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International Journal of Current Research in Life Sciences
Vol. 07, No. 11, pp.2816-2821, November, 2018

ISSN: 2319-9490

RESEARCH ARTICLE

NACL INDUCED CHANGES IN ANTIOXIDANT METABOLISM OF *SESAMUM INDICUM* L.

Usha Kiran, D. and *Ranganayakulu, G. S.

Dept of Botany, Rayalaseema University, Kurnool-518007, Andhra Pradesh, INDIA

Received 28th September, 2018; Accepted 20th October, 2018; Published 30th November, 2018

ABSTRACT

In the present study we analyzed the extent of oxidative damage as the rate of lipid peroxidation and antioxidative response in 5-day-old seedlings of two sesamum cultivars (cultivarYML-17 and Sweetha) under different NaCl stress levels (50, 100 and 150Mm). Salt stress resulted a significant increase in the rate of lipid peroxidation as showed in the levels of malondialdehyde content coupled with the increase in the activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione reductase (GR) and glutathione S-transferase (GST) in both Sesamum cultivars with increasing slat stress compared to their controls. However the cultivar YML-17 registered high antioxidant activity and lower lipid peroxidation than cultivar sweetha. These results suggest that salt stress causes oxidative stress in plants and the antioxidative enzymes SOD, CAT, POD, GR, and GST could play a pivotal role against oxidative injury.

Key words: *Salt stress, Sesamum, CAT, SOD, POD and, MDA.*

Citation: ***Usha Kiran, D. and Ranganayakulu, G. S.*****, 2018.** "Nacl induced changes in antioxidant metabolism of sesamum indicum L." *International Journal of Current Research in Life Sciences*, 7, (11), 2816-2821.

INTRODUCTION

Soil salinity is one of the most important agricultural problems in arid and semiarid regions in different parts of the world. Limited rainfall, high evapo-transpiration, high temperature and inadequate water management contribute to increase in soil salinity (Dionisio-Sese and Tobita, 1998; Mehta, 2011). The economic prosperity of a nation like India, where a majority of population is primarily dependent on agriculture depends on the crop productivity (Pitman, 2002). So better understanding of the mechanism that enables plants to adapt to salt stress was necessary for exploiting saline soils. Therefore, the study of the effects of salinity on the plant growth, development and yield is not only of great academic interest but also of economic importance. The cellular mechanisms are especially important to glycophytes, by which physiological and biochemical processes contribute to the stress adaptation (Lauchli, 1990). One of the biochemical changes occurring when plants are subjected to biotic or abiotic stresses is the production of reactive oxygen species (Allen, 1995 and Dionisio-Sese, 1998). Exposure of plants to unfavorable environmental conditions such as temperature, high light intensity, water availability, air pollutants or salt-stress can increase the production of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide radical (O^-_2), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-). Like other abiotic stresses, salinity also induces oxidative stress in plants (Bartosz,, 1997 and Rout, 2001). Superoxide dismutases (SOD) are a family of metalloenzymes that catalyze the disproportionation of O_2 radicals into H_2O_2 and O_2, and play an important role in protecting cells against the toxic effects of superoxide radicals produced in different cellular compartments like chloroplasts, cytosol and mitochondria (Fridovich, 1986; Elstner, 1991; Del Rio, 1992; Bowler, 1994; Halliwell, 2000). The presence of SOD in peroxisomes was demonstrated for the first time in plant tissues by del Rio *et al*., (2003). The balance between SOD and APX or CAT activities in cells is crucial for determining the steady-state level of superoxide radicals and hydrogen peroxide (Bowler, 1994). Catalase is found to be present only in peroxisomes and absent in chloroplasts. It dismutates H_2O_2 into water and molecular oxygen (Willekens, 1997; Mehta, 2011 and Meloni, 2003). Benavides *et al*., (Benavides, 2000), reported that the enzymes ascorbate peroxidase and catalase are responsible for hydrogen peroxide detoxification in *Solanum tuberosum* and suggested that ascorbate peroxidase was found to be more important than catalase in the detoxification. Enhanced productions of oxygen free radicals are responsible for peroxidation of membrane lipids and the degree of peroxidative damage of cells was controlled by the potency of antioxidative peroxidase enzyme system. Peroxidase decomposes H_2O_2 by oxidation of co-substrates such as phenolic compounds and antioxidants. An increase in the peroxidase activity was a common response to oxidative and abiotic stresses (Olmos, 1997 and Fieldes, 1998). Peroxidase was used as a parameter to screen salt tolerance in *Brassica* species by Stevens *et al*. (Fieldes, 1978). Glutathione

