

ISSN 1819-1894

Asian Journal of  
**Agricultural**  
Research

## **Development of Salt Stress-tolerant Plants by Gene Manipulation of Antioxidant Enzymes**

Baby Joseph and D. Jini

Interdisciplinary Research Unit, Department of Biotechnology, Malankara Catholic College, Mariagiri, Kaliakkavilai-629153 Kanyakumari District, Tamil Nadu, India

*Corresponding Author: Baby Joseph, Interdisciplinary Research Unit, Department of Biotechnology, Malankara Catholic College, Mariagiri, Kaliakkavilai-629153 Kanyakumari District, Tamil Nadu, India  
Tel: 04651244153,+9197904032221*

### **ABSTRACT**

Salinity toxicity is a worldwide agricultural and eco-environmental problem. Salt stress is one of the major abiotic stresses faced by plants, which adversely affect their productivity. Salt stress causes reduction of crop yield and alterations in plant metabolism, including a reduced water potential, ion imbalances and toxicity and sometimes severe salt stress may even threaten survival. Salinity also leads to oxidative stress in plants due to the production of Reactive Oxygen Species (ROS) such as the super oxide radical, hydrogen peroxide and hydroxyl radical. Reactive Oxygen Species (ROS) generated during metabolic processes damage cellular functions and consequently lead to disease, senescence and cell death. Plants have evolved an efficient defense system by which the ROS is scavenged by antioxidant enzymes. Attempts to reduce oxidative damages under the salt stress conditions have included the manipulation of ROS scavenging enzymes by gene transfer technology. It is important to increase the productivity under stressful environment by developing plants that have well adapted to environmental stress through manipulating antioxidant system. In this study, the rational approaches to develop stress-tolerant plants by gene manipulation of antioxidant enzymes will be introduced to provide solutions for the global food and environmental problems in the 21st century.

**Key words:** Salinity, transgenic plants, salt stress, antioxidant enzymes, oxidative stress

### **INTRODUCTION**

Many arid and semi-arid regions in the world contain soils and water resources that are too saline for most of the common economic crops, which affect plants through osmotic effects, ion specific effects and oxidative stress (Munns, 2002; Pitman and Lauchli, 2002). High concentrations of salt in soil are causing large decreases in yields for a wide variety of crops all over the world (Sekmen *et al.*, 2007). Currently, 20% of the world's cultivated land is affected by salinity, which results in the loss of 50% of agricultural yield (Zhu, 2001; Bartels and Sunkar, 2005). Many crop plants such as barley, maize and rice, are often subject to salinity stress (Sairam and Tyagi, 2004).

Abiotic pressures like salt stress and chemical insultance can impose limitations on crop productivity and also limit land available for farming, often in regions that can ill afford such constraints, thus highlighting a greater need for understanding how plants respond to adverse conditions with the hope of improving tolerance of plants to environmental stress (Joseph *et al.*, 2010). The effects of various environmental stresses in plants are known to be mediated, at least

in part, by an enhanced generation of Reactive Oxygen Species (ROS) including  $O_2^-$ ,  $H_2O_2$  and  $OH^-$  (Hernandez *et al.*, 2000; Benevides *et al.*, 2000). These ROS are highly reactive and can alter normal cellular metabolism through oxidative damage to membranes, proteins and nucleic acids; they also cause lipid peroxidation, protein denaturation and DNA mutation (Imlay, 2003). To prevent damage to cellular components by ROS, plants have developed a complex antioxidant system. The primary components of this system include carotenoids, ascorbate, glutathione and tocopherols, in addition to enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), peroxidases and the enzymes involved in ascorbate-glutathione cycle (Foyer and Halliwell, 1976), such as ascorbate peroxidase (APX) and Glutathione Reductase (GR). Many components of this antioxidant defense system can be found in various subcellular compartments (Hernandez *et al.*, 2000).

The scavenging of ROS by increased activation of antioxidant enzymes can improve salt tolerance (Alscher *et al.*, 2002). A relationship between salt tolerance and increased activation of antioxidant enzymes has been demonstrated in *Plantago* (Sekmen *et al.*, 2007), pea (Hernandez *et al.*, 2000), Arabidopsis, rice (Dionisio-Sese and Tobita, 1998), tomato, soybean and maize (Azevedo-Neto *et al.*, 2006). However, this antioxidative mechanism seems to be not enough to protect plants from the elevated environmental stresses such as salinity. To maintain the productivity of plants under the salt stress condition, it is important to fortify the antioxidative mechanism of the chloroplasts by manipulating the antioxidant enzymes and small antioxidant molecules in the chloroplast. Transgenic plants over expressing single transgene of SOD, APX and GR separately in chloroplast or other compartment of plant cell were generated and displayed increased tolerance against the oxidative stress. It seems that multiple expressions of tolerance genes in target organelle and their efficient regulation of expressions using a stress-inducible promoter are required for the ideal transgenic plants with enhanced tolerance to environmental stresses.

