



ISSN: 0975-833X

REVIEW ARTICLE

ANTIOXIDATIVE DEFENCE AGAINST REACTIVE OXYGEN SPECIES IN PLANTS UNDER SALT STRESS

*Anirudha Rishi and Sonam Sneha

Department of Biotechnology and Food Sciences, Jayoti Vidyapeeth Women's University, Jaipur-303007

ARTICLE INFO

Article History:

Received 25th April, 2013
Received in revised form
10th May, 2013
Accepted 18th June, 2013
Published online 18th July, 2013

Key words:

Salinity stress, Antioxidant enzymes,
Transgenic crops, ROS.

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.

Copyright, IJCR, 2013, Academic Journals. All rights reserved.

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⁺ 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 recent 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 uptake. Salt stress causes excessive uptake of ions (Na⁺ and Cl⁻) 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 (O₂⁻), hydroxyl radical (OH) as well as non radical molecules like hydrogen peroxide (H₂O₂), singlet oxygen (¹O₂). ROS are always formed in plants due to unavoidable leakage of electrons onto O₂ 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].

*Corresponding author: Anirudha Rishi

Department of Biotechnology and Food Sciences, Jayoti Vidyapeeth Women's University, Jaipur-303007

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 O₂ 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 CO₂ 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. O₂⁻ 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 H₂O₂ content in the roots was most highly correlated with the CAT activity, indicating an increased role of CAT in H₂O₂ 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 O₂ by catalyzing its dismutation, one O₂ being reduced to H₂O₂ and another oxidized to O₂. It removes O₂ 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 H₂O₂ into H₂O and O₂ and is indispensable for ROS detoxification during stressed conditions [Garg, 2009]. Catalase removes H₂O₂ 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 ⁺ /H ⁺ antiporter	Arabidopsis	Zhu, 2000
AtNHX1 & AtNHX2	Vacuolar Na ⁺ /H ⁺ 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 ⁺ /H ⁺ antiporter	Cotton	Wu <i>et al.</i> , 2004
OsNHX1	Vacuolar Na ⁺ /H ⁺ antiporter	Rice	Fukuda <i>et al.</i> , 2004
OsSOS1	Plasma membrane Na ⁺ /H ⁺ exchanger	Rice	Martinez-Atienza <i>et al.</i> , 2007
SOD2	Vacuolar Na ⁺ /H ⁺ antiporter	Rice	Zhao <i>et al.</i> , 2006
SsVP-2	Vacuolar Na ⁺ /H ⁺ antiporter	Arabidopsis	Guo <i>et al.</i> , 2006
SsNHX1	Vacuolar Na ⁺ /H ⁺ antiporter	Rice	Zhao <i>et al.</i> , 2006
TsVP	Vacuolar Na ⁺ /H ⁺ 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⁺ 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.

