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ANTIOXIDANT METABOLISM IN *CALAMUS TENUIS* LEAVES UNDER SHORT TERM NaCl TREATMENT

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The effect of short term NaCl (0, 100, 200 and 300 mM) induced oxidative stress and antioxidant metabolism in rattan (*Calamus tenuis* Roxb.) leaves were investigated. Alterations in the activated oxygen metabolism of the leaves were detected as evidenced by the increase in H_2O_2 content and lipid peroxidation due to the accumulation of thiobarbituric acid reactive substances (TBARS) with the increasing NaCl concentrations. Antioxidative enzymes superoxide dismutase (SOD) and peroxidase (POX) activities decreased, whereas, catalase (CAT), and glutathione reductase (GR) activities increased. The non-enzymic antioxidants ascorbate and glutathione increased significantly in response to NaCl treatment. Na^+ ion increased, whereas, K^+ ion increased in lower concentrations followed by a decline in higher concentrations with no significant change in growth with the increase in NaCl treatments.

Key words: *Calamus tenuis,* catalase, glutathione reductase, NaCl, Na^+, K^+, peroxidase, superoxide dismutase.

Increased salinity lead to significant decrease in crop productivity. Salinity limits plant growth and productivity affecting water deficit, ionic imbalance, osmotic stress and inducing secondary oxidative stress by the production of reactive oxygen species (ROS) (Munns, 2002; Bor *et al.,* 2003; Panda and Khan, 2003). Any imbalance in the cellular redox homeostasis can be called oxidative stress which results in the production of reactive oxygen species (ROS) such as superoxide radical (O_2), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\cdot}OH$), alkoxyl radical ($RO^{\cdot}$) and singlet oxygen (O_2) formation via enhanced leakage of electron to oxygen. Reactive oxygen species attack and oxidize proteins, lipids and nucleic acids (Alscher *et al.*, 1997; Noctor and Foyer, 1998; Grant and Loake, 2000; Panda and Khan, 2003). Plants have developed a complex antioxidative defence systems to alleviate the damage caused by ROS and the degree of damage depends on the balance between the formation of ROS and its removal by the antioxidative scavenging systems that defend against them. The antioxidative systems include carotenoids, ascorbate, glutathione, a-tocopherols and enzymes such as superoxide dismutase (EC. 1. 15. 1.1.), catalase (EC.1. 11. 1. 6.), glutathione peroxidase (EC. 1. 11. 1.9.) peroxidases and enzymes involved in ascorbate glutathione cycle (ASC-GSH Cycle), ascorbate peroxides (EC. 1. 11. 1.1.), dehydroascorbate reductase (EC. 1. 8. 5.1.) monodehydrosacorbate reductase (EC. 1. 6. 5.4.) and glutathione reductase (EC. 1. 6. 4.2.). (Alscher *et al.,* 1997; Noctor and Foyer, 1998; Jimenez *et al.,* 1997; Shalata and Tal, 1998; Gomez *et al.,* 1999; Hernandez *et al.,* 2000; Jose *et al.,* 2002; Khan *et al.,* 2002; Bor *et al.,* 2003; Panda and Khan, 2003; Meloni *et al.,* 2003; Tsaia *et al.,* 2004; Demiral and Turkan, 2005, Mandhania *et al.,* 2006).

Calamus tenuis (rattan) is a slow growing tropical climbing palm that has multiple economic uses. The effect of NaCl-salinity induced oxidative stress and antioxidant metabolism in rattan are relatively less known. The present investigation was carried out to evaluate the oxidative stress, antioxidant metabolism in response to short NaCl treatments in rattan leaves.

