

## REVIEW

# A review of ascorbic acid potentialities against oxidative stress induced in plants

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Received: 4<sup>th</sup> August 2011

Revised: 16<sup>th</sup> December 2011

Published online: 31<sup>st</sup> January 2012

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### Abstract

Ascorbic acid (AA) currently holds a significant position in plant physiology, mainly due to its possession of antioxidant and cellular reductant etc. properties and its diverse roles in plant growth and development and the regulation of a broad spectrum of plant cellular mechanisms against environmental stresses. Some researchers suggest that endogenous AA has been implicated in the promotion of plant growth and development by involvement in a complex and enigmatic array of phytohormone-regulated signalling networks that ties together different environmental stresses. As it is evident from the present review, recent progress on AA potentiality in the tolerance of plants to environmental stresses has been impressive. Indeed, AA plays an important role in resistance to oxidative stresses such as heavy metal, saline, ultra-violet etc. Rapidly increasing evidence indicates that AA is centrally involved in several physiological processes but there has been much disagreement regarding the mechanism(s) by which AA reduces the damaging effects of such stresses in plants. Perhaps the role of AA in mediating tolerance to abiotic stress (e.g. UV, salinity and temperature, etc.) will lead to a greater research focus in the near future. In addition, AA might provide a suitably attractive target for the enhancement of crop production.

**Key words:** environmental stress; heavy metal stress; redox state; plant hormones; saline stress; UV-stress

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### Abbreviations:

**AA** – ascorbic acid; **ABA** – abscissic acid; **APX** – ascorbate peroxidase; **CAT** – catalase; **DHA** – dihydroascorbic acid; **DHAR** – dihydro-

ascorbate reductase; **ET** – ethylene; **ETS** – electron transport system; **GA** – gibberellic acid; **GAL** – L-galactono-1,4-lactone; **GR** – glutathione reductase; **GSH** – glutathione; **GSSG** – oxidized glutathione; **JA** – jasmonic acid; **MAPK** – mitogen-activated protein kinase; **MDHA** – monodehydroascorbate; **MDHAR** – monodehydroascorbate reductase; **PS** – photo system; **ROS** – reactive oxygen substances; **SA** – salicylic acid; **SAG** – senescence associated genes; **SOD** – superoxide dismutase

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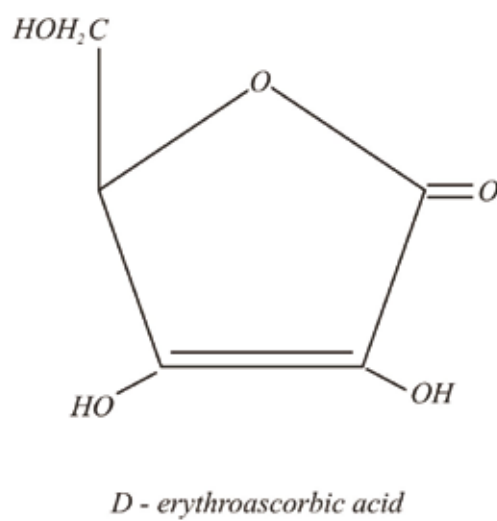
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## INTRODUCTION

L-ascorbic acid (L-AA) fulfils essential metabolic functions in the life of animals and plants. Some fungi can synthesize erythroascorbic acid, a vitamin C analogue with similar metabolic functions, but among prokaryotes, only blue green algae have been reported to have a small amount of AA (Arrigoni and De Tullio 2002). It also acts directly to neutralize superoxide radicals ( $O_2^{\cdot-}$ ), singlet oxygen ( $^1O_2$ ) or hydroxyl radical ( $\cdot OH$ ) simply by acting as a secondary antioxidant during the reductive recycling of the oxidized form of  $\alpha$ -tocopherol (Noctor and Foyer

1998). L-AA serves as a co-factor of many enzymes (Arrigoni and De-Tullio 2000), for example,  $\alpha$ -ketoglutarate-dependent dioxygenases (e.g. prolyl hydroxylases), important for the formation of covalent adducts with electrophilic secondary metabolites in plants (Traber and Stevens 2011). The antioxidant activity of AA is associated with resistance to oxidative stress and longevity in plants. By adding various natural and synthetic compounds such as PPGs (phenylpropanoid glycosides), AA is used as a reference compound in a large number of studies associated with stress mitigation/tolerance in plants (Lopez-Munguia et al. 2011) (Fig. 1) (Table 1).



**Fig. 1.** Chemical structure of D-erythroascorbic acid. It is the most widely existing form of ascorbic acid in plant system

**Table 1.** Physical properties of L-ascorbic acid (vitamin C)

Properties	Comments
Molecular formula	$C_6H_8O_6$
Appearance	White or light yellow solid
Melting point	190–192 °C/463–465 K/374–378 F
Solubility in water	33 g 100 ml <sup>-1</sup>
Solubility in ethanol	2 g 100 ml <sup>-1</sup>
Solubility in others solvent	Insoluble in diethylether, chloroform, benzene, petroleum ether, oils and fats
Acidity (pKa)	4.10 (I) 11.6 (II)
pH	3 (5 mg ml <sup>-1</sup> ), 2 (50 mg ml <sup>-1</sup> )

The endogenous level of AA has also recently been suggested to be of importance in the regulation of developmental senescence (Pavet et al. 2005). Plants have several L-AA biosynthetic pathways including routes via L-galactose and L-gulose (Wolucka and Van Montagu 2003), but the contribution of each one varies between different species, organs and developmental stages (Cruz-Rus et al. 2011). Also, genes encoding the biosynthetic enzymes of these pathways have been identified (Gatzek et al. 2002). A recent plethora of evidence suggests that AA may play a role in the protection of plants against several environmental stresses such as heavy metal action, salinity and temperature, etc. (Shalata and Neumann 2001, Vwioko et al. 2008).