**Oxidative damage by salt stress:** Oxygen is essential for the existence of aerobic life, but toxic Reactive Oxygen Species (ROS), which include the superoxide anion radical ( $O_2^-$ ), hydroxyl radical (OH) and hydrogen peroxide ( $H_2O_2$ ), are generated in all aerobic cells during metabolic processes (Foyer *et al.*, 1994; Asada, 1999). Injury caused by these ROS is known as oxidative stress, which is one of the major damaging factors to plants exposed to environmental stress.

Production of ROS is increased under saline conditions (Hasegawa *et al.*, 2000) and ROS-mediated membrane damage has been demonstrated to be a major cause of the cellular toxicity by salinity in rice, tomato and citrus (Mittova *et al.*, 2004). The effects of various environmental stresses in plants are known to be mediated, at least in part, by an enhanced generation of Reactive Oxygen Species (ROS) including  $O_2^-$ ,  $H_2O_2$  and  $OH^-$  (Hernandez *et al.*, 2000; Benevides *et al.*, 2000). These ROS are highly reactive and can alter normal cellular metabolism through oxidative damage to membranes, proteins and nucleic acids; they also cause lipid peroxidation, protein denaturation and DNA mutation (Imlay, 2003).

Salt stress induces cellular accumulation of ROS which can damage membrane lipids, proteins and nucleic acids (Hernandez *et al.*, 1993, 1999; Mansour *et al.*, 2005; Ben-Amor *et al.*, 2007; Eyidogan and Oz, 2007). Much of the injury to plants exposed to stress is connected with oxidative damage at the cellular level (Foyer and Noctor, 2003). During oxidative stress, the excess production of ROS causes membrane damage that eventually leads to cell death. For protection against ROS, plants contain antioxidant enzymes as well as a wide array of non-enzymatic antioxidants (Blokhina *et al.*, 2003).

**Regulation of Reactive Oxygen Species (ROS) at normal environment:** Under normal growth conditions, low amounts of ROS such as superoxide radical ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ), hydroxyl radical ( $OH^-$ ) and singlet oxygen ( $^1O_2$ ) are metabolic byproducts of plant cells (Cai-Hong *et al.*, 2005). Plants have developed the scavenging mechanism of ROS categorized as enzymatic and non-enzymatic (Reddy *et al.*, 2004; Demiral and Turkan, 2005). When ROS increases, chain reactions start, in which superoxide dismutase (SOD) catalyzes the dismutation of  $O_2^-$  radicals to molecular  $O_2$  and  $H_2O_2$  (Meloni *et al.*, 2003). The  $H_2O_2$  is then detoxified in the ascorbate-glutathione cycle (Asada, 1999; Mittler, 2002), which involves the oxidation and re-reduction of ascorbate and glutathione through the ascorbate peroxidase (APX) and Glutathione Reductase (GR) action (Noctor and Foyer, 1998). If there is a serious imbalance in any cell compartment between the production of Reactive Oxygen Species (ROS) and antioxidant defense, oxidative stress and damage occurs (Mittler, 2002).

**Role of antioxidant enzymes for salt tolerance in plants:** To minimize the effects of oxidative stress, plant cells have evolved a complex antioxidant system, which is composed of low-molecular mass antioxidants (glutathione, ascorbate and carotenoids) as well as ROS-scavenging enzymes, such as: superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and Glutathione Reductase (GR) (Apel and Hirt, 2004). Moreover, plants with the high activity of peroxidase and active proline accumulation could acclimate to salts stress (100 mM NaCl, 24 h) independently of SOD activity (Kartashov *et al.*, 2008). Several studies have pointed out that salt-tolerant species increased their antioxidant enzyme activities and antioxidant contents in response to salt treatment, whereas salt-sensitive species failed to do so (Shalata *et al.*, 2001; Demiral and Turkan, 2005). The scavenging of ROS by increased activation of antioxidant enzymes can improve salt tolerance (Alscher *et al.*, 2002). A relationship between salt tolerance and increased activation of antioxidant enzymes has been demonstrated in *Plantago* (Sekmen *et al.*, 2007), pea (Hernandez *et al.*, 2000), tomato, maize (Azevedo-Neto *et al.*, 2006), sorghum (Costa *et al.*, 2005; Heidari, 2009), soybean (Cicek and Cakýrlar, 2008) and mulberry (Harinasut *et al.*, 2003). The antioxidant enzymes present in different organelles of the plant cell is shown in the Fig. 1.

The generation of ROS and increased the activity of many antioxidant enzymes during salt stress have been reported in cotton (Desingh and Kanagaraj, 2007), mulberry (Sudhakar *et al.*, 2001; Harinasut *et al.*, 2003), wheat (Sairam *et al.*, 2002), tomato (Mittova *et al.*, 2002), rice (Vaidyanathan *et al.*, 2003), sugar beet (Bor *et al.*, 2003) and maize (Azevedo-Neto *et al.*, 2006). In general, the activities of antioxidant enzymes were increased in the root and shoot under saline stress. But the increase was more significant and consistent in the root (Kim *et al.*, 2005).

