Impact of Exogenous Abscisic Acid Pretreatment on the Antioxidant Activity of *Suaeda maritima* under Salinity Stress

G. anbarasi[#1], S.T. Somasundaram[*2]

** Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai-608 502, Tamilnadu, India.*

[1]anbarasi.gk08@gmail.com

[2]drstscas@yahoo.com

** Corresponding Author. E-mail: anbarasi.gk08@gmail.com*

Abstract **— Salinity stresses are a serious menace to agriculture productivity. Salt stress exerts its negative effects first and foremost by causing water shortfall which further leads to the formation of Reactive Oxygen Species (ROS). These ROS are capable of causing oxidative damage to different cellular compounds which includes membrane lipids, protein and nucleic acids. Plants generally utilize different antioxidant enzymes such as superoxide dismutases (SOD), Catalase (CAT), Ascorbate peroxidase (APX), Glutathione-S- transferase (GST) and glutathione peroxidases (GPX) to scavenge ROS. The plant hormone abscisic acid (ABA) also acts as a free radical scavenger and more important in overcoming stress than in acting as a simple osmolyte. Thus, this present study focused on the effect of exogenous abscisic acid on enzymatic scavenging activities of antioxidant enzymes such as SOD, POD, CAT, APX and GST to control ROS levels were studied in the leaves of salt tolerant variety of *S.maritima*.**

Keywords**— Exogenous abscisic acid, salinity tolerance, antioxidant enzymes, halophytes.**

I. INTRODUCTION

Researchers across the globe have clarified that salinity adversely affects the production of most crops. Salt stress exerts its negative effects first and foremost by causing water shortfall which further leads to the formation of Reactive Oxygen Species (ROS) (1). Impressive reports from worldwide researchers highlight that ROS incorporate signaling pathways concerned in plant growth, development, gravitropism, hormonal action and many other physiological functions (2) (3) (4) (5). The relation between various saline or drying environments, endogenous levels of water-soluble antioxidants and anti-oxidative enzymes has been reported by researchers across the world (6) (7).

Reports also highlight that the plants generates ROS like superoxide radicals, hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-) in increased levels upon exposure to conditions like biotic and abiotic stresses. These ROS are capable of causing oxidative damage to different cellular compounds which includes membrane lipids, protein and nucleic acids (8). Plants generally utilize different antioxidant enzymes such as superoxide dismutases (SOD), Catalase (CAT), Ascorbate peroxidase (APX), Glutathione-S- transferase (GST) and glutathione peroxidases (GPX) to scavenge ROS. Halophytes have an exceptional ability to utilize antioxidant enzymes to protect themselves from extreme environmental changes (9) (10).

The plant hormone abscisic acid (ABA) has long been known to play role in various environmental stresses particularly drought and salinity (11). It also acts as a free radical scavenger and more important in overcoming stress than in acting as a simple osmolyte (12) (13). Under stress conditions, ABA alters ion fluxes in guard cells to promote stromatal closure. There are possibilities for stimulating the plants enzymatic and non-enzymatic antioxidant defense system by the exogenous application of ABA, which alternatively triggers the increase of these metabolites (14) (15). Thus, this present study focused on the effect of exogenous ABA on enzymatic scavenging activities of antioxidant enzymes such as SOD, POD, CAT, APX and GST to control ROS levels were studied in the leaves of salt tolerant variety of *S.maritima*.

II. MATERIALS AND METHODS

A. Collection of plant seeds and growth condition parameters

The collected seeds of *Suaeda maritima* were sown in paper cups containing vermiculite in moisture condition with half-strength Murashige and Skoog (½ MS) medium. The seeds were daily sprinkled with distilled water. Later, only six weeks old seedlings was transferred to hydroponic media and were further maintained in culture room with a dark/light cycle of 8/16h at 37°C for 5days. At every one day of gap, the nutrient solution was replaced in order to avoid depletion of nutrients. After 5 days of adaptation, the plants were divided in four groups at random as (i) Control: Nutrient solution without NaCl and ABA addition), (ii) ABA treatment: Control solution supplemented with 50µM ABA (iii) NaCl treatment: Control solution supplemented with 300mM NaCl, (iv) ABA+ NaCl treatment: 50µM ABA applied daily for a period of 3days prior to stress exposure. After three days of placement with 50µM ABA, salt stress was imposed by adding 300mM NaCl to half strength of the nutrient solution. After 3days of treatment exposure, all the plants were harvested and washed quickly with distilled water. The plants were blotted dried on filter papers and stored in liquid nitrogen at -70°C for further analysis.

