quinone formed was measured at 495 nm (ε = 1150 M-1cm-1) at intervals of 5 s. One unit of enzyme activity is defined as the amount of enzyme that produces 1 µmol of t-butylquinone per minute under the assay conditions[11].

RESULTS AND DISCUSSION

Effect of salinity on relative water content (RWC): Metabolic activity and leaf survival is determined by the relative water content (RWC) of the plant[12]. Imposition of salt stress reduced the RWC in brinjal plants (Table 1, Fig 1a). Stomata closure caused by the accumulation of abscisic acid produced in roots and then accumulated in guard cells in response to salt stress are the causes of decrease in RWC[13, 14]. Re-

watering plants showed recovery in terms of RWC illustrating the importance of plasticity in the leaf area for maintaining control over water use, [15] studied the crop responses to drought and the interpretation of adaptations.

Effect of salinity on Carotenoids: Many reports have emphasized the role of accessory pigments, carotenoids (especially xanthophylls) in direct deactivation of ROS[16, 17]. Carotenoids influence many plant processes including the protection of photosynthetic components against oxidative damage and the dissipation of excess absorbed energy **(Table 1, Fig 1b)**.

These functions moderate the effects of varying temperatures and ultraviolet (UV) light intensity, thus maintaining plant productivity in times of environmental stress. Carotenoids are spatially associated with chlorophyll[18] and sustain photochemical functions by protecting chlorophyll against oxidative destruction[17]. In all photosynthetic organisms, the carotenoids β-carotene and zeaxanthin serve an important photo-protective role, either by dissipating excess excitation energy as heat or by scavenging ROS and suppressing LPO[19].Decrease in the carotenoid content at higher salinity levels has been reported to be due to degradation of β-carotene and formation of zeaxanthin in barley and sorghum[20].It has also been reported decreased carotenoid and chlorophyll contents in *Phyllanthus amarus* and *Vigna mungo* plants with increasing Cd concentrations respectively[21,22]. However, an increased carotenoid content was also reported following Cd stress[23].

Table 1: Levels of relative water content (RWC) and Carotenoids in leaves of Brinjal plants

Sample	Relative Water Content (RWC)	Carotenoids (µg/g FWt)
Control	65.64±2.90	240±60
Sample A	62.93±3.08	300±96
Sample B	60.54±3.00	270±56
Sample C	55.8 ±3.10	528±66
Sample D	52.18±3.9	434±41

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Effect of salinity stress on Antioxidant enzyme activity: Plants have multiple strategies to prevent oxidative damage to cells, employing enzymatic and non-enzymatic antioxidants. Superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), glutathione reductase (GR), and ascorbate peroxidase (APX) are among the enzymatic antioxidants.

Catalase (CAT): It is well known fact that dismutation of O^{2-} catalyzed by SOD produces H_2O_2 and O_2[24]. CAT, the main scavenger of the strong oxidant H_2O_2 produced during photorespiration, β-oxidation of lipids in peroxisomes and purine catabolism under normal conditions is generally accumulated under stressful conditions. Apart from the reaction with hydrogen peroxide CAT also reacts with hydroperoxides such as methyl hydrogen peroxide[25]. The induction of CAT activity under salt stress is well documented and a positive relationship has been found between its up-regulation and stress tolerance

through signal transduction[26, 27, 28]. CAT activity was found to increase followed by a decline with time of progression of salinity stress in Brinjal **(Table 2, Fig. 2a)** .