In recent years, remarkable progress has been made in the understanding of the biosynthesis of AA. It has been recently shown that AA biosynthesis may be regulated by jasmonates (Wolucka et al. 2005) and studies report (Shan et al. 2011) that jasmonic acid (JA) induces an increase in the transcript levels and activities of APX, GR, MDHAR, DHAR, the contents of AA, GSH, ratio of AA/DHA and GSH/GSSG and reduces the GSSG/GSH (for the abbreviations see their list above). They also suggest that JA could induce the activation of mitogen protein kinase 2 (MAPK2) by increasing the phosphorylation level, which, in turn, results in the regulation of ascorbate and GSH content in *Agropyron cristatum*. Similarly, the results of Talla et al. (2011) suggest that the levels and redox state of AA modify the pattern of modulation of photosynthesis by mitochondrial metabolism. The extent of the alternative pathway as a percentage of the total respiration in *Arabidopsis* mesophyll protoplasts is much higher in *Vtc1* than in the wild type and becomes pronounced at high light and/or when the alternative pathway is inhibited. As well as acknowledging the importance of the cytochrome-C oxidase pathway they hypothesize that AA and the alternative pathway may complement each other to protect photosynthesis against photo-inhibition.

Because AA also serves as an important co-factor in the biosynthesis of many plant hormones, including ethylene (ET), JA, salicylic acid (SA), abscissic acid (ABA) and gibberellic acid ( $GA_3$ ); one has to assume that the endogenous level of AA will affect not only biosynthesis, but also the levels and the signalling of these hormones under stressful circumstances. Treatment with

a plant hormone (brassinolide) as foliar spray, has mitigated salt stress by inducing the enzyme activities responsible for antioxidation and detoxification as well as by elevating AA content (El-Mashad and Mohamed 2011). Furthermore, despite the correlation between environmental stresses and the level of AA in higher plants, the physiological explanation for such attention in AA levels remains unknown. As reported by various researchers during the last two decades, oxidative stress is a central and serious emerging issue responsible for a heavy reduction in crop productivity. Therefore, the elucidation of intracellular signalling processes mediating AA signalling is of potential significance to any programme targeted on improving crop resistance against abiotic stresses.

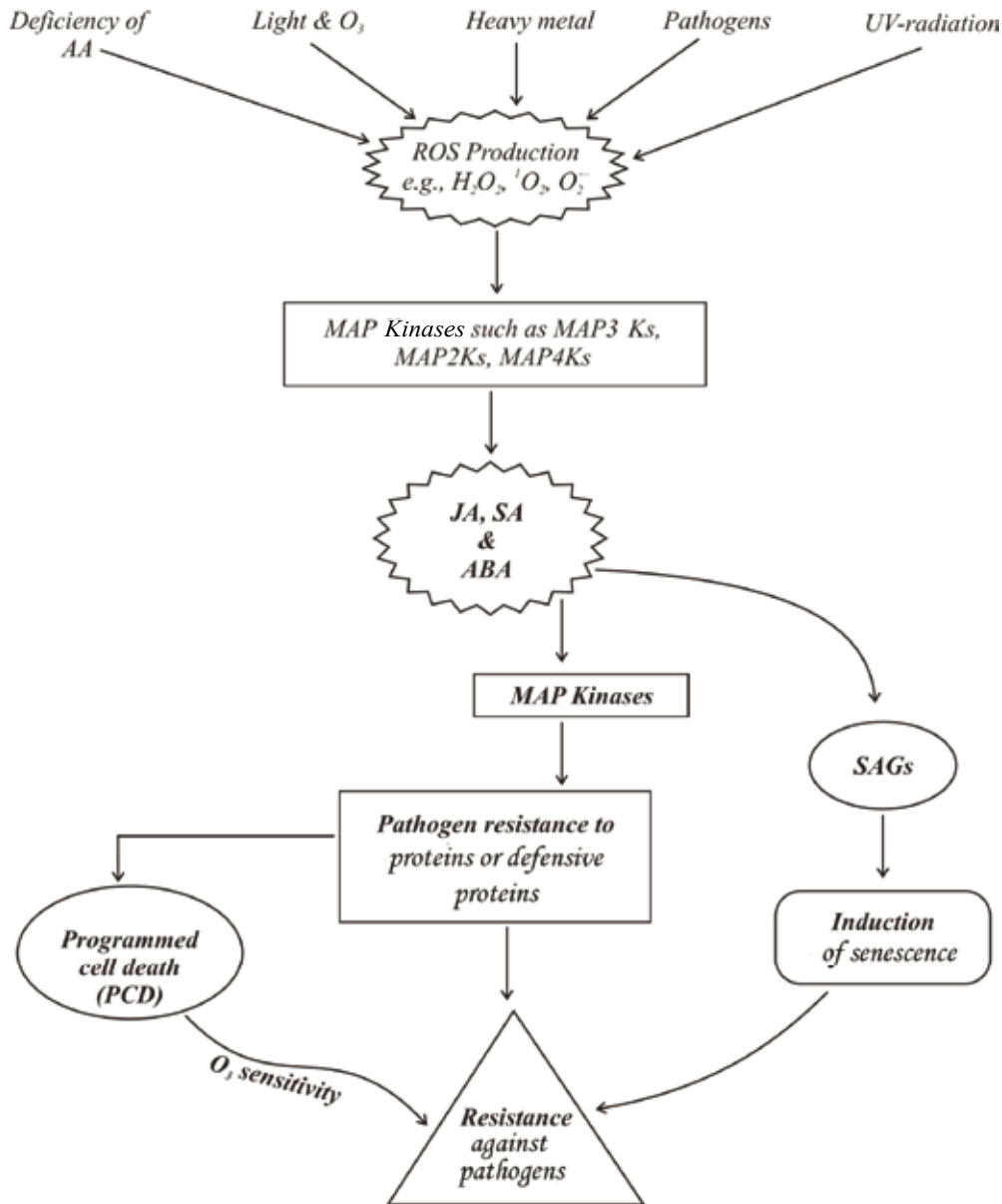
This review will highlight the recent advances in our understanding of how AA may regulate responses against abiotic environmental stresses, particularly temperature, salinity and ultra-violet radiation via a complex network of phytohormone signalling pathways.