B. Extraction and analysis of antioxidant enzymes

For enzyme extraction leaf samples of *S.maritima* were homogenized in pre-cooled mortar and pestle in 5ml extraction mixture (1mM EDTA, 0.05% Triton X-100, 2% PVP, 1mM ascorbate in 50mM phosphate buffer of pH 7). The homogenate was centrifuged at 13,500rpm for 25min at 4^{O}C. Protein concentration was determined for the leaves of *S.maritima* by bicinchoninic acid protein assay kit (BCA, Sigma Aldrich) following the manufacturer's manual using the LAMBDA 25 UV/Vis Spectrophotometer (Perkin Elmer). BSA was used as the standard (1µg/µl) and the absorbance was measured at 562nm.

C. Superoxide Dismutase (SOD) Assay

Superoxide Dismutase was assayed by the method of Marklund and Marklund (1974) (16). To 1ml of tissue homogenate 0.25ml of ethanol and 1.2ml of chloroform were added, kept it in a mechanival shaker for 15min and centrifuged for 15min. To 0.5ml of the supernatant, 2.0ml of 0.1M tris-Hcl buffer pH 8.2 was added then 1.5ml of distilled water and 0.5ml of pyrogallol were added. Change in optical density was read at 420nm for 0, 1, 2, 3minutes intravels. Control tube contain 1ml of distilled water were also treated in a similar manner against a buffer blank. The enzyme activity is expressed as Units/mg protein. One Unit of SOD was defined as the amount of enzyme required to bring about 50% inhibition of pyrogallol auto-oxidation.

D. Catalase Assay

The enzyme activity was assayed by the method of maely and change, 1954 (17). The tissue was homogenized in 2ml phosphate buffer at 4^{o}C and centrifuged at 5000rpm. Estimation was done spectro photometrically following the decrease in absorbance at 240nm. 3ml of H_2O_2-phosphate buffer and about 0.01ml of enzyme solution was pipette into experimental cuvette. Read after every 30sec for about 3min against a control cuvette containing enzyme solution in H_2O_2 free phosphate buffer. The enzyme activity of catalase was expressed as µmols of H_2O_2 consumed/min/mg protein.

E. Peroxidase Assay

Peroxidase activity was assyed by the method of Kochba *et al*., 1977 (18). To 1.8ml of buffer, 1ml of 2% H_2O_2, 1ml of 50mM guaiacol were added and incubated at 37^{0}C for 10min. After incubation, 0.2ml of homogenate was added. The increase in optical density was measured against that of the blank at 470nm at 30sec intervals for 3mins.

F. Ascorbate Peroxidase Assay

Ascorbate Peroxidase activity was assyed by the method of Asada, 1984 (19). To 1ml of buffer, 0.1ml of homogenate, 1.7ml of distilled water and 0.1ml of CDNB were added and incubated at 37^{0}C for 10min. After incubation, 0.1ml of reduced glutathione was added. The increase in optical density was measured against that of the blank at 360nm. Enzyme activity is expressed as µmols of CDNB utilized 1min/mg protein at 37^{0}C.

G. Glutathione S Transferase Assay

The enzyme activity was assayed by the method of Habig *et al*., 1974 (20). To 1ml of buffer, 0.1ml of homogenate, 1.7ml of distilled water and 0.1ml of CDNB were added and incubated at 37^{0}C for 10min. After incubation, 0.1ml of reduced glutathione was added. The increase in optical density was measured against that of the blank at 360nm. Enzyme activity is expressed as µmols of CDNB utilized 1min/mg protein at 37^{0}C.