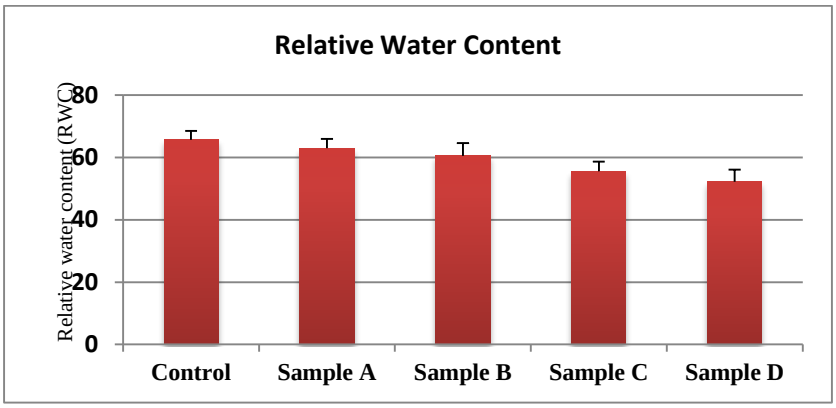

Fig 1a.: Levels of Relative water Content of leaf tissue of Brinjal after treatment with 25 mM NaCl 3DAS (Sample A) and 5DAS (Sample B) interval and 50 mM NaCl 3DAS (Sample C) and 5DAS (Sample D). Data plotted are mean ± SE of duplicates of three separate replicates, mean values were compared by one way ANOVA ($P \leq 0.05$).

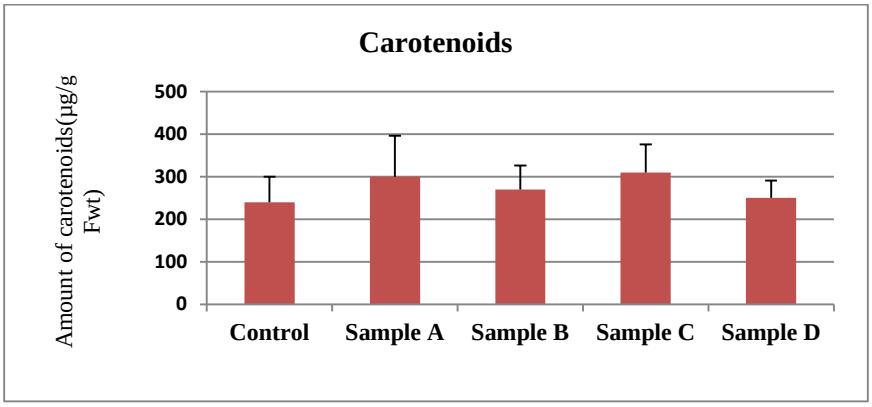

Fig .1b: Levels of Carotenoids of leaf tissue of Brinjal after treatment with 25 mM NaCl 3DAS (Sample A) and 5DAS (Sample B) interval and 50 mM NaCl 3DAS (Sample C) and 5DAS (Sample D). Data plotted are mean ± SE of duplicates of three separate replicates, mean values were compared by one way ANOVA ($P \leq 0.05$).

This phenomenon occurs in many plant species under oxidative stress and is related to the accumulation of salicyclic acid in oxidatively-stressed plants. A variable response has been observed under metal stress. Its activity was found to decline in *Glycine max*[29], *Capsicum annum*[30]and *A.thaliana*[31].whereas, an increase was seen in *Oryza sativa*[32], *T. aestivum*[14], and *V. mungo* roots[22] under Cd stress. [33]Also that pre-treatment of rice seedlings with hydrogen peroxide under non heat shock conditions resulted in an increase in the CAT activity and protected rice seedlings from subsequent Cd stress.

Peroxidase (POX): Under sub lethal salinity conditions, level of POX activity has been used as a potential biomarker to evaluate the intensity of stress **(Table 2, Fig.2b)**. Like CAT, POX is involved in detoxifying H_2O_2, but at the expense of another substrate being oxidized such as ascorbate. POX is also reported to be enhanced by salt stress and this was positively correlated with salt stress tolerance[34,28] . Levels of POX in salt-stressed leaves exhibited concentration-dependent elevation .The activity of peroxidase varies considerably depending upon the plant species and stress conditions. Increased peroxidase has been reported in Cd-exposed plants of *T. aestivum*[35], *A. thaliana*[31] and *C. demersum*[36]. An increase in peroxidase activity under drought stress was reported in liquorice[37], sun flower[38], and polar[39].

Besides their role in the removal of H_2O_2 from cytosol and chloroplasts, POX are involved in a variety of other physiological and metabolic functions including biosynthesis of cell wall, oxidation of toxic compounds, growth and development processes, and formation of isodityrosine bridges that are believed to crosslink structural proteins[40, 41]. CAT has low substrate affinities when compared to POX and requires simultaneous access to two H_2O_2 molecules at the active site, stressing the importance for the need for POX in ROS removal[42].

