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Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675
E- ISSN: 2249-4538

Volume: 12
Issue: 05

Res Jr of Agril Sci (2021) 12: 1558–1563

CARAS

Res. Jr. of Agril. Sci. (Sep-Oct 2021) 12(5): 1558–1563
www.rjas.org

Research Review

An Insight on the Ascorbate Peroxidase and Glutathione Reductase Activities in Plants under Salinity Stress: Mini Review

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Received: 10 Jun 2021 | Revised accepted: 09 Aug 2021 | Published online: 11 Sep 2021

A B S T R A C T

Abiotic stresses adversely affect growth, physio-biochemical processes and yield of crop plants. Among abiotic stresses, salinity stress is a major environmental threat that reduced crop productivity. It causes osmotic stress, ionic toxicity, metabolic imbalance and generation of reactive oxygen species (ROS) that disturb cellular homeostasis and lead to cell death. To cope with such adversities, plants develop certain internal mechanism to tolerate salt stress. Among these, the enhancement of antioxidant enzyme activities is a well-known intrinsic defence mechanism of plants. In the present mini review article, the focus has been given on the role of ascorbate peroxidase (APX) and glutathione reductase (GR) under salinity stress in plants.

***Key words*:** Salinity, Growth, Physio-biochemical processes, Reactive oxygen species, Antioxidant enzymes

Plant's productivity is adversely affected by various environmental stresses. The excess amount of salt in the soil greatly affects plant growth and development. Soil salinity is one of the major environmental stresses that reduce agricultural productivity worldwide. The processes like seed germination, seedling growth, vigour, vegetative growth, flowering and fruit set are deleteriously affected by high salt concentration and eventually diminish the economic yield and quality of product. Salinity stress affects the growth and development of plants by the way of osmotic stress, the toxic effects of Na^+ and Cl^- ions and to some extent Cl^- and SO_4^{2-} (ionic stress) and imbalance of nutrients caused by the excess of Na^+ and Cl^- ions.[53] In accordance to this, plants are classified as halophytes and glycophytes according to their capacity to grow in high salt medium. Large majority of plant species are glycophytes, which are not salt-tolerant and damaged easily by high salinity. Firstly, osmotic potential of the soil solution decreases due to high salt concentrations and it creates water stress in plants. Secondly, high salt concentration causes severe ion toxicity since Na^+ is not readily sequestered into the vacuoles [36].

The nutrient imbalance and toxicity ultimately lead to death of the plant as a result of growth arrest and molecular damage. In addition to these, another consequence of salinity stress in plants is the excessive generation of reactive oxygen species (ROS). Reactive oxygen species consist of both free radicals such as superoxide anion $(O_2)^-$, and hydroxyl radicals ($OH^\cdot$) and non-radicals like hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$). The chloroplast and mitochondria are the major sites for the generation of ROS [45,41,21]. Reactive oxygen species cause damage to lipids (lipid peroxidation), proteins, carbohydrates, DNA, membrane disorganisation and ultimately cell death [25]. Under stress plants produce certain defence mechanisms to combat with harmful effects of stress. To scavenge ROS is the common defence response against abiotic stresses [64]. Scavenging of reactive oxygen species (ROS) provided by an integrated system of enzymatic and non-enzymatic antioxidants [57]. The non-enzymatic antioxidant (ascorbate (AsA) and glutathione (GSH) etc.) and enzymatic antioxidants (ascorbate peroxidase (APX), monodehydroascorbate reductase, dehydroascorbate reductase (DAsAR), glutathione peroxidase (GPX), glutathione reductase (GR), catalase (CAT) and superoxide dismutase (SOD) etc.) are key enzymes of ascorbate-glutathione cycle and have complex response against ROS [51]. The ROS scavenging pathways, which are in different compartments of cell, are coordinated [42], [49].

The glutathione (GSH)-ascorbate (AsA) pathway is a crucial part of the network of reactions involving metabolites and enzymes with redox properties for detoxification of ROS and thus to prevent the ROS-emanated oxidative damage in plants. Ascorbate and GSH are located in cell compartments such as cytoplasm, peroxisomes, mitochondria and chloroplast, the AsA is additionally found in the apoplast. Ascorbate and