MATERIALS AND METHODS

Plant material and NaCl treatment : Rattan (*Calamus tenuis* Roxb.) seeds were sown and germinated in a plastic tray containing sand moistened with tap water for four weeks. The germinated seeds one in each was transferred to

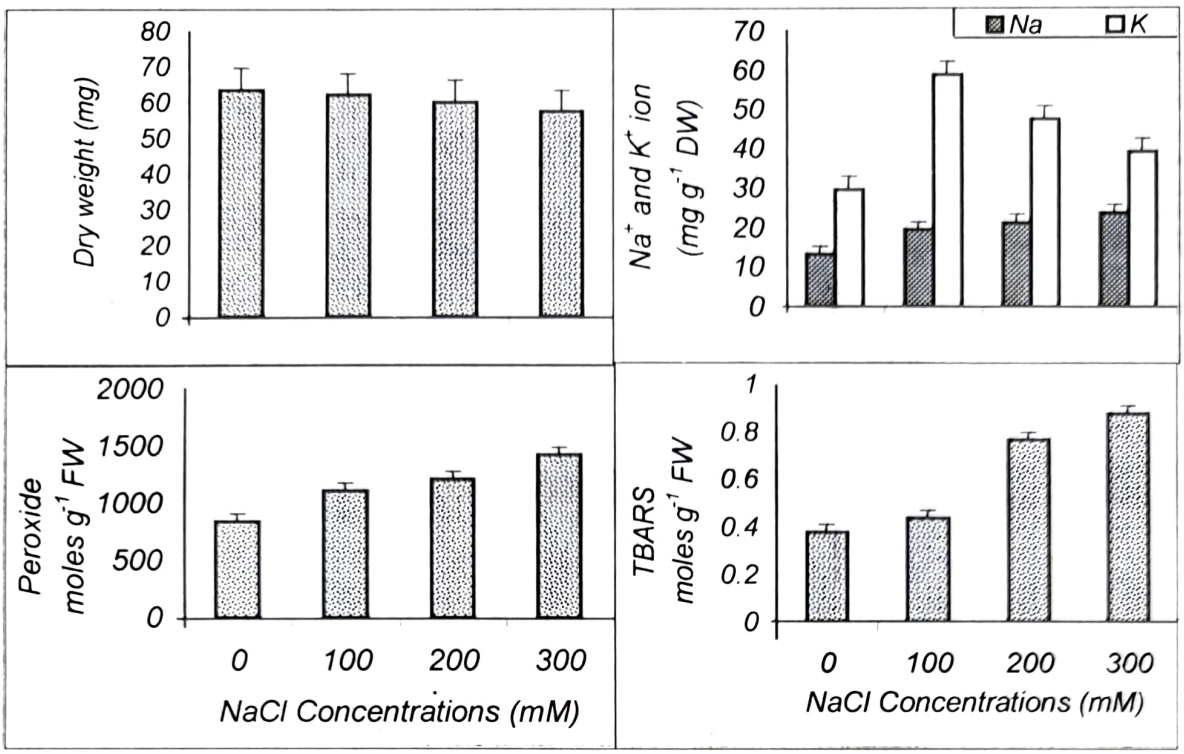

Figure 1. Changes in (A) dry weight, (B) sodium (Na^+) and potassium (K^+) ion content, (C) peroxide levels and (D) thiobarbituric acid reactive substance (TBARS) content in *Calamus tenuis* leaves under short-term NaCl treatment. Data presented means ± SE.

plastic pots containing sand and grown with nutrient Hoagland's solution (Hoagland and Arnon, 1950) in green house for 9 (nine) months. The environmental conditions in the green house containing the germinated plants were 28 ^{0}C / 24 ^{0}C (day / night) temperature, 80 % relative humidity and 7000 lux of light intensity with 18h photoperiod. The 9 months old plants consisted of 5 compound leaves. The plants were treated with different concentrations of NaCl (0, 100, 200 and 300 mM L^{-1}) in soil for 5 (five) days. Seedling grown in soil with Hoagland's nutrient solution only was used as control. On 6th day, the leaves were used for various biochemical estimations.

