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**REVIEW ARTICLE**

**ANTIOXIDATIVE DEFENCE AGAINST REACTIVE OXYGEN SPECIES IN PLANTS  
UNDER SALT STRESS**

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**ABSTRACT**

Salinity is one of the major constraints in agriculture which affects the plant growth and productivity. The high concentration of salt in soil causes ion imbalance leading to osmotic stress in plants which further leads to oxidative damage in the plant cell via ROS production. ROS caused damage to the lipids, proteins and nucleic acids. Plants have developed certain mechanisms to resist the oxidative damage caused due to salinity. The genes conferring salinity stress resistances provide foundation for scientific improvement of the plants productivity under arid condition and contribute to improvement and stabilization of plant yield. Molecular genetics and plant transformation has helped in generating salt tolerant plant by modifying the signal cascades, biochemical pathways, specific protein expression or by altering the gene response under natural condition. Salt tolerant transgenic crops have been developed and more work is going on in this direction.

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**INTRODUCTION**

Environmental factors such as temperature, light intensity, salinity, drought and availability of nutrients play an important role in plants growth and development. Any variation in these environmental factors which leads to decline in the growth and productivity of plants is termed as abiotic stress [Levitt 1980]. Salinity affects more than 10% arable land on global scale [Bray *et al.*, 2000]. Year after year more land under irrigation is becoming increasingly saline. About 20% of worlds cultivated land and nearly half of the worlds irrigated area are inflicted to salinity [FAO, 2007]. Mostly plants are extremely susceptible to salt and are unable to tolerate a concentration higher than 100 mM NaCl. Salinity adversely affects the plant growth and development leading to low yield [Bernstein, 1975; Ashraf, 1999]. The salt stress causes reduced water potential, ion imbalance and toxicity which results in decreased growth, development and productivity and sometimes high salinity even causes death [Hasegwa *et al.*, 2000]. Ionic, osmotic and associated secondary stress is caused due to high salt concentration which leads to homeostasis, detoxification and growth control response in plants. To re-establish homeostasis in plants under stressful condition both ionic and osmotic homeostasis must be restored by activation of various ion transporters which reduces or prevents the entry of Na<sup>+</sup> in the plant cell or in organelles other than the vacuoles. Complex molecular responses like production of stress proteins and compatible osmolytes is the detoxification response in plants under salt stress. Growth control response includes coordinated stress adaptation like slower growth, decreased rate of cell division etc [Zhu, 2001]. The technology for combating the problem of salinity is extremely costly which requires large expenditure of energy to reclaim land and maintain soil balances. The ability of crop to tolerate a given level of salinity becomes paramount in managing water and soil resources. An alternative to expensive large scale irrigation and drainage schemes is the development of salt tolerant plants and for this reason there has been an upsurge of interest towards tailoring crop plants to suit more

saline environment. A substantial research is needed to understand the plant salt tolerance mechanism, though resent knowledge about the salt stress signaling and mechanism to prevent oxidative damage caused due to salinity generated ROS. Certain genes have been isolated which are responsible for over expression of antioxidant enzymes (SOD, APX, CAT, GR), osmolytes or osmoprotectant (proline, glycine betain, Mannitol) under salt stress condition have been studied and these genes have been used to producing transgenic salt tolerant plants.

**Salinity and Ros**

Salinity can be defined as the degree to which soil contains salts. There are basically two main causes of salinity primary and secondary salinity. Primary salinity is caused due to weathering of rocks containing soluble salts (chlorides of calcium, sodium and magnesium) and through saline water intrusion via tides. Secondary salinity is mainly caused due to human activities such as use to chemical fertilizers which contains salts, using improper methods of irrigation and using salt rich water for irrigation. Presence of salt in soil leads to reduced availability of water to plants due to physiological drought condition i.e. causes osmotic or water deficit effect of salinity [Romero Aranda *et al.*, 2001]. Salinity imposes osmotic stress by decreasing the soil water potential which limits the water uptak. Salt stress causes excessive uptake of ions (Na<sup>+</sup> and Cl<sup>-</sup>) that ultimately interferes with many metabolic processes occurring in plants. ROS is produced from photosynthesis, photorespiration and respiration in plant cell under normal condition. Still scientists are technically unable to determine the major sources of ROS as well as the mechanisms responsible for the generation of ROS under salt stress. ROS includes free radicals such as superoxide anions (O<sub>2</sub><sup>-</sup>), hydroxyl radical (OH) as well as non radical molecules like hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), singlet oxygen (<sup>1</sup>O<sub>2</sub>). ROS are always formed in plants due to unavoidable leakage of electrons onto O<sub>2</sub> from the electron transport activities of mitochondria and chloroplast and as by products of various metabolic pathways localized in different cellular compartments such as chloroplasts, mitochondria and peroxisomes [Mittler, 2002; del Rio *et al.*, 2006; Blokhina and Fagerstedt, 2010].

