



OCCURRENCE, BIOSYNTHESIS AND POTENTIALITIES OF ASCORBIC ACID IN PLANTS

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ABSTRACT : Ascorbic acid (AA) is found in all eukaryotes including animals and plants and lacks completely in prokaryotes except cyanobacteria, have been reported to have a small amount of AA. It is an antioxidant and, in association with other components of the antioxidant system, protects plants from oxidative damage resulting from aerobic metabolism, photosynthesis and a range of pollutants like ozone, heavy metal and saline stress. In addition, it is not only an antioxidant; it also appears as a co-factor for several metabolic enzymes involved in the fundamental developmental process of plants and a well known cellular reductant with an intimate and comprehensive role in the response to environmental stress. Also, some studies suggests that the endogenous AA has been implicated in the promotion of plant growth and development by involving in a complex array of phytohormone-mediated signaling network that ties together different environment stress. Indeed, in addition to acting simply as an antioxidant and cellular reductant, AA influences transition from the vegetative to the reproductive phase and the final stage of development, senescence. Since the biosynthetic pathway of AA in plants has not been identified and evidence for the proposed pathway is reviewed slightly in this review. Therefore, there are a need to increase our understanding of this enigmatic molecule since, it could be involved in a wide range of developmental phenomenon's as well as works against actual stress in order to regulate better growth and development.

Keywords: Ascorbic acid, oxidative stress, heavy metal stress, environmental stress, redox state and plant hormones.

Abbreviations: AO, ascorbate oxidase; ETS, electron transport system; GAL, L-galactono-1,4-lactone; PS, photo system; QC, quiescent center.

INTRODUCTION

AA is a small antioxidant molecule, vitamin C (L-ascorbic acid), fulfils essential metabolic functions in the life of animals and plants. It is found in plants, animals and single cell organisms. Some fungi can synthesize erythro-ascorbic acid, a vitamin-C analogue with similar metabolic functions. Among prokaryotes, only blue green algae have been reported to have a small amount of AA. As well as it is a small, water soluble, reductone sugar acid with antioxidant properties and acts as a primary substrate in the cyclic pathway for enzymatic detoxification of a number of reactive oxygen species (ROS) such as H₂O₂, and many other, harmful to normal functioning of plant metabolism. In addition, it acts directly to neutralize superoxide radicals (O₂⁻), singlet oxygen (O⁻) or hydroxyl radical (OH⁻) simply by acting as a secondary antioxidant during reductive recycling of the oxidized form of α -tocopherol (Noctor and Foyer 1998). L-AA serves as a co-factor for many enzymes (Arrigoni and De-Tullio 2000) and, it contributes to the detoxification of ROS (Smirnoff and Wheeler 2000, Conklin 2001, Conklin and Barth 2004) (Table 1).

AA is an essential cofactor for α -ketoglutarate-dependent dioxygenases (e.g. prolyl hydroxylases) important for formation of covalent adducts with electrophilic secondary metabolites in plants (Traber and Stevens 2011). This antioxidant activity of AA is associated with resistance to oxidative stress and longevity in plants. In a large number of studies associated with stress mitigation/tolerance in plants by adding the different natural and synthetic compounds such as PPGs (phenylpropanoid glycosides), AA is used as a reference compound (Lopez-Munguia et al. 2011). Similarly, studies of Da Silva et al. (2011) suggesting that in phosphonolibdenium assay, the *Anadenanthera Colubrina* (ACHE) *Libidibia ferrea* (LFHE) and *Pityrocarpa moniliformis* (PMHE) showed increased antioxidant activity in relation to AA against ROS respectively.

Table 1. Enzyme 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
Violaxanthine de-epoxidase	increase	Zeaxanthin biosynthesis
Gibberellin 3- β -dioxygenase	increase	Gibberellin biosynthesis
Thioglucoside glucohydrolase	increase	Catabolism of glucosinolate

Moreover, the endogenous level of AA has recently been suggested to be important in the regulation of developmental senescence and plant defense against pests (Pastori et al. 2003, Barth et al. 2004, Pavet et al. 2005). Results of Dias et al. (2011) confirmed that AA is the main precursor of oxalic acid in susceptible and resistant cacao (*Theobroma cacao* L.) infected by the hemibiotrophic fungus *Moniliophthora perniciosa*. Oxalic acid help in synthesis of H_2O_2 in plant-pathogen interaction play a role in inhibition of growth of biotrophic pathogens and could help in prevention of the infection/colonization process of plants by necrotrophic pathogens. Katay et al. (2011) reported that the effect of ascorbigen and 1-methyl ascorbigen on the disease resistance in bean against fungal pathogen *Uromyces phaseoli* and also suggests that effectiveness of protection depended on the dosage of the applied 1-methylascorbigen and on the time interval between the chemical pre-treatment and inoculation. Studies of Bala and Thukral (2011) established that AA along with glycerol found to be most effective in increasing the phytoremediating potential of *spirodela polyrrhiza* L. In addition Belide et al. (2011) also suggested that hyperhydration and necrosis of Agrobacterium-infected cotyledons found to be effectively controlled by using AA along with L-cysteine and iota-carrageenan.

A recent plethora of evidences suggests that it may play a role in protection of plant against several environmental stresses such as heavy metal action, pesticides, ozone etc (Shalata and Neumann 2001, Vwioko et al. 2008). Due to fact that AA also serves as an important co-factor in the biosynthesis of many plant hormones, including SA, ET, JA, GA and ABA one has to assume that the endogenous level of AA will affect not only the biosynthesis, but also the levels and the signaling of these hormones under the stressful circumstances. Shan et al. (2011) studies investigated that JA induced increases in the transcript levels and activities of APX, GR, MDHAR, DHAR, the contents of AA, GSH and ratio of AA/DHA and GSH/GSSG and reduced the GSSG/GSH. They also suggest that JA could induce the activation of MAPK2 by increasing the phosphorylation level, which, in turn, resulted in the p-regulation of Ascorbate and GSH content in *Agropyron cristatum*. High levels of AA in tomato fruits provide health benefits for humans and also play an important role in several aspects of plant life. The transcriptional control of AA levels in fruits can be investigated by combining the advanced genetic and genomic resources currently available for tomato (Di-Matteo et al. 2011). This will have profound effect to induce tolerance against environmental stresses and regulation of developmental processes including flowering and senescence.