The quantitative and qualitative aspects of changes are often related to the levels of resistance to salinity. Meratan *et al.* (2008) showed a remarkable increase in Peroxidase and Polyphenol oxidase between 50 to 200 mM NaCl in three *Acanthophyllum* species. In tomato and citrus, salt-tolerance is attributed to the increased activities of SOD, APX and CAT (Gueta-Dahan *et al.*, 1997; Mittova *et al.*, 2002). In rice, the salt-tolerant varieties have higher SOD activity and lower lipid peroxidation than the salt-sensitive varieties (Dionisio-Sese and Tobita, 1998). It was suggested that the ratio between superoxide dismutase and  $H_2O_2$ -scavenging enzyme activities could be used as a working hypothesis for a biochemical marker for salt tolerance in sorghum (Costa *et al.*, 2005).

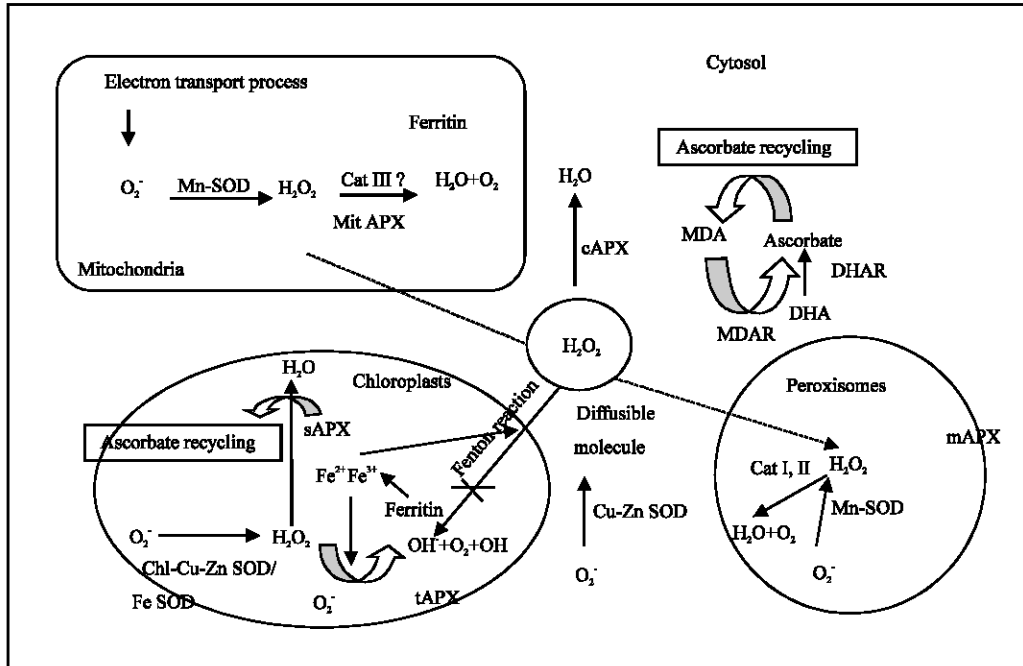


Fig. 1: Antioxidant network in different organelles of the plant cell (Jithesh *et al.*, 2006)

Further supporting evidence on the involvement of antioxidant enzymes in salt tolerance has been provided by transgenic plants with a reduced or an increased expression of antioxidant enzymes (Willekens *et al.*, 1997). Increased protection to salt stress has been demonstrated by the over expression of cytosolic APX (Torsethaugen *et al.*, 1997).

### Transgenic plant for salt tolerance

**Expression of superoxide dismutase in salt tolerant transgenic plants:** Superoxide dismutases catalyze the breakdown of superoxide radicals and provide the first line of defense against oxygen toxicity. They are essential for aerobic life and are involved in stress resistance and longevity (Carlioz and Touati, 1986; Orr and Sohal, 1994). Three groups of superoxide dismutases (SODs) can be distinguished on the basis of the metal cofactor at the active site: Cu/Zn SOD, Mn-SOD and Fe-SOD (Fridovich, 1989). Mn-SOD and Fe-SOD appear to be closely related both in structural and in evolutionary aspects, but have no resemblance to Cu/ZnSOD (Bowler *et al.*, 1994). Cu/Zn SODs occur in the cytoplasm and chloroplasts; Mn-SODs are usually found in the mitochondrial matrix and in peroxisomes; and Fe-SODs are present within the chloroplasts of some plants (Raychaudhuri and Deng, 2000).

Zhao *et al.* (2009) introduced the transcription factor *YAP1*, originally from yeast (*Saccharomyces cerevisiae*), into *Arabidopsis thaliana* (ecotype Columbia). When treated with various NaCl concentrations, transgenic plants showed increased activities of antioxidant enzymes catalase, superoxide dismutase, ascorbate peroxidase, peroxidase, glutathione *S*-transferase and glutathione reductase compared with the wild-type *Arabidopsis*. This demonstrated that an active oxygen scavenging system was enhanced to protect plants from salt stress by equilibrating ROS metabolism. Transgenic *Arabidopsis* maintained higher photosynthesis levels and lower amounts of  $H_2O_2$ , suggesting that ROS production was reduced (Zhao *et al.*, 2009). Wang *et al.* (2010)

introduced a *MnSOD* gene (*TaMnSOD*) from *Tamarix androssowii*, under the control of the *CaMV35S* promoter, was introduced into poplar (*Populus davidiana*×*P. bolleana*). They showed that SOD activity was enhanced in transgenic plants and the MDA content were significantly decreased when exposed to NaCl stress (Wang *et al.*, 2010).