H. Statistical analysis

Statistical analysis was performed by one way ANOVA followed by post Hoc Duncan method and the values are expressed as mean ± SD, n=3 in each group. Different letters on the column represent significant difference ($P < 0.05$) based on Duncan's test.

III. RESULTS

A. Effect of exogenous ABA on SOD activity under salinity stress

SOD activity was significantly affected by salt stress ($1.92X10^4$ U mg^{-1} protein) as compared with control ($3.03\ X10^4$ U mg^{-1} protein). ABA pretreatment showed significant increase in SOD activity ($2.67\ X10^4$ U mg^{-1} protein) over NaCl treated leaves (Fig 1).

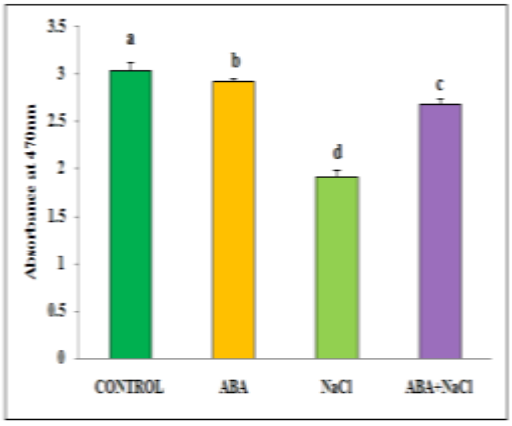

Fig 1 Individual and combined effect of salinity and ABA on SOD activity

B. Effect of exogenous ABA on CAT activity under salinity stress

The interaction between abscisic acid and salinity significantly affected the CAT activity (Fig 2). The highest value of CAT activity of $0.3X10^4$ µmols/ mg of protein was recorded in NaCl treated leafs which was significantly higher than control ($0.1X10^4$ µmols per mg of proteins) and single ABA treated leaves ($0.28\ X10^4$ µmols per mg of proteins). Treatment with ABA+NaCl resulted in significant reduction of CAT activity

($0.21X10^4$ µmols per mg of protein) as compared with NaCl treatment.

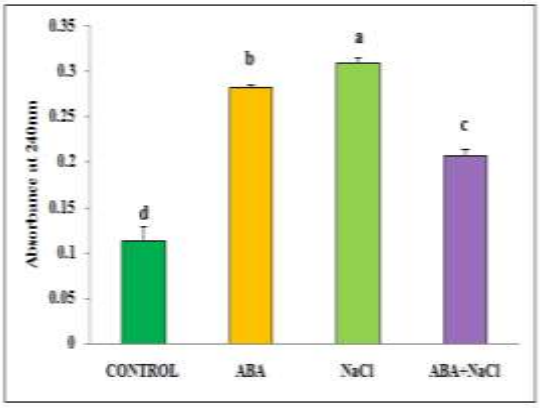

Fig 2 Individual and combined effect of salinity and ABA on CAT activity

C. Effect of exogenous ABA on POX activity under salinity stress

Highest POX activity observed in control (23.16 U mg^{-1} protein) and lowest peroxidase activity noted in ABA (16.67 U mg^{-1} protein) treated leafs. NaCl Treatment significantly decreased the Peroxidase activity as 21.86 U mg^{-1} protein and there were no significant changes observed in ABA+NaCl (21.5 U mg^{-1} protein) (Fig 3)

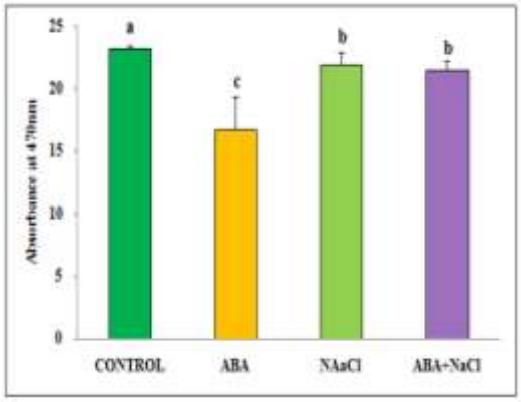

Fig 3 Individual and combined effect of salinity and ABA on POX activity

D. Effect of exogenous ABA on APX activity under salinity stress

The results of the present study highlight that significant increase in APX activity on imposition of salt stress (0.88 U mg^{-1} protein) over control (0.49 U mg^{-1} protein) was noted (Fig 4). Upon comparing with salt stress, ABA pretreatment under salt stress resulted more APX activity (1.13 U mg^{-1} protein) and not much effective changes noted in single ABA treated leafs (0.31 U mg^{-1} protein).