Chemistry of ascorbic acid

Structurally, L-AA is one of the simplest vitamins. It is related to the C₆ sugars, being the aldono-1, 4-lactone of a hexonic acid (L-gluconic acid) and contains an enediol group on C₂ and C₃. The stereo-isomer of L-AA, D-iso-ascorbic acid has little any anti-ascorbic activity and should not be confused with D-erythro-ascorbic acid, which is the C₅ analogue of L-AA found in many yeasts and fungi. Delocalization of the π -electron over the C₂-C₃ conjugated enediol system stabilizes the molecule and causes the hydrogen of the C₃ hydroxyl to become highly acidic, and to dissociate with a Pka of 4.13. Therefore, at physiological PH, L-AA exists as a monovalent anion (L-AA⁻). Dissociation of the second hydroxyl takes place at PH 11.6. The occurrence in the cell wall is not accidental since high affinity carriers for both ascorbate (K_m=90 μ M) and dehydroascorbate (K_m=20 μ M) occur on the plasma membrane of barley leaf protoplasts. DHA, the oxidized form of AA is taken up more rapidly than AA (figure 1).

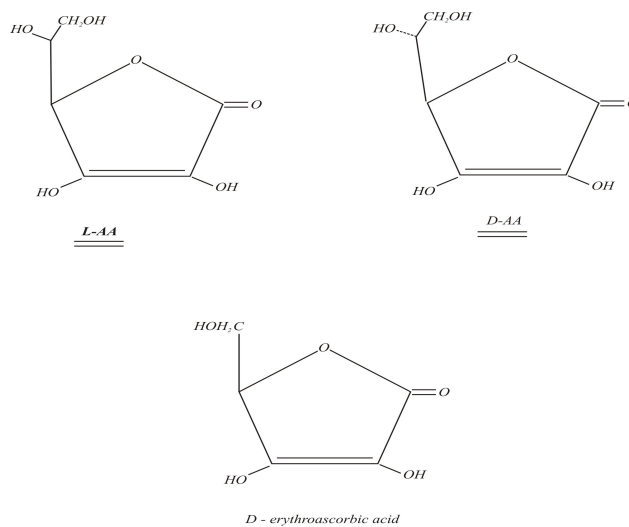


Figure 1. Different forms of ascorbic acid exist in plant systems.

The effects of ionophores and channels blockers on ascorbate uptake suggest a requirement for a H⁺-gradient across the PM. Isolated spinach chloroplasts take up ascorbate with a surprisingly low affinity (K_m=18-40 mM) via a saturable carrier. Uptake in to chloroplasts is inhibited by DHA (Beck et al. 1983). Generally, high affinity carrier's form on the PM would facilitate movement of ascorbate in to the cell wall and allow movement from cell to cell through apoplasts. Several natural derivatives of L-AA are also known as ascorbate-2-sulphate, ascorbic acid-2-O- β -glucuronide, 2-O- α -glucoside and several C₅-linked glucosides of 6-deoxyascorbate have been identified in mushroom (Okamura 1994). However, the possible existence and function of such derivatives in plant tissues received very little attention.

In addition, the synthetic derivatives ascorbate-2-phosphate and ascorbate-6-palmitate have *in vitro* antioxidant properties and are used in preservation of foods. Farmers commonly use L-AA supplemented foods for optimum growth and health of checks, piglets, calves and animals under stress (Madej and Grzeda 2000). L-AA is also frequently added to feeds in aquaculture because several commercial fish species are unable to synthesize it *de novo* (Maeland and Waagbo 1998). The richest elucidation of the plant biosynthetic pathway represents new opportunities not only for the direct synthesis of L-AA by fermentation but also for the production of human crop plants and animal fodder with the enhanced nutritional value. Oxidation of AA results initially in formation of the MDA radical. MDA disproportionate to form ascorbate and DHA. DHA is unstable above PH 7 so it is necessary to maintain the total ascorbate pool in a reduced state to prevent rapid loss. Recent evidence suggests that MDA can act as an electron acceptor from PS-I *in vivo* (Miyake and Asada 1992) and that it could act as both electron donor and acceptor in transmembrane electron transport (Asard et al. 1995).

While AA is a familiar molecule because of its antioxidant and cellular reductant properties, most aspects of its biosynthesis are very poorly understood. AA occurs in all plants tissues, usually being higher in photosynthetic cells and meristems and some fruits. Its concentration is reported to be highest in mature leaves with fully developed chloroplast. It has been reported that AA mostly remain available in reduced form in leaves and chloroplast under normal physiological conditions (Smirnoff 2000). About 30 to 40% of the total AA (as Ascorbate) is in the chloroplast and stromal concentration as high as 50 mM have been reported (Foyer and Noctor 2005). More than 45 years ago Isherwood et al. (1954) proposed a route for the biosynthesis of L-AA in plants based on the conversion of derivatives of D-galactose. This route involves the conversion of derivatives of D- galactose by an “inversion” route.

D-Gal \longrightarrow D-GalUA (ester) \longrightarrow L-galactonic acid \longrightarrow L-galactono-1, 4 lactone \longrightarrow L-AA

Support for this route is largely centered around the last step of the pathway where L-galactono-1, 4-lactone is oxidized to L-AA by the enzyme L-galactose-1, 4-lactone dehydrogenase (GLDH, EC 1.3.2.3). It extracted from a number of plant species (Oba et al. 1994) and has recently been cloned from cauliflower (Ostergaard et al. 1997). Radiolabelling studies have demonstrated that D-galacturonic acid methyl ester is directly converted to L-AA without entering central carbohydrate metabolism, and a soluble enzyme activity catalyzing the NADP-dependent reduction of D-galacturonic acid methyl ester to L-galactonic acid was also identified, although, the affinity for its substrate is low (Mapson and Isherwood 1956). Moreover, extensive radio-tracer studies have demonstrated that the majority of D-glucose is incorporated into L-AA by a pathway that does not involve inversion of the C-skeleton (Saito et al. 1990). Other features of the plant L-AA biosynthetic pathway are that the hydroxyl methyl group at C₆ is conserved during synthesis and that there are a core of label from [5-³H]-D-glucose, suggesting that this is the site of epimerization which causes the conversion from D to L configuration. Thus, in plants, L-AA biosynthesis from B-glucose proceeds via a non inversion type pathway.