#### **Ascorbic acid and plant hormones: A cross talk**

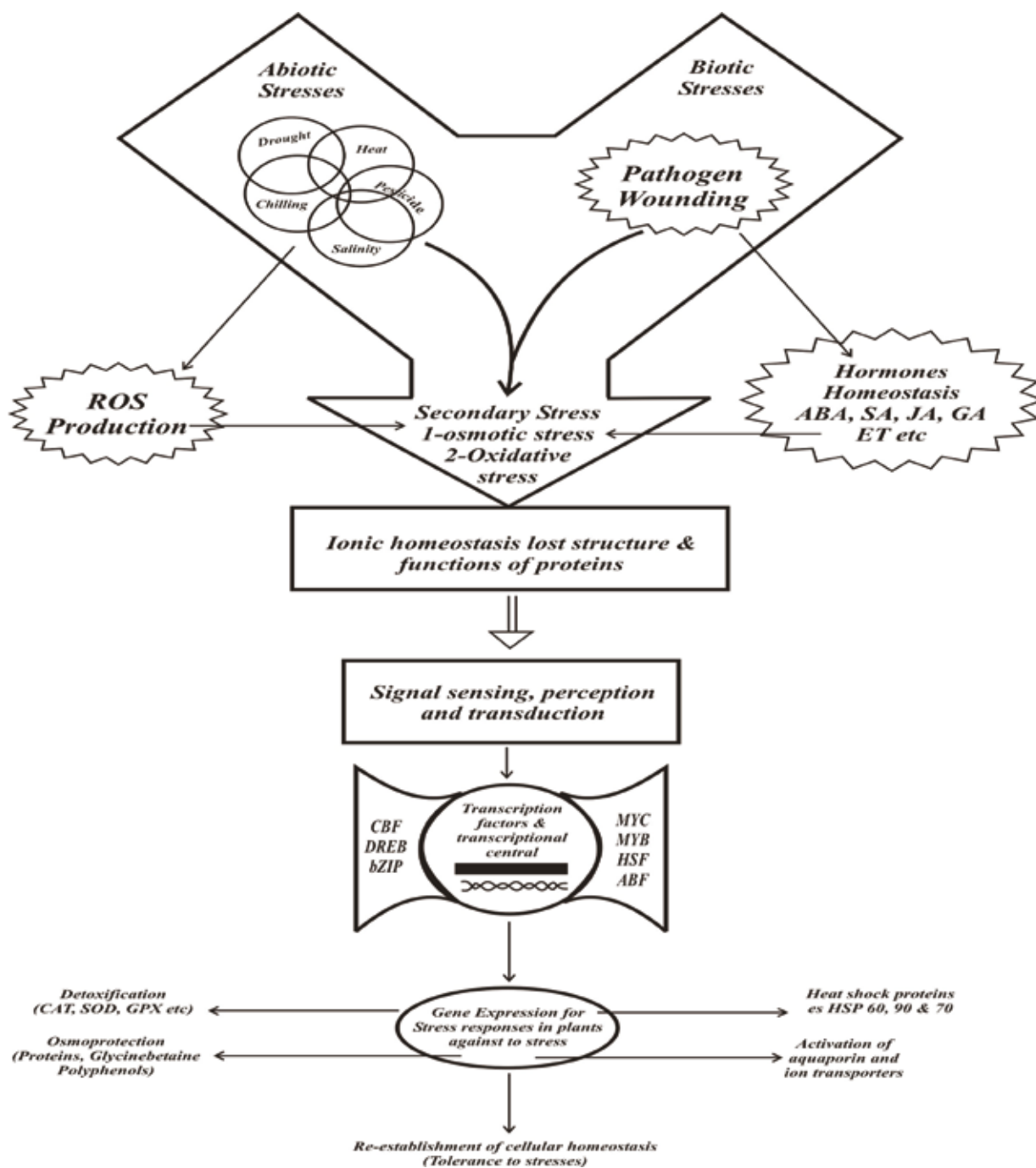
Plant responses to environmental stress can be viewed as being orchestrated through a network that integrates signalling pathways characterized by the production of ET, JA, SA, ABA and  $GA_3$ . The identified regulatory step in the network involves transcription, protein interaction and targeted protein damage. In plants, the MAPK cascade plays a key role in various abiotic stress responses and in phyto-hormone responses that include ROS signalling (Fujita et al. 2006) (Fig. 2). Molecular and genetic studies present the notion that cross talk between AA and various plant hormones exists. It includes alternation in the expression of hormones biosynthetic genes and/or signalling intermediates. The two most significant of the numerous signalling molecules integrated in the regulation of abiotic environmental stress and plant development, are GA and ABA. The biosynthesis of GA regulated GA20-oxidase, require AA for its activity. *Arabidopsis thaliana* has three GA20-oxidase genes, AtGA20ox1, AtGA20ox2, and AtGA20ox3. Transgenic *Arabidopsis* expresses an antisense copy of AtGA20ox1, and displays a delayed flowering phenotype, but only under short-day conditions (Coles et al. 1999). Moreover, high levels of ABA in *vtc1*, presumably caused

by the up-regulation of the AA requiring ABA biosynthetic enzyme, NCED dioxygenase (Pastori et al. 2003), may contribute to the late-flowering phenotype under short days. This result seems

somewhat supportive and contradictory, as the ABA biosynthetic pathway requires AA. ABA is known to act antagonistically to GA (Pastori et al. 2003) (Fig. 2).