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Though ROS causes various destructions in the cell but it also acts as a secondary messenger in various cellular processes which includes tolerance to environmental stress [Desikan *et al.*, 2001; Yan *et al.*, 2007; Neill *et al.*, 2002]. Beneficial or harmful effect of ROS in plants highly depends on the concentration, at high concentration it causes damage in cell but at low concentration it acts as signal molecule. It has been calculated that out of total O<sub>2</sub> consumed by plants 1% is converted into ROS [Asada and Takahashi, 1987]. Salinity stress leads to stomatal closure in leaves of plants which in turn reduces CO<sub>2</sub> availability in leaves and inhibits carbon fixation which causes exposure of chloroplasts to excessive excitation energy and over-reduction of photosynthetic electron transport system leading to enhanced ROS generation. It has been evident that high salinity induces oxidative stress in response to this the plants have activated the mechanism of antioxidant resistance which provides enhanced salt tolerance [Gomez *et al.*, 1999]. The nature of the damage that high salt concentrations inflict on plants is not clear. One of the important causes of damage is reactive oxygen species (ROS) generation which disrupts the cellular homeostasis by affecting the integrity of cellular membranes, the activities of various enzymes, peroxidation of lipids, oxidation of proteins as well as causes damage to nucleic acid as studied in *Fragaria ananassa*, *Pisum sativum* L. and *Carthamus tinctorius* plants [Tanou *et al.*, 2009; Hernandez *et al.*, 2000; Karray Bouraoui *et al.*, 2011].

ROS when generated in excess amount results in damage of protein, lipids, nucleic acid and cell structures [Valko *et al.*, 2006]. Damage caused to DNA due to abiotic or biotic stress is known as genotoxic stress, which includes base deletion, pyrimidine dimers, cross links, strand breaks and base modification either by alkylation or oxidation [Tuteja *et al.*, 2001; Tuteja and Tuteja 2001]. The ·OH is the most reactive free radical that causes damage to both purine and pyrimidine bases and also the deoxyribose backbone [Halliwell and Gutteridge, 1999]. DNA damage caused due to ROS results in reduced protein synthesis, photosynthetic proteins and cell membrane destruction which ultimately leads to impaired growth and development of plants. Mostly ROS or its by products reacts with the lipids and causes lipid peroxidation by covalent modification and these are mostly irreversible [Ghezzi and Bonetto, 2003]. The ROS attacks on the ester linkage between the glycerol and the fatty acid and on the unsaturated double bonds between the carbon atoms. O<sub>2</sub><sup>-</sup> and ·OH reacts with methylene groups which forms the conjugated dienes and lipid peroxides. In many cases membrane damage is taken as the parameter to determine the level of lipid peroxidation under stress.

### Antioxidants and Salt Stress

Salinity induced ROS disrupts normal metabolism through lipid peroxidation, denaturing proteins and nucleic acid in several plant species. Different proteins and enzymes are responsible for the detoxify ROS, antioxidants such as Superoxide dismutase, ascorbate, tocopherol and glutathione plays an important role in the regulation of the cellular ROS homeostasis. Differential genomic and proteomic screenings carried out in *Physcomitrella patens* plant showed that they responded to salinity stress by upregulating a large number of genes involved in antioxidant defense mechanism suggesting that the antioxidative system may play a crucial role in protecting cell from oxidative damage following exposure to salinity stress in *P. patens* [Wang *et al.*, 2008]. A major category of organic osmotic solutes consists of simple sugars (fructose, glucose), and complex sugars (trehalose, raffinose and fructans) [Bohnert and Jensen, 1996]. Glycine betaine preserves thylakoid and plasma membrane integrity after exposure to salt stress or freezing or high temperatures [Rhodes and Hanson, 1993]. Many of the osmoprotectants enhances salt stress tolerance of plants when expressed as trans gene products [Bohnert and Jensen, 1996]. An adaptive biochemical function of osmoprotectants is the scavenging of ROS that are byproducts of hyperosmotic and ionic stresses and cause membrane dysfunction and cell death [Bohnert and Jensen, 1996]. A common feature of compatible solutes is that these compounds can accumulate to high levels without disturbing intracellular biochemistry [Bohnert and