REFERENCES

- Aghaei, K., Ehsanpour, A. A. & Komatsu, S. (2009). Potato responds to salt stress of antioxidant enzymes. *Journal of Integrative Plant Biology*, 51, 1095-1103.
- Ahmad, P. & Jhon, R. (2005). Effect of salt stress on growth and biochemical parameters of *Pisum sativum* L. *Archives of Agronomy and Soil Science*, 51, 665-672.
- Ai-Ke, B., Suo-Min, W., Guo-Qiang, W., Jie-Jun, X., Jin-Lin, Z. & Chun-Mei, W. (2009). Overexpression of the arabidopsis H⁺-PPase enhanced resistance to salt and drought stress in transgenic alfalfa (*Medicago sativa* L.). *Plant Science*, 176, 232-240.
- Asada, K. & Takahashi, M. (1987). Production and scavenging of active oxygen in photosynthesis," in Photoinhibition: Topics of Photosynthesis, D. J. Kyle, C. B. Osmond, and C. J. Arntzen, Elsevier, Amsterdam: The Netherlands.
- Ashraf, M. (1999). Breeding for salinity tolerance proteins in plants. *Critical Reviews in Plant Sciences*, 13, 17-42.
- Bernstein, L. (1975). Effects of salinity and sodicity on plant growth. *Annual Review of Phytopathology*, 13, 295-312.
- Blokhina, O., & Fagerstedt, K. V. (2010). Reactive oxygen species and nitric oxide in plant mitochondria: Origin and Regulatory System. *Physiologia Plantarum*, 138, 447-462.
- Bohnert, H.J. & Jensen, R.G. (1996). Strategies for engineering water stress tolerance in plants. *Trends in Biotechnology*, 14, 89-97.
- Bowler, C., Slooten, L., Vandenbranden, S., De Rycke, R., Botterman, J., Sybesma, C., Van Montagu, M. & Inze, D. (1991). Manganese superoxide dismutase can reduce cellular damage mediated by oxygen radicals in transgenic plants. *EMBO Journal*, 10, 1723-1732.
- Bray, E.A., Bailey-Serres, J., Weretilnyk, E. (2002). Responses to abiotic stresses, *Biochemistry and Molecular Biology of Plants*, Grissem, W., Buchnann, B., Jones, R. *Rockville, Maryland: American Society of Plant Physiologists*.
- del Rio, L. A., Sandalio, L. M., Corpas, F. J., Palma, J. M. & Barroso. (2006). Reactive oxygen species and reactive nitrogen species in peroxisomes, production, scavenging and role in cell signaling. *Plant Physiology*, 141, 330-335.
- Desikan, R., Mackerness, S. A.H., Hancock, S., J. T. & Neill, S. J. (2001). Regulation of the Arabidopsis transcriptome by oxidative stress. *Plant Physiology*, 127, 159-172.
- Desingh, R. & Kanagaraj, G. (2007). Influence of salinity stress on photosynthesis and antioxidative systems in two Cotton Varieties. *General and Applied Plant Physiology*. 33, 221-234.
- Edwards, E.A., Rawsthorne, S. & Mullineaux, P.M. (1990). Subcellular distribution of multiple forms of glutathione reductase in leaves of pea (*Pisum sativum* L.). *Planta*, 180, 278-284.
- FAO Land and Plant Nutrition Management Service. (2007). Retrieved from, <http://www.fao.org/ag/agl/agll/spush>.
- Foyer, C. H., & Harbinson, J. (1994). Oxygen metabolism and the regulation of photosynthetic electron transport, in causes of photooxidative stresses and amelioration of defense systems in plants, Foyer, C. H. and Mullineaux, P., Florida: CRC press.
- Fukuda, A., Nakamura, A., Tagiri, A., Tanaka, H., Miyao, A., Hirochika, H. & Tanaka Y. (2004). Function, intracellular localization and the importance in salt tolerance of a Vacuolar Na⁺/H⁺ antiporter from rice. *Plant and Cell Physiology*. 45, 146-159.