Polyphenoloxidase (PPO): A major focus of research in polyphenol oxidase has been its potential role in defense mechanism in plants especially to biotic stresses (Table 2, Fig.2c). PPO has been proposed to function in the Mehler reaction, photoreduction of molecular oxygen by PSI[43]. PPO activity is known to increase during the progression of stress and could be a possible tolerance mechanism due to its role in phenolic compound synthesis[44]. PPO levels in Brinjal showed concentration-dependent enhancement under salinity stress. Such increase in activity was demonstrated in salt-stressed *C. angustifolia*[45] and bean seedlings[46]. Decline in PPO levels beyond 5DAS suggested that the oxidative stress set in at this point is inhibitory to the enzyme or the signals which induce PPO may become disrupted. Our results are in conformity with those reported in olive[47], groundnut[48], rice[49] and yam[50].

Table 2: Activity of CAT, POX, PPO in leaves of Brinjal subjected to NaCl stress

Sample	Catalase (IU/g FWt	Peroxidase (IU/g FWt)	Polyphenoloxidase (IU/g FWt)
Control	0.008±0.001	0.987±0.023	0.086±0.001
Sample A	0.023±0.004	1.670 ±0.053	0.124±0.004
Sample B	0.021±0.003	1.540 ±0.042	0.102±0.002
Sample C	0.069±0.005	3.518±0.092	0.162±0.005
Sample D	0.043±0.005	2.129±0.082	0.152±0.004

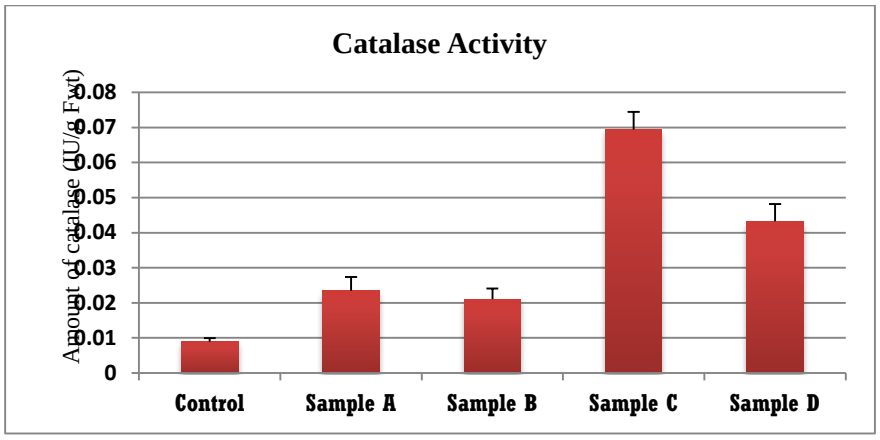

Fig 2a: Activity of CAT in leaf tissue of Brinjal after treatment with 25 mM NaCl 3DAS (Sample A) and 5DAS (Sample B) interval and 50 mM NaCl 3DAS (Sample C) and 5DAS (Sample D) respectively. Data plotted are mean ± SE of duplicates of three separate replicates, mean values were compared by one way ANOVA ($P \leq 0.05$)