Study of growth, Na^+ and K^+ ions estimation : Leaves were separated from the seedlings and their constant fresh weight (mg) was taken. Dry weight (mg) of the leaves was determined after placing samples in hot air oven at 55-60^{0}C for 72h using balance (Sartorius, Germany). The dried leaves were acid digested in a ratio of HNO_3-HCl (3:1 v/v) mixture and Na^+ and K^+ concentrations were determined as per the method described by Humpries (1956) using Flame Photometer (Systronics, India).

Estimation of hydrogen peroxide and lipid peroxidation levels : Leaves (0.5 g) were homogenized in 5 % trichloroacetic acid (TCA) and the homogenate was used for the determination of hydrogen peroxide (H_2O_2) levels by the method of Sagisaka (1976). The level of lipid peroxidation in the root tissues was determined as thiobarbituric reactive substances (TBARS) as described by Heath and Packer (1968).

Determination of ascorbate and total glutathione content : For the extraction and estimation of ascorbate, the method of Oser (1979) was used. Glutathione was extracted and estimated as per the method of Griffith (1980).

Extraction and assay of antioxidative enzymes : Leaves were excised from the seedlings. Fresh

weight (0.2 g) was homogenized at 4 ^{0}C in 5 ml of extraction buffer (0.1 M phosphate buffer, pH 6.8) with mortar and pestle. The homogenate was then centrifuged at 15,000 g for 20 min and the homogenate was used as the crude extract for the superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) and glutathione reductase (GR) assay using the methods of Giannopolitis and Reis (1977), Chance and Maehly (1955) and Smith *et al* (1988) respectively. The experiments were repeated twice with three replicates ($n = 6$) and data presented are mean ± standard errors (SE).

RESULTS AND DISCUSSION

There is relatively little change in the growth of the plants as measured in terms of dry weight in all concentrations of NaCl (figure 1 a). The Na^+ ion content increased uniformly in all concentrations of NaCl with respect to the control values, whereas, the K^+ ion content increased followed by a decline in higher NaCl concentrations (figure 1 b). The results showed that NaCl-salinity increased the Na^+ ion content and decreased the K^+ ion uptake. The increase in the Na^+ ion content and decrease in K^+ ion uptake disturbs ionic balance as observed in most species exposed to salt stress (Al Zahrani and Hajar, 1998; Heuer, 2003). High Na^+ accumulation in salt-sensitive foxtail millet cultivar, in tomato and rice have been reported to result in an enhanced membrane damage, electrolyte leakage and oxidative damage, whereas significantly lower Na^+ accumulation in salt tolerant cultivars showed maintenance of cellular intactness (Qadar, 1991; Sreenivasulu *et al.*, 2000; Racagni *et al.*, 2003/4; Mandhania *et al.*, 2006).

The NaCl (0, 100, 200, and 300 mM) treatments showed significant increase in peroxide content. As an indicator of lipid peroxidation, the thiobarbituric acid reacting substances (TBARS) content was measured. Increasing concentrations of NaCl caused an enhancement of TBARS (figure 1 c, d). Salt treatments increase lipid peroxidation or induce oxidative stress in plant tissues. Lipid peroxidation measured as amount of TBARS is produced when polyunsaturated fatty acids in the membrane undergo peroxidation by the accumulation of free oxygen radicals. Salt stress produced ion leakage indicating injury to membrane integrity, which could be affected by ROS formed during leaf photosynthesis or respiration, enhancing lipid peroxidation of the membrane (Gomez *et al.*, 1999; Savoure *et al.*, 1999; Hernandez *et al.*, 2000). There are reports that salt treatment increases the content of H_2O_2 and peroxidation of the lipid membrane, thus disrupting its permeability or induce oxidative stress in plant tissues (Gomez *et al.*, 1999; Khan and Panda, 2002; Khan *et al.*, 2003; Panda and Khan, 2003; Tsai *et al.*, 2004; Demiral and Turkan, 2005; Mandhania *et al.*, 2006). As lipid peroxidation is the symptom mostly ascribed to oxidative damage, it is often used as an indicator of increased damage (Gomez *et al.*, 1999; Hernandez *et al.*, 2000). The results reported here show that the degree of accumulation of TBARS due to salt stress (Gomez *et al.*, 1999; Hernandez et al., 2001; Meloni *et al.*, 2003; Bor *et al.*, 2003).