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glutathione serve as substrates for ascorbate peroxidase (APX) and glutathione reductase (GR), respectively. APX and GR are the key enzymes of the AsA-GSH cycle [3]. Ascorbate peroxidase and glutathione reductase play a key role in regulating the tolerance of plants under environmental stress including salinity stress. The activity of GRs and APXs is different in response to salinity. Under salinity stress, increase in the activity of APX and GR was reported in pea [24], *Casuarina* [16], soybean [31] *Cicer arietinum* [33] and cotton [17]. The activities of APX and GR increased in the root and shoot of barley under NaCl stress. But the increase was consistent and significant in the root, indicating thereby rapid response of antioxidant enzymes to salt stress in the root of barley [50]. Salinity stress remarkably enhanced GR and APX activity in the shoots of salt-tolerant potato while their activity decreased in salt-sensitive variety [1]. GR and APX activity remained unchanged in the root of salt-tolerant maize and significantly reduced in salt-sensitive genotype [15]. APX and GR activity in the chloroplast of *Suaeda salsa* (halophyte) in leaves markedly increased with 200mM NaCl treatment [8]. Due to the diverse roles of APX and GR enzymes in plants, the present article emphasizes on the role of APX and GR in AsA-GSH cycle and their activities under salinity stress is reviewed.

Function of ascorbate peroxidase and glutathione reductase in AsA-GSH cycle in plants

In plants, the ascorbate-glutathione cycle operates in the cytosol, plastids, mitochondria and peroxisomes [29]. The cycle begins with the reduction of Hydrogen peroxide (H_2O_2) into H_2O by APX using Ascorbate (AsA) as an electron donor. Simultaneously AsA was oxidized to monodehydroascorbate (MDAsA). This MDAsA is reduced into AsA by monodehydroascorbate reductase (MDAsAR) or part of this spontaneously converted into dehydroascorbate (DAsA). Later DAsA is reduced to AsA again by dehydroascorbate reductase (DAsAR) by using GSH, yielding GSSG (oxidized glutathione). Finally, GSSG regenerated GSH by the activity of GR using NADPH as electron donor. Both AsA and GSH are strong antioxidant but the maintenance of their redox state is significant in response to stress tolerance in plant which largely depends upon the activity of the enzymes associated with the AsA-GSH cycle. Thus, AsA and GSH are not consumed; the net flow of electron is from NADPH to H_2O_2 [23, 59, 49, 5]. A diagrammatic representation of AsA-GSH cycle is depicted in (Fig 1).

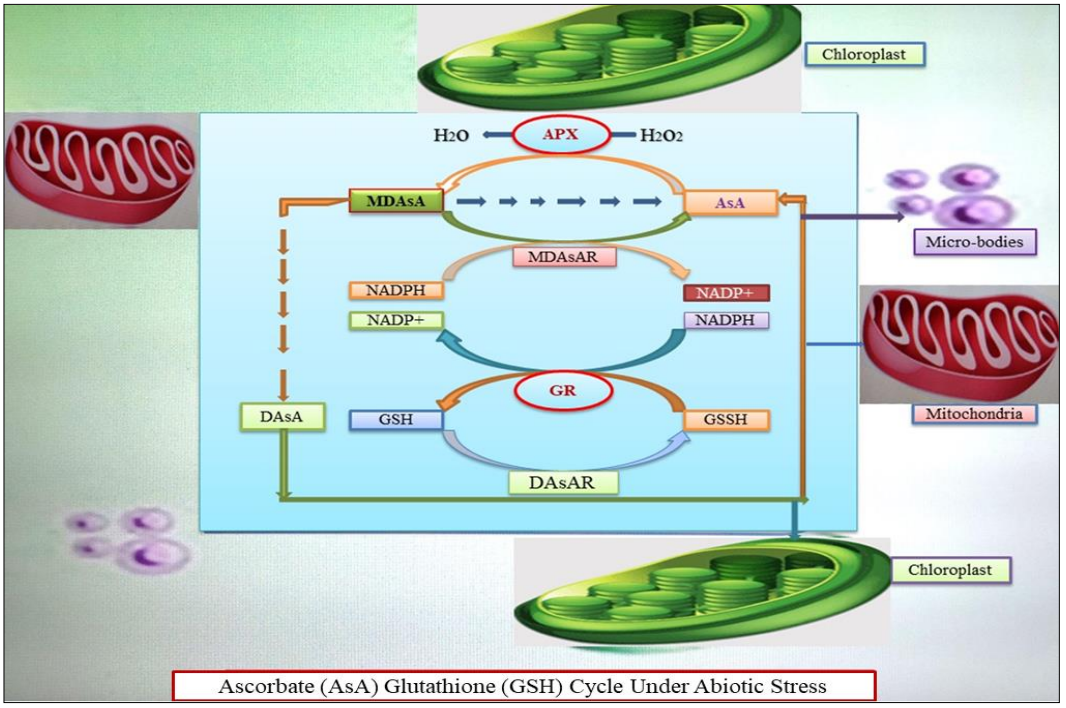

Fig 1 Ascorbate (AsA) and glutathione (GSH) cycle under abiotic stress

Ascorbate peroxidase (EC 1.11.1.11)

Ascorbate peroxidase is the most important peroxidase that detoxifies H_2O_2 using AsA as a substrate. The reaction it catalysed is the transfer of electrons from AsA to a H_2O_2 to produce deyhdroascorbate and water [46,52]. The reaction is as follows:

Ascorbate + Hydrogen peroxide ⋯⋯▶ Dehydroascorbate + Water

APX isoenzymes have high affinity for AsA, utilizes as a specific electron donor. One of the characteristic properties of ascorbate peroxidase, which differs from other peroxidases is its specificity with AsA and APX is instable in the absence of AsA. The APX in plants are found in many compartments of cell including cytosol, mitochondria, chloroplast, peroxisomes and microbodies [28], [48].