**Expression of catalase in salt tolerant transgenic plants:** Catalases (CAT) are haem-containing tetrameric enzymes involved in the removal of H<sub>2</sub>O<sub>2</sub> (Guan and Scandalios, 1996). Plant catalases are involved in photorespiratory functions (Canvin, 1990) and scavenging of H<sub>2</sub>O<sub>2</sub> during  $\beta$ -oxidation of fatty acids in germinating seeds (Willekens *et al.*, 1995) and also during salt stress and other abiotic stress conditions (Willekens *et al.*, 1997). Catalase enzymes that can remove the Reactive Oxygen Species (ROS) produced by the salt stress play an important role in tolerance. Thus quenching of H<sub>2</sub>O<sub>2</sub> was an important factor for salt tolerance as observed in *cyanobacteria* (Kaku *et al.*, 2000). Nagamiya *et al.* (2007) were able to produce salt tolerant japonica rice (at 100 mM salt concentration) by over expressing the catalase gene *katE*. Proadhan *et al.* (2008) introduced *katE*, a catalase gene of *Escherichia coli*, into the indica rice cultivar Kasalath. Transgenic rice plants at a very young stage (three-four days) were able to grow up to 15 days in 100 mM NaCl solution and seven days in 250 mM NaCl solution whereas, control plants died within five days in 100 mM and seven days in 50 mM NaCl. Moriwaki *et al.* (2008) integrated the *katE* gene into BR5 rice plants using an *Agrobacterium tumefaciens*-mediated method. The introduced *katE* gene was actively expressed in the transgenic BR5 rice plants and catalase activity in transgenic rice was approximately 150% higher than in non transgenic plants. Under NaCl stress conditions, the transgenic rice plants exhibited high tolerance compared with non transgenic rice plants (Moriwaki *et al.*, 2008).

**Expression of glutathione s-transferase:** Glutathione S-transferases (GSTs) are a family of multifunctional enzymes that play important roles in oxidative stress resistance. The Key Laboratory of Plant Stress, P. R. China, obtained a GST from *Suaeda salsa* cDNA library (accession number BE859255) and showed that it played an important role in salt stress resistance (Wang *et al.*, 2002; Qi *et al.*, 2004). Zhao and Zhang (2006) proved that the expression of *Suaeda salsa* GST gene in transgenic rice (*Oryza sativa* L. cv) could confer resistance to salt stress. Overexpression of a tobacco glutathione S-transferase with glutathione peroxidase activity (GST/GPX) in transgenic tobacco (*Nicotiana tabacum* L.) enhanced seedling growth under salt stress conditions (Roxas *et al.*, 2000). Qi *et al.* (2010) introduced the *Suaeda salsa* glutathione S-transferase gene (GST) into *Arabidopsis* under the control of the cauliflower mosaic virus 35S promoter. The expression of the GST gene promoted a higher level of salt tolerance *in vivo* in transgenic *Arabidopsis* plants. Southern and northern blot analyses confirmed that GST was transferred into the *arabidopsis* genome and the GST and GPX activities in transgenic plants (GT) were much higher than in wild-type plants (WT) (Qi *et al.*, 2010).

**Expression of other enzymes in transgenic plants:** The transgenic plants overexpressing GR have higher ascorbate contents and improved tolerance to oxidative stress (Foyer *et al.*, 1991, 1995; Aono *et al.*, 1993). On the other hands, reduced GR activity resulted in increased stress sensitivity (Aono *et al.*, 1993). Ascorbate-deficient mutant of *Arabidopsis* is sensitive to oxidative stress such as UV and pollutants (Conklin *et al.*, 1997).

Ascorbate acts as an important antioxidant in both enzymatic and non-enzymatic (reacting directly with hydroxyl radicals, superoxide and singlet oxygen) reactions in plant cells. Some of the monodehydro ascorbate (MDHA), oxidized form of ascorbate, is re-reduced by monodehydro ascorbate reductase (MDHAR) using NAD(P)H, but the remainder undergoes spontaneous dismutation to AsA (reduced ascorbate) and dehydro ascorbate (DHA, oxidized ascorbate). DHAR (EC 1.8.5.1) catalyzes the re-reduction of DHA to AsA with simultaneous oxidation of GSH to GSSG. Thus, DHAR, as well as MDHAR, is critical for protection of cellular components against oxidative injury (Asada, 1999). Also, DHAR activity is enhanced in response to various environmental stresses (Urano *et al.*, 2000). The DHAR-overexpressing tobacco plants have been recently developed in our laboratory and have elevated level of tolerance to oxidative stress derived from various sources (Kwon *et al.*, 2000).