Corresponding author: **Ranganayakulu, G. S.**
Dept of Botany, Rayalaseema University, Kurnool-518007, Andhra Pradesh, INDIA

(GSH) is known to be found at higher concentrations in chloroplasts and other cellular compartments are crucial for plant defense against oxidative stress (Noctor, 1998). The glutathione biosynthesis in chloroplasts resulted in oxidative damage and altering the overall redox state of chloroplasts (Karpinski, 1999) Above studies suggested that the oxidized: reduced ratio of the different antioxidants served as a signal for the modulation of ROS-scavenging mechanisms. Plant responses under salt stress are only suggestive of interesting abiotic modulation of plant metabolism but probably also signify, at least to some extent, the plant's potential to adjust to salt stress environment. Knowledge of such responses is pre requisite for systematic identification and development of plant genotypes. Genotypic variations in the magnitude of salt induced responses in several crop plants including sesamum have provided significant information concerning their adaptability or tolerance to salinity.

Sesamum indicum L. is an important oilseed crop in the tropics and subtropics belongs to the family pedoliaceae. It is grown mainly in developing countries by small holder farmers who rarely apply fertilizer. Among the oil seed plants, sesamum is an important crop for both oil and food for many people in the semi-arid regions where the rainfall is inadequate. It is one of the oldest cultivated plants in the world, sesame oil is not mentioned in the Bible, but appears to have been important in non-Hebrew cultures 2,000 to 4,000 years ago. It was a highly prized oil crop of Babylon and Assyria at least 4,000 years ago. Today, India and China are the world's largest producers of sesame, followed by Burma, Sudan, Mexico, Nigeria, Venezuela, Turkey, Uganda and Ethiopia (Irbrahim and Aliyu, 2012). World production in 1985 was 2.53 million tons on 16.3 million acres. Sesame seeds are approximately 50% oil and 25% protein. They are used in baking, candy making, and other food industries. The oil contains about 47% oleic and 39% linoleic acid. Sesame oil and foods fried in sesame oil have a long shelf life because the oil contains an antioxidant called sesamol. Sesame oil is also used in the manufacture of soaps, paints, perfumes, pharmaceuticals and insecticides. In the present investigation, therefore, an attempt is made to understand the biochemical and molecular responses of two sesamum cultivars with differential sensitivity to salt stress and to identify the traits, which contribute for better performance during periods of salt stress. Information on salt-responsive proteins/genes is crucial for improving salt-tolerance through genetic engineering techniques.

METERIAL AND METHODS

Seeds of *Sesamum indicum L.* cultivars namely YLM-17 and sweetha, were procured from Acharya N. G. Ranga Agricultural University (ANGRAU) Guntur, Andhra Pradesh India. Seeds were surface sterilized with 0.1% (w/v) sodium hypochlorite solution for 5 minutes, thoroughly rinsed with distilled water, germinated in Petri plates lined with filter papers. Salt stress was induced by using sodium chloride solution at different concentrations (50, 100, and 150mM) prepared from half strength Hoagland nutrient solution. Half strength nutrient solution alone served as control. The Petri plates were kept at room temperature of 25± 4^{oC} at normal light conditions. 5-day old seedlings were taken for morphological and biochemical analysis at different NaCl stress levels).