There is a significant decrease in the SOD activity in all the treatments (figure 2 a). SOD catalyzes the conversion of the superoxide anion ($O_2^{\cdot}$) to hydrogen peroxide (H_2O_2), which is very damaging to the chloroplasts, nucleic acids and proteins. The results showed a decreased SOD activity (figure 2 a), may result from an increased inactivation by H_2O_2 there by lowering the dismutation of H_2O_2 and unabling the plant to resist the potential oxidative damage caused by NaCl salinity exposure (Shalata and Tal, 1998; Hernandez *et al.*, 2000; Khan and Panda, 2002; Panda and Khan, 2003). Lee *et al.* (2001) observed that NaCl treatment induced a significant increase of SOD activity of rice leaves, however, activity in rice roots were affected by NaCl. A significant increase of SOD activity occurred in pea leaves after short-term NaCl stress (Hernandez and Almansa, 2002). Tsai *et al.* (2004) observed that NaCl had no effect on the activity of SOD and isozymes of SOD in rice roots. An increased CAT activity and decreased POX activity was observed with the increase in the concentrations of NaCl treated leaves (figure 2 b,

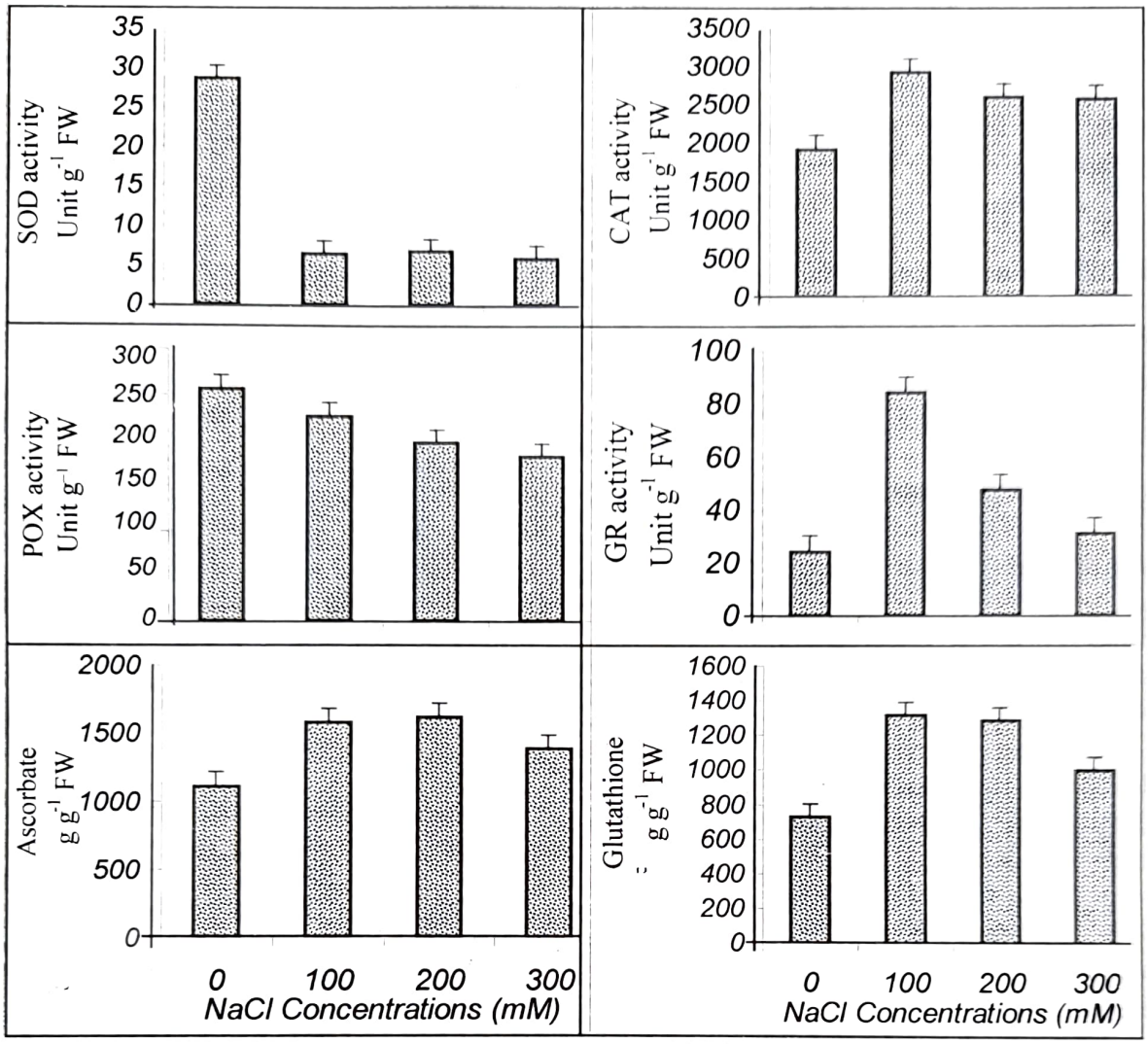

Figure 2. Changes in (A) superoxide dismutase (SOD), (B) catalase (CAT), (C) peroxidase (POX), (D) glutathione reductase (GR) activities, (E) ascorbate and (F) glutathione content in *Calamus tenuis* leaves under short-term NaCl treatment. Data presented means ± SE