Under conditions where concentration of AsA is lower than 2 0µM, the APX activity is rapidly lost [56]. The chloroplast APX (ChlAPX) has more specificity with their substrate [28]. Ascorbate peroxidase is a class I haem-containing enzyme whose prosthetic group is protoporphyrin. Iron plays a significant role in the catalytic site; this was investigated in sugar beet plant by applying iron deficiency condition to the plants [69]. Azide and cyanide are the inhibitors of APX. It is also inhibited by p-chloromercuribenzoate, thiol and suicide inhibitors such as hydroxyurea and p-aminophenol [11].

In the catalytic domain of APX some of the functional amino-acid residues undergo site-directed mutagenesis. Due to this Arg172 is changed to glutamine, lysine and asparagine. APX variants are incapable to oxidize

AsA. It is revealed that Arg172 of pea cAPX have important role in the utilization of ascorbate [7].

[37] of the opinion that membrane bound class III secretory peroxidase exhibit an almost 1000-fold higher affinity for H_2O_2 as catalase and their activities can be modified in the presence of different stress factors, they play a key role in detoxification of ROS yet without utilization of proton released from the breakdown of H_2O_2 can detoxified by AsA-GSH cycle only. The Arg172 and Lys30 are the position on APX, it acts as binding site for ascorbate and interactions of hydrogen bonding [55].

The isoenzymes of APX are distributed in different subcellular compartments like cytosolic APX (cAPX), chloroplast APX (chlAPX), stromal APX (sAPX), thylakoid membrane-bound APX (t APX), micro-body membrane bound APX (m APX, including peroxisomes and glyoxysomes APX) and mitochondrial APX (mit APX). In *Arabidopsis thaliana* there are eight isoenzymes APX: APX1, APX2, APX6 (Cytosolic); APX3, APX4, APX5 (microsome membrane bound) and sAPX and tAPX (chloroplastic) stromal and thylakoidal. [61] suggested that, the localization of isoforms of APX in subcellular compartments is decided by the peptidyl signal and transmembrane domains on N- and C- terminal region. The novel spinach glyoxysomes APX was found on the external side of the organelle [26] and pumpkin APX isoenzyme was localized on the membrane of microbodies [67].

[66] classified plant peroxidases into three classes on the basis of sequences of amino acid. Class I comprises intracellular peroxidases having prokaryotic origin like yeast cytochrome *c* peroxidase (CCP). APXs belongs to class I family [56,14], class II contains the secretory fungal peroxidases including lignin peroxidases (LiPs), and manganese dependent peroxidases (MnPs). The class III consists of secretory plant peroxidases. Under extreme environmental condition when H_2O_2 is produced in subcellular compartments it is efficiently scavenged in the same compartments themselves. The cytosolic APX first isolated from pea shoot after purification, exhibits stability even in the absence of AsA [41]. APX isoforms have been isolated and characterized from many other species of plants including cotton [6], tobacco [38], rice [54] and tea [34]. APX in plants differ in optimal pH, molecular weight, stability and substrate specificity.

APXs synthesizing genes were identified in the tomato genome comprising of three cytosolic isoforms, two chloroplastic and two peroxisomal isoforms [44]. APX genes were also suggested in *Arabidopsis thaliana* which include three cytosolic, three peroxisomal and two chloroplastic [12]. Eight APX genes were identified in *Oryza sativa* L genome through *in silico* analysis: two cytosolic isoforms, two putative peroxisomal isoforms and four putative chloroplastic ones. It has been confirmed also by southern blot hybridization [60].

Glutathione reductase

Glutathione reductase (GR, EC 1.6.4.2), is a substrate specific ubiquitous antioxidant enzyme which convert oxidized glutathione (GSSG) to reduced glutathione (GSH) using NADPH. It plays an important role in AsA-GSH cycle to lead scavenging of ROS and confer abiotic stress tolerance [68]. GR catalyse the following reaction:

$$GSSG + NADPH + H^+ \dashrightarrow 2\ GSH + NADP^+$$

GR is a flavin adenine dinucleotide (FAD)-bound homodimer. GR has been identified and well characterized from the leaves of several plants such as *Pisum sativum* [30], *Spinacia oleracea* and *Zea* mays [39]. Glutathione reductase (GR) is mainly found in chloroplast. Although a small amount of the enzyme isoforms is also found in cytosol, mitochondria and peroxisomes [29,19]. GR found in both prokaryotes and eukaryotes. GR in plants is from 60 to 190 kDa [43]. It has different quaternary structure in different organisms.