Lipid peroxidation assay: Lipid peroxidation was determined by measuring of malondialdehyde (MDA) formation using thiobarbituric acid (TBA) method followed by Peever and Higgins (Peever, 1989), 1 gr of tissue (FW) homogenized in 5 ml of 0.1% (w/v) TCA. The homogenate was centrifuged at 10000 rpm for 10 minutes and 4 ml of 20% TCA containing 0.5% (w/v) TBA was added to 1 ml of supernatant. The mixture was heated at 95°C for 30 minutes and then quickly cooled on ice. The content was centrifuged at 10000 rpm for 15 minutes and absorbance of the supernatant was read at 532nm and 600nm, the MDA concentration was determined by its extinction coefficient of 155 mM^{-1} cm^{-1}.

Superoxide dismutase (SOD) (EC: 1.15.1.1): The activity of superoxide dismutase was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium as described by Beauchamp and Fridovich (Fridovich, 1986).

Extraction: Leaf material was homogenized in 50 mM phosphate buffer (pH 7.0) containing 1% poly vinyl pyrrolidine. The homogenate was filtered and then centrifuged in a refrigerated centrifuge at 15000×g for 15 min, and the supernatant obtained was used as source of enzyme. All steps in the preparation of enzyme extract were carried out at $4\ ^0$ C.

Assay: The reaction mixture (3 ml) consisting of 50mM phosphate buffer (pH 7.8), 13mM methionine, 75µM nitroblue tetrazolium, 0.1mM EDTA, 2µM riboflavin and 0.1 ml of enzyme extract. Riboflavin was added lastly, and test tubes were shaken and placed 30 cm below a light source (30 W fluorescent lamps). The reaction was started by switching-on the lights. The reaction was allowed for 30 min and then stopped by switching-off the lights. The tubes were covered with black cloth. The reaction mixture which was not exposed to light did not develop colour and served as control. The absorbance was measured at 560 nm in a Shimadzu-1601, UV-Vis spectrophotometer. Log A_{560} was plotted as a function of the volume of enzyme extract used in the reaction mixture. From the resultant graph, the volume of the enzyme extract corresponding to 50% inhibition of the reaction was read and considered as one enzyme unit and expressed as unit g^{-1} fresh weight min^{-1}.

Catalase (CAT) (EC: 1.11.1.6) and Peroxidase (POD) (EC: 1.11.1.7)

Extraction: The plant material was placed in a precooled mortor and ground with cold 50 mM Tris-HCl buffer, pH 7.0. The extract was passed through muslin cloth and centrifuged in a refrigerated centrifuge at 1000×g to remove cellular debris. The supernatant solution was centrifuged again at 10,000×g for 20 min. The supernatant was passed through a Sephadex G-25 column and fractions containing enzyme were pooled and used as enzyme source for the assay of catalase and peroxidase. All the steps were carried out in cold room.

Catalase assay: Catalase activity was assayed as per the method of Barber (Barber, 1980). The reaction mixture consisted of enzyme extract, 5 mM H_2O_2 and 50 mM Tris-buffer, pH 7.0. After incubating it for 1 min at 25^0 C, the reaction was stopped by adding 1.0 ml of 2.5 N H_2SO_4. The residual H_2O_2 was titrated with 0.01 N $KMnO_4$. A blank was maintained with the reaction mixture at zero time. Catalase activity was expressed as mg H_2O_2 oxidized g^{-1} fresh weight min^{-1}.

Peroxidase assay: Total peroxidase activity in the extracts was assayed as described by Hammer Schimdt *et al* (Hammerschmidt, 1982). The reaction mixture (3 ml) consisted of 0.25% (v/v) guaiacol in 10 mM sodium phosphate buffer (pH 6.0) containing 10 mM hydrogen peroxide. 25 µl of the crude enzyme extract was added to initiate the reaction which was measured spectrometrically at 470 nm (Shimadzu 1601). Total peroxidase activity was expressed as the increase in absorbance at 470 nm min^{-1} g^{-1} F.W (0.01 OD = 1 EU). Proteins in the extracts were quantified by the method of Lowry *et al.*, (Lowry, 1951), using BSA as the standard as described earlier.

Glutathione reductase (GR) (EC: 1.6.4.2): The plant material was extracted in 100 mM potassium phosphate buffer (pH 7.0). The homogenate was centrifuged for 10 min at 10,000×g in a refrigerated centrifuge. The supernatant was passed through Sephadex G-25 column; active fractions were collected and used as enzyme source for the assay of glutathione reductase.

