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Review article

ASCORBIC ACID: AN ENIGMATIC MOLECULE TO DEVELOPMENTAL AND ENVIRONMENTAL STRESS IN PLANT

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ABSTRACT: Ascorbic acid (AA) is present in all eukaryotes including animals and plants and lack completely in prokaryotes with exception of cyanobactaria. Today, AA has gained significant place in plant science, mainly due to its properties (antioxidant and cellular reductant etc.), and multifunctional roles in plant growth, development, and regulation of large spectrum of plant defense mechanisms against environmental stresses. 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 networks that ties together various developmental and environmental stresses. In the last few years, the role of AA in tolerance of plants to environmental stress has established much consideration. As it is evident from the present review, recent progress on AA potentiality in tolerance of plants to environmental stresses has been impressive. Indeed, AA also plays an important role in resistance to developmental programmes (stresses, e.g., bud induction, flowering and senescence) and environmental stresses (e.g., osmotic, temperature, pathogenesis and weedicides and herbicides).

Keywords: Oxidative stress, heavy metal stress, saline stress, UV-stress, herbicides and plant hormones.

INTRODUCTION

Structurally, L-AA is one of the simplest vitamins. It is related to the C_6 sugars, being the aldono-1, 4-lactone of a hexonic acid (L-gluonic acid) and contains an enediol group on C_2 and C_3. The stereo-isomer of L-AA, D-iso-ascorbic acid has little any anti-ascorbutic activity and should not be confused with D-erythro-ascorbic acid, which is the C_5 analogue of L-AA found in many yeasts and fungi. Vitamin C (L-ascorbic acid), fulfils essential metabolic functions in the life of animals and plants (figure 1). 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[1]. AA is a familiar molecule because of its antioxidant and cellular reductant properties, most aspects of its biosynthesis are very poorly understood. Still, 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[2].

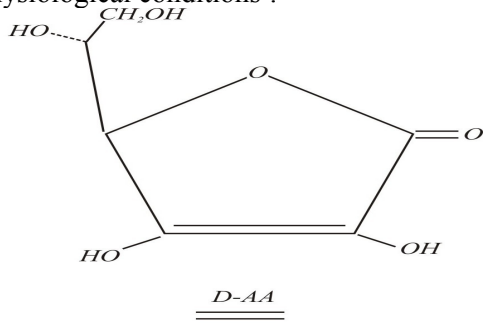

Figure 1. Dextro-rotatory form of ascorbic acid.

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[3]. 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 (phenypropanoid glycosides), AA is used as a reference compound[4]. Similarily, studies of Da Silva et al. (2011)[5] 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. 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[6]. 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 (Table-1).

Table 1. Physical properties of L-AA (Vitamin C)

Properties	**Comments**
Molecular formula	$C_6H_8O_6$
Molar mass	176.12 $gmoL^{-1}$
Appearance	White or light yellow solid
Density	1.65 g/cm^3
M.P.	190-192 °C/463-465 K/374-378 F
Solubility in water	33 g/100 ml
Solubility in ethanol	2 g/100 ml
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 $mgml^{-1}$), 2 (50 $mgml^{-1}$)
Spectral properties	
UV pH 2	Emax (1%, 1 cm), 695 at 245 nm
pH 6.4	Emax (1%, 1 cm), 940 at 265 nm

AA 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 H_2O_2, and many other, harmful to normal functioning of plant metabolism. In addition, it acts directly to neutralize superoxide radicals ($O_2^{\cdot-}$), singlet oxygen ($O^{\cdot-}$) or hydroxyl radical ($OH^{\cdot-}$) simply by acting as a secondary antioxidant during reductive recycling of the oxidized form of α-tocopherol[7]. L-AA serves as a co-factor for many enzymes[1] and it contributes to the detoxification of ROS[8]. This antioxidant activity of AA is associated with resistance to oxidative stress and longevity in plants. Moreover, the endogenous level of AA has recently been suggested to be important in the regulation of developmental senescence and plant defense against pests[9].

A recent plethora of evidences suggests that it may play a role in protection of plant against several environmental stresses such as metal action, salinity, weedicides, O_3, UV-B and pathogenesis[10]. The endogenous level of AA is determined by both *de novo* AA biosynthesis and recycling of the oxidized forms of AA, monodehydroascorbate (MDA) and dehydroascorbate (DHA) via MDA reductase and DHA redctase, respectively[8]. 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[6]. 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.

In recent years, there are remarkable progress has been made in the understanding of the biosynthesis of AA. Plant synthesizes AA via several distinct pathways including routes via L-galactose[11] and L-gulose[12]. Genes encoding the biosynthetic enzymes of these pathways have been identified[13]. It has been recently shown that AA biosynthesis may be regulated by jasmonates (JA)[14].