- Fukushima, E., Arata, Y., Endo, T., Sonnewald, U. & Sato, F. (2001). Improved salt tolerance of transgenic tobacco expressing apoplastic yeast-derived invertase. *Plant and Cell Physiology*. 42, 245-249.
- Gao, F., Gao, O., Duan, X., Yue, G., Yang, A. & Zhang, J. (2006). Cloning of an H⁺-PPase gene from *Thellungiella halophila* and its heterologous expression to improve tobacco salt tolerance. *Journal of Experimental Botany*, 57, 3259 - 3270.
- Gao, S., Ouyang, C., Wang, S., Xu, Y., Tang, L. & Chen, F. (2008). Effects of salt stress on growth, antioxidant enzyme and phenylalanine ammonia-lyase activities in *Jatropha curcas* L. seedlings. *Plant, Soil and Environment*, 54, 374-381.
- Gapinska, M., Skłodowska, M. & Gabara, B. (2008). Effect of short- and long-term salinity on the activities of antioxidative enzymes and lipid peroxidation in tomato roots. *Acta Physiologiae Plantarum*, 30, 11-18.
- Garg, N., Manchanda, G. (2009). ROS generation in plants: boon or ban. *Plant Biosystems*, 143, 8-96.
- Ghezzi, P. & Bonetto, V. (2003). Redox Proteomics: Identification of oxidatively modified proteins. *Journal of Proteomics*, 3, 1145-1153.
- Gill, T., Kumar, S., Ahuja, P. S. & Sreenivasulu, Y. (2010). Overexpression of *Potentilla* Superoxide dismutase improves salt stress tolerance during germination and growth in *Arabidopsis thaliana*. *Journal of Plant Genetics and Transgenics*, 1, 1-10.
- Gómez, J.M., Hernández, J.A., Jiménez, A., Del Río, L.A., & Sevilla, F. (1999). Differential response of antioxidative enzymes of chloroplasts and mitochondria to long-term NaCl stress of pea plants. *Free Radical Research*, 31, 11- 18.
- Guo, S., Yin, H., Zhang, X., Zhao, F., Li, P., Chen, S., Zhao, Y. & Zhang, H. (2006). Molecular cloning and characterization of a Vacuolar H⁺-pyrophosphatase gene, SsVP, from the halophyte *Suaeda salsa* and its overexpression increases salt and drought tolerance of *Arabidopsis*. *Plant Molecular Biology*, 60, 41-50.
- Halliwel, B., Gutteridge J. M. C. (1999). *Free Radicals in Biology and Medicine*. Oxford : Oxford University Press.
- Harinasut, P., Poonsopa, D., Roengmongkol, K. & Charoensataporn, R. (2003). Salinity effects on antioxidant enzymes in mulberry cultivar. *Science Asia*, 29, 109-113.
- Hasegawa, P.M., Bressan, R.A., Zhu, J.K. & Bohnert, H.J. (2000). Plant cellular and molecular responses to high salinity. *Annual Review of Plant Physiology and Plant Molecular*, 51, 463-499.
- Hernández, J. A., Jiménez, A., Mullineaux, P., & Sevilla, F. (2000). Tolerance of Pea (*Pisum sativum* L.) to long-term salt Stress is associated with induction of antioxidant defences. *Plant, Cell and Environment*, 23, 853-862.
- Hmida-Sayari, A., Gargouri-Bouزيد, R., Bidani, A., Jaoua, L., Savouré, A. & Jaou, S. (2005). Overexpression of 1-pyrroline-5-carboxylate Synthetase increases proline production and confers salt tolerance in transgenic potato plants. *Plant Science*, 169, 746-752.
- Holmstrom, K. O., Somersalo, S., Mandal, A., Palva, T. E. & Welin, B. (2000). Improved tolerance to salinity and low temperature in transgenic Tobacco producing Glycine betaine. *Journal of Experimental Botany*, 51, 177-185.
- Hong, B., Barg, R. & Ho, T. H. (1992). Developmental and organ specific expression of an ABA and stress induced protein in Barley. *Plant Molecular Biology*, 18, 663-674.
- Horie, T., Horie, R., Chan, W.Y., Leung, H.Y. & Schroeder, J.I. (2006). Calcium regulation of sodium hypersensitivities of