Biosynthetic pathways for ascorbic acid

Since, plants have several L-AA biosynthetic pathways but the contribution of each one of the synthesis of AA varies between different species, organs and developmental stages (Cruz-Rus et al. 2011). The transcription of genes encoding biosynthetic enzymes such as D-galacturonate reductase and myo-inositol oxygenase and the AA recycling enzymes MDHAR are positively correlated with the increase in AA during plant ripening. Ascorbate is a major metabolite thus it comes as a surprise that a critical appraisal of the evidence suggests that the biosynthetic pathway in plants is not known. However, three pathways have been suggested.

(a) Inversion pathway

This pathway suggests that the immediate precursor to ascorbate is L-galactono-1, 4-lactone (GAL) (Isherwood and Mapson 1962). This pathway is supported by rapid conversion of exogenous GAL to ascorbate (De-Gara et al. 1994) and characterization of a mitochondrial enzyme (GAL dehydrogenase) which catalyses GAL oxidation to ascorbate (Oba et al. 1994). Further support for the pathway is provided by the action of lycorine. This alkaloid can induce scurvy in animals and decreases ascorbate levels in plants (Arrigoni et al. 1975). De-Gara et al. (1994) suggest that lycorine inhibits GAL dehydrogenase and GUL oxidase. However, the effect of lycorine has only been tested *in vivo* and its effect on purified GAL dehydrogenase is not known so, its specificity must be questioned. Even, it GAL is a precursor, it is not known how it is synthesized (Isherwood and Mapson 1962).

(b) Non-inversion pathway

Loewos et al. (1990) proposed a new biosynthetic route from D-glucose in which no inversion of configuration occurs by as following equation

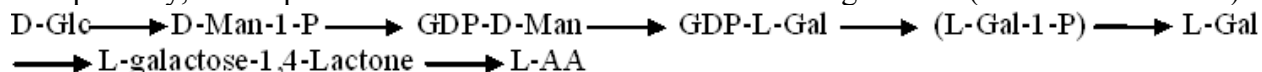
D-glucose \longrightarrow D-glucosone \longrightarrow L-sorbose \longrightarrow L-AA

Here, D-glucose oxidized at C₂ to D-glucosone by a pyranose-2-oxidase activity. D-glucosone is then epimerizes at C₅ to give L-sorbose (L-xylo-hexo-2-ulose), and C₁ oxidation of L-sorbose then yields L-AA. It involving oxidation of glucose at C₂ to produce the unusual osone-D-glucose has been proposed based on the labeling pattern. Glucosone is found in some basidiomycetes fungi which produce it by oxidation of glucose by pyranose-2-oxidase. This enzyme generates H₂O₂ which may used by white root fungi to degrade lignin (Daniel et al. 1994). So far, no attention has been paid to phosphorylated or UDP-derivatives of sugars, sugar acids or lactones on intermediates of ascorbate biosynthesis.

Supporting evidence is based on the non-inversion pathway of incorporation of radiolabel from D-glucose and D-glucosone in to L-AA and the partial purification of an enzyme analyzing the NADP-dependent oxidation of L-sorbose to L-AA. In addition, non-labelled D-glucosone and L-sorbose both completed with radiolabelled D-glucose for L-AA biosynthesis. Wheeler et al. (1998) reported that a newly identified enzyme, L-galactose dehydrogenase also slowly catalyzed the oxidation of L-sorbose, possibly accounting for earlier results. Recently, the conversion of D-glucosone and L-sorbose to L-AA has been re-examined, also supporting the conclusion that this pathway is not physically relevant (Pallanca and Smirnov 1999).

(c) L-galactose pathway

Many of the contradictory data of the past decades have now been resolved by the proposal of a new pathway, which proceeds via GDP-D-mannose and GDP-L-galactose (Wheeler et al. 1998)



Interestingly, this pathway utilizes the same terminal enzyme GLDH, as the route originally proposed by Isherwood et al. (1954), so that previous observations on the conversion of L-galactono-1, 4-lactone to L-AA apply. However, here, the conversion of D-glucose to L-AA occurs without inversion of the hexose C-skeleton, thus reconciling the crucial radio labeling data (Saito et al. 1990). Then L-galactose is an effective precursor of L-AA *in vivo*, and particularly purified a new enzyme, L-galactose dehydrogenase from pea and *Arabidopsis thaliana*. This enzyme catalyses the NAD-dependent oxidation of the C₁ of L-galactose to L-galactono-1, 4-lactone, with a *K_m* for L-galactose of 0.3 mM. The same enzyme extract is also able to slowly oxidize L-sorbose to L-AA at low affinity thus possibly accounting for earlier results (Saito et al. 1990). GDP-L-galactose is synthesized from the double epimerization of GDP-D-mannose and incorporated as a minor component of certain cell wall polysaccharides (Baydoun and Fry 1988). This reaction is catalyzed by a known, but poorly characterized enzyme, GDP-D-mannose-3, 5-epimerase, that is originally identified in *Chlorella pyrenoidosa* and flax (Feingold et al. 1980). The enzyme converting GDP-L-galactose to L-galactose still have to be identified in plants. The significance of this new biosynthetic route is that it integrates L-AA biosynthesis in to the pathways of central carbohydrate metabolism and provides connections to polysaccharides biosynthesis and proper glycosylation. Since, little is known about the regulation of ascorbate biosynthesis. The ascorbate pool is increased in leaves grown at high light intensity and low temperature. However, unlike GSH, it does not seem to be strongly responsive to oxidative stress (Smirnov and Pallanca 1996). In barley leaves, the ascorbate pool is correlated with photosynthetic capacity and with the supply of soluble carbohydrate (Smirnov and Pallanca 1996).