**Fig. 2.** Model of ascorbic acid induced response to environmental stress (e.g. drought, oxidation, heavy metals, temperature, pesticides and pathogens) in plants. This model can be evaluated to understand the responsive natural sympathetic behaviour of ascorbic acid under drastic circumstances affecting plant growth and development. From Fujita et al. (2006).



**Fig. 3.** Both kinds of environmental stresses (biotic and abiotic) affect the natural growth patterns of plants to survive under such circumstances. Above mentioned signalling cascades operating in plants in response to various kinds of environmental stresses.

It is now well understood that the endogenous level of AA will affect not only biosynthesis, but also that the levels and therefore, the signalling of these plant hormones play a tremendously significant role in the removal of a number of environmental stresses. However, GA can also promote cell death in this system (Bethke et al. 1999), and a protectant involves ROS, and, notably, H<sub>2</sub>O<sub>2</sub> which is produced in glyoxysomes by the activity of a flavin-containing acyl CoA oxidase. Moreover, ABA can present cell death by promoting high activities of enzymes that destroy ROS induced by oxidative stress produced by heavy metal action. In addition, GA<sub>3</sub> also plays a vital role in the detoxification of heavy metal and in tolerance to salt stress by improving plant growth, chlorophyll synthesis, the activities of antioxidant enzymes and by preventing lipid peroxidation (Maggio et al. 2010). ABA represents a good example of a combination of genetic, molecular and biochemical approaches that can lead to the elucidation of a complex biosynthetic pathway. Moreover, APX is synergistically induced by ABA and oxidative stress (Fryer et al. 2003) while another peroxidase (2CPA) is oxidatively induced by wounding and photo-oxidation. Studies of ET signalling in *Arabidopsis* indicate that receptor gene families in plants may function in a way similar to many of their animal counterparts, increasing their flexibility in responding to stressful environments (Fig. 3).

### Response to heavy metal stress

The effects of heavy metals on plants result in growth inhibition, structural damage, and decline in physiological and biochemical activities as well as the function of plants. Also, heavy metal toxicity causes the blocking of functional groups of important molecules, e.g. enzymes, polynucleotides, transport systems for essential nutrients and ions, displacement and/or substitution of essential ions from cellular sites, denaturation and inactivation of enzymes, and disruption of cell and organelle membrane integrity. Moreover, elevated levels not only decrease soil microbial activity and crop production, but also threaten human health through the food chain (McLaughlin et al. 1999). Heavy metals such as Cd, Pb, Hg, Cu, Zn and Ni at supra-optimal concentrations affect plant growth, development and yield (Sresty and Madhava Rao 1999) (Fig. 3).

Heavy metal toxicity can elicit a variety of adoptive responses in plants. Cells are normally protected against free oxy-radicals by the operation

of intricate antioxidant systems, comprising both enzymic systems such as SOD, CAT and APXs and non-enzymic systems, acting as free radical scavengers such as AA and GSH and phenolic compounds (Foyer et al. 1994). Enzymes requiring AA as a co-factor in the detoxifying enzymic processes are listed in Table 2. The oxidative damage to different cellular components by H<sub>2</sub>O<sub>2</sub> could be minimized either by CAT and peroxidase activities or by a reaction sequence known as the ascorbate-glutathione cycle involving the redox pairs of ascorbate-dehydroascorbate and glutathione-glutathione disulfide (Foyer et al. 1994). Much information is available on the effect of redox heavy metals on various antioxidant processes in plants (Mazhoudi et al. 1997). However, the information on the effect of excess concentrations of some metals (e.g. Zn and Ni) on anti-oxidative processes is rare (Schickler and Caspi 1999), but they have been found to be useful to plants in lower concentrations while affecting them drastically at elevated concentrations. In addition, the symptoms of Zn and Ni toxicity appeared as a reduction in seedling growth. The growth of the main root is considerably affected and as a result, it exhibits the function of fibrous roots.

Previous studies report that the AA content of the roots and shoots of two cultivars of pigeon pea (*Cajanus cajan*) decreased with an increasing concentration of externally supplied Zn and Ni and registered lower values when compared to their respective controls. Moreover, the AA content of roots and shoots of the two pigeon pea cultivars showed a significant negative correlation with the increasing concentrations of metal ions supplied and a positive correlation with dry matter accumulation (Madhava Rao and Sresty 2000). Also, the study showed that between Zn and Ni treatments, the Ni-treated pigeon pea seedlings had a lower AA content. It was demonstrated that tolerant plants accumulate a higher level of heavy metals in their roots (Verkleij et al. 1990). Toxic levels of heavy metal ions have been reported to induce negative effects on some key metabolic processes coupled to growth in higher plants (Van Assche and Clijsters 1990). The induced oxidative stress by Zn and Ni in pigeon pea cultivars is evident from the increased lipid peroxidation in their roots and shoots. Moreover, dry matter accumulated in roots and shoots of the treated plants (Madhava Rao and Sresty 2000). A similar pattern of response together with an elevation in the photosynthesis was observed in mustard plants exposed to Cd through a

nutrient medium. Perhaps a constitutively high antioxidant capacity or increase in the levels of one or more antioxidants could prevent oxidative damage and improve resistance to oxidative stress.