In addition, due to fact that AA also serves as an important co-factor in the biosynthesis of many plant hormones, including salicylic acid (SA), ethylene (ET), jasmonic acid (JA), gibberellic acid (GA_3) and abscissic acid (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. This will have profound effect to induce tolerance against environmental stresses and regulation of developmental processes including flowering and senescence. Furthermore, despite the correlation between environmental stresses and AA level in higher plants, the physiological rationale for such attention in AA levels is still not known. This review will highlight the recent advances in our understanding of how AA may regulate responses against developmental and environmental stress via a complex network of phytohormone signaling pathways.

Stress, ascorbic acid and plant hormones

Plant responses to stress can be viewed as being orchestrated through a network that integrates signaling 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 mitogen activated protein kinase (MAPK) cascade plays a key role in various stress responses and in phyto-hormone responses that include ROS signaling[15]. Molecular and genetic studies present the notion that the cross talk between AA and plant hormones includes alternation in the expression of hormones biosynthetic genes and/or signaling intermediates. Two of the numerous signaling molecules which integrated in the regulation of environmental stress response 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[16]. AtGA20ox1 is mainly expressed in the stem and inflorescence. Transgenic Arabidopsis express an antisense copy of AtGA20ox1, display a delayed flowering phenotype, but only under short-day conditions[17]. The delayed flowering phenotype of vtc1 under short day conditions may, therefore, be expressed be a possible deficiency in GA, because of limiting GA20-oxidase activity. In addition, a second GA biosynthetic enzyme, gibberellins-3-β-hydroxylase, also requires AA as a co-factor, and a deficiency in this activity could further contribute to a possible GA deficiency in vtc1. Kiddle and Foyer have evidences suggesting that vtc1 has a significant deficiency in GA_4[18,19], lending concrete support for this hypothesis. Moreover, high levels of ABA in vtc1, presumably caused by the up-regulation of the AA requiring ABA biosynthetic enzyme NCED dioxygenase, may contribute to the late-flowering phenotype under short days. This result seems somewhat supporting and contradictory, as the ABA biosynthetic pathway requires AA.

Moreover, ABA is known to act antagonistically to GA[20]. Thus ABA may indirectly contribute to the down-regulation of LEAFY, and hence to the late flowering phenotype. The exogenous treatment of AA serves as an important factor in biosynthesis regulation of tissue levels and therefore the signaling of various plants hormones including GA_3, ET, ABA, SA, cytokinin (CK) and JA. These all plant hormones collectively have a critical influence on the regulation of developmental processes including senescence, as both ET and ABA are known to promote senescence, whereas GA delays it[21]. SA and CK are also involved in regulating the developmental senescence process.

More direct evidence for the role of AA in regulating the expression of senescence associated genes (SAGs) is provided by a study that revealed an up-regulation of select SAG (senescence associated genes) transcripts in the AA-deficient Arabidopsis mutant vtc1 when grown under a long-day photoperiod[22], suggesting that AA-deficiency induces a senescent phenotype. However, the developmental senescence marker SAG12 is not induced, suggesting that other SAGs that play critical roles in cellular degradation and nutrient remobilization accumulates during senescence (e.g., SAG13 and SAG15)[22]. Similarly, it has been reported that SAG12 is not induced in response to O_3, a known promoter of senescence, whereas other SAGs are up-regulated during O_3 exposure[23]. Due to the fact that AA also serves as an important co-factor in the biosynthesis of many plant hormones, including ET, GA_3, and ABA. Now, it is well cleared that the endogenous level of AA will affect not only the biosynthesis, but also the levels and therefore, the signaling of these plant hormones, play a tremendously significant role in removal of a number of environmental stresses and management of several developmental programmes. In addition, the redox status of AA may play a role in signaling of the interconnected phytohormone network. However, there are obviously still gaps to fill in order to elucidate the precise role of GA_3, ABA and SA in AA regulated plant responses to developmental and environmental stress in plant.

The SA, and ubiquitous plant phenolic compound, was recognized as an endogenous regulator in many plant physiological processes[24]. In this context, Janda et al. (1999)[25] observed that SA pre-treatment at normal growth temperature induced protection against low-temperature stress in young maize plants, probably due to increased antioxidant activity. Results of El-Hariri et al (2010)[26] show that salinity levels showed a gradual significant increase in total phenol contents. This increase may be due to total phenols play a significant role in mechanism of regulation of plant metabolic processes and consequently overall plant growth as well as lignin biosynthesis[27]. Moreover, phenols act as a substrate for many antioxidants enzymes, so, it mitigates the salinity injuries[28]. Rivero et al (2001)[29] suggested that an accumulation of phenol compounds (e.g. SA) in response to abiotic stress would be attributed to activation of phenyl alanine ammonia lyase (PAL), would be beneficial to achieve acclimatization and tolerance to drought stress. Since many kinds of plant phenolic have been considered to the main lines of cell acclimatization against stress in plant.