- SOS3 and athkt1 mutants. *Plant & Cell Physiology*, 47,622-633.
- Hoshida, H., Tanaka, Y., Hibino, T., Hayashi, Y., Tanaka, A., Takabe, T. & Takabe, T. (2000). Enhanced tolerance to salt stress in transgenic rice that overexpress chloroplast Glutamine Synthetase. *Plant Molecular Biology*, 43, 103-111.
- Ishizaki Nishizawa, O., Fujii, T., Azuma, M., Sekiguchi, K., Murata, N., Ohtani, T. & Toguri, T. (1996). Low temperature resistance in higher plants is significantly enhanced by a non-specific *Cyanobacterial desaturase*. *Nature Biotechnology*, 14, 1003-1006.
- Jalali-e-Emam Seyed Mohammad Shahab, Alizadeh Bahram, Zaefizadeh Mohammad, Asghari Zakarya Rasool & Khayatnezhad Majid, Superoxide Dismutase (SOD) Activity in NaCl Stress in Salt-Sensitive and Salt-Tolerance Genotypes of Colza (*Brassica napus* L.). *Middle-East Journal of Scientific Research*, 7, 07-11.
- Karray Bouraoui, N., Harbaoui, F., & Rabhi, M. (2011). Different antioxidant responses to salt stress in two different provenances of *Carthamus tinctorius* L. *Acta Physiologiae Plantarum*, 33, 1435-1444.
- Kasuga, M., Liu, Q., Miura, S., Yamaguchi- Shinozaki, K., & Shinozaki, K. (1999). Improving plant drought, salt and freezing tolerance by gene transfer of a single stress- inducible transcription factor. *Nature Biotechnology*, 17, 287-291.
- Kukreja, S., Nandval, A.S., Kumar, N., Sharma, S.K., Sharma, S.K., Unvi, V. & Sharma, P.K. (2005). Plant water status, H₂O₂ scavenging enzymes, ethylene evolution and membrane integrity of *Cicer arietinum* roots as affected by salinity. *Biology Plant*. 49, 305-308.
- Kumar, S., Dhingra, A. & Daniell, H. (2004). Plastid-expressed Betaine Aldehyde dehydrogenase gene in carrot cultured cells, roots, and leaves confers enhanced salt tolerance. *Plant Physiology*, 135, 2843- 2854.
- Levitte J. (1980). Responses of Plants to Environmental Stress Chilling, Freezing and High temperature Stresses. New York: Academic Press.
- Lilius, G., Holmberg, N. & Bulow, L. (1996). Enhanced NaCl stress tolerance in transgenic Tobacco expressing bacterial Choline dehydrogenase. *Journal of Biotechnology*, 14, 177-180.
- Liu, Q., Ishitani, M., Halfter, U., Kim, C. S. & Zhu, J. K. (2000). The *Arabidopsis thaliana* SOS2 gene encodes a protein kinase that is required for salt tolerance. *Proceedings of the National Academy of Sciences of the United States of America*, 97, 3730-3734.
- Luo, G.Z., Wang, H.W., Jian, H., Tian, A.G., Wang, Y.J., Zhang, J.S. & Chen, S.Y. (2005). A Putative Plasma Membrane Cation/proton Antiporter from Soybean Confers Salt Tolerance in Arabidopsis. *Plant Molecular Biology*, 59, 809-820.
- Mahalakshmi, S., Christopher, G.B.S., Reddy, T.P., Rao, K.V. & Reddy, V.D. (2006). Isolation of a cDNA clone (PcSrp) encoding Serine-rich- protein from *Porteresia coarctata* T. and its expression in Yeast and Finger Millet (*Eleusine coracana* L.) affording salt tolerance. *Planta*, 224, 347-359.
- Martinez-Atienza, J., Jiang, X., Garciadablas, B., Mendoza, I., Zhu, J.K., Pardo, J.M. & Quintero, F.J. (2007). Conservation of the salt overly sensitive pathway in rice. *Journal of Plant Physiology*, 143, 1001-1012.
- Md. Amir, H., Jung-II, C., Muho, H., Chul-Hyun, A., Jong-Seong, J., Gynheung, A. & Phun, B.P. (2010). The ABRE-binding bZIP transcription factor OsABF2 is a positive regulator of abiotic stress and ABA signaling in rice. *Journal of Plant Physiology*, 167, 1512-1520.
- Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science*, 7, 405-410.
- Nagesh Babu, R. & Devaraj, V. R. (2008). High temperature and salt stress response in French bean (*Phaseolus vulgaris*). *Australian Journal of Crop Science*, 2, 40-48.
- Neill, S., Desikan, R., & Hancock, J. (2002). Hydrogen peroxide signaling. *Current Opinion in Plant Biology*, 5, 388-395.