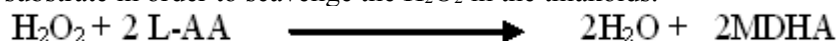
It is likely that molecular genetics, combined with further biochemical studies will provide an opening in to identification of the pathway. Mutants with altered ascorbate levels could be isolated by mass screening or by selecting plants that are hypersensitive or resistant to oxidative stress. This has recently been achieved in *Arabidopsis thaliana*. *soz1*, a mutant is selected for ozone sensitivity has 30% of wild type ascorbate levels (Conklin et al. 1996). Labeling studies suggests that *soz1* is defective in ascorbate biosynthesis rather than turnover.

Developmental role of ascorbic acid

(a) Photosynthesis

Ascorbate and APX, a peroxidase with specificity for ascorbate as reductant, appear to be universal in photosynthetic eukaryotes including algae and bryophytes (Loewus 1980, Miyake et al. 1991). Amongst prokaryotes, APX occurs in some cyanobacteria (Miyake and Asada 1992). Ascorbate occurs in cytosol, chloroplasts, vacuoles, mitochondria and cell wall (Rauten-Kranz et al. 1994). The concentration in chloroplasts can be high (up to 50mM in spinach) and is probably related to its central role in photosynthesis (Foyer 1993) and it has been shown to have important functions in photosynthesis such as in the protection of the photosynthetic apparatus against the oxygen radicals and H₂O₂ that are formed during photosynthetic activity (Asada 1994) and against photo-inactivation, since it is a factor of carotenoid-de-epoxidation (Siefermann and Yamamoto 1974).

AA works in its three biochemical modes. Firstly, it acts as an anti oxidant by removing H_2O_2 (Chloroplast lack CAT) formed by oxygenic photo-reduction in PS-I (Mehler reaction), catalyzed by APX. Some APX bound to thylakoid, there it can scavenge (Miyake and Asada 1992). Secondly, MDA can act as a direct electron acceptor to PS-I (Foyer and Lelandais 1993). Thirdly, it is a co-factor for violaxanthin de-epoxidase. Since, ascorbate is well known as an *in vitro* electron donor for photosynthetic and mitochondrial electron transport. Additionally, it regenerates the lipophilic antioxidant α -tocopherol (Asada 1994). Recent evidence suggests that MDA can act as an electron acceptor from PS-II *in vivo* (Miyake and Asada 1992) and that it could act as both electron donor and acceptor in trans-membrane electron transport (Asard et al. 1995). Ascorbate can be cleaved to form oxalate and tartrate (Saito 1996). In bright light or when low temperature and drought limit CO_2 fixation, the excess excitation energy is dissipated as heat by zeaxanthin in the light harvesting antennae. Zeaxanthin is formed by successive de-epoxidation of the xanthophyll cycle pigments violaxanthin and antheroxanthin. The de-epoxidase, which is bound to the lumen side of the thylakoid membrane, is dependent on ascorbate as a co factor (Neubauer and Yamamoto 1992, 1993). APX isozymes are present in the chloroplast as a thylakoid and stromal form, as well as in the cytosol, mitochondria and Peroxisome (Jimenez et al. 1997). APX requires L-AA as a substrate in order to scavenge the H_2O_2 in the thlakoids.



Moreover, MDHA is reduced back to L-AA either by acting as a direct electrons acceptor to PS-I at the reduced ferredoxin on the outside of the thylakoid membrane (Miyake and Asada 1994) or alternatively by the ascorbate- glutathione cycle, which links H_2O_2 to the oxidation of light generated NADPH. In this cycle MDHA generated from the reduction of H_2O_2 by APX is reduced back to L-AA by a stromal, NADPH dependent enzyme, MDHAR. The L-AA-GSH pathway depends ultimately on reducing power derived from the light dependent electron transport reactions of the chloroplast, or on secondary activities such as glucose-6-phosphate dehydrogenase and malate dehydrogenase for the generation of NADPH. It may well be that in the chloroplast; MDHA is primarily regenerated via reduced ferredoxin, and that ascorbate-glutathione cycle, at least in the scavenging DHA formed from the disproportionation of any MDHA that escape photo reduction at the thylakoids.

Furthermore, the quantitative importance of the Mahler peroxidase reaction *in vivo* (Foyer and Lelandais 1993) and the extent to which it increases in response to limitation of CO_2 fixation by low temperature and drought remain to be determined. Activities of enzyme in the ascorbate-glutathione cycle are increased by drought and low temperature suggesting the requirement for increased activities the cycle under these conditions (Smirnoff 1993, 1995). L-AA is also a co factor for the enzyme violaxanthin de-epoxidase, attached to the lumen side of the thylakoid membrane and form zeaxanthin. Zeaxanthin under CO_2 limiting conditions serves to dissipate excess excitation energy as heat in the light-harvesting antennae. To date, however, there are no any carrier to transport L-AA across the thylakoid membrane has been identical. Therefore, L-AA fulfills several functions in photosynthesis: It is a co-substrate for enzymes of zeaxanthin (A photo-protectant) biosynthesis pathway; it is a substrate for APX in the detoxification of H_2O_2 and an electron acceptor (as MDHA) for reduced ferredoxin in the electron transport chain (E.T.C) through Mehler reaction. In last, authors suggest that manipulation of the ascorbate-glutathione cycle in chloroplasts by targeted over expression of APX, GR, MDAR and DHAR should provide further evidence for the role of ascorbate (Foyer et al. 1994) in photosynthesis.

(b) Cell division

Several reports suggest that role of AA in cell division and differentiation (Arrigoni 1994, Edgar 1970). Histochemical staining with silver nitrate usually reveals high levels of ascorbate in meristems and its involvement in cell division has been suggested for both plant and animals cells. Exogenous L-AA has been found to accelerate the onset of cell proliferation in root premordia of *Allium*, *pisum* and *Lupinus* due to an increased proportion of cells progressing through the G1/S transition (Arrigoni et al. 1997, Citterio et al. 1994, Navas et al. 1995). Studies on the maize root quiescent centre, which comprises non-dividing cells arrested in G1 phase, have shown that it contains high level of ascorbate oxidase mRNA protein and activity (Kerk and Feldman 1995) and that this is correlated with low or undetectable levels of L-AA.

The addition of exogenous L-AA stimulates the cells of the quiescent centre to re-enter the cell cycle in both maize (Kerk and Feldmann 1995) and in *Allium* (Liso et al. 1988). Recently, in synchronized tobacco BY-2 suspension culture, L-AA content is found to increase during cell division, and it is concluded that the concentration of DHA during G1 phase may contribute to a shortening of the cell cycle and to cell elongation (Kato and Esaka 1999).