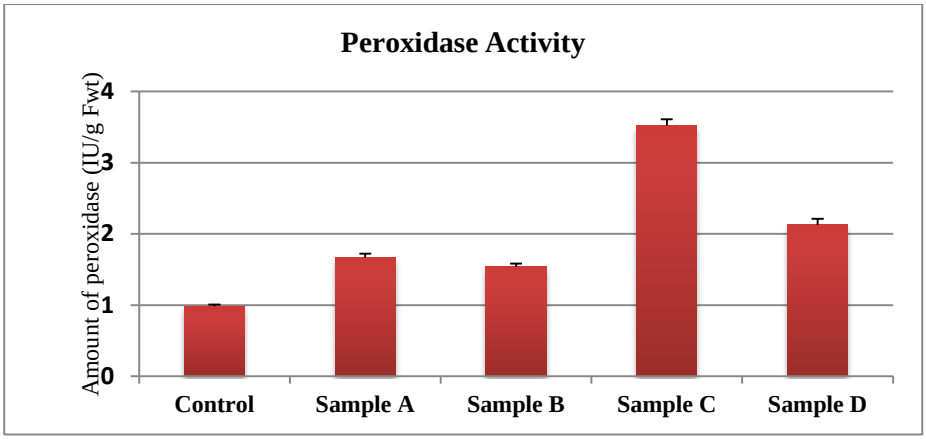

Fig 2b: Activity of POX in leaf tissue of Brinjal after treatment with 25 mM NaCl 3DAS (Sample A) and 5DAS (Sample B) interval and 50 mM NaCl 3DAS (Sample C) and 5DAS (Sample D) respectively. Data plotted are mean ± SE of duplicates of three separate replicates, mean values were compared by one way ANOVA ($P \leq 0.05$).

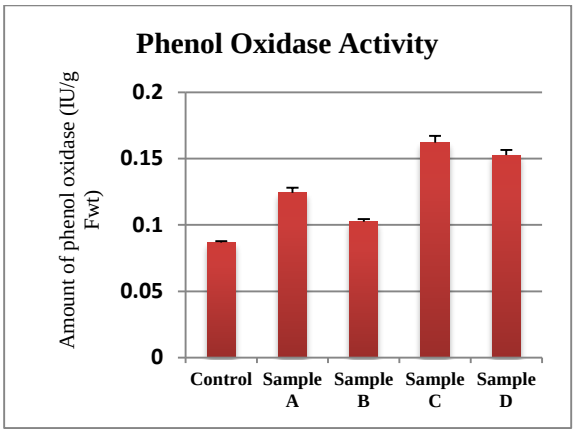

Fig 2c: Activity of PPO in leaf tissue of Brinjal after treatment with 25 mM NaCl 3DAS (Sample A) and 5DAS (Sample B) interval and 50 mM NaCl 3DAS (Sample C) and 5DAS (Sample D) respectively. Data plotted are mean ± SE of duplicates of three separate replicates, mean values were compared by one way ANOVA ($P \leq 0.05$).

CONCLUSION

Soil salinity, one of the most serious problems on planting areas, has the most obstructive impact on crop production in the world. As more land becomes saline by poor irrigation practices the impact of salinity is becoming more important. Salinity poses many adverse effects on plant growth and developmental processes such as germination, seedling growth and vigor, vegetative growth, flowering and fruit set by causing cytotoxicity and osmotic stress. Depending on the species of the plant and source of stress, the plant will respond in different ways. When a certain tolerance level is reached, the plant will die eventually.

Antioxidant enzymes also play a very important role in counteracting the damage caused by reactive oxygen species. Catalases are enzymes that catalyze the conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese cofactor. Here, its cofactor is oxidized by one molecule of hydrogen peroxide and then regenerated by transferring the bound oxygen to a second molecule of substrate. Peroxidases are a set of enzymes that act on hydrogen peroxide converting it to water and oxygen. It is a very important antioxidant which is the first to act in case of any stress. Polyphenol oxidase enzymes are also an important antioxidant that is found to have a potential role in defense metabolism in plants especially to biotic stress. All these enzymes were found to show considerable increase in their activity in response to salinity stress. Low levels of antioxidants, or inhibition of the antioxidant enzymes, causes oxidative stress and may damage or kill cells.

Carotenoids influence many plant processes including the protection of photosynthetic components against oxidative damage and dissipation of excess absorbed energy. These functions moderate the effects of varying temperature and UV light intensity, thus maintaining plant productivity in times of environmental stress. The response of plants to salt stress is based on the transcriptional action of many defense proteins, and research has not discovered the basis for them all. Osmotic stress and ion toxicity are the problems stemming from salt stress, and the resulting decrease in chemical activity causes cells to lose turgor. Cell growth depends on turgor to stretch the cell walls, and lack of turgor implies danger for

cell survival. The plant's defense against this salinity attack requires osmotic adjustment, and, to a certain degree this can be done through synthesis of intracellular solutes.

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