In addition, evidence suggests that these hormones are perceived at the plasma membrane and that phospholipases and G-proteins are involved in the early signaling events. Down streams are Ca^{2+} and calmodulin, which, in turn, target various ion channels, protein kinases and phosphatases. Through these systems, GA_3 and ABA regulate the expression of a number of proteins in the aleorone layer, including α-amylase. However, GA can also promote cell death in this system[30], a protectant involves ROS, and notably H_2O_2 which is produced in glyoxysomes by the activity of a flavin-containing acyl CoA oxidase. ABA can present cell death by promoting high activities of enzymes that destroy ROS. Plant hormones GA_3 also play a vital role in the detoxification of heavy metal and in tolerance to salt stress by improving plant growth, chlorophyll synthesis and activities of antioxidant enzymes and by preventing lipid peroxidaton[31]. ABA biosynthesis increases when plant cells lose turgor, raising the question of which step in the pathway is activated by water stress. The promoters of the peroxidases respond to ABA and to redox signals[32]. APX is synergistically induced by ABA and oxidative stress[33], while other peroxidases (2CPA) is oxidatively induced by wounding and photo-oxidation. Stress via one MAPK2 but strongly suppressed by ABA via an antagonistically responding MAPK2[32].

Bud induction and flowering

L-AA is stable when dry, but solutions readily oxidize, especially in the presence of trace amounts of cupper, iron and alkali. The first oxidation product of L-AA is the MDHA. *In vivo*, it is reduced back to L-AA by activity of the NADP-dependent enzyme, MDHAR or by electron transfer. The rates of L-AA oxidation and DHA hydrolysis will be influenced by factors such as concentration, temperature, light, pH etc. One clearly defined function of L-AA is to modulate a number of important enzymatic reactions induced bud induction and finally, flowering or induced transition from the vegetative to the reproductive and to the final developmental stage, senescence, is of vital importance for the survival of flowering plants. Moreover, 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[34].

This will have profound effect to induce tolerance against environmental stresses and regulation of developmental processes including flowering and senescence. Flowering is controlled by the developmental age of the plant and environmental signals, including photoperiod, vernalization, light quality and the availability of water and nutrients. The strongly inductive long-day pathway that only operates in long day conditions involves photoreceptors and the circadian clock. The GA pathway is a more weakly inductive pathway and is essential in short-day conditions. Effort to determine the physiological role of GA and to elucidate the biosynthetic pathway have been greatly facilitated by the availability of GA-deficient (i.e., dwarf) mutants.

Metabolic studies have been conducted with systems that are rich sources of GAs, such as the fungus *gibberella fujikuroi* and immature seeds of pumpkin, pea and bean. However, maize is the only higher plant in which the entire biosynthetic pathway has been demonstrated in vegetative tissues by feeding various intermediates. The autonomous pathway is required in both long and short photoperiods. The vernalization pathway requires cold temperatures for flower development. Floral induction causes the up regulation of floral-meristems identity genes such as LEAFY. Although the strong correlation between flowering and seasons is common knowledge, the phenomenon poses fundamental questions for plant physiologists. The transition to flowering involves major changes in the pattern of morphogenesis and cell differentiation at the shoot apical meristems.

During vegetative development, the fates of the meristematic cells become altered in ways that cause them to produce new types of structures. This phenomenon is known as phase change. The events occurring in the shoot apex that specifically commits the apical referred to as floral evocation. There are two general categories of developmental states associated with floral evocation: competent and determined. The development signals that bring about floral evocation include endogenous factors, such as hormones, circadian rhythms, and external factors, such as day length (photoperiod) and temperature. The evolution of internal control systems enables plants to carefully regulate the timing of flowering. It is a critical for survival that plants flower at the optimal time for reproductive success (figure 2).