Moreover, AA is the widely known compound used as an antioxidant and the most effective compound increasing the tolerance of plants to oxidative stresses. The results obtained by using the transgenic plants and mutants, confirmed the role of AA in oxidative stress or scavenging free-oxy-radicals (Smith et al. 1989). In addition, AA affects the physiological activities of the plants. Also, there is evidence that the tolerance of plants is correlated with the increased amount of AA. The antioxidant defense system in the plant cells includes both enzymatic antioxidants such as

SOD, CAT, APX and non-enzymatic antioxidants like AA, GSH and tocopherol. When plants are subjected to environmental stresses, oxidative damage may result because the balance between the production of ROS and their detoxification by the antioxidative system is altered (Gomez et al. 1999). Tolerance to damaging environmental stresses is correlated with an increased capacity to scavenge or detoxify ROS (Foyer et al. 1994). Taking all these observations together, it may be suggested as a hypothetical framework that Cd induces a transient loss in antioxidative capacity perhaps accompanied by a stimulation of oxidant producing enzymes, which results in intrinsic ascorbic acid accumulation. AA then would act as a signalling molecule triggering secondary defences.

**Table 2.** Enzymes requiring L-ascorbate

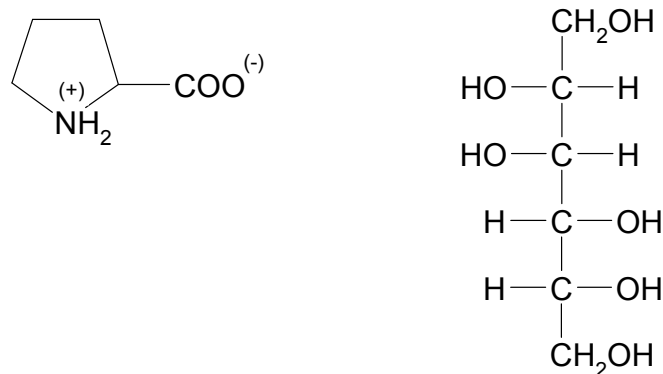
Enzyme	Change in activity	Physiological role
Thymine dioxygenase	increase	Pyrimidine metabolism
Pyrimidine deoxynucleoside	increase	Pyrimidine metabolism
Deacetoxy cephalosporin C synthase	increase	Antibiotic metabolism
1-aminocyclopropane-1-carboxylate oxidase	increase	Ethylene biosynthesis
Violaxanthin de-epoxidase	increase	Zeaxanthin biosynthesis
Gibberellin 3-β-dioxygenase	increase	Gibberellin biosynthesis
Thioglucoside glucohydrolase	increase	Catabolism of glucosinolates

### Response of saline stress

Today, soil salinity has become a serious environmental problem which affects the growth and productivity of many crops. High salt content in the soil affects the soil porosity and also decreases the soil water potential that results in a physiological drought. High salt content also affects the physiology of plants, both at the cellular as well as whole plant levels (Murphy and Durako 2003). An excess amount of ions like Na<sup>+</sup>, Cl<sup>-</sup> in cells also cause enzyme inhibition of for example, nitrate reductase (Shafea 2003), ribulose-bi-phosphate carboxylase oxygenase (Rubisco) and phosphoenolpyruvate (PEP), carboxylase (Soussi et al. 1999), and metabolic dysfunction such as degradation of photosynthetic pigments (Soussi et al. 1999). Moreover, high exogenous salt concentrations cause an imbalance of the cellular ions resulting in ion toxicity, osmotic stress and the production of ROS. Plant salt tolerance has generally been studied in relation to regulatory

mechanisms of ionic and osmotic homeostasis (Ashraf and Harris 2004) (Fig. 4).

The capacity to scavenge ROS and to reduce their damaging effects on macromolecules such as proteins and DNA appears to represent an important stress tolerance trait. For instance, an *Arabidopsis* mutant vst 1, which exhibits increased tolerance to salt stress, was found to have an increased capacity to scavenge ROS (Tsugane et al. 1999). Elimination of ROS is mainly achieved by antioxidant compounds such as AA, GSH, thioredoxin and carotenoids and by ROS scavenging enzymes (e.g. SODs, GPXs, and CAT). The expression of genes encoding many of these enzymes is also regulated by other stresses as well as by ABA (Guan and Scandalios 1998). Upon ABA treatment, there is an increase in H<sub>2</sub>O<sub>2</sub> levels in maize cultured cells (Guan et al. 2000) and in *Arabidopsis* guard cells (Pei et al. 2000). Transgenic plants with reduced CAT activity showed increased sensitivity to salt stress (Willekens et al. 1997).



**Fig. 4.** Molecular structure of osmoprotectants (proline and D-mannitol) synthesized during salinity in plants as a protector or inhibitor of harmful effects of high salt concentrations in soil in which the plants grow

Foliar application of AA at 200 mg l<sup>-1</sup> counteracted the adverse effect of salinity that was accompanied by a significant increase in plant growth of flux cultivars (El-Hariri et al. 2010). Also, AA affects many physiological processes including the regulation of growth, differentiations and metabolism of plants under saline conditions and the increasing physiological availability of water and nutrient (Barakat 2003). In addition, AA protects metabolic processes against H<sub>2</sub>O<sub>2</sub> and other toxic derivatives of oxygen affecting many enzyme activities, minimizes the damage caused by oxidative processes through synergistic function with other antioxidants, and stabilizes membranes (Shao et al. 2008). Studies by Hassanein et al. (2009) and Abd-El Hamid (2009) suggest that AA increases the content of IAA, which stimulates cell division and/or cell enlargement and this, in turn, improves plant growth. Also, AA was more effective at 400mg l<sup>-1</sup> and attributed to an increase in nutrient uptake and assimilation. One possibility is that additional AA would inhibit a stress-induced increase in the leakage of essential electrolytes following peroxidative damage to plasma membranes. However, additional AA did not inhibit an increase in the leakage of electrolytes from roots of salt-stressed tomato seedlings. Nor did additional AA significantly reduce the undesirable accumulation of Na<sup>+</sup> in the stress of salt-stressed plants. Moreover, the increase in yield and its compounds might be due to the effect of the antioxidant role on enhancing protein synthesis and delaying senescence (Hammam et al. 2001).