Jensen, 1996]. Compatible solutes have the capacity to persevere the activity of enzymes that are in saline solutions. high antioxidant activity could be interpreted as higher tolerance to oxidative stress (the plant suffers less oxidative stress because it has higher antioxidant activity). Efficient antioxidative defense has often been viewed as upregulation of a full set of antioxidant enzymes (SOD, CAT, POD, GR, etc.) although each of these enzymes performs a specific function and its activity should be assigned to a specific role in ROS detoxification i.e., efficient antioxidative activity does not necessarily mean the strong upregulation of the full set of antioxidant enzymes and vice versa. Many comparative studies using salt tolerant and sensitive genotypes have correlated the salt tolerance to an increase in the activity of antioxidant enzymes in the examples mentioned below, the catalase (CAT), ascorbate peroxidase (APX), polyphenoloxidase (PPO) and peroxidase activity (POD) and proline content increased as a result of salinity stress in Soybean (*Glycine max* L.). However addition of Zinc resulted in significant reduction in lipid peroxidation and hydrogen peroxide concentration [Weisany *et al.*, 2012]. The SOD and APX increased in both the varieties of *Pisum sativum* EC33866 and Puget under salt stress. GR and DHAR were increased in both the varieties at 150 mM NaCl however at 200 mM NaCl decline was observed [Ahmad and Jhon, 2005]. Guaiacol-specific peroxidase (POX) was significantly elevated Glutathione reductase (GR) and Catalase (CAT) less increased under salt stress in French bean (*Paseolus vulgaris*) however no variation was observed in AP, POX and CAT isozymes [Nagesh Babu and Devaraj, 2008]. The effect of salinity on antioxidative system was studied in liquorice seedlings (*Glycyrrhiza uralensis* Fisch) the result showed that SOD and POD activity was upregulated while CAT activity decreased [Yan Pan *et al.*, 2006]. An increased in activity of antioxidant enzyme such as Ascorbate peroxidase, Catalase and Glutathione reductase contribute to salt tolerance in Kennebec, a salt resistant cultivar of potato [Aghaei *et al.*, 2009]. Increasing salt concentration on *Jatropha curcas* L. seedlings showed enhanced activity of CAT, PAL in cotyledons, hypocotyls and radicles [Gao *et al.*, 2008]. Photosynthetic rate and activities of RuBP carboxylase and sucrose phosphate synthase (SPS) decreased but activity of Superoxide dismutase, Ascorbate peroxidase (APX) and Glutathione reductase increased significantly with increased salt concentration [Desingh and Kanagaraj, 2007]. Analysis of increasing salt concentration on *Brassica napus* L. showed that amount of SOD activity also increased linearly in both the genotype of Colza [Jalali-Emam *et al.*, 2011]. The activities of SOD, CAT, APX, POX and GR were increased significantly in the roots of Barley within 1 day after treatment and so on. Among these CAT activity showed drastic increase under salt stress condition. The H<sub>2</sub>O<sub>2</sub> content in the roots was most highly correlated with the CAT activity, indicating an increased role of CAT in H<sub>2</sub>O<sub>2</sub> detoxification [Sang Yong *et al.*, 2005].

SOD has been proposed to be important in plant stress tolerance and provide the first line of defense against the toxic effects of elevated levels of ROS. The SODs remove O<sub>2</sub> by catalyzing its dismutation, one O<sub>2</sub> being reduced to H<sub>2</sub>O<sub>2</sub> and another oxidized to O<sub>2</sub>. It removes O<sub>2</sub> and hence decreases the risk of OH formation via the metal catalyzed Haber Weiss reaction. This reaction has a 10,000 fold faster rate than spontaneous dismutation. Significant increase in SOD activity under salt stress has been observed in various plants viz. mulberry, *C. arietinum* and *Lycopersicon esculentum* [Gapinska *et al.*, 2008; Kukreja *et al.*, 2005; Harinasut *et al.*, 2003]. Catalase has the potential to directly dismutate H<sub>2</sub>O<sub>2</sub> into H<sub>2</sub>O and O<sub>2</sub> and is indispensable for ROS detoxification during stressed conditions [Garg, 2009]. Catalase removes H<sub>2</sub>O<sub>2</sub> generated in peroxisomes by oxidases involved in β-oxidation of fatty acids, photorespiration and purine catabolism. Glutathione Reductase is a flavo-protein oxidoreductase, potential enzyme of the ASH-GSH cycle and plays an essential role in defense system against ROS by sustaining the reduced status of GSH. It is localized predominantly in chloroplasts, but small amount of this enzyme has also been found in mitochondria and cytosol [Edwards *et al.*, 1990; Romero Puertas *et al.*, 2006].