Role of ascorbate peroxidase and glutathione reductase under salinity stress

Salinity is one of the most devastating abiotic stress factors that reduces crop production. It caused ionic and osmotic stress both disturb growth, photosynthesis related parameters and yield of crop plant. Under such circumstances, plant cellular signaling cascade upregulated at molecular level and ultimately increased in the activities of APX and GR that reduced the damages of salinity. Ascorbate peroxidase and glutathione reductase play a key role in regulating the tolerance and survival of the plants under environmental stress conditions such as drought, salt, high temperature and high light. Though, results about the changes in activity of APX and GR in plants under stress conditions are debatable. Various studies suggested that the activity of APX and GR increased in plants under stress conditions. But several studies showed that the activity of APX and GR decreased. Changes in the activities of these enzymes may be due to protein synthesis or may be due to change in the kinetic properties [35]. Hence findings of various worker regarding the activities of APX and GR in number of plants under salinity stress is reviewed.

Activity of APX and GR responded differently to salinity stress depending on MDHAR and DHAR activities and availability of AsA and GSH pool. [17] examine in two cotton varieties that the four different concentrations of mixed salts: NaCl, $MgSO_4$ and $CaCl_2$ (0, 50 mM, 100 mM and 150 Mm) were applied, the photosynthetic rate, RuBP carboxylase and sucrose phosphate synthase activities decreased while activities of key antioxidant enzymes, APX and GR significantly increased. Further, in *Morus sp.* exposed to increasing salinity level up to 150 mM, the activities of APX and GR slightly increased to confer tolerance against salt stress [22,9]. have investigated in *Calendula officinalis* L. under 50 and 100 mM NaCl concentrations, high salinity reduced growth parameters, lipid peroxidation and hydrogen peroxidase accumulation but APX and GR activities increased to reduce lipid peroxidation and accumulation of hydrogen peroxide.

Subsequently, *Oryza sativa* cultivars treated with 50 and 100 NaCl showed higher APX and GR activity over the control plant [47], [62]. In C3 and C4 plants the activity of APX increase in both maize and wheat, whereas GR activity showed increased in the leaves of maize under salt stress (50, 100 and 150 mM NaCl) condition. [58,40] observed that with increasing salinity up to 100 mM NaCl, activities of APX and GR increased in *Triticum aestivum* and hence mitigates salt adverse effects. [20] suggest when *Cicer arietinum* L. were subjected to 0.1, 0.2 and 0.5 M NaCl the activities of APX and GR exhibited significant increases in leaf tissue under all stress treatments. They play protective role against salt stress. The activity of ascorbate peroxidase increased in *Catharanthus roseus* L. under salinity stress and hence decreased salt effects [27]. Similarly, three

strawberry cultivars like 'Camarosa', 'Tioga' and 'Chandler' were irrigated with the nutrient solution containing 0, 8.5, 17.0 and 34.0 mM NaCl for 30 days, the APX and GR activities sharply increased and ameliorated salinity induced toxic effects. [63,4,32] observed that *Zea mays* L. exposed to high salinity stress showed decreased chlorophyll content, carotenoid contents and relative water content, and increased the APX and GR activities to alleviate salt stress damages. [13] evaluated that the 10-day old seedlings of *Lablab purpureus* L. treated with 100-500 mM NaCl for 72 h. The growth parameter of the plant reduced but antioxidant enzymes; GR and APX activities increased under salinity. In *Glycine max* L. antioxidant response have been tested, when grown under 0, 50, 100 and 150 mM NaCl concentrations. It has been reported that fresh and dry weight are reduced while proline concentration increased at high salinity. Moreover APX, GR and other antioxidant enzymes activities decreased [18]. The growth parameters of *Glycine max* L. decreased under salinity stress (99 mM NaCl) and APX-GR activities increased [65]. Similarly, activities of APX and GR were significantly higher in *Oryza sativa* L. with increasing salinity from 0 to 100 mM [10]. The APX and GR activities enhanced under 100 and 200 mM NaCl concentrations in *Brassica juncea* L. and they alleviated toxic effects of salinity [2].

In summary, to mitigate adverse effects of salt stress, plants have been naturally equipped with antioxidant defence system. However, APX and GR are crucial components of enzymatic antioxidant defence system that help to reduce ROS production, maintain ion homeostasis, improve membrane stability and, balance metabolic pathways and ultimately curtail crop plant damages and increase productivity. An overview of antioxidants mediated salinity stress tolerance in plants is represented in (Fig 2).