In plants, the evidence is based on the increased proportion of cells progressing to from G1 to S phase in onion root meristems and pericycle in response to exogenous ascorbate (Liso et al. 1988, Arrigoni et al. 1989, Citterio et al. 1994). More evidence for the role of ascorbate in controlling the transition from G1 to S phase is provided by studies on maize root quiescent centers (QCs). As stated in a large number of various studies that AA also serve as a factor in biosynthesis of ET, GA and ABA one has to assume that AA endogenously affect the signaling levels of these hormones. Barth et al. (2006) assumed that AA affects phytohormone-mediated signaling processes during the transition from the vegetative to reproductive phase and the final stage of development. GA induces stem growth in many rosette plants and dwarf mutants. This growth response can be quite dramatic and is the combined results of enhanced cell division activity in the sub-apical meristems and increased cell elongation.

Sachs et al. (1959) provided a well documented example of GA-promoted mitosis in the sub-apical meristems of the long day plants, *Samolus parviflorus*. In the intercalary meristems of deep water rice, GA promotes cell division and cell elongation (Sauter and Kende 1992). This leads to internodal growth rates up to 5 mm/hr. Therefore, it has been proposed that the primary action of GA in the intercalary meristems of rice is on cell elongation and that entry in to the cell cycle is a function of cell size, a phenomenon that has been well documented in yeast (Nurse 1991). Moreover, GA promotes the activity of a P34^{cdc-2} like protein kinase and the expression of genes encoding a P34^{cdc-2} like protein kinase and cyclin homologs in the intercalary meristems of deepwater rice (Sauter et al. 1995). However, there are no case has been shown that plant hormone regulate directly the expression of genes that code for regulatory proteins of the cell cycle.

Moreover, histochemical detection of ascorbate with silver nitrate and immunolocalization of AO in meristems show that ascorbate is not detectable in the QC while AO levels are high. High AO activity could oxidize any ascorbate transported from cells neighboring the QC. lycorine, a alkaloid, decreases the ascorbate content of tissues and also inhibits cell division and cell elongation in *Avena* Coleoptiles and Pea internodes (De Leo et al. 1973) and in onion roots, it induces the disappearance of cells in S-phase (Arrigoni 1994). Furthermore, there are several lines of evidence indicates that ascorbate or more likely, MDA stimulates cell proliferation in cultures of animals cells by shortening the cell cycle (Navas and Gomez-Diaz 1995). Another suggestion is that ascorbate increases deoxyribonucleotide reductase activity, required for DNA replication. In addition, the role of AO and L-AA/DHA ratio in determining exit from the cell division cycle (Finazzi-Argo 1987). In *Arabidopsis* root, however, L-AA GSH and non-specific reducing agents such as dithiothreitol are all found to stimulate cell division in the root primary meristems and to extend the range of meristematic divisions (Sanchez-Fernandez 1997).

(c) Cell wall development

Growth in higher plants is the results of two different processes, cell proliferation and cell elongation. Growth of the plant cells is driven by water uptake which, in turn, results from stress relaxation of the cell wall (Cosgrove 1993, 1997). To promote growth, plant hormones are expected to cause loosening of the cell wall. The acid growth theory postulates that secretion of H⁺ in to the cell wall is stimulated by auxin and that the lowered PH in the apoplasts activates wall-loosening processes (Rayle and Cleland 1970, Hager et al. 1971). For a cell to elongate, its load-bearing cellulose micro-fibrils must be oriented perpendicular to the direction of growth (Green 1980). Induction of cell elongation by GA may be confined to meristematic and young cells because their cellulose micro-fibrils are oriented transversely under the influence of GA, this transverse orientation of the cellulose micro-fibrils is maintained over a longer distance, thus extending the elongation zone of the organs (Sauter et al., 1993). Another AA oriented plant hormone, ET inhibits the elongation of terrestrial plants and causes thickening of their stems. This effect has been ascribed to a re-orientation of both the cortical microtubules and the newly deposited cellulose micro-fibrils from mostly transverse to mostly oblique/longitudinal (Lang et al. 1982).

By contrast, the rapid elongation of many semi-aquatic plants upon submergence is mediated by ET, which accumulates in the submerged tissue (Voesenek et al. 1992). It has been shown in case of aquatic plants that ET acts by increasing the tissue responsiveness to GA and immediate growth-promoting hormone. In addition, the mechanism of cell wall development is also directly affected by L-AA, AO and its other oxidation products. By involvement of ascorbate oxidase, which have consistently been found to influence plant cell expansion by a number of proposed mechanisms (Smirnoff 1996). These includes the co-substrate requirements of the iron-deoxygenases required for the post-translational modification of cell wall proteins (Arrigoni et al. 1997, Arrigoni et al. 1994) and possibly the direct reaction of DHA with lysine and arginine side chains to prevent cross-linking (Lin and Varner 1991).

Moreover, AO, which oxidizes ascorbate to water and MDA, is a member of the blue copper oxidase family, which also includes laccase and ceruloplasmin (Esaka et al. 1990, O'Malley et al. 1993). AO and L-AA have been directly/indirectly involved in the modulation of both cell proliferation and cell elongation. High AO activity is associated with tissues containing rapidly expanding cells in a wide range of plants. High AO activity correlates with the beginning of rapid expansion in germinating seeds and can be localized to regions where cells are expanding (Suzuki and Ogiso 1973). High AO activity is found in rapidly expanding cucurbits fruits. This developmental regulation of AO expression is supported by the effect of light and auxin. Continuous far-red light stimulates AO activity (Hayashi and Morohashi 1993). High wall AO activity might be expected to increase wall MDA and DHA. Higher ascorbate and DHA levels would cause higher steady state levels of MDA as predicted from the equilibrium constant of the disproportionation reaction (Takahama 1994). This observation suggests a close connection between cell expansion, wall ascorbate and AO.