c). Peroxide produced during oxidative stress can easily permeate membranes and are removed by catalase (CAT) or by peroxidases (POX) or catalase (CAT) (Meloni *et al.*, 2003). The results indicated a decreased POX activity. Demiral and Turkan (2005) showed that the POX decreased in rice cultivar Pokkali whereas increased in rice cultivar IR 28. There are reports of increased POX activity in rice (Oidaira *et al.*, 2000; Meloni *et al.*, 2003). The results showed increased CAT activity. The induction of catalase activity under water stress is well documented and a positive relationship has been found between its up-regulation and stress tolerance (Hernandez *et al.*, 2000; Shalata *et al.*, 2001; Ushimaru *et al.*, 2001; Demiral and Turkan, 2005). The results obtained in this study are in accordance with those of Sairam *et al.* (2002) and Gueta-Dahan *et al.* (1997) who reported enhancement in CAT activity in both salt sensitive and salt-tolerant cultivars of wheat. Vaidyanathan *et al.* (2003) reported enhancement in CAT activity in salt-tolerant rice cultivar. Savoure *et al.* (1999) found that NaCl stimulated catalase activity through activation of the *Cat2* and *Cat3* genes. Demiral and Tukan (2005) reported that the CAT activity increased under NaCl stress in rice cultivar Pokkali, whereas the CAT activity decreased in rice cultivar IR 28. Fadzilla *et al.* (1997) reported that NaCl had no effect on CAT

activity on rice shoots. Tsai et al. (2004) reported that NaCl does not influence the activity and isozymes of CAT in rice roots. On the other hand, decrease in CAT by NaCl has been shown in rice leaves (Lee *et al.*, 2001) and in *Malus domestica* explants (Molassiotis *et al.*, 2006).