Assay: Glutathione reductase activity was assayed as per the method of Foster and Hess (Foster, 1980). The reaction mixture consists of enzyme extract, 100 mM potassium phosphate buffer, (pH 7.0) containing 1.0 mM EDTA, 150µM NADPH and 500µM oxidized glutathione. The enzyme activity was measured at 340 nm. Activity was calculated using the extinction coefficient for NADPH of 6.22 mM^{-1} cm^{-1} and expressed as mmol NADPH oxidized mg^{-1} protein min^{-1}.

Statistical Analysis

The data obtained in all cases were subjected to Analysis of Variance (ANOVA), and the mean values were compared by Duncan's Multiple Range (DMR) test at 0.05% level (Duncan, 1955).

RESULTS

Lipid peroxidation: Lipid peroxidation in the seedlings of the control and stressed samples were measured in both Sesamum cultivars. The MDA content gradually increased with increase in stress intensity from mild to severe stress in both cultivars. MDA content was significantly higher more pronounced in cultivar Sweetha than cultivar YML it is indicating that a high rate of lipid peroxidation in cultivar Sweetha due to NaCl stress. Whereas cultivar YML showed no change in MDA content at the end of the experiment under NaCl stress results were represented in (Figure 1).

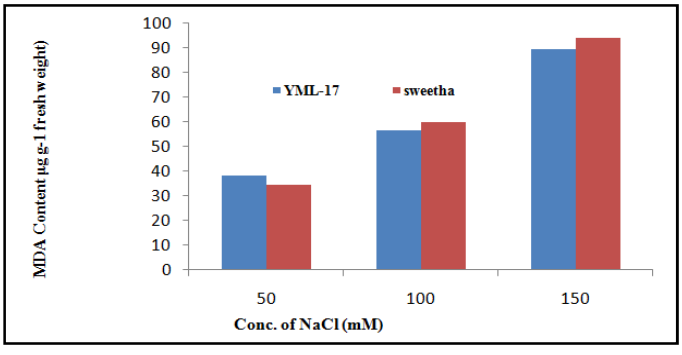

Figure 1. Malondialdehyde content (µ mol g^{-1} fresh wt) in 5-day-old seedlings of control and NaCl stressed Sesamum cultivars. Values are mean from five replications (±SD)

Lipid peroxidation in the leaves of the control and stressed samples were measured in both cultivars. The MDA content gradually increased with increase in stress intensity from mild to severe stress in both cultivars. MDA content was significantly higher more pronounced in drought-sensitive than drought-tolerant cultivar indicating a high rate of lipid peroxidation in drought-sensitive due to water stress. Drought-tolerant (A-1) showed no change in MDA content at the end of the experiment under water stress.

Superoxide dismutase: The enzyme superoxide dismutase activity was assayed in the 5-day-old seedlings of control and salt stressed plants and the results are presented in figure 2. Superoxide dismutase activity was significantly elevated in the stressed plants over control plants of both the cultivars. The superoxide dismutase activity did not appreciably increased at 50mM NaCl stress in both cultivars, but recorded a significant elevation in its activity at 100 and 150mM NaCl treatments in both cultivars. Nevertheless, the magnitude of increase in SOD activity was relatively more in the cultivar YML-17S1, than in Sweetha, 100 and 150mM NaCl stress levels.

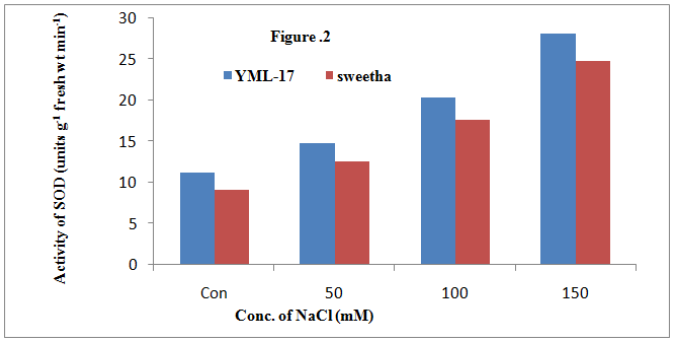

Figure 2. Activity of superoxide dismutase (units g^{-1} fresh wt min^{-1}) in 5-day-old seedlings of control and NaCl stressed Sesamum cultivars. Values are mean from five replications (±SD)