**Strategies to improve salt stress tolerance:** Recent advances in plant genome mapping and molecular biology techniques offer a new opportunity for understanding the genetics of salt stress-resistance genes and their contribution to plant performance under salt stress. These biotechnological advances will provide new tools for breeding in salt stress environment. Molecular genetic maps have been developed for major crop plants, including rice, wheat, maize, barley, sorghum and potato, which make it possible for scientists to tag desirable traits using known DNA landmarks. Molecular genetic markers allow breeders to track genetic loci controlling stress resistance without having to measure the phenotype, thus reducing the need for extensive field-testing over time and space. Moreover, gene pyramiding or introgression can be done more precisely using molecular tags. Together, molecular genetic markers offer a new strategy known as marker assisted selection. Another molecular strategy which depends on gene cloning and plant transformation technology is genetic engineering of selected genes into elite breeding lines. What makes a particular goal attainable or unattainable in genetic engineering experiments is the availability of the following three inputs: (1) the gene of interest, (2) an effective technique for transferring the desired gene from one species to another and (3) promoter sequences for regulated expression of that gene. Amongst these, the first is considered a rate-limiting factor. Arrays of salt stress-induced genes have been isolated. Salt stress-responsive genes can be analyzed following targeted or non-targeted strategy. The targeted approach relies upon the availability of relevant biochemical information (i.e., in terms of defined enzyme, protein, a biochemical reaction or a physiological phenomenon). The non-targeted strategy to obtain a desired gene is indirect. This strategy, for instance, includes differential hybridization and shotgun cloning. The list of genes, whose transcription is upregulated in response to stress, is rapidly increasing. Understanding of the mechanisms which regulate gene expression and the ability to transfer genes from other organisms into plants will expand the ways in which plants can be utilized. To exploit the full potential of these approaches, it is essential that the knowledge is applied to agriculturally and ecologically important plant species.

## CONCLUSION AND FUTURE PROSPECTS

Salinity in the soil and irrigation water is an environmental problem and a major constraint for crop production. Salt stress (NaCl) has both osmotic (cell dehydration) and toxic (ion accumulation) effects on plants. Although a wide range of genetic adaptations to saline conditions has been observed and a number of significant physiological responses have been associated with tolerance, underlying mechanisms of salt tolerance in plants are still poorly understood.

The ROS, especially hydrogen peroxide, have been proved as a central component of plant adaptation to biotic and abiotic stresses (50) (Mittler, 2002). Under the stress conditions, ROS may play in damaging the cellular components or signaling the activation of defense responses. To allow for these different roles, cellular levels of ROS must be tightly controlled. In first, precise understanding the roles of each ROS scavenging enzyme and small molecular antioxidants in stress adaptation and accurate characterization of the complex stress tolerance phenotypes is necessary to develop stress tolerant plants.

Cloned plant genes and transgenic plants have become a standard tool in plant-stress biology. These technologies have mainly been applied to model systems and have greatly enlarged the knowledge of mechanisms of tolerance. The various abiotic stresses cause changes in plant processes at all levels of organization (morphological, physiological, biochemical and molecular). In recent years, attention has focused on alterations in gene expression. The list of genes whose transcription is upregulated in response to stress is rapidly increasing. Functions for some of these polypeptides are close to being identified and their likely role in stress physiology is being determined. The understanding of mechanisms that regulate gene expression and the ability to transfer genes from other organisms into plants will expand the ways in which plants can be utilized. The exploitation of cloned genes to alter the function of gene products in transgenic plants provides novel opportunities to assess their biological role in a salt stress response. We are trying to develop ideal transgenic plants with enhanced tolerance to salt stress by expression of multiple antioxidant enzymes in target organelle of a plant cell under the control of salt stress-inducible promoter.

#### ACKNOWLEDGMENTS

The authors gratefully acknowledged to our Malankara Catholic College Correspondent Fr. Prem Kumar (MSW) given encouragement and support for preparation of this manuscript. We wish to express our sincere thanks to Dr. Sujatha (ICBM), Mr. K. Suresh and the research scholars in the Interdisciplinary Research centre for updated article collection and all the efficient supports of this review preparation.

#### REFERENCES

- Alscher, R.G., J.L. Donahue and C.L. Cramer, 2002. Reactive oxygen species and antioxidants: Relationships in green cells. *Physiol. Plant.*, 100: 224-233.
- Aono, M., A. Kubo, H. Saji, K. Tanaka and N. Kondo, 1993. Enhanced tolerance to photooxidative stress of transgenic *Nicotiana tabacum* with high chloroplastic glutathione reductase activity. *Plant Cell Physiol.*, 34: 129-135.
- Apel, K. and H. Hirt, 2004. Reactive oxygen species: Metabolism, oxidative stress and signal transduction. *Annu. Rev. Plant Biol.*, 55: 373-399.
- Asada, K., 1999. The water-water cycle in chloroplasts: Scavenging of active oxygens and dissipation of excess photons. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 50: 601-639.
- Azevedo-Neto, A.D., J.T. Ptisco, J. Eneas-Filho, C.E.B. Abreu and E. Gomez-Filho, 2006. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *Environ. Exp. Bot.*, 56: 87-94.
- Bartels, D. and R. Sunkar, 2005. Drought and salt tolerance in plants. *Crit. Rev. Plant. Sci.*, 24: 23-58.
- Ben-Amor, N., A. Jimenez, W. Megdiche, M. Lundqvist, F. Sevilla and C. Abdelly, 2007. Kinetics of the anti-oxidant response to salinity in the halophyte *Cakile maritima*. *J. Integr. Plant Biol.*, 49: 982-992.