The most direct test for a role of AO in growth would be to down-regulate its expression in transgenic plants by antisense technology using the cloned AO. There are also appears to be a cell wall plasticity by decreasing the availability of H_2O_2 for other apoplastic peroxidase reactions. The addition of exogenous MDHA (not L-AA) has been found to promote growth and rooting in *Allium* (Gonzalez-Reyes et al. 1994, De Cabo et al. 1996) and this effect has been linked to increased cell expansion and solute uptake by stimulation of the plasma-membrane- H^+ -ATPase as a result of transmembrane electron transport to extracellular MDHA via cytochrome-b (cyto-b) (Asard et al. 1995). Because of the involvement of AO, any hypothesis to explain the role of wall ascorbate in cell expansion must include MDA or DHA. MDAR and DHAR are not usually present in the wall so there must be another method to maintain reduction of the ascorbate pools. The wall therefore has a system to regenerate MDA via AO activity and a means of reducing MDA to ascorbate. Ascorbate is transported in to the apoplasts by a carrier. DHA, formed by disproportionation of any MDA which escapes reduction by cyto-b, can be transported by a high affinity carrier in to the cytosol (RautenKranz et al. 1994) where it is reduced to ascorbate by GSH-dependent DHAR. The net result of this system is electron transport across the PM with NAD(P)H as the reductant and extracellular oxygen is the ultimate electron acceptor.

It has been suggested that electron transport stimulates the plasma-membrane H^+ -ATPase (Carrasco-Luna et al. 1995), which lead to increased cell expansion and solute uptake (Rayle and Cleland 1992). Treatment with MDA caused increased growth rate by stimulating cell expansion, vacuolization and solute uptake (Gonzalez-Reyes et al. 1995). The MDA treatment also causes membrane hypo-polarization which suggests that the treatment increases H^+ -ATPase activity (Gonzalez-Reyes et al. 1995). This suggests the relationship between AO and growth but a number of other ways in which ascorbate and AO could interact with wall function. In addition, L-AA can also serve as a substrate for oxalic acid biosynthesis (Loewus 1988). It has been suggested that apoplastic oxalate could be responsible for the sequestering of Ca^{+2} , important for the cross-linking of pectin's and cell wall strengthening. Oxalate oxidase catalyzes the breakdown of oxalate to CO_2 and H_2O_2 , thus liberating Ca^{+2} and H_2O_2 , both of which would promote cell wall stiffening.

Although, it is difficult to assess the relative contributions of these various mechanisms to cell wall expansion, it is clear that L-AA is able to modulate fundamental aspects of cell wall metabolism. In this respect the redox status of apoplastic L-AA and the balance between L-AA and H_2O_2 level will directly or indirectly influence the degree of lignifications and cross-linking of cell wall components.

Lignifications are associated with high wall peroxidase (POD) activity and formation of H_2O_2 in the wall. POD uses H_2O_2 as oxidant to form monolignol radicals from monolignol precursors such as coniferyl alcohol (Otter Polle 1994). This reaction is inhibited by ascorbate which may primarily act by scavenging the monolignol radicals (Takahama and Oniki 1994). Otter and Polle (1994) therefore suggest that understanding the role of ascorbate requires a method to measure it specifically in lignifying walls. The wall ascorbate pool is smaller and more oxidized in lignifying spruce needle (more DHA) than in mature needles/(less DHA). However the average combination of ascorbate in the apoplasts of lignifying needles is still high enough to inhibit POD.

In summary, it is strongly evident that opposite MDA generated by AO activity, could have fundamental role in regulating cell expansion by affecting H^+ pumping. DHA may also have a role in reducing interaction between wall proteins and polysaccharides and could generate wall oxalate which might then influence free Ca^{2+} levels. So far the possible involvement of ascorbate and ascorbate oxidase has only been investigated in a limited number of systems and more work is needed to examine this hypothesis.

(d) Senescence

With ageing, an impairment of physiological functions occurs. This is called senescence. It is well known that senescence correlates with the loss of antioxidant capacity and consequently with an increase in ROS (reactive oxygen species) (Leshem 1988, Zimmermann and Zentgraf 2005). Photosynthetic organisms are particularly vulnerable to ROS since large amounts of O_2 are produced in the immediate vicinity of a powerful oxidation-reduction system, capable of reducing O_2 to O_2^- . In the light, the chloroplasts of higher plants produce ROS as a consequence of the transfer of high-energy electrons from reduced ferredoxin of the photosynthetic electron transport chain to oxygen instead of to NADP. This photo-reduction of oxygen in PS-I (Mehler reaction) and the overall transfer of electrons from water to molecular oxygen (Pseudocyclic electron flow) by which the plant is able to dissipate excess reducing power under conditions when CO_2 is limited (Alscher et al. 1997). In addition, studies of Kumari et al. (2011) suggests that UV-B generates oxidative stress in plant cells due to excessive generation of ROS and also reported that stimulation of activities of SOD, CAT, APX and GR observed at initial growth stage while the activities of CAT and SOD decreased at later stage but there are no definite trend of change observed for AA.

It is hypothesized that low levels of AA cause accelerated flowering and senescence under long-day conditions and delayed flowering and senescence under short-day conditions through alterations in phytohormone levels that are at least partially dependent on photoperiod. Yamane et al. (2011) suggests that treatment with AA suppressed both H_2O_2 accumulation and the changes in chloroplast ultra-structure which is in support of the fact that light-induced production of excess H_2O_2 under salinity is responsible for the changes in chloroplast ultra-structure. It is hypothesized that the low levels of AA cause GA deficiency and that the GA flowering pathway is favoured under short-day conditions, leading to delayed senescence. Also, GA induces stem growth in many rosette plants and dwarf mutants. This growth response can be quite dramatic and is the combined result of enhanced cell division activity in the sub-apical meristems and increased cell elongation. Moreover, under short days, senescence is delayed. By contrast, under long days, flowering and senescence are accelerated in *vtc1*. High SA content and decreased levels of GA may, in a photoperiod-dependent manner, result in early flowering and senescence under low AA conditions.