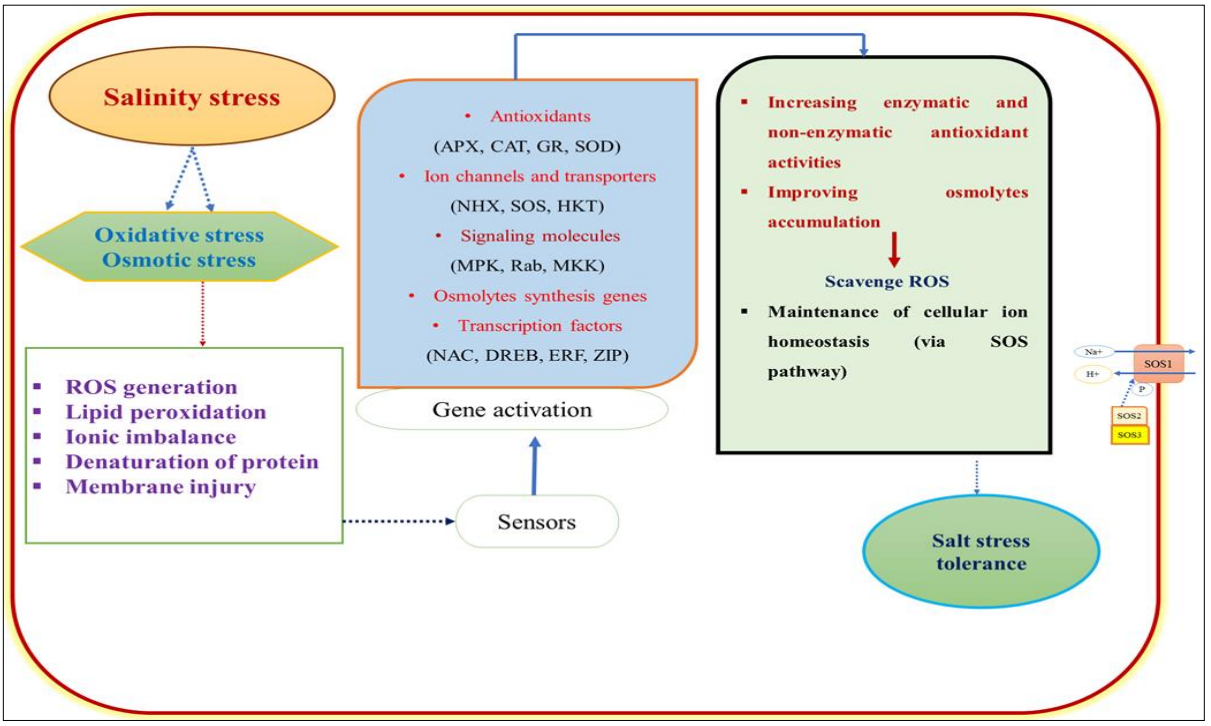

Fig 2 Antioxidants- mediated salinity stress tolerance in plants

CONCLUSION

It may be concluded from the above appraisal of literature that ascorbate peroxidase and glutathione reductase, components of antioxidants especially pool of AsA and GSH, play a pivotal role in ascorbate-glutathione cycle to detoxify reactive oxygen species. They also interact with other defence systems in plants and protect from various abiotic stresses including salinity stress. The damages in plant growth parameters and physiobiochemical processes caused by salinity stress are ameliorated by antioxidants and provide protection to crop plants. A number of strategies have been applied to enhance the activities of antioxidants under varied environmental challenges and good progress has been achieved. Yet scientific efforts are needed to significantly boost antioxidant defense system that helps to improve crop productivity.

LITERATURE CITED

1. Aghaei K, Ehsanpour AA, Komatsu S. 2009. Potato responds to salt stress by increased activity of antioxidant enzymes. *Jr. of Int. Plant Biology* 51: 1095-1103.
2. Ahmad P, Ahanger MA, Alyemeni MN, Wijaya L, Egamberdieva D, Bhardwaj R, Ashraf M. 2017. Zinc application mitigates the adverse effects of NaCl stress on mustard [*Brassica juncea* (L.) Czern & Coss] through modulating compatible organic solutes, antioxidant enzymes, and flavonoid content. *Jr. of Plant Inter*. 12(1): 429-437.
3. Anjum NA, Umar S, Chan MT. 2010. Ascorbate-glutathione pathway and stress tolerance in plants.; Springer Science, Business Media.
4. Azooz MM, Ismail AM, Elhamd MA. 2009.Growth, lipid peroxidation and antioxidant enzyme activities as a selection criterion for the salt tolerance of maize cultivars grown under salinity stress. *Int. Jr. of Agric. Biology* 11(1): 21-26.