Catalase: The activity of catalase was assayed in control and NaCl stressed seedlings of both Sesamum cultivars. Relative to controls, the activity of catalase was registered an increase in the stressed plants of both varieties. However it was significantly elevated during moderate and severe stress treatments in both cultivars. The rate of increase in enzyme activity was dependent on stress severity. Nevertheless, a greater per cent increase was recorded in cultivar YML-17 compared to sweetha at all stress regimes results were predicted in Figure 3.

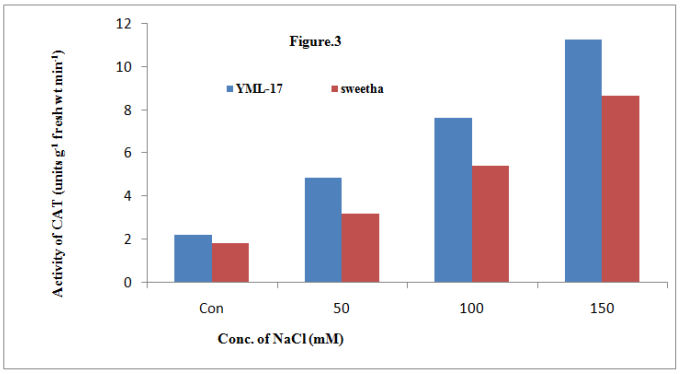

Figure 3. Catalase activity (mg of H_2O_2 oxidized g^{-1} fresh wt min^{-1}) in 5-day-old seedlings of control and NaCl stressed Sesamum cultivars. Values are mean from five replications (±SD)

Peroxidase: The peroxidase activity was assayed in the 5-day-old seedlings of control and salt stressed cultivars of Sesamum. The peroxidase activity was elevated in the stressed plants of both cultivars. However, the per cent increase in POD activity was relatively more in cultivar YML-17 than in cultivar sweetha data was represented in Figure 4.

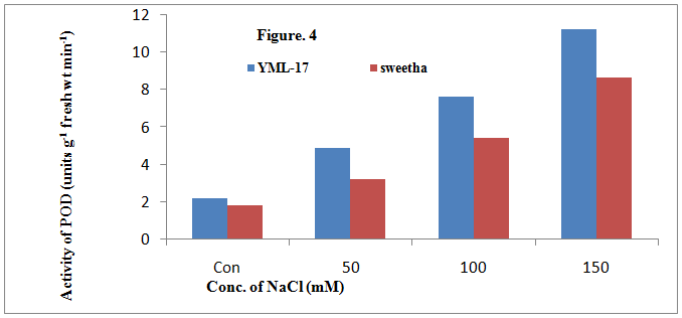

Figure 4. Activity of peroxidase (units g^{-1} fresh wt min^{-1}) in 5-day-old seedlings of control and NaCl stressed Sesamum cultivars. Values are mean from five replications (±SD)

Glutathione reductase: Glutathione reductase activity was assayed in 5-day-old seedlings of two sesamum cultivars under control and NaCl stressed conditions. The enzyme glutathione reductase was significantly increased in both cultivars at all stress levels. The rate of increase in enzyme activity was found to be dependent on salt concentration in both cultivars. But the per cent increase in the enzyme activity was more in cultivar YML-17 than in cultivars results were placed in Figure 5.