The remarkable protective effect of exogenous AA appears to be specially related to its antioxidant activity, rather than its possible utility as an organic substrate for energy respiratory metabolism (Shalata and Neumann 2001). The study also supports the notion that an additional exogenous supply of AA to seedlings might decrease the build up of ROS. and consequently, resistance to salt stress increase. Such increase in resistance to salt stress is associated with the antioxidant activity of AA and a partial inhibition of salt- induced increase in lipid peroxidation by ROS. Possibly, the protective effect of AA is more related to a reduction in ROS damage to essential proteins and/or nucleic acids (Noctor and Foyer 1998). Finally, the fact that new roots and leaves are produced by the seedlings which have recovered from 9h of salt treatment with AA, suggests that additional AA may have affected meristematic cells in the salt stressed root and shoot tissues. Similarly, the research of Upadhyaya et al. (2011) has proposed that genetic engineering of the ascorbate pathway enzyme (galacturonic acid reductase, GalUR) in potato, enhanced its ascorbic acid content and subsequently plants suffered minimal oxidative-stress-induced damage under salinity. The transgenic plants under salinity maintained a higher reduction in the oxidized GSH:GSSG ratio together with an enhanced activity of GSH dependent antioxidative and glyoxalase enzymes. These results suggest the engineering of ascorbate pathway enzymes as a major step towards the development of salinity tolerant crop plants.



Foliar spray of AA increased the percentage of cellulose and cellulose/lignin and at the same time decreased the proportion of lignin. This was attributed to the synthesis of chlorophyll involved in an increase of photosynthetic metabolites, which lead to the accumulation of different fractions of soluble sugars and nitrogen content in plant tissues under saline conditions. This evidence points to AA as an important part of the plant defence system maintaining the integrity and normal function of the photosynthetic apparatus (Liu et al. 2008) acting directly to neutralize  $O_2^{\cdot-2}$  or  $^1O_2$  in plant cells. The adverse effects of salt stress treatments on cellulose and lignin contents in shoots or roots are partially or completely alleviated by soaking grains in AA: this could perhaps alleviate the inhibitory effects of salinity on glucose incorporation to cell wall polysaccharides (Al-Hakimi and Hamada 2001). Grain soaking in AA had an inhibitory effect on the accumulation of sodium in different organs under various concentrations of NaCl (Al-Hakimi and Hamada 2001). In general, the effect of AA in mitigating partially or completely the adverse effects of salt stress may be one aspect of the role of this compound in the activation of some enzymatic reactions. Among the positive effects of AA in the counteraction of the adverse effects of salt stress are the stabilization and protection of the photosynthetic pigments and the photosynthetic apparatus from oxidisation (Hamada 1998).

In addition, apart from water stress, ROS such as  $O^{\cdot-2}$ ,  $H_2O_2$  and  $\cdot OH$  are also produced during salinity stress, and are responsible for the damage to membrane and other essential macromolecules such as photosynthetic pigments, proteins, DNA and lipids (Fahmy et al. 1998). The parallel increase in AA found resembles the response of maize to water deficit (Ashraf and Foolad 2006). ROS produced as a result of abiotic environmental stresses need to be scavenged for maintenance of normal growth. The primary scavenger is SOD (superoxide dismutase; EC 1.15.1.1), converting  $O_2^{\cdot-}$  to  $H_2O_2$  which is eliminated by APX (EC 1.11.1.11) in association with DHAR (EC 1.8.5.1) and GR (EC 1.6.4.2), and finally regenerates the AA (Asada 1994). However, little information is available on the effects of salt stress on the ROS metabolism and antioxidant enzymes activities in tolerant/susceptible genotypes of wheat. This knowledge could supply information on the possible involvement of antioxidants as a defence against ROS in the mechanism of salt sensitivity, thus allowing an insight into the molecular

mechanisms of plant tolerance to salt induced oxidative stress.

Furthermore, the results of Sairam et al. (2005) suggest that both constitutive as well as salt-stress-induced increases in antioxidant enzyme activity are important for providing protection against ROS. The constitutive level provides protection from oxidative stress arising from normal oxidative metabolism, and the salinity-induced increase in activities of antioxidants in response to the increase in oxidative stress actually decides the level of tolerance of a plant. Plants possess antioxidant systems in the form of enzymes such as APX, GSH, and compounds such as  $\alpha$ -tocopherol, flavonoids, etc. The antioxidant enzymes and metabolites are reported to increase under various environmental stresses (Yu and Rengel 1999) and also comparatively higher activity has been reported in tolerant cultivars than in the susceptible ones (Sreenivasulu et al. 2000), suggesting that higher antioxidant enzyme activity has a role in imparting tolerance to these cultivars against environmental stresses. The work of Nagesh and Devaraj (2008) suggests that, under the influence of salinity, oxidative stress indicators such as  $H_2O_2$ , GSH, AA and proline are significantly elevated. A remarkable increase in AA levels indicated the induction of an antioxidant mechanism such as glutathione-ascorbate cycle, as reported for a number of plants (Koca et al. 2007). Further research work is required to decipher the mechanisms through which AA acts and how antioxidant enzymes might be connected with salt resistance tolerance.