- Benevides, M.P., P.L. Marconi, S.M. Gullego, M.E. Comba and M.L. Tomaro, 2000. Relationship between antioxidant defense system and salt tolerance in *Solanum tuberosum*. *Aust. J. Plant Physiol.*, 27: 273-278.
- Blokhina, O., E. Virolainen and K.V. Fagerstedt, 2003. Antioxidants, oxidative damage and oxygen deprivation Stress: A review. *Ann. Bot.*, 91: 179-194.
- Bor, M., F. Ozdemir and I. Turkan, 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.
- Bowler, C., W. Camp, M. Montagu and D. Inze, 1994. Superoxide dismutase in plants. *Cri. Rev. Plant Sci.*, 13: 199-218.
- Cai-Hong, P., Z. Su-Jun, G. Zhi-Zhong and W.W. Bao-Shan, 2005. NaCl treatment markedly enhances H<sub>2</sub>O<sub>2</sub>-scavenging system in leaves of halophyte *Suaeda salsa*. *Physiol. Plant.*, 125: 490-499.
- Canvin, T., 1990. Photorespiration and CO<sub>2</sub> Concentrating Mechanisms. In: *Plant Physiology, Biochemistry and Molecular Biology*, Dennis, D.T. and D.H. Turpin (Eds.). Longman Scientific and Technical, Harlow, pp: 253-273.
- Carlioz, A. and D. Touati, 1986. Isolation of superoxide dismutase mutants in *Escherichia coli*: Is superoxide dismutase necessary for aerobic life. *EMBO J.*, 5: 623-630.
- Cicek, N. and H. Cakýrlar, 2008. Changes in some antioxidant enzyme activities in six soybean cultivars in response to long-term salinity at two different temperatures. *Gen. Appl. Plant Physiol.*, 34: 267-280.
- Conklin, P.L., J.E. Pallanca, R.L. Last and N. Smirnov, 1997. L-Ascorbic acid metabolism in the ascorbate-deficient Arabidopsis mutant vtcl. *Plant Physiol.*, 115: 1277-1285.
- Costa, P.H.A., A. Neto, M. Bezerra, J. Prisco and E. Filho, 2005. Antioxidant-enzymatic system of two sorghum genotypes differing in salt tolerance. *Braz. J. Plant Physiol.*, 17: 353-361.
- Demiral, T. and I. Turkan, 2005. Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. *Environ. Exp. Bot.*, 53: 247-257.
- Desingh, R. and G. Kanagaraj, 2007. Influence of salinity stress on photosynthesis and antioxidative systems in two cotton varieties. *Gen. Appl. Plant Physiol.*, 33: 221-234.
- Dionisio-Sese, M.L. and S. Tobita, 1998. Antioxidant responses of rice seedlings to salinity stress. *Plant Sci.*, 135: 1-9.
- Eyidogan, F. and M.T. Oz, 2007. Effect of salinity on antioxidant responses of chickpea seedlings. *Acta Physiol. Plant.*, 29: 485-493.
- Foyer, C.H. and B. Halliwell, 1976. The presence of glutathione and glutathione reductase in chloroplasts: A proposed role in ascorbic acid metabolism. *Planta*, 133: 21-25.
- Foyer, C.H., M. Lelandais, C. Galap and K.J. Kunert, 1991. Effects of elevated cytosolic glutathione reductase activity on the cellular glutathione pool and photosynthesis in leaves under normal and stress conditions. *Plant Physiol.*, 97: 863-872.
- Foyer, C.H., P. Descourvieres and K.J. Kunert, 1994. Protection against oxygen radicals: An important defense mechanism studied in transgenic plants. *Plant Cell Environ.*, 17: 507-523.
- Foyer, C.H., N. Souriau, S. Perret, M. Lelandais, K.J. Kunert, C. Pruvost and L. Jouanin, 1995. Overexpression of glutathione reductase but not glutathione synthetase leads to increases in antioxidant capacity and resistance to photoinhibition in poplar trees. *Plant Physiol.*, 109: 1047-1057.