Oxidative stress has been demonstrated to promote the expression of SAGs. Treatment with AA, in addition, to silver nitrate abolished an increase in LSC54 and LSC94 expression. This suggests that AA is involved in the transcriptional regulation of some SAGs during oxidative-stress-induced senescence. More direct evidence for the role of AA in regulating the expression of SAGs is provided by a study that revealed an up-regulation of select SAG transcripts in the AA-deficient Arabidopsis mutant *vtc1* when grown under a long day photoperiod (Barth et al. 2004), suggesting that AA-deficiency induces a senescent phenotype. Pavet and co-workers concluded that the abundance of AA modifies the threshold for activation of plant defence responses via redox mechanisms that are independent of the natural senescence programme.

Functional role of ascorbic acid under abiotic stress**(a) Heavy metal stress**

Plants have a remarkable ability to take up and accumulate heavy metal from their external, for example, aquatic environment. Heavy metals such as Cd, Pb, Hg, Cu, Zn, and Ni at supra-optimal concentrations affect plants growth, development and yield (Woolhouse 1983, Sresty and Madhava Rao 1999). The presence of elevated concentrations of heavy metals in the growth medium of the germinating seeds suppresses mobilization and translocation of reserve materials from the reserve tissue to the growing regions and their subsequent utilization there (Mishra and Choudhuri 1997). Heavy metals stress results in the production of O_2^- , H_2O_2 and OH^- , which affect various cellular processes mostly the functioning of membrane systems (Foyer et al. 1997, Weckx and clijsters 1997). A regulated balance between oxygen radical production and destruction is required if metabolic efficiency and function are to be maintained in both optimal and stress conditions (Foyer et al. 1994). Most contamination of the aquatic environment occurs as a result of human activities and affects organisms at the biochemical, cellular, population and community level. Aquatic plants are primary producers of most aquatic food chains and account for much of the production base of fresh water and manure ecosystems.

Heavy metals toxicity is considered to induce the production of ROS and may result in significant damage to cellular constituents (Weckx and clijsters 1997, Vangronsveld and clijsters 1994) but, however, phenomenon of radical formation found to be least explored (Smirnoff 1995). The Zn and Ni induced oxidative stress in pigeon pea cultivars is evident from the increased lipid per oxidation in their roots and shoots (Madhava and Sresty 2000). A similar pattern of response together with an elevation in the photosynthesis is observed in the plants of mustard exposed to Cd through nutrient medium. Perhaps a constitutively high antioxidant capacity or increase in the levels of one or more antioxidants could prevent the oxidative damage and improve resistance to oxidative stress. AA is the best well known compound used for antioxidant and most effective compound which increases the tolerance of the 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, but in addition, it affects the physiological activities of the plants. Studies of Madhava Rao and Sresty (2000) suggest that Zn and Ni treatments decrease the AA contents of roots and shoots of the two pigeon pea cultivars. This decrease depends on the Zn or Ni concentration supplied and it is evident by the establishment of a significant negative correlation between AA content and increasing concentrations of externally supplied metals ions. The ROS are assumed to be involved in the oxidation of AA and to DHA leading to reduction in the AA content of plants (Fridovich and Handler 1961).

(b) Saline stress

Salt stress can affect several physiological processes, from seed germination to plant development and therefore act as a one of the most limiting factor to plant productivity (Bohnert et al. 1995). Salt stress is becoming even more prevalent as the intensity of the agriculture increases. Therefore, elucidation of the mechanism by which plant perceive and transduce this stress is critical if we are to understand plant response and introduce genetic or environmental improvement to stress tolerance. In previous studies, many plants species indicate that salt tolerance are developmentally regulated stage specific phenomena because tolerance at one stage of plant development is not necessarily correlated with tolerance at other stages (Johnson et al. 1992). The complexity of the plant response to salt stress can be partially explained by the fact that salinity imposes both an ionic and an osmotic stress (Pasternak 1987). Photosynthesis, a key metabolic pathway in plants, is a target for salt stress. The ABA produced in response to salt stress decreases turgor in guard cell and limits the CO_2 available for photosynthesis (Leung et al. 1994).

Foliar application of AA at 200mg/l counteracted the adverse effect of salinity that accompanied by significant increase in plant growth of flux cultivars (El-Hariri et al. 2010). AA has effects on many physiological processes including the regulation of growth and metabolism of plants under saline conditions and increasing physiological availability of water and nutrient (Barakat 2003). In addition, AA protect metabolic processes against H_2O_2 and other toxic derivatives of oxygen affected many enzyme activities, minimize the damage caused by oxidative processes through synergistic function with other antioxidants and stabilize membranes (Shao et al. 2008).

Studies of Hassanein et al. (2009) and Abd-El Hamid (2009) suggested that AA increase the content of IAA, which stimulates cell division and /cell enlargement and this, in turn, improve plant growth. Also, AA is more effective at 400mg/l and attributed to the increase in nutrient uptake and assimilation. Moreover, studies of EL-Hariri et al. (2010) also suggested the effect of salinity on decreasing chlorophyll synthesis which ultimately reduces the biosynthesis of carbohydrates and also, foliar application of AA counteracted the adverse effect of salinity, this accompanied by significant increase in plant growth, IAA contents, and yield components while decrease in total phenol contents. In addition, Shukry et al. (2007) also suggested that the percentage of fibers, cellulose% and cellulose/lignin% were decreased in flux grew in calcareous soil comparing with the central soil. Meanwhile, the increase in lignin% by increasing salinity levels are concurrent with the increase in total phenols and thus, also may be due to phenol which is used in biosynthesis of lignin (Shukry et al. 2007). Moreover, the gradual increase in lignin% due to salinity levels stimulates the generation of ROS, used for lignifications processes and function as signals in the defence to salt (Lee et al. 2007). Meanwhile, addition of AA and 400mg/l at normal conditions or under salinity levels caused significant increase in cellulose% and cellulose/lignin% and decrease in lignin % as compared with the control plants or the corresponding salinity levels. In respect to foliar spray of AA as 400mg/l caused an increase in fiber diameter compared with control plants or the corresponding salinity levels.

Moreover, AA can scavenge O_2^- and H_2O_2 non-enzymatically, and also takes part in APX mediated scavenging of H_2O_2 (Asada 1992) as well as also involved in the regulation of another important non-enzymatic antioxidant α -tocopherol (Hess 1993). H_2O_2 is toxic ROS and has deleterious effects in plant tissues (Sairam et al. 1998). Higher H_2O_2 accumulation and lipid per-oxidation in sensitive cultivars of pea and rice (Dionisio-Sese and Tobita 1998) have been reported earlier.