### **Response to light and UV radiations**

UV-B is a key environmental signal, regulating diverse responses in plants which promotes UV protection, and survival in sunlight, and influencing metabolism, development and defense responses. A study by Kumari et al. (2010) suggests that UV-B generates oxidative stress in plant cells due to the excessive generation of ROS. They also reported that stimulation of the activities of SOD, CAT, APX and GR was observed at the initial growth stage, while the activities of CAT and SOD decreased at a later stage. However, there was no definite trend of changes observed in AA. Also, the direct effects of UV radiation are mostly damaging, because UV photons have enough energy to create lesions in important UV-absorbing biomolecules such as nucleic acids and proteins. UV radiation induced the degradation of AA in a model apple juice. AA degradation was more rapid at higher radiation dose levels and

the reaction accelerated with increasing exposure time. Tikekar et al. (2011) showed the effect of UV processing on AA and suggested that process developers and researchers could use this study as a model for designing experiments to identify factors that influence the stability of AA and other bioactive compounds during UV processing.

A study by Younis et al. (2011) has suggested that UV-B containing light conditions reduced DNA and RNA contents. This appeared in association with marked changes in protein patterns, providing information concerning the structural genes and their regulatory system control of the varied biosynthetic pathways. In addition, many of the effects of UV-B involve the different regulation of gene expression. Responses to UV-B are mediated by both non-specific signalling pathways involving DNA damage, ROS (e.g.  $H_2O_2$ ), and various other defensive signalling molecules. Even though a number of studies have shown that UV-B radiation inhibits plant growth and regulates cell cycle progress, little is known about the molecular and cellular mechanisms involved. Similarly, a study by Jiang et al. (2011) implied that UV-B induced DNA damage resulted in the delay of G1 to S transition of the plant cell cycle. UV-B induced G1 to S arrest may be a protective mechanism that prevents cells with damaged DNA from dividing and may explain plant growth inhibition under increased solar UV-B radiation. AA treatment did not change the expression pattern of cell cycle regulation genes affected by a high level of UV-B. In the leaves of tropical trees, the ambient UV-B radiation might contribute to a reversible decline in potential PS-II efficiency observed upon exposure to full and direct sunlight. UV-(A+B) radiation also produces oxidative stress (Panagopoulos et al. 1990), although the mechanism of ROS generation in irradiated plants is not known (Rao et al. 1996) and thus requires further research (Fig. 2).

In the combined conditions of high light and low temperature where metabolism is slowed relative to photochemistry, the Mehler reaction may occur at increased rates. Hence, providing that the resulting  $O_2^{\cdot-}$  and  $H_2O_2$  can be efficiently metabolized,  $O_2$  reduction would play an important role in allowing the ongoing utilization of light energy absorbed by the photosynthetic apparatus. Enhanced operation of the Mehler reaction may thus diminish the extent of photo-inhibition, the slowly reversible reduction in photosynthetic efficiency and capacity which occurs when light energy is in excess. In addition, the AA/DHA and GSH/GSSG ratio may protect

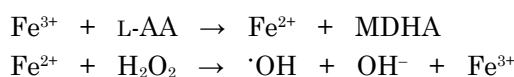
the thio-modulated enzymes of the Benson-Calvin cycle from oxidation by  $H_2O_2$  and allow photosynthesis to proceed at relatively high rates even during oxidative stress.

In peroxisomes,  $H_2O_2$  can be destroyed either by CAT or APX. APX has a high affinity for  $H_2O_2$  but it requires a reducing substrate; ascorbate. In the APX reaction,  $H_2O_2$  is reduced to water and ascorbate is oxidized to monodehydroascorbate (MDHA), the univalent product of ascorbate oxidation. In the chloroplast, MDHA is rapidly reduced to ascorbate by reduced ferredoxin. Other membrane associated ETSs such as those on the plasmalemma may also be instrumental in re-reducing MDHA non-enzymatically. In addition, MDHA reductases (MDHAR) rapidly reduce MDHA to ascorbate using NADPH. The presence of these membranes for recycling MDHA has thrown doubt on the requirement for a GSH-dependent reduction of DHA in ensuring ascorbate regeneration. When MDHA is not reduced, it rapidly disproportionates to AA and DHA, the divalent oxidized product. Although DHA is reduced to AA by DHAR, DHA is always detectable in plant tissues (Foyer et al. 1983) and AA/DHA ratios are relatively low compared to GSH/GSSG ratios, particularly under field conditions.

It has recently been argued that DHA detected in extracts is artefactual and that *in vivo* its levels are much lower or negligible (Morell et al. 1997). This connection is principally supported by the observation that inclusion of DHA in enzyme assays, at a concentration thought to exist in the chloroplast *in vivo*, led to oxidation inactivation of two enzymes known to be regulated by the thioredoxin system (Morell et al. 1997). The authors therefore concluded that a significant formation of DHA *in vivo* must be avoided (Morell et al. 1997). It is well established that the soluble stromal enzymes regulated by the thioredoxin system require ongoing reduction to remain active (Noctor and Mills 1988). Data obtained by the addition of oxidants to enzymes removed from the light and the membrane dependent thioredoxin reduction system lack any relevance whatsoever to *in vivo* conditions, where the activation state of thiol-regulated enzymes will reflect the differences between reductive and oxidative fluxes. This means that it is important that DHA is reduced back to ascorbate, not that DHA cannot exist *in vivo*. A monodehydroascorbate reductase (MDHAR) has been found to be associated with the inner (cytoplasmic) side of the plasma membrane in spinach. This enzyme

has been shown to preferentially utilize NADH as an electron donor (Berczi and Moller 1998). Models have been produced in which this MDHAR is involved in recycling of AA that is oxidized to MDHA on the cytoplasmic side of the plasma membrane by the activation of the plant PMcytb561 protein (Pignocchi and Foyer 2003).