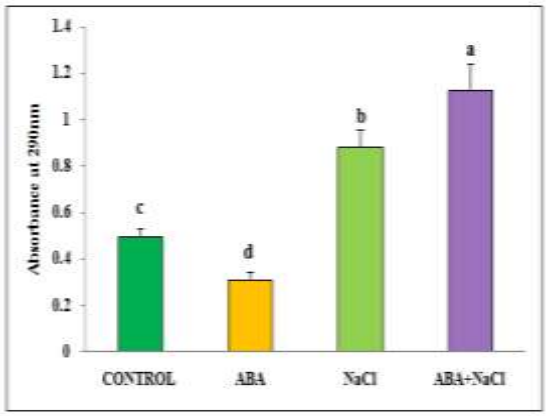

Fig 4 Individual and combined effect of salinity and ABA on APX activity

E. Effect of exogenous ABA on GST activity under salinity stress

NaCl treatment caused significant decrease in GST activity (3958 µmoles/min/mg protein) than the control (6204 µmoles/min/mg protein) and single ABA treated plant leafs (5277 µmoles/min/mg protein). In case of ABA pretreatment (ABA+NaCl) showed significant increase of GST (4750µmoles/min/mg protein) than the NaCl (Fig 5).

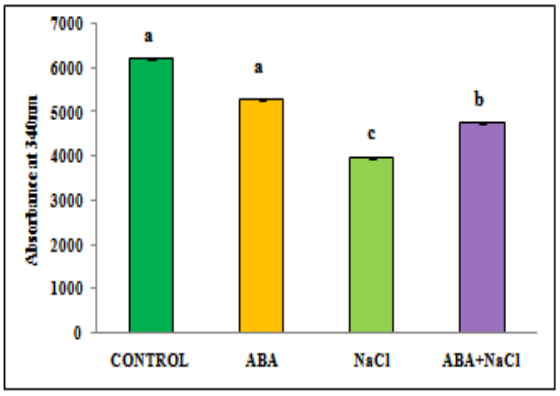

Fig 5 Individual and combined effect of salinity and ABA on GST activity

IV. Discussion

Salt damage has a broad physiological spectrum affecting metabolic processes. Salt stress induces the over production of ROS and cause the oxidative damage (21). Thus, the extent of oxidative stress is determined by the amount of ROS (O^-, HO and OH^-) in plant cells and the balance in the activity of antioxidant enzymes (SOD, APOX, POX and CAT) is crucial for suppressing toxic ROS levels (22) (23). In the present study, increased activity of antioxidant enzymes especially SOD, POX, APX and GST in leaves of *Suaeda maritima* plants treated with ABA indicate that the growth hormone greatly activates defense systems in order to alleviate oxidative damage induced by salt stress.

ABA treatment significantly increased SOD activity than the control. Exogenous ABA increasing the production of H_2O_2, O_2^- and OH^- and induces the activities of SOD, POD, CAT and glutathione reductase (GR) (24) (25). Induced antioxidant enzyme activities were reported in *Oryza sativa* (26), *Zea mays* (24), *Cynodon dactylon* (27), *Triticum aestivum* (28) by ABA applications. However, in *Picea aspartata* plants exposed to ABA, elevated SOD activity and unchanged CAT and APX activities (29). The current study found that SOD, GST and CAT activities increased in leaves which also highlight that ABA can cause the induction of antioxidant enzymes in *S.maritima* plants.

An increase in the activity of antioxidative enzymes under salt stress could be indicative of an increased production of ROS and a build-up of a protective mechanism to reduce oxidative damage triggered by stress experienced by plants. Even though a high SOD activity protects the plant against the superoxide radical, it cannot be considered solely responsible for membrane protection against peroxidation because it converts O_2^- to H_2O_2, which is also ROS. Intracellular levels of H_2O_2 are mainly regulated by CAT and POX (30). Numerous plants have been shown to express increased activity of CAT and POX in order to cope with toxic levels of H_2O_2 (31) (32) (33).