Table 1. List of genes and gene products in transgenic plants for improved salt tolerance

Gene	Gene Product(s)/ Gene action	Transgenic Plant	References
SOS1	A putative Na <sup>+</sup> /H <sup>+</sup> antiporter	Arabidopsis	Zhu, 2000
AtNHX1 & AtNHX2	Vacuolar Na <sup>+</sup> /H <sup>+</sup> antiporter	Arabidopsis	Yokoi <i>et al.</i> , 2002
GmCAX1	Cation/Proton antiporter	Arabidopsis	Luo <i>et al.</i> , 2005
AtHKT1	Reduction of Sodium in roots	Arabidopsis cell	Horie <i>et al.</i> , 2006
GhNHX1	Vacuolar Na <sup>+</sup> /H <sup>+</sup> antiporter	Cotton	Wu <i>et al.</i> , 2004
OsNHX1	Vacuolar Na <sup>+</sup> /H <sup>+</sup> antiporter	Rice	Fukuda <i>et al.</i> , 2004
OsSOS1	Plasma membrane Na <sup>+</sup> /H <sup>+</sup> exchanger	Rice	Martinez-Atienza <i>et al.</i> , 2007
SOD2	Vacuolar Na <sup>+</sup> /H <sup>+</sup> antiporter	Rice	Zhao <i>et al.</i> , 2006
SsVP-2	Vacuolar Na <sup>+</sup> /H <sup>+</sup> antiporter	Arabidopsis	Guo <i>et al.</i> , 2006
SsNHX1	Vacuolar Na <sup>+</sup> /H <sup>+</sup> antiporter	Rice	Zhao <i>et al.</i> , 2006
TsVP	Vacuolar Na <sup>+</sup> /H <sup>+</sup> antiporter	Tobacco	Gao <i>et al.</i> , 2006
PcSrp	Serine rich protein	Finger millet	Mahalakshmi <i>et al.</i> , 2006
OsABF2	ABRE- binding bZIP transcription factor	Rice	Hossain <i>et al.</i> , 2010
DREB1A	Transcription factor	Arabidopsis	Kasuga <i>et al.</i> , 1999
ALFIN1	Zinc finger transcription factor	Alfalfa	Winicov & Bastola, 1999
PP2B	Signaling regulator	Arabidopsis	Padro <i>et al.</i> , 1998
SOS3	Calcium binding protein with N myristoylation signature sequence	Arabidopsis	Liu <i>et al.</i> , 2000
PpDHNA	Dehydrin protein accumulation	Moss	Saavedra <i>et al.</i> , 2006
HVA1	Group 3 late embryogenesis abundant protein gene	Oat	Oraby <i>et al.</i> , 2005
Gly1 and Gly2	Glutathione based detoxification of methyl glyoxal	Tobacco	Singla Pareek <i>et al.</i> , 2006
AtGSK1	Homologue of GSKS3/Shaggy like protein kinase	Arabidopsis	Piao <i>et al.</i> , 2001
Atnoa1	Impaired Nitric Oxide synthesis	Arabidopsis	Zhao <i>et al.</i> , 2007
AtSZF1 & AtSZF2	CCCH-type zinc finger protein	Arabidopsis	Sun <i>et al.</i> , 2005
SCABP8	Interacts with SOS2	Arabidopsis	Quan <i>et al.</i> , 2007
Apo-Inv	Apoplastic invertase	Tobacco	Fukushima <i>et al.</i> , 2001
bet A	Choline dehydrogenase	Tobacco	Lilius <i>et al.</i> , 1996
mt1D	Mannitol-1-phosphate dehydrogenase	Tobacco	Tarazynski <i>et al.</i> , 1993
CDH,BADH	Glycine betain synthesis	Tobacco	Holmstrom <i>et al.</i> , 2000
Cod A	Glycine betain synthesis	Brassica	Prasad <i>et al.</i> , 2000
COX	Choline oxidase (glycine betaine synthesis)	Rice	Su <i>et al.</i> , 2006
mt1D	Mannitol-1-phosphate dehydrogenase	Arabidopsis	Thomas <i>et al.</i> , 1995
p5csF	Proline synthesis	Tobacco	Hong <i>et al.</i> , 2000
mt1D & Gut D	Mannitol-1-phosphate dehydrogenase and Glucitol-6-phosphate dehydrogenase	Ioblolly pine	Tang <i>et al.</i> , 2005
P5C5	Pyrroline carboxylate synthase (proline synthesis)	Potato	Hmida-Sayari <i>et al.</i> , 2005
BADH-1	Betaine aldehyde dehydrogenase	Carrot	Kumar <i>et al.</i> , 2004
Cu-Zn SOD	Copper Zinc Superoxide dismutase	Tobacco	Lee <i>et al.</i> , 2007
MnSOD	Manganese superoxide dismutase	Tobacco	Bowler <i>et al.</i> , 1991
FeSOD	Iron superoxide dismutase	Arabidopsis	Van Camp <i>et al.</i> , 1996
Pa SOD	Superoxide dismutase	Arabidopsis	Gill <i>et al.</i> , 2010
Am SOD	Superoxide dismutase	Rice	Sarangi <i>et al.</i> , 2011
Mn-SOD	Mn superoxide dismutase	Arabidopsis	Wang <i>et al.</i> , 2004
GS2	Glutamine synthetase	Rice	Hoshida <i>et al.</i> , 2000
SOS2	Serine/Threonine type protein Kinase	Arabidopsis	Liu <i>et al.</i> , 2000
ZmSPK1	Sucrose non-fermenting-1-related protein kinase	Arabidopsis	Zou <i>et al.</i> , 2011