- Foyer, C.H. and G. Noctor, 2003. Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. *Physiol. Plant.*, 119: 355-364.
- Fridovich, I., 1989. Superoxide dismutases: An adaptation to a paramagnetic gas. *J. Biol. Chem.*, 264: 7761-7764.
- Guan, L.Q. and J.G. Scandalios, 1996. Molecular evolution of maize catalases and their relationship to other eukaryotic and prokaryotic catalases. *J. M. Evol.*, 42: 570-579.
- Gueta-Dahan, Y., Z. Yaniv, B.A. Zilinskas and G. Ben-Hayyim, 1997. Salt and oxidative stress: Similar and specific responses and their relation to salt tolerance in citrus. *Planta*, 204: 460-469.
- Harinasut, P., D. Poonsopa, K. Roengmongkol and R. Charoensataporn, 2003. Salinity effects on antioxidant enzymes in mulberry cultivar. *Sci. Asia*, 29: 109-113.
- Hasegawa, P.M., R.A. Bressan, J.K. Zhu and H.J. Bohnert, 2000. Plant cellular and molecular responses to high salinity. *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, 51: 463-499.
- Heidari, M., 2009. Antioxidant activity and osmolyte concentration of sorghum (*Sorghum bicolor*) and wheat (*Triticum aestivum*) genotypes under salinity stress. *Asian J. Plant Sci.*, 8: 240-244.
- Hernandez, J.A., J. Francisco, F.J. Corpas, L.A. Del Rio and F. Sevilla, 1993. Salt induced oxidative stresses mediated by activated oxygen species in pea leaf mitochondria. *Physiol. Plant.*, 89: 103-110.
- Hernandez, J.A., A. Campillo, A. Jimenez, J.J. Alarcon and F. Sevilla, 1999. Response of antioxidant systems and leaf water relations to NaCl stress in pea plants. *New Phytol.*, 141: 241-251.
- Hernandez, J.A., A. Jimenez, P. Mullineaux and F. Sevilla, 2000. Tolerance of pea (*Pisum sativum* L.) to long term salt stress is associated with induction of antioxidant defences. *Plant Cell Environ.*, 23: 853-862.
- Imlay, J.A., 2003. Pathways of oxidative damage. *Annu. Rev. Microbiol.*, 57: 395-418.
- Jithesh, M.N., S.R. Prashanth, K.R. Sivaprakash and A.K. Parida, 2006. Antioxidative response mechanisms in halophytes: their role in stress defence. *J. Genet.*, 85: 237-254.
- Joseph, B., D. Jini and S. Sujatha, 2010. Biological and physiological perspectives of specificity in abiotic salt stress response from various rice plants. *Asian J. Agric. Sci.*, 2: 99-105.
- Kaku, N., T. Hibino, Y. Meng, Y. Tanaka, E. Araki, T. Takabe and T. Takabe, 2000. Effects of overexpression of *Escherichia coli* katE and bet genes on the tolerance for salt stresses in a fresh water *Cyanobacterium synechococcus* sp. PCC 7942. *Plant Sci.*, 159: 281-288.
- Kartashov, A.V., N.L. Radyukina, Y.V. Ivanov, P.P. Pashkovskii, N.I. Shevyakova and V.V. Kuznetsov, 2008. Role of antioxidant systems in wild plant adaptation to salt stress. *Russ. J. Plant Physiol.*, 55: 463-468.
- Kim, S., J. Lim, M. Park, Y. Kim, T. Park, Y. Seo and S. Yun, 2005. Enhanced antioxidant enzymes are associated with reduced hydrogen peroxide in barley roots under saline stress. *J. Biochem. Mol. Biol.*, 38: 218-224.
- Kwon, S.Y., Y.O. Ahn, S.M. Choi, H.S. Lee and S.S. Kwak, 2000. Enhanced stress tolerance of DHAR transgenic plants. *Proceedings of the 2000 Japan Korean Joint Symposium of Plant Science, (JKJSPS'2000)*, Japan, pp: 150-150.
- Mansour, M.M.F., K.H.A. Salama, F.Z.M. Ali and A.F. Abou Hadid, 2005. Cell and plant responses to NaCl in *Zea mays* L. cultivars differing in salt tolerance. *Gen. Applied Plant Physiol.*, 31: 29-41.
- Meloni, D.A., M.A. Oliva, C.A. Martinez and J. Cambraia, 2003. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Environ. Exp. Bot.*, 49: 69-76.