Our results do show that leaf CAT activity increase with salt stress. Similar results have been observed when *Beta maritima* (halophyte) and the non-halophyte *Beta vulgaris* were studied in *B. Gymnorrhiza* (34) (35). However, when the plants were subjected ABA, activity of catalase was decreased. Ramkishan Choudhary *et al*., (2012) reported that CAT activity decreased gradually with increasing concentration of ABA in *Syzygium cumini* plant.

This study result showed more POX activity in NaCl treated samples than the control. Dionisio Sese and Tobita (1998) reported that peroxidase activity increase in most of the rice seedling in response to salinity stress and may cause retarded growth due to inhibition of cell elongation. A transient increase of POX activity was also observed in halophytes like *Suaeda nudiflora* (38) *Crithmum maritimum* (39) at more NaCl concentrations. POD activity was significantly elevated by ABA pretreatment under salinity conditions. Similar result was obtained by Yang *et al*., (2012) in Perennial Grass Species. But no significant difference was found between NaCl and ABA pretreatment in leaves.

In the present study, salt stress caused significant increase of CAT and APX activities in the leaves of *Suaeda maritima*. APX and CAT activities induced by salinity have also been reported in salt- tolerant sugar beet (34), rice (41), plantain (42), potato (43), canola (44) and tobacco (45) which was consistent with the results obtained in this study. In case of ABA pre-treatment under salt stress also strongly increased the APX activity. Similar results have been observed in exogenously applied ascorbic acid in common bean under salinity stress (46). According to the present `study APOX and POX probably had more important role in H_2O_2 detoxifying than CAT. Similar results were obtained by Samia *et al*., (2009) in Maize Plants under salt stress. However, Induction of APOX in ABA pretreatment may have even dramatic effect on the protection of plants against salinity stress.

Significant increase of GST expression was recorded in *S.maritima* exposed to both ABA and ABA+NaCl exposure. GSTs may have important functions in the stress response due to their role in detoxification of toxic stress metabolites (eg. Lipid peroxides), so preventing the membrane damages. Consequently the GST enzyme can participate in the maintenance of the redox state, metabolism, structure and function of cells (48). This study results indicate that increased GST enzyme activities with diverse functions may hold a part of the effect with ABA pretreatment and improve the salt stress tolerance of plants in a complex way.

Based on the results, it is clear that ABA plays a role in the enhancement of tolerance to salt induced oxidative stress by increasing the activity of antioxidant enzymes.

REFERENCES

1 Parida, A.K., and Das A.B. (2005). Salt tolerance and salinity effect on plants: a review. Ecotoxicol. Environ. Saf., 60:324-34.

2 Apel, K. and Hirt, H (2004). Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant. Biol., 55:373-399.

3 Mittler, R., Vanderauwera, S., Gollery, M. and Breusegem, F.V. (2004). Reactive oxygen gene network of plants. Trends. Plant Sci., 9:490–498.

4 Foyer, C.H., Noctor, G. (2003). Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria, Physiol. Plantarum., 119:355–364.

5 Miller, G., Shulaev. V. and Mittler, R. (2008). Reactive oxygen signaling and abiotic stress. Physiol. Plant., 133:481-489.

6 Shalata, A. and Tal, M. (1998). The effect of salt stress on lipid peroxidation and antioxidants in the leaf of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii*. Physiol. Plant .,104:169–74.

7 Tsugane, K., Koboyashi, K., Niwa, Y., Ohba, Y., Wada, K. and Koboyashi, H. (1999). A recessive arabidopsis mutant that grows photo autotrophically under salt stress shows enhanced active oxygen detoxification. The Plant Cell., 11:1195–1206.

8 Halliwell, B. and Gutteridge, J.M.C. (1986). Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. Arch. Biochem.Biophys., 246:501–514.