Glutathione reductase (GR) a key enzyme of the ascorbate–glutathione cycle increased in lower NaCl treatments, however, declined in higher concentrations of NaCl (300 mM) (figure 2 d). GR also plays a key role in oxidative stress by converting the oxidised glutathione (GSSG) to reduced glutathione (GSH) and maintaining a high GSH/GSSG ratio (Fadzilla *et al.*, 1997; Irishimovitch and Shapira, 2000). In our study, increased GR activity was observed. Increased GR activity in leaves of sugar beet plant have been reported, may be closely related with salt tolerance capacity of these plants (Bor *et al.*, 2003). Increased GR activity facilitates improved stress tolerance and has the ability to alter the redox poise of improved components of electron transport chain (Tyystjarvi *et al.*, 1999). Hernandez and Almansa (2002) demonstrated that GR activity increased in pea leaves during short-term NaCl stress. Tsai *et al.* (2004) showed that increased GR activity and are enhanced by NaCl in the roots of rice seedlings. Since NaCl-induced enzyme activity indicates a specific role in coping with the stress, constitutive and/or induced activities of SOD, CAT, AP, and GR further suggest improved tolerance to salt stress (Gueta-Dahan *et al.* 1997). Enhanced GR activity has been reported in plants by NaCl treatment (Savoure *et al.*, 1999; Kawasaki *et al.*, 2001).

The non-enzymic antioxidants ascorbate and glutathione increased significantly with the increasing NaCl concentrations (figure 2 e, f). The non-enzymic antioxidant ascorbate and glutathione react directly with the ROS in photosynthetic tissues, recycles a-tocopherol and protect enzymes with prosthetic metal ions and is utilized as a substrate for ascorbate peroxidase (APX), catalyzes H_2O_2 detoxification (Noctor and Foyer, 1998). The results observed showed increased ascorbate and glutathione content allowing better antioxidant protection as reported for other plants (Meneguzzo *et al.*, 1999; Khan and Panda, 2002; Khan *et al.*, 2002; Tsai *et al.*, 2004). However, there are reports that ascorbate and glutathione levels decreased in plants in response to NaCl stress (Hernandez *et al.*, 2000; Shalata *et al.*, 2001). It appears that the increase in ascorbate levels in rice roots treated with NaCl depends on the rates of its synthesis as well as on the rates of its regeneration (Tsai *et al.*, 2004). Changes in processes that regulate GSH concentration and / or redox status are considered to be one of the important adaptive mechanisms of plant exposed to stress conditions (Fadzilla *et al.*, 1997). It has been suggested that salinity induce glutathione synthesis in *Brassica napus* (Ruiz and Blumwald, 2002).

The better NaCl tolerance in rattan leaves observed during present investigation may be due to restriction of Na^+ accumulation and ability to maintain high K^+/Na^+ ratio in tissue. Though TBARS and H_2O_2 content accumulated indicating NaCl-induced oxidative stress, activities of the antioxidative enzymes like SOD and POX decreased, whereas, CAT and GR activities increased and the non-enzymic antioxidants like ascorbate and glutathione increased, may explain the higher NaCl-tolerance of rattan against salt stress.

REFERENCES

Al-Zahrani H S & Hajar A S 1998 Salt tolerance in the halophyte *Halopeplis perfoliata* (Forssk) Beg Ex Schweint: Effect of NaCl salinity on growth and ion uptake. *Ind J Plant Physiol* **3** 32-35.

Bor M, F Ozdemir & 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.

Chance B & Maehly AC 1955 Assay of catalase and peroxidase. *Methods Enzymol* **2** 764-775.

Demiral T & Turkan I 2004 Does exogenous

glycinebetaine affect antioxidative system of rice seedlings under NaCl treatment? *J Plant Physiol* **161** 1089-1100.

Fadzilla N M, Finch R P & Burdon R H 1997 Salinity, oxidative stress and antioxidant responses in shoot cultures of rice. *J Exp Bot* **48** 325-331.