- Meratan, A.A., S.M. Ghaffari and V. Niknam, 2008. Effects of salinity on growth, proteins and antioxidant enzymes in three *Acanthophyllum* species of different ploidy levels. JSUT, 33: 1-8.
- Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci., 7: 405-410.
- Mittova, V., M. Tal, M. Volokita and M. Guy, 2002. Salt stress induces up-regulation of an efficient chloroplasts antioxidant system in the salt-tolerant wild tomato species *Lycopersicon pennellii* but not in the cultivated species. Plant Physiol., 115: 393-400.
- Mittova, V., M. Guy, M. Tal and M. Volokita, 2004. Salinity upregulates the antioxidative system in root mitochondria and peroxisomes of the wild salt-tolerant tomato species *Lycopersicon pennellii*. J. Exp. Bot., 55: 1105-1113.
- Moriwaki, T., Y. Yamamoto, T. Aida, T. Funahashi and T. Shishido and M. Asada *et al.*, 2008. Overexpression of the *Escherichia coli* catalase gene, katE, enhances tolerance to salinity stress in the transgenic indica rice cultivar, BR5. Plant Biotechnol. Rep., 2: 41-46.
- Munns, R., 2002. Comparative physiology of salt and water stress. Plant Cell Environ., 25: 239-250.
- Nagamiya, K., T. Motohashi, K. Nakao, S.H. Prodhan and E. Hattori *et al.*, 2007. Enhancement of salt tolerance in transgenic rice expressing an *Escherichia coli* catalase gene, *Kat E*. Plant Biotechnol. Rep., 1: 49-55.
- Noctor, G. and C.H. Foyer, 1998. Ascorbate and glutathione: Keeping active oxygen under control. Annu. Rev. Plant Physiol. Mol. Biol., 49: 249-279.
- Orr, W.C. and R.S. Sohal, 1994. Extension of life span by over expression of superoxide dismutase and catalase in *Drosophila melanogaster*. Science, 263: 1128-1130.
- Pitman, M.G. and A. Lauchli, 2002. Global Impact of Salinity and Agricultural Ecosystems. In: Salinity Environment-Plants Molecules, Lauchli, A. and V. Lutge (Eds.). Kluwer, Netherlands, pp: 3-20.
- Prodhan, S.H., A. Hossain, K. Nagamiya, A. Komamine and H. Morishima, 2008. Improved salt tolerance and morphological variation in indica rice (*Oryza sativa* L.) transformed with a catalase gene from *E. coli*. Plant Tissue Cult. Biotechnol., 18: 57-63.
- Qi, Y.C., S.M. Zhang, L.P. Wang, M.D. Wang and H. Zhang, 2004. Overexpression of GST gene accelerates the growth of transgenic Arabidopsis under salt stress. J. Plant Physiol. Mol. Biol., 30: 517-522.
- Qi, Y.C., W.Q. Liu, L.Y. Qiu, S.M. Zhang, L. Ma and H. Zhang, 2010. Overexpression of glutathione S-transferase gene increases salt tolerance of arabidopsis. Russian J. Plant Physiol., 57: 233-240.
- Raychaudhuri, S.S. and X.W. Deng, 2000. The role of superoxide dismutase in combating oxidative stress in higher plants. Bot. Rev., 66: 89-98.
- Reddy, A.R., K.V. Chaitanya and M. Vivekanandan, 2004. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. J. Plant Physiol., 161: 1189-1202.
- Roxas, V.P., S.A. Lodhi, D.K. Garrett, J.R. Mahan and R.D. Allen, 2000. Stress tolerance in transgenic tobacco seedlings that overexpress glutathione S-transferase/glutathione peroxidase. Plant Cell Physiol., 41: 1229-1234.
- Sairam, R.K., K.V. Roa and G.C. Srivastava, 2002. Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. Plant Sci., 163: 1037-1046.
- Sairam, R.K. and A. Tyagi, 2004. Physiology and molecular biology of salinity stress tolerance in plants. Curr. Sci., 86: 407-421.

- Sekmen, A.H., I. Turkana and S. Takiob, 2007. Differential responses of antioxidative enzymes and lipid peroxidation to salt stress in salt-tolerant *Plantago maritime* and salt-sensitive *Plantago media*. *Physiol. Plant*, 131: 399-411.
- Shalata, A., V. Mittova, M. Volokita, M. Guy and M. Tal, 2001. Response of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii* to salt-dependent oxidative stress: The root antioxidative system. *Physiol. Plant.*, 112: 487-494.
- Sudhakar, C., A. Lakshmi and S. Giridarakumar, 2001. Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. *Plant Sci.*, 161: 613-619.
- Torsethaugen, G., L.H. Pitcher, B.A. Zilinskas and E.J. Pell, 1997. Overproduction of ascorbate peroxidase in the tobacco chloroplast does not provide protection against ozone. *Plan Physiol.*, 114: 529-537.
- Urano, J., T. Nakagawa, Y. Maki, T. Masumura, K. Tanaka, N. Murata and T. Ushimaru, 2000. Molecular cloning and characterization of a rice dehydro ascorbate reductase. *FEBS Lett.*, 466: 107-111.
- Vaidyanathan, H., P. Sivakumar, R. Chakrabarsty and G. Thomas, 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.
- Wang, L.P., Y.C. Qi, Y.X. Zhao and H. Zhang, 2002. Cloning and sequencing of GST gene of *Suaeda salsa* and its expression characteristics. *J. Plant Physiol. Mol. Biol.*, 28: 133-136.
- Wang, Y.C., G.Z. Qu, H.Y. Li, Y.J. Wu, C. Wang, G.F. Liu and C.P. Yang, 2010. Enhanced salt tolerance of transgenic poplar plants expressing a manganese superoxide dismutase from *Tamarix androssowii*. *Mol. Bio. Rep.*, 37: 1119-1124.
- Willekens, H., M. van Montagu and W. van Camp, 1995. Catalase in plants. *Mol. Breed.*, 1: 207-228.
- Willekens, H., S. Chamnongpol, M. Davey, M. Schraudner and C. Langebartels *et al.*, 1997. Catalase is a sink for H<sub>2</sub>O<sub>2</sub> and is indispensable for stress defense in C3 plants. *EMBO J.*, 16: 4806-4816.
- Zhao, F. and H. Zhang, 2006. Expression of *Suaeda salsa* glutathione S-transferase in transgenic rice resulted in a different level of abiotic stress resistance. *J. Agric. Sci.*, 144: 547-554.
- Zhao, J., S. Guo, S. Chen, H. Zhang and Y. Zhao, 2009. Expression of yeast YAP1 in transgenic arabidopsis results in increased salt tolerance. *J. Plant Biol.*, 52: 56-64.
- Zhu, J.K., 2001. Plant salt tolerance. *Trends Plant Sci.*, 6: 66-71.