5. Bartoli CG, Buet A, Grozeff GG, Galatro A, Simontacchi M. 2017. Ascorbate-glutathione cycle and abiotic stress tolerance in plants. *In*: Ascorbic acid in plant growth, development and stress tolerance. Springer, Cham. pp 177-200.
6. Bunkelmann JR, Trelease RN. 1996. Ascorbate peroxidase (A prominent membrane protein in oilseed glyoxysomes). *Plant Physiology* 110(2): 589-598.
7. Bursey EH, Poulos TL. 2000. Two substrate binding sites in ascorbate peroxidase: the role of arginine 172. *Biochemistry* 39(25): 7374-7379.
8. Cai-Hong P, Su-Jun Z, Zhi-Zhong G, Bao-Shan W. 2005. NaCl treatment markedly enhances H_2O_2-scavenging system in leaves of halophyte Suaeda salsa. *Physiology Planta* 125(4): 490-499.
9. Chaparzadeh N, D'Amico ML, Khavari-Nejad RA, Izzo R, Navari-Izzo F. 2004. Antioxidative responses of *Calendula officinalis* under salinity conditions. *Plant Physiology and Biochemistry* 42(9): 695-701.
10. Chawla S, Jain S, Jain V. 2013. Salinity induced oxidative stress and antioxidant system in salt-tolerant and salt-sensitive cultivars of rice (*Oryza sativa* L.). *Jr. of Plant Biochem. Biotechnology* 22(1): 27-34.
11. Chen GX, Asada K. 1990. Hydroxyurea and p-aminophenol are the suicide inhibitors of ascorbate peroxidase. *Jr. of Biol. Chemistry* 265(5): 2775-2781.
12. Chew O, Whelan J, Millar AH. 2003. Molecular definition of the ascorbate-glutathione cycle in Arabidopsis mitochondria reveals dual targeting of antioxidant defenses in plants. *Jr. of Biol. Chemistry* 278(47): 46869-46877.
13. D'Souza MR, Devaraj VR. 2010. Biochemical responses of Hyacinth bean (*Lablab purpureus*) to salinity stress. *Acta Physiol. Planta* 32(2): 341-353.
14. Dąbrowska G, Kata A, Goc A, Szechyńska-Hebda M, Skrzypek E. 2007. Characteristics of the plant ascorbate peroxidase family. *Acta Biol. Cracovi Ser. Bot*. 49(1): 7-17.
15. de Azevedo Neto AD, Prisco JT, Enéas-Filho J, de Abreu CEB, Gomes-Filho E. 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. Botany* 56(1): 87-94.
16. Desingh R, Jutur PP, Reddy AR. 2006. Salinity stress-induced changes in photosynthesis and antioxidative systems in three casuarina species. *Jr. of Plant Biology* 33: 155-161.
17. Desingh R, Kanagaraj G. 2007. Influence of salinity stress on photosynthesis and antioxidative systems in two cotton varieties. *Gen. App. Plant Physiology* 33(3/4): 221-234.
18. Doğan M. 2011. Antioxidative and proline potentials as a protective mechanism in soybean plants under salinity stress. *Afr. Jr. of Biotechnology* 10(32): 5972-5978.
19. Edwards EA, Rawsthorne S, Mullineaux PM. 1990. Subcellular distribution of multiple forms of glutathione reductase in leaves of pea (*Pisum sativum* L.). *Planta* 180(2): 278-284.
20. Eyidogan F, Öz MT. 2007. Effect of salinity on antioxidant responses of chickpea seedlings. *Acta Physiol. Planta* 29(5): 485-493.