Giannopolitis C N & Ries S K 1997 Superoxide dismutase. I. Occurrence in higher plants. *Plant Physiol* **59** 309-314.

Gomez J M, Hernendez J A, Jimenez A, del Rio LA & Sevilla F 1999 Differential response of antioxidative enzymes of chloroplasts and mitochondria to long term NaCl stress of pea plants. *Free Rad Res* **31** S11-S18.

Grant J J & Loake G J 2000 Role of reactive oxygen intermediate and cognate redox signaling in disease resistance. *Plant Physiol* **124** 21-29.

Griffith O W 1980 Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Anal Biochem* **106** 207 – 221.

Gueta-Dahan Y, Yaniv Z, Zilinskas B A & Ben-Hayyim G M 1997 Salt and oxidative stress: similar and specific responses and their relation to salt tolerance in citrus. *Planta* **203** 460-469.

Heath RL & Packer L 1968 Photoperoxidation in isolated chloroplasts. I. Kinetic and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys* **125:** 189-198.

Hernandez JA, J Jimenez, P Mullineaux & F Sevilla 2000 Tolerance of pea (*Pisum sativum* L.) to long term stress is associated with induction of antioxidant defenses. *Plant Cell Environ* **23** 583-862.

Hernandez J A, Ferrer M A, Jimenez J, Barcelo AR & Sevilla F 2001 Antioxidant systems and $O_2^{\cdot-}$/H_2O_2 production in the apoplast of pea leaves. Its relation with salt-induced necrotic lesions in minor veins. *Plant Physiol* **127** 817-831.

Hernandez J A & Almansa M S Short-term effect of salt stress on antioxidant systems and leaf water relations of pea leaves. *Physiol Plant* **115** 251-257.

Heuer B 2003 Influence of exogenous application of proline and glycine betaine on growth of salt-stressed tomato plants. *Plant Sci* **165** 693-699.

Hoagland D R & Arnon D I 1950 The water culture method for growing plants without soil. California Agricultural Experimental Station Circular, report 374.

Humpries E C 1956 Modern methods of Plant Analysis. In: Peach K & NV Tracey (eds.). Vol I, pp.468, Springer-Verlag, Berlin.

Irihimovitch V & Shapira M 2000 Glutathione redox potential modulated by reactive oxygen species regulates translation of Rubisco large subunit in the chloroplast. *J Biol Chem* **275** 16289-16295.

Jose A, Hernandez A , Maria S & Almansa B 2002 Short term effects of salt stress on antioxidant systems and leaf water relations of pea leaves. *Physiol Plant* **115** 251-257.

Kawasaki S, Borchert C, Deyholos M, Wang H, S Brazille H, Kawai K, Galbraith D & Bohnert H J 2001 Gene expression profiles during the initial phase of salt stress in rice. *Plant Cell* **13** 889-905.

Khan M H & Panda S K 2002 Induction of oxidative stress in roots of *Oryza sativa* L. in response to salt stress. *Biol Plant* **45** 625-627.

Khan M H, Singha L B & Panda S K 2002 Changes in antioxidant levels in *Oryza sativa* L. roots subjected to NaCl-salinity stress. *Acta Physiol Plant* **24** 145-148.

Lee D H, Kim Y S & Lee C B 2001 The inductive responses of the antioxidant enzymes by salt stress in the rice (*Oryza sativa* L.). *J Plant Physiol* **158**

737-745.

Mandhania S, Madan S & Sawhney V 2006 Antioxidant defense mechanism under salt stress in wheat seedlings. *Biol Plant* **227** 227-31.

Meloni D A, Oliva M A, Martinez C A & Cambraia J 2003 Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Environ Exp Bot* **49** 69-76.

Meneguzzo S, Navari-Izzo F & Izzo R 1999 Antioxidative responses of shoots and roots of wheat to increasing NaCl concentrations. *J Plant Physiol* **155** 274-280.