9 Jithesh, M.N., Prashanth, S.R., Sivaprakash, K.A. and Parida, A.K. (2006). Antioxidative response mechanisms in halophytes: their role in stress defence. J. Genet., 85:237-254.

10 Ozgur, R., Uzilday, B., Sekmen, A.H. and Turkan I. (2013). Reactive oxygen species regulation and antioxidant defence in halophytes. Functional. Plant. Biol., 40:832–847.

11 Zhu, J.K., (2001). Plant salt stress tolerance. Trends. Plant Sci., 6:166-171.

12 Ramachandra Reddy, A., Chaitanya, K.V. and Vivekanandan, M. (2004). Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. J. Plant. Physio., 161:1189-1202.

13 Versules, P.E. and Bray, E.A. (2006). Role of abscisic acid and *Arabidopsis thaliana* ABA-insensitive loci in low water potential included ABA and proline accumulation. J. Exp. Bot., 57: 201-212.

14 Jiang, M. and Zhang, J. (2002). Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and upregulates the activities of antioxidant enzymes in maize leaves. J. Exp. Bot., 53:2401-2410.

15 Jiang, M. and Zhang, J. (2004). Abscisic acid and antioxidant defense in plant cells, Acta. Botanica. Sinic., 46(1):1-9.

16 Marklund, S. and Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Europian. J. Biochem., 47(3):469-474.

17 Maehly, A. and Chance, B. (1954). The assay of catalases and peroxidases. Methods. Biochem. Anal., 1:357-424.

18 Kochba, J., Lavee S. and Roy, S.P. (1977). Orange ovular callus lines. Plant. Cell. Physiol., 18: 463-467.

19 Asada, K., (1984). Chloroplasts: formation of active oxygen and its scavenging. Methods. Enzymol., 105:422-429.

20 Habig, W.H., Pabst M.J. and Jakoby, W.B. (1974). Glutathione *S*-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249:7130-7139.

21 Ashraf, M., (2009). Biotechnological approach of improving plant salt tolerance using antioxidants as markers. Biotechnol. Advs., 27:84-93.

22 Blokhina, O., Virolainen, E. and Fagerstedt, K.V. (2003). Antioxidants, oxidative damage and oxygen deprevation stress: a review. Ann. Bot., 91:179-194.

23 Nguyen, T., Sherratt, P.J., Nioi, P., Yang, C.S. and Pickett, C.B. (2005). Nrf2 controls constitutive and inducible expression of ARE-driven genes through a dynamic pathway involving nucleocytoplasmic shuttling by Keap1. J. Biol. Chem., 280:32485–92.

24 Jiang, M. and Zhang, J. (2001). Effect of abscisic acid on active oxygen species, antioxidative defence system and oxidative damage in leaves of maize seedlings. Plant. Cell. Physiol., 42:1265-1273

25 Li, X.J., Yang., Chen, H., Qu, L.Q., Chen, F. and Shen, S.H. (2010). Abscisic acid pretreatment enhances salt tolerance of rice seedlings: Proteomic evidence. Biochim. Biophys. Acta., 1804:929-940.

26 Hung, K.T. and Kao, C.H. (2008). Abscisic acid-induced hydrogen peroxide is required for anthocyanin accumulation in leaves of rice seedlings. J. Plant Physiol., 165:1280-1287.

27 Lu, S., Su, W., Li H. and Guo, Z. (2009). Abscisic acid improves drought tolerance of triploid bermudagrass and involves H_2O_2 and NO-induced antioxidant enzyme activities. Plant. Physiol. Biochem., 47pp.

28 Agarwal, S., Sairama, R.K., Srivastava, G.C., Tyagi, A. and Meena, R.C. (2005). Role of ABA, salicylic acid, calcium and hydrogen peroxide on antioxidant enzymes induction in wheat seedlings. Plant. Sci., 169:559-570.

29 Duan, B., Yang, Y., Lu, Y., Korpelainen, H., Berninger, F. and Li, C. (2007). Interactions between water deficit, ABA and provenances in *Picea asperata*. J. Exp. Bot., 58:3025-3036.

30 Blokhina, O., Virolainen, E. and Fagerstedt, K.V. (2003). Antioxidants, oxidative damage and oxygen deprevation stress: a review. Ann. Bot., 91:179-194.