21. Gill SS, Tuteja N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochemistry* 48(12): 909-930.
22. Harinasut P, Poonsopa D, Roengmongkol K, Charoensataporn R. 2003. Salinity effects on antioxidant enzymes in mulberry cultivar. *Sci Asia* 29(2): 109-113.
23. Hasanuzzaman M, Hossain MA, da Silva JAT, Fujita M. 2012. Plant response and tolerance to abiotic oxidative stress: antioxidant defense is a key factor. In Crop stress and its management: Perspectives and strategies. Springer, Dordrecht. pp 261-315.
24. Hernandez JA, Jiménez A, Mullineaux P, Sevilia F. 2000. Tolerance of pea (*Pisum sativum* L.) to long-term salt stress is associated with induction of antioxidant defences. *Plant Cell Environ*. 23(8): 853-862.
25. Imlay JA. 2003. Pathways of oxidative damage. *Ann. Rev. Microbiology* 57(1): 395-418.
26. Ishikawa T, Yoshimura K, Sakai K, Tamoi M, Takeda T, Shigeoka S. 1998. Molecular characterization and physiological role of a glyoxysome-bound ascorbate peroxidase from spinach. *Plant Cell Physiology* 39(1): 23-34.
27. Jaleel CA, Gopi R, Manivannan P, Panneerselvam R. 2007. Responses of antioxidant defense system of *Catharanthus roseus* (L.) G. Don. to paclobutrazol treatment under salinity. *Acta Physiol. Planta* 29(3): 205-209.
28. Jespersen HM, Kjaersgård IV, Ostergaard L, Welinder KG. 1997. From sequence analysis of three novel ascorbate peroxidases from *Arabidopsis thaliana* to structure, function and evolution of seven types of ascorbate peroxidase. *Biochemistry Journal* 326(2): 305-310.
29. Jimenez A, Hernandez JA, del Río LA, Sevilla F. 1997. Evidence for the presence of the ascorbate-glutathione cycle in mitochondria and peroxisomes of pea leaves. *Plant Physiology* 114(1): 275-284.
30. Kalt-Torres W, Burke JJ, Anderson JM. 1984. Chloroplast glutathione reductase: purification and properties. *Physio Planta* 61(2): 271-278.
31. Khan F, Siddiqi TO, Mahmooduzzafar, Ahmad A. 2009. Morphological changes and antioxidant defence systems in soybean genotypes as affected by salt stress. *Jr. Plant Inter*. 4(4): 295-306.
32. Kholova J, Sairam RK, Meena RC, Srivastava GC. 2009. Response of maize genotypes to salinity stress in relation to osmolytes and metal-ions contents, oxidative stress and antioxidant enzymes activity. *Biol. Planta* 53(2): 249-256.
33. Kukreja S, Nandwal AS, Kumar N, Sharma SK, Unvi V, Sharma PK. 2005. Plant water status, H_2O_2 scavenging enzymes, ethylene evolution and membrane integrity of Cicer arietinum roots as affected by salinity. *Biol Planta* 49(2): 305-308.
44. Kvaratskhelia M, Winkel C, Thorneley RN. 1997. Purification and characterization of a novel class III peroxidase isoenzyme from tea leaves. *Plant Physiology* 114(4): 1237-1245.
45. Lascano HR, Gomez LD, Casano LM, Trippi VS. 1999. Wheat chloroplastic glutathione reductase activity is regulated by the combined effect of pH, NADPH and GSSG. *Plant Cell Physiology* 40(7): 683-690.