Molassiotis A N, Sotiropoulos T, Tanou G, Kofidis G, Diamantidis G & Therios E 2006 Antioxidant and anatomical responses in shoot culture of the apple rootstock MM 106 treated with NaCl, KCl, mannitol or sorbitol. *Biol Plant* **50** 61-68.

Munns R 2002 Comparative physiology of salt and water stress. *Plant Cell Environ* **25:** 239-150

Noctor G & Foyer C 1998 Ascorbate and glutathione: keeping active oxygen under control. *Ann Rev Plant Physiol Plant Mol Biol* **49** 249-279.

Oidaira H, Sano S, Koshiba T & Ushimaru T 2000 Enhancement of oxidative enzyme activities in chilled rice seedlings. *J Plant Physiol* **156** 811-913.

Oser B L 1979 *Hawks Physiological Chemistry. McGraw Hill. N.Y. USA,* pp. 702-705.

Panda S K & Khan M H 2003 Salt stress influences lipid peroxidation and antioxidants in the leaf of an indica rice (*Oryza sativa* L). *Physiol Mol Biol Plants* **9** 273-278.

Qadar A 1991 Potassium status of the rice shoot as index for salt tolerance. *Ind J Plant Physiol* **31** 388-393.

Racagni H, Pedranzani H, Alemano S, Taleisnik E, Abdala G & Machado-Domenech E 2003/4 Effect of short-term salinity on lipid metabolism and ion accumulation in tomato roots. *Biol Plant* **47** 373-7.

Ruiz J M & Blumwald E 2002 Salinity-induced glutathione synthesis in *Brassica napus. Planta* **214** 965-969.

Sairam R K, Rao K V & Srivastava G C 2002 Differential response of wheat genotypes to long-term salinity stress in relation to oxidative stress, antioxidant activity and osmolytes concentration. *Plant Sci* **1631**037-1046.

Savoure A, Thorin D, Davey M, Hua X J, Mauro S, Van Motagu M, Inze D & Verbruggen N 1999 NaCl and $CuZnSO_4$ treatments trigger distinct oxidative defense mechanism in *Nicotiana plumbaginifolia* L. *Plant Cell Physiol* **22** 387-396.

Sagisaka S 1976 The occurrence of peroxide in a perennial plant *Populas gelrica. Plant Physiol* **57** 308-309.

Shalata A & Tal M 1998 The effect of salt stress on lipid peroxidation and antoxidants in the leaf of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii. Physiol Plant* **104** 169-174.

Shalata A, Mittova V, Volokita M, Guy M & M Tal 2001 Response of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennilli* to salt-dependent oxidative stress: The root antioxidant system. *Physiol Plant* **112** 487-494.

Smith I K, Vierheller T L & Thorne C A 1988 Assay of glutathione reductase in crude tissue homogenates using 5, 5′ - dithiobis (2-nitrobenzoic acid). *Anal Biochem* **175** 408-413.

Sreenivasulu N, Grimm B, Wobus U & Weschke W 2000 Differential response of antioxidant compounds to salinity stress in salt-tolerant and salt-sensitive seedlings of foxtail millet (*Setaria italica*). *Physiol Plant* **109** 435-42.

Tsai Y, Hongb C, Liua L, Kao C H 2004 Relative

importance of Na^+ and Cl^- in NaCl-induced antioxidant systems in roots of rice seedlings. *Physiol Plant* **122** 86-94.

Tyystjarvi E, Riikenon M, CM Arisi A, Kettunen R, Jouanin L & Foyer C H 1999 Photoinhibition of photosystem II in tobacco plants overexpressing glutathione reductase and poplars overexpresing superoxide dismutase. *Physiol Plant* **105** 405-416.

Ushimaru T, Kanematsu S, Katayama M & Tsuji H 2001 Antioxidative enzymes in seedlings of *Nelumbo nucifera* germinated under water stress. *Physiol Plant* **112** 39-46.

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