31 Agarwal, S. and Pandey, V. (2004). Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia*. Biologia. Plantarum., 48:555-560.

32 Rahnama, H. and Ebrahimzadeh, H. (2005). The effect of NaCl on antioxidant enzyme activities in potato seedlings. Biol. Plantarum., 49:93-97.

33 Nagesh Babu, R. and Devaraj, V.R. (2008). High temperature and salt stress response in french bean *Phaseolus vulgaris*. Aust J Crop Sci 2:40-48.

34 Bor, M.F., Ozdemir, F. and Turkan, I. (2003). The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L. and wild beet *Beta maritima* L. Plant Sci., 164:77-84.

35 Takemura, T., Hanagata, N., Sugihara, K., Baba, S., Karube, I. and Dubinsky, Z. (2000). Physiological and biochemical responses to salt stress in the mangrove, *Bruguiera gymnorrhiza*. Aquat. Bot., 68:15-28.

36 Ramkishan Choudhary., Ajaya Eesha., Saroha. and Swarnkar, P.L. (2012). Effect of abscisic acid and hydrogen peroxide on antioxidant enzymes in *Syzygium cumini* plant. J. Food. Sci. Technol., 49(5):649-652.

37 Dionísio-Sese, M.L. and Tobita, S. (1998). Antioxidant responses of rice seedlings to salinity stress. Plant. Sci., 135:1-9.

38 Cherian, S. and Reddy, M.P. (2003). Evaluation of NaCl tolerance in the callus cultures of *Suaeda nudiflora*. Mol. Biol. Plant., 46:193-198.

39 Ben Amor, N., Ben Hamed, K., Debez ,A., Grignon, C. and Abdelly, C. (2005). Physiological and antioxidant responses of the perennial halophyte *Crithmum maritimum* to salinity. Plant. Sci., 68:889-899.

40 Yang, Z., Jingjin Yu., Emily Merewitz. and Bingru Huan. (2012). Differential Effects of Abscisic Acid and Glycine Betaine on Physiological Responses to Drought and Salinity Stress for Two Perennial Grass Species. J. Am. Soc. Hort. sci., 137(2):96-106.

41 Vaidyanathan, H., Sivakumar, P., Chakrabarty, R. and Thomas, G. (2003). Scavenging of reactive oxygenspecies in NaCl-stressed rice (*Oryza sativa* L.) differential response in salt-tolerant and sensitive varieties. Plant. Science., 165:1411-1418.

42 Sekmen, A.H., Türkan, I. and Takio, S. (2007). Differential responses of antioxidative enzymes and lipid peroxidation to salt stress in salt-tolerant *Plantago maritima* and salt sensitive *Plantago medi*a. Physiologia. Plantarum., 131:399- 411.

43 Aghaei, K., Ehsanpour, A.A. and Komatsu, S. (2008). Proteome analysis of potato under salt stress. J. Proteome. Res., 7:4858-4868.

44 Bybordi, A., Tabatabaei, S.J. and Ahmedov, A. (2010). The influence of salinity stress on antioxidant activity in canola cultivars (*Brassica napus* L.). J. Food. Agricul. Environ., 8(1):122-127.

45 Celik, O. and Atak, C. (2012). The effect of salt stress on antioxidative enzymes and proline content of two Turkish tobacco varieties. Turk. J. Biol., 36:339-356.

46 Aria, D. and Ramin, S.J. (2009). Impact of exogenous ascorbic acid on antioxidant activity and some physiological traits of common bean subjected to salinity stress. Not. Bot. Hort. Agrobot. Cluj., 37(2):165-117.

47 Samia, M., El-Khallal, A., Hathout, A., Ahsour A. and Abd-Almalik, A. (2009). Brassinolide and salicylic acid inducedantioxidant enzymes, hormonal balance and protein profile of maize plants grown under salt stress. Res. J. Agricul. Biolo. Sci., 5:491-402.

48 Dixon, D.P, Skipsey, M. and Edwards, R. (2010). Roles for glutathione transferases in plant secondary metabolism. Phytochem., 71:338-350.