### Salt Tolerant Transgenic Plants

Scientists across the world have studied the response and regulatory mechanisms occurring in plants under saline condition. Whenever a plant is subjected to salt stress a complex response is observed such as over expression of stress proteins, antioxidant enzymes and osmolytes, which functions to both lower the production of ROS or scavenging the ROS and also prevent cellular damage caused by ROS. There are various mechanisms by which plants can protect themselves from abiotic stresses by accumulation of osmoprotectants, exclusion of ions, compartmentation of ions, transporter and symporter systems, water channels, chaperons, ROS scavenging machinery and signaling molecules. Molecular genetics and plant transformation has helped in generating salt tolerant plants by modifying the cascades, biochemical pathways, specific protein expression or by altering the genes responses under natural conditions. Numerous transgenic salt stress tolerant plants have been reported till date which involves modification in the genes responsible for over expression of antioxidant enzymes (glutathione peroxidase, ascorbate, superoxide dismutase, glutathione reductase), osmolytes (proline, Mannitol, glycinebetaine), expression of DREB proteins which shows detoxifying, re-establishing homeostasis by various ion transporters which prevents or reduces Na<sup>+</sup> entry and regulatory proteins NPK1, mitogen- activated protein kinase. A list of few genes

and their products has been listed for the production of salt tolerant plants (Table I).

### Concluding remarks

Salinity is causing a major problem in plant productivity. Salt tolerance in plants is a complex trait involving the coordinated action of many gene that perform a variety of functions which controls water loss through stomata, ion homeostasis, metabolic adjustments, osmotic adjustment and antioxidative defense. ROS are unavoidable by products in plant cell metabolism under salinity stress. These are generated by electron transport activities of chloroplast and mitochondria, during light ROS is produced in triplet chlorophyll formation in the antenna system and in reaction center of PS-II. Under normal growth conditions ROS production in various cell compartments is low but under salinity stress ROS production increases which disrupt the cellular homeostasis. Both enzymatic and non-enzymatic antioxidants plays an important role in protecting plant cells against the toxicity of ROS by virtue of their capacity to scavenge the free oxygen radicals. Plants maintain a balance between the rate of ROS generation and removal by regulatory a mechanism which controls the synthesis of enzymatic as well as non- enzymatic antioxidant in response to salinity stress. But still now little information and understanding of the molecular mechanism for the

salinity induced ROS which activates the antioxidant defense in the plant cell is available. Mechanism of salt tolerance and expression of these salt resistance gene in plants will help to improve salinity condition. The gene conferring salinity stress resistance provides foundation for scientific improvement of the plants productivity under arid condition and contributes to improvement and stabilization of plant yield. This review summarizes the recent effort to improve abiotic stress tolerance in crop plants by employing some of the stress related genes and transcription factors. There is a clear and urgent need to begin to introduce stress tolerance genes in crop plants. Although progress in improving stress tolerance has been slow, there are a number of reasons for optimism. The use of trans genes to improve the tolerance of crops to a biotic stresses remains an attractive option. A well focused approach combining the molecular physiological and metabolic aspects of a biotic stress tolerance is required for bridging the knowledge gaps between the molecular or cellular expression of the genes and the whole plant phenotype under stress.

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