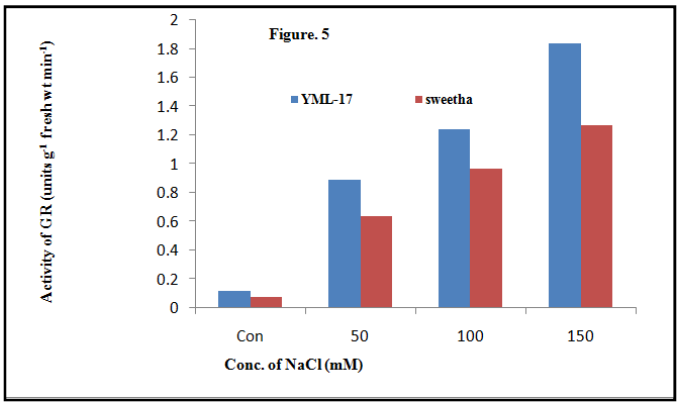

Figure 5. Activity of Glutathione reductase (m mol NADPH min^{-1} mg $protein^{-1}$) in 5-day-old seedlings of control and NaCl stressed Sesamum cultivars. Values are mean from five replications (±SD)

DISCUSSION

To avoid oxidative damage under unfavorable conditions such as high/low temperatures, water deficit, salinity etc. plants possess efficient antioxidant system. Plants possess antioxidant systems in the form of enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), glutathione reductase (GR), dehydroascorbate (DHAR) and metabolites *viz*., ascorbic acid, glutathione, α-tocopherol, carotenoids and flavanoids etc. The role of these antioxidative enzymes is responsible for scavenging of ROS in plants (Bowler, 1994; Moran, 1994; Smirnoff, 1989). The increase of antioxidant enzymes and metabolites are reported to increase under various environmental stresses (Hernandez, 1995 and Yu, 1999). Role of cellular antioxidant system in relation to water and/or temperature stress tolerance has been reported by many workers (Sairam, 1998 and Sairam, 2000). Some investigators have also reported on the generation of ROS and plant antioxidants in relation to salt stress (Hernandez, 1995 and Gueta-Dahan, 1997). POD activity in tolerant plant species enable plants to protect themselves against oxidative stress, where as such activity was not observed in sensitive plants (Pastori, 1992). The salt induced enhancement of POD activity in cultivar YML-17 indicated that it had a higher capacity for the decomposition of H_2O_2 generated by SOD. Elevated SOD activity without an accompanying increase in the ability to scavenge H_2O_2 can result in enhanced cytotoxicity by the destructive hydroxyl radical generated from H_2O_2 in a metal catalyzed Haber-Weiss reaction (Gossett, 1994). The enhanced activities of catalase and GR were noticed in cucumber leaves under NaCl stress (Lechno, 1997). GR could play a key role in the protection against oxidative stress, in contrast the negative correlation between the accumulations of SOD, CAT, POD activities which do not conferred protection against oxidative damage in salt-stressed cowpea leaves were reported by Cavalcanti *et al*., (Cavalcanti, 2007).

Salt tolerant varieties exhibit protection mechanism against increased radical production by maintaining the specific activity of antioxidant enzymes (Dionisio-Sese, 1998). Several studies indicated that acquisition of salt tolerance was a consequence of improved resistance to oxidative stress (Hernandez, 2000; Hernandez, 2002; Gossett, 1994 and Gomez, 1998). The antioxidative enzyme activity was found to be higher in salt tolerant cultivars than the susceptible ones (Sudhakar, 2001 and Hernandez, 2002). Rout and Shaw (Rout, 2001), have been suggested that salt-tolerant plants besides being able to regulate the ion and water movements also have a better antioxidative system for effective removal of ROS. Similarly, in the present study a higher accumulation of antioxidative enzymes was observed in tolerant cultivar YML-17 than in the susceptible cultivar sweetha, suggesting that higher antioxidant enzyme activity have a role in imparting tolerance to tolerance against various environmental stresses.

Conclusion

In the present investigation, the diverse responses of SOD, CAT, POD and GR enzyme activities to NaCl stress on 5-day-old seedlings suggest that oxidative stress may be an influential component of environmental stresses on Sesamum species. Salinity led to significant increases in SOD, CAT, POD and GR activities in both sesamum cultivars but the activity was higher in cultivar YML-17 compared to sweetha. Similar increase in the activities of these enzymes has been reported in other tolerant cultivars subjected to salt stress (Sudhakar, 2001; Rout, 2001; Thippeswamy, 2013).

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