(c) Oxidative stress (Ozone stress)

In plant cells, it is not very clear how environmental stress induces the accumulation of ROS. ROS as ubiquitous messenger of stress responses likely play a signaling role in various adoptive processes. Plants can sense, transduce and translate ROS signal into appropriate cellular responses with the help of some redox sensitive proteins. The oxidative stress, during which large quantities of ROS like O_2^- , H_2O_2 , OH^- , peroxy radicals, alkoxy radicals and 1O_2 etc. are generated, is one of the earliest response of plant cells under various biotic and abiotic stresses and natural courses of senescence (Gapper and Dolan 2006). In fact, reaction involving ROS are an inherent feature of plant cells and contribute to a process of oxidative deterioration that may lead ultimately to cell death (PCD). Resources of ROS include leakage of electrons from ETS, decompartmentalization of iron (Fe) which facilitates generation of highly reactive OH^- , and also various biological reactions. The imposition of both biotic and abiotic stresses causes over production of ROS, which ultimately imposes a secondary oxidative stress in plant cells (Alscher et al. 1997). Degradation of membrane lipids, resulting in free fatty acids, initiates oxidative deterioration by providing a substrate for enzyme lipoxygenase, causing membrane lipid per-oxidation. Since lipid per-oxidation is known to produce alkoxy, peroxy radicals as well as 1O_2 , these reactions in the membrane are a major source of ROS in plant cells (figure 2).

AA play an essential role in plant growth and development, have been implicated in many physiological responses (El-Hariri 2010). As well as, it is a well-known anti oxidant and cellular reductant with an intimate and complex role in the response of plants to O_3 (Conklin and Barth 2004). However, little is known about the role of AA in the plant responses to oxidative stress induced by O_3 . It was shown that exogenous application of AA modified antioxidant enzymes such as SOD, CAT, GPX and APX and non-enzymatic antioxidants, such as tocopherols, carotenoids, glutathione etc in plants under different stress conditions and induced various defensive signaling mechanisms by interacting/stimulating the signaling of different plant hormones like SA, JA, ET, GA_3 and ABA etc. Moreover, it is clear from a number of studies that sensitivity of plants to O_3 is correlated with total AA levels, and that a first line of defense against the reactive oxygen species generated in the apoplastic space by O_3 is AA. For activity, AA must be in the fully reduced state. Therefore, both the rate of AA synthesis and recycling via DHA and MDHAR are critical in the maintenance of a high AA redox state. Active transport of AA across the plasma membrane is necessary to achieve reduction of oxidized AA by cytoplasm-localized reductases (Conklin and Barth 2004).

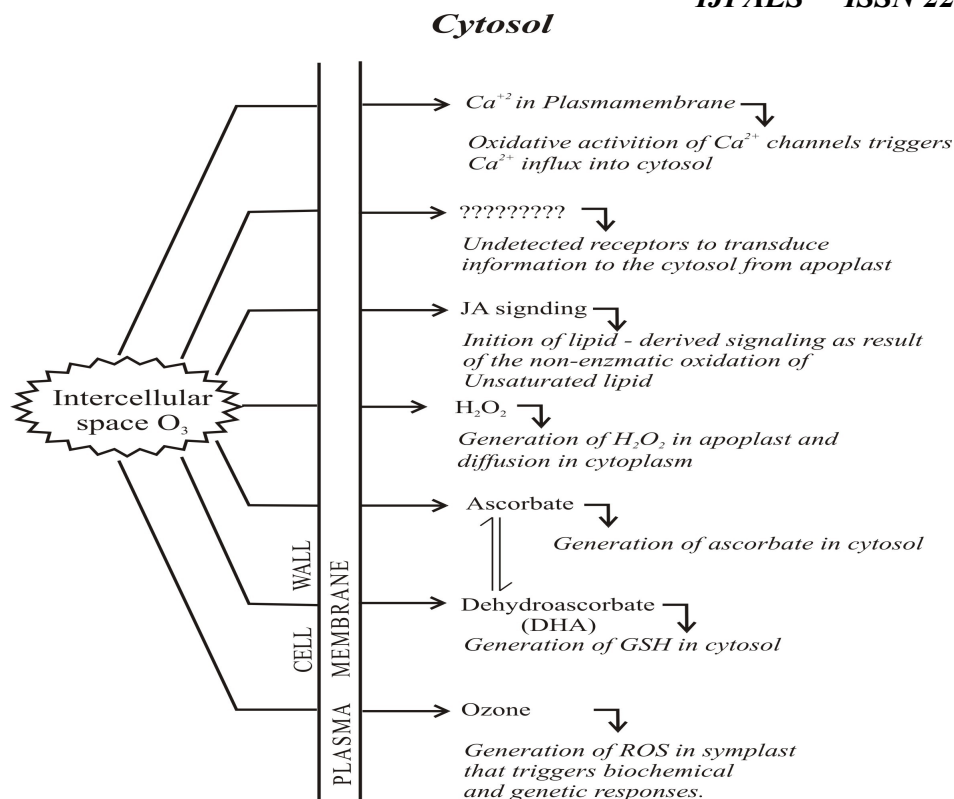


Figure 2. A model to demonstrate the complexity interaction between the deficiency of AA, light, ozone, pathogens, heavy metal stress and UV-stress can induce several plant defence pathways.

CONCLUSION

AA can act efficiently in plants as immunomodulators when applied at the appropriate concentration and the current stage of plant development. Ascorbate is implicated in plant responses to abiotic environmental stresses and to undergo profound changes in plants interacting with pathogens. AA regulated 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 few developmental phenomenon. Due to its essential function as a co-factor for the biosynthesis of GA, ABA, SA and ET, AA appears to influence not only the endogenous level but also signaling of these plant hormones, and thus affect responses against the abiotic environmental stresses. Also, redox status of AA may play a role in signaling of this interconnected phytohormone network. In addition, AA also open up new approaches for plant resistance against hazardous environmental conditions. However, there are obviously still large gaps to fill in order to elucidate the precise role of AA in enhancing the tolerance of plant to a number of environmental stresses during development of plant systems.

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