- Oraby, H.F., Ransom, C.B., Kravchenko, A.N. & Sticklen, M.B. (2005). Barley HVA1 gene confers salt tolerance in R3 transgenic Oat. *Crop Science*, 45, 2218-2227.
- Orlene, G.P., Ha, T.N., Peter, S. & Leipner, J. (2009). ZmCOI6.1, a novel, alternatively spliced Maize gene, whose transcript level changes under abiotic stress. *Plant Science*, 176, 783-791.
- Padro, J. M., Reddy, M. P., Yang, S., Maggio, A., Huh, G. H., Matsumoto, T., Coca, M. A., Paino-D'Urzo, M., Koiwa, H., Yun, D. J., Watad, A. A., Bressan, R. A. & Hasegawa, P. M. (1998). Stress signaling through Ca²⁺/ Calmodulin- dependent protein phosphatase Calcineurin mediates salt adaptation in plants. *Proceedings of the National Academy of Sciences of the United States of America*, 16, 9681-9686.
- Piao, H. L., Lim, J. H., Kim, S. J., Cheong, G. W. & Hwang, I. (2001). Constitutive over-expression of AtGSK1 induces NaCl stress responses in the absence of NaCl stress and results in enhanced NaCl tolerance in Arabidopsis. *The Plant Journal*, 27, 305-314.
- Prasad, K. V. S. K., Sharmila, P., Kumar, P. A. & Pardha Saradhi, P. (2000). Transformation of *Brassica juncea* L. Czern with bacterial coda gene enhances its tolerance to salt stress. *Molecular Breeding*, 6, 489-499.
- Pyo Lee, Y., Hyung Kim, S., Wook Bang, J., Soon Lee, H., Soo Kwak, S. & Yoon Kwon, S. (2007). Enhanced tolerance to oxidative stress in transgenic Tobacco plants expressing three antioxidant enzymes in chloroplasts. *Plant Cell Reports*, 26, 591-598.
- Quan, R., Lin, H., Mendoza, I., Zhang, Y., Cao, W., Yang, Y., Shang, M., Chen, S., Pardo, J.M. & Guo, Y., SCABP8/CBL10, a putative calcium sensor, interacts with the protein kinase SOS2 to protect *Arabidopsis* shoots from salt stress. *The Plant Cell*, 19, 1415-1431.
- Rhodes, D. & Hanson, A.D. (1993). Quaternary ammonium and tertiary sulfonium compounds in higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, 44, 357-384.
- Romero Aranda, R., Soria, T. & Cuartero, J. (2001). Tomato plant water uptake and plant water relationships under saline growth conditions. *Plant Science*, 160, 265-272.
- Romero Puertas, M.C., Corpas, F.J., Sandalio, L.M., Leterrier, M., Rodriguez Serrano, M., del Rio, L.A., Palma, J.M. (2006). Glutathione reductase from pea leaves: response to abiotic stress and characterization of the peroxisomal isozyme. *New Phytologist*, 170, 43-52.
- Saavedra, L., Svensson, J., Carballo, V., Izmendi, D., Welin, B. & Vidal, S. (2006). A dehydrin gene in *Physcomitrella patens* is required for salt and osmotic stress tolerance. *The Plant Journal*, 45, 237-249.
- Sang Yong, K., Jung-Hyun, L., Myoung Ryoul, P., Young Jin, K., Yong Won, S., Kyeong Gu, C., Song Joong, Y. & Tae, I. P. (2005). Enhanced antioxidant enzymes are associated with reduced hydrogen peroxide in Barley roots under saline stress. *Journal of Biochemistry and Molecular Biology*, 38, 218-224.
- Sarangi, S., Ghosh, J., Bora, A., Das, S. & Mandal, A. B. (2011). Agrobacterium mediated genetic transformation of *indica* rice varieties involving Am-SOD gene. *Indian Journal of Biotechnology*, 10, 9-18.
- Singla Pareek, S. L., Reddy, M. K. & Sopory, S. K. (2003) Genetic engineering of the Glyoxalase pathway in Tobacco leads to enhanced salinity tolerance. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 14672-14677.
- Su, J., Hirji, R., Zhang, L., He, C., Selvaraj, G. & Wu, R. (2006). Evaluation of the stress-inducible production of Choline oxidase in transgenic rice as a strategy for producing the stress-protectant Glycine betaine. *Journal of Experimental Botany*, 57, 1129-1135.
- Sun, J., Jiang, H., Xu, Y., Li, H., Wu, X., Xie, Q. & Li, C. (2007). The CCCH-type zinc finger proteins AtSZF1 and AtSZF2 regulate salt stress responses in Arabidopsis. *Plant & Cell Physiology*, 48, 1148-1158.