46. Lauchli A, Grattan SR. 2007. Plant growth and development under salinity stress. *In*: Advances in molecular breeding toward drought and salt tolerant crops. Springer, Dordrecht. pp. 1-32
47. Lüthje S, Martinez-Cortes T. 2018. Membrane-bound class III peroxidases: unexpected enzymes with exciting functions. *Int. Jr. of Mol. Science* 19(10): 2876.
48. Madhusudhan R, Ishikawa T, Sawa Y, Shigeoka S, Shibata H. 2003. Characterization of an ascorbate peroxidase in plastids of tobacco BY-2 cells. *Physio Planta* 117(4): 550-557.
49. Mahan JR, Burke JJ. 1987. Purification and characterization of glutathione reductase from corn mesophyll chloroplasts. *Physio. Planta* 71(3): 352-358.
50. Mandhania S, Madan S, Sawhney V. 2006. Antioxidant defense mechanism under salt stress in wheat seedlings. *Biol. Planta* 50(2): 227-231.
51. Mittler R, Zilinskas BA. 1991. Purification and characterization of pea cytosolic ascorbate peroxidase. *Plant Physiology* 97(3): 962-968.
52. Mittler R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trend Plant Science* 7(9): 405-410.
53. Mullineaux PM. 1997. Glutathione reductase: regulation and role in oxidative stress. Oxidative stress and the molecular biology of antioxidant defence. pp 667-713.
54. Najami N, Janda T, Barriah W, Kayam G, Tal M, Guy M, Volokita M. 2008. Ascorbate peroxidase gene family in tomato: its identification and characterization. *Mol. Gen. Geno*. 279(2): 171-182.
55. Neill S, Desikan R, Hancock J. 2002. Hydrogen peroxide signalling. *Cur. Opinion Plant Biology* 5(5): 388-395.
56. Noctor G, Foyer CH. 1998. Ascorbate and glutathione: keeping active oxygen under control. *Ann. Res. Plant Biology* 49(1): 249-279.
57. Pal M, Singh DK, Rao LS, Singh KP. 2004. Photosynthetic characteristics and activity of antioxidant enzymes in salinity tolerant and sensitive rice cultivars. *Ind. Jr. of Plant Physiology* 9(4): 407-412.
58. Panchuk II, Volkov RA, Schöffl F. 2002. Heat stress-and heat shock transcription factor-dependent expression and activity of ascorbate peroxidase in Arabidopsis. *Plant Physiology* 129(2): 838-853.
59. Pang CH, Wang BS. 2008. Oxidative stress and salt tolerance in plants. *In*: Progress in Botany. Springer, Berlin, Heidelberg. pp 231-245
60. Park MR, Kim YJ. 2005. Enhanced antioxidant enzymes are associated with reduced hydrogen peroxide in barley roots under saline stress. *Jr. of Biochem. Mol. Biology* 38(2): 218-224.
61. Prochazkova D, Sairam RK, Srivastava GC, Singh DV. 2001. Oxidative stress and antioxidant activity as the basis of senescence in maize leaves. *Plant Science* 161(4): 765-771.
62. Raven EL. 2000. Peroxidase-catalyzed oxidation of ascorbate structural, spectroscopic and mechanistic correlations in ascorbate peroxidase. *In*: Enzyme-Catalyzed Electron and Radical Transfer. Springer, Boston, MA. pp 317-349.
63. Sairam RK, Tyagi A. 2004. Physiology and molecular biology of salinity stress tolerance in plants. *Current Science*. pp 407-421.
64. Sharma P, Dubey RS. 2004. Ascorbate peroxidase from rice seedlings: properties of enzyme isoforms, effects of stresses and protective roles of osmolytes. *Plant Science* 167(3): 541-550.
65. Sharp KH, Mewies M, Moody PC, Raven EL. 2003. Crystal structure of the ascorbate peroxidase–ascorbate complex. *Nat. Structural Mol. Biology* 10(4): 303-307.
66. Shigeoka S, Ishikawa T, Tamoi M, Miyagawa Y, Takeda T, Yabuta Y, Yoshimura K. 2002. Regulation and function of ascorbate peroxidase isoenzymes. *Jr. of Exp. Botany* 53(372): 1305-1319.
67. Srivalli B, Chinnusamy V, Khanna-Chopra R. 2003. Antioxidant defense in response to abiotic stresses in plants. *Jr. of Plant Biol*. 30(2): 121-140.
68. Stepien P, Klobus G. 2005. Antioxidant defense in the leaves of C_3 and C_4 plants under salinity stress. *Physio. Planta* 125(1): 31-40.
69. Szarka A, Tomasskovics B, Bánhegyi G. 2012. The ascorbate-glutathione-α-tocopherol triad in abiotic stress response. *Int. Jr. of Mol. Sci*. 13(4): 4458-4483.
70. Teixeira FK, Menezes-Benavente L, Galvão VC, Margis R, Margis-Pinheiro M. 2006. Rice ascorbate peroxidase gene family encodes functionally diverse isoforms localized in different subcellular compartments. *Planta* 224(2): 300-314.
71. Teixeira FK, Menezes-Benavente L, Margis R, Margis-Pinheiro M. 2004. Analysis of the molecular evolutionary history of the ascorbate peroxidase gene family: inferences from the rice genome. *Jr. of Mol. Evol*. 59(6): 761-770.
72. Tsai YC, Hong CY, Liu LF, Kao CH. 2005. Expression of ascorbate peroxidase and glutathione reductase in roots of rice seedlings in response to NaCl and H_2O_2. *Jr. of Plant Physiology* 162(3): 291-299.
73. Turhan E, Gulen H, Eris A. 2008. The activity of antioxidative enzymes in three strawberry cultivars related to salt-stress tolerance. *Acta Physio. Planta* 30(2): 201-208.
74. Vranová E, Inzé D, Van Breusegem F. 2002. Signal transduction during oxidative stress. *Jr. of Exp. Botany* 53(372): 1227-1236.
75. 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 (*G. max*). *Plant Omics*. 5(2): 60-67.
76. Welinder KG. 1992. Superfamily of plant, fungal and bacterial peroxidases. *Cur. Opinion Str. Biology* 2(3): 388-393.
77. Yamaguchi K, Mori H, Nishimura M. 1995. A novel isoenzyme of ascorbate peroxidase localized on glyoxysomal and leaf peroxisomal membranes in pumpkin. *Plant Cell Physiology* 36(6): 1157-1162.
78. Yousuf PY, Hakeem KUR, Chandna R, Ahmad P. 2012. Role of glutathione reductase in plant abiotic stress. *In*: Abiotic stress responses in plants. Springer, New York, NY. pp 149-158
79. Zaharieva TB, Abadía J. 2003. Iron deficiency enhances the levels of ascorbate, glutathione, and related enzymes in sugar beet roots. *Protoplasma* 221(3/4): 269-275.