We are aware of no evidence indicating that MDHAR activity in general is regulated in response to the eliminated ROS generated by UV. The abundance of a putative cytosolic MDHAR mRNA from tomato (*Lycopersicon esculentum* Mill) is shown to be inversely proportional to total AA and greatly elevated in response to UV radiation. Under certain circumstances, L-AA can also have a prooxidant effect, in particular by maintaining the transition metal ions, Fe(III), Cu(II), in their reduced forms. These metal ions then react with H<sub>2</sub>O<sub>2</sub> to form the highly reactive OH<sup>•</sup> radicals in the Fenton reaction (Halliwell 1996).



There is currently no clear evidence that these reactions are of significance *in vivo* (Diplock et al. 1998) (Fig. 2).

Moreover, it is well known that the over-production of ROS in living organisms, including photoautotrophs under UV stress conditions, is partially toxic, may attack biomolecules such as lipid, protein, DNA and some small molecules and finally result in oxidative damage, even the death of the organisms (Halliwell and Gutteridge 1999). Of the antioxidant small molecules, AA is perhaps the most important antioxidant in plants and plays a pivotal role in the destruction of ROS, particularly H<sub>2</sub>O<sub>2</sub>, as well as the extremely reactive OH<sup>•</sup> and the regeneration of α-tocopherol (Foyer et al. 1994). The exogenous addition of ascorbate (5 and 10 mM) to the culture of green algae *Chlamydomonas reinhardtii* prevented the ROS increase and therefore, the ROS-mediated down-regulation of large subunits of Rubisco translation induced by excess light stress (Irihimovitch and Shapira 2000). Yamane et al. (2011) have suggested that treatment with AA suppresses both H<sub>2</sub>O<sub>2</sub> accumulation and the changes in chloroplast ultra-structure, which is supported by the fact that light-induced production of excess H<sub>2</sub>O<sub>2</sub> under salinity is responsible for the changes in chloroplast ultra-structure.

A study by He and Hader (2002) suggests that AA also exhibited a significant protective effect on lipid peroxidation and DNA strand break. They

detected a significant decrease in ds DNA levels under UV-stress and their study also indicated the elevated formation of ROS and oxidative stress from UV-B treatment. Under moderate UV-B radiation, a significant loss in survival occurred in cyanobacteria. The protective effect of AA indicates that survival of the irradiated organisms by UV-B is associated with the extent of DNA damage (He and Hader 2002). Previously, DNA damage was considered as closely related to membrane lipid peroxidation (Zastawny et al. 1995). There was no direct evidence observed in the study of He and Hader (2002) in respect to lipid peroxidation and DNA damage. However, the role of AA in intracellular signalling in ultraviolet-stressed plants and in the putative regulation of antioxidant enzymes synthesis needs to be investigated. Finally, it has been concluded that, consistently with a signalling role for this compound, some reports also have shown that endogenously synthesized ascorbic acid in response to abiotic stresses, can protect plants against UV-B stress and can induce stress response genes.

### Temperature stress

Under conditions of high and low temperature where metabolism is slowed relative to photochemistry, the Mehler reaction may occur at increased rates. Hence, providing that the resulting O<sub>2</sub><sup>•-</sup> and H<sub>2</sub>O<sub>2</sub> can be efficiently metabolized, O<sub>2</sub> reduction would play an important role in allowing the ongoing utilization of light energy absorbed by the photosynthesis apparatus.

The mutual effects of temperature × salinity or radiation were discussed in previous chapters.

The addition of AA decreased the ROS levels detected by dichlorofluoresceine (DCF) fluorescence. In particular, AA did not demonstrate a significantly higher ROS scavenging efficiency than other antioxidants such as N-acetylcysteine (NAC) during the start of the irradiation while there was a significantly higher antioxidant effect from AA than that of NAC after 24 h of irradiation (He and Hader 2002). AA is involved in the *in vivo* ROS scavenging action in multiple ways, i.e. direct scavenging or via the ascorbate glutathione cycle (Smirnoff 2000). Also, the exogenous added AA was taken up significantly and no efflux of AA occurred during the uptake of AA as demonstrated by [14C]-labelling in potato leaves (Imai et al. 1999). It is suggested that a transient increase in AA signals activation of the protective mechanism for acclimation to temperature tolerance in plant system.

## CONCLUSION

AA can act efficiently in plants as an immunomodulator when applied at the appropriate concentration and the current stage of plant development. Ascorbate is implicated in plant responses to abiotic environmental stresses and regulates the stress response as a result of a complex sequence of biochemical reactions such as activation or suppression of key enzymatic reactions, induction of stress responsive proteins synthesis, and the production of various chemical defense compounds. In addition, an attempt has been made to connect some very intriguing observations that have been reported for the AA-deficient mutant *vtc1* in terms of some factors such as temperature, salinity and ultraviolet radiation etc. to cause oxidative stress. Due to its essential function as a co-factor for the biosynthesis of various plant hormones, AA appears to influence not only the endogenous level but also the signalling of these plant hormones, and thus affects responses against the above mentioned environmental stresses. Also, the redox status of AA may play a role in the signalling of this interconnected phytohormone network. However, there are obviously still large gaps to be filled in order to elucidate the precise role of AA in enhancing the tolerance of plant to environmental stresses during development as well as normal conditions.

## ACKNOWLEDGEMENTS

The authors are highly thankful for the facilities obtained at AMU Aligarh. Financial support from the Department of Science and Technology, New Delhi in the form of project (SR/FT/LS-087/2007) is gratefully acknowledged.

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