- Tang, W., Peng, X. & Newton, R.J. (2005). Enhanced tolerance to salt stress in transgenic loblolly Pine simultaneously expressing two genes encoding Mannitol-1-phosphate dehydrogenase and Glucitol-6-phosphate dehydrogenase. *Plant Physiology and Biochemistry*, 43, 139-146.
- Tanou, G., Molassiotis, A., & Diamantidis, G. (2009). Induction of reactive oxygen species and necrotic death-like destruction in Strawberry leaves by salinity. *Environmental and Experimental Botany*, 65, 270–281.
- Thomas, J. C., Sepahi, M., Arendall, B. & Bohnert, H. J. (1995). Enhancement of seed germination in high salinity by engineering mannitol expression in *Arabidopsis thaliana*. *Plant, Cell & Environment*, 18, 801-806.
- Traczynski, M. C., Jensen, R. G. & Bohnert, H. J. (1993). Stress protection of the transgenic tobacco by production of the osmolytes mannitol. *Science*, 259, 508-510.
- Tuteja, N., & Tuteja, R. (2001). Unravelling DNA repair in Human: Molecular mechanisms and consequences of repair defect. *Critical Reviews in Biochemistry and Molecular Biology*, 36, 261-290.
- Tuteja, N., Singh, M.B., Misra, M.K., Bhalla, P.L. & Tuteja, R. (2001). Molecular mechanisms of DNA damage and repair: Progress in plants. *Critical Reviews in Biochemistry and Molecular Biology*, 36, 337-397.
- Valko, M., Rhodes, C.J., Moncol, J., Izakovic, M. & Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced Cancer. *Chemico-Biological Interaction*, 160, 1-40.
- Van Camp, W., Capiu, K., Van Montagu, M., Inze, D. & Slooten, L. (1996). Enhancement of oxidative stress tolerance in transgenic Tobacco plants overproducing Fe-Superoxide dismutase in chloroplasts. *Plant Physiology*, 112, 1703-1714.
- Wang, X., Yang, P., Gao, Q., Liu, X., Kuang, T., Shen, S. & He, Y. (2008). Proteomic analysis of the response to high-salinity stress in *Physcomitrella patens*. *Planta*, 228, 167–177.
- Wang, Y., Ying, Y., Chen, J. & Wang, X. (2004). Transgenic *Arabidopsis* over expressing Mn-SOD enhanced salt-tolerance. *Plant Science*, 167, 671-677.
- Weisany, W., Sohrabi, Y., Heidari, G., Siosemardeh, A., & Ghassemi Golezani K. (2012). Changes in antioxidant enzymes activity and plant performance by salinity stress and zinc application in Soybean (*Glycine max* L.). *Plant Omics Journal*, 5, 60-67.
- Winicov, I. I. & Bastola, D. R. (1999). Transgenic overexpression of the transcription factor alfin 1 enhances expression of the endogenous MsPRP2 gene in Alfalfa and improves salinity tolerance of the plants. *Plant Physiology*, 110, 249-257.
- Wu, C.A., Yang, G.D., Meng, Q.W. & Zheng, C.C. (2004). The cotton GhNHX1 gene encoding a novel putative Tonoplast Na⁺/K⁺ antiporter plays an important role in salt stress. *Plant & Cell Physiology*, 45, 600-607.
- Wu, W., Su, Q., Xia, X. Y., Wang, Y., Luan, Y.S. & An, L.J. (2008). The *Suaeda liaotungensis* Kitag Betaine aldehyde dehydrogenase gene improves salt tolerance of transgenic Maize mediated with minimum linear length of DNA fragment. *Euphytica*, 159, 17-25.
- Yan, J., Tsuichihara, N., Etoh, T., & Iwai, S. (2007). Reactive oxygen species and Nitric oxide are involved in ABA inhibition of stomatal opening. *Plant Cell and Environment*, 30, 1320–1325.
- Yan, P., Li, J. W. & Zeng, L. Y. (2006). Effect of salt and drought stress on antioxidant enzymes activities and SOD isoenzymes of Liquorice (*Glycyrrhiza uralensis* Fisch). *Plant Growth Regulation*, 49, 157–165.
- Yokoi, S., Cubero, F.J.Q.B., Ruiz, M.T., Bressan, R.A., Hasegawa, P.M. & Pardo, J.M. (2002). Differential Expression and function of *Arabidopsis thaliana* NHX Na⁺/H⁺ antiporters in the salt stress response. *Plant Journal*, 30, 529-536.
- Zhao, F., Guo, S., Zhang, H. & Zhao, Y. (2006). Expression of yeast SOD2 in transgenic rice results in increased salt tolerance. *Plant Science*, 170, 216-224.
- Zhao, F., Wang, Z., Zhang, Q., Zhao, Y. & Zhang, H. (2006). Analysis of the physiological mechanism of salt-tolerant transgenic rice carrying a Vacuolar Na⁺/H⁺ antiporter gene from *Suaeda salsa*. *Journal of Plant Research*, 119, 95-104.
- Zhao, M.G., Tian, Q.Y. & Zhang, W.H. (2007). Nitric oxide synthase- dependent Nitric oxide production is associated with salt tolerance in *Arabidopsis*. *Plant Physiology*, 144, 206-217.
- Zhu, J. K. (2000). Genetic analysis of plant salt tolerance using *Arabidopsis*. *Plant Physiology*, 124, 941-948.
- Zhu, J.K. (2001). Plant salt tolerance. *Trends Plant Science*, 6, 66-71.
- Zou, H., Li, C., Liu, H., Zhao, M., Tian, X., Ma, G. & Li, Z. (2011). ZmSPK1, a Member of plant SnRK2 subfamily in Maize enhances tolerance to salt in transgenic *Arabidopsis*. *Australian Journal of Crop Sciences*, 5, 1179-1184.