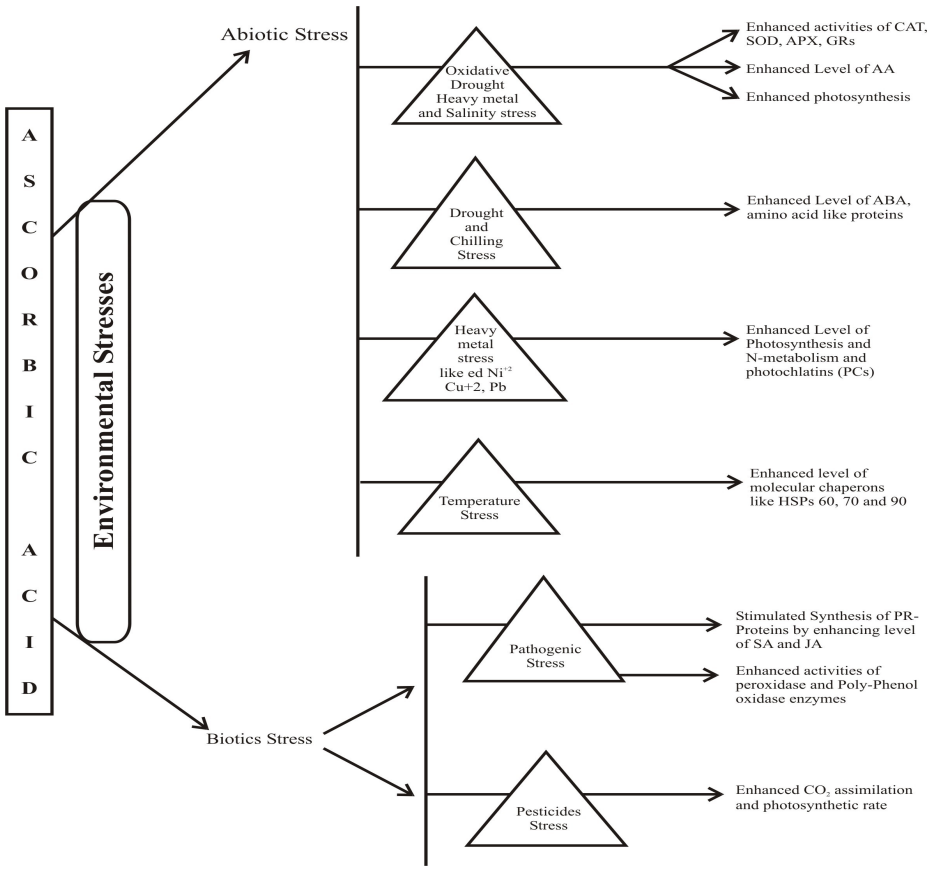

Figure 2 : Diagramatic representation of responses induced in response to various kinds of biotic and abiotic environmental stresses and their later consequences in plant system

Moreover, there are two independent lines of evidence suggest a role for AA in floral induction. When grown under long-day conditions (16h photoperiod), AA-deficit vtc1 mutants enter the flowering stage before the wild type[8], suggestive of an inhibitory effect of AA on the timing of floral induction. In addition, support of the involvement of AA in floral induction, many laboratories have very recently shown that elevation of AA content in Arabidopsis via feeding with the AA precursor L-galactono-1,4-lactone leads to an average delay of 5d in the time of flowering. Furthermore, expression of a LEAFY:GUS transgene in the vegetative apex of Arabidopsis is significantly delayed in plants pre-treated with L-galactono-1,4-lactone. As GAs activates the LEAFY promoter, these results suggest an antagonistic role for AA in LEAFY expression[20].

Moreover, among AA interacted plant hormones, GA has a strong influence on flowering. Exogenous GA can substitute for photoperiodic induction when applied to long day plant (LDP) that grows as rosettes in short days. In these plants, the flowering response is accompanied by elongation of the flowering stem. In addition, GAs application can evoke flowering in a few short day plant (SDP) in non-inductive conditions and can substitute partly or completely for a low-temperature signal in several cold-requiring plants. The delayed flowering phenotype may be explained by a defect in the GA-mediated flowering pathway that induces flowering under short days through the induction of LEAFY. The delayed flowering phenotype of vtc1 under short-day conditions may, therefore be explained by a possible deficiency in GA because of limiting GA_{20}-oxidase activity. In addition, a GA biosynthetic enzyme, gibberellins-3-β-hydroxylase, also requires as a cofactor, and a deficiency of its activity could further contribute to a possible GA deficiency in vtc1.

Finally, high levels of ABA in vtc1 presumably caused by the up-regulation of the AA-requiring ABA biosynthetic enzyme NCED dioxygenases, may contribute to the late-flowering phenotype under short days. This result seems somewhat surprising and contradictory, as the ABA biosynthetic pathways require AA.ABA is known to act antagonistically to GA. Thus ABA may contribute to the down-regulation of LEAFY and hence to the late-flowering phenotype.

Furthermore, AA may play an important yet indirect role in floral induction because of its necessity for GA and ABA biosynthesis. ABA delays flowering via its binding to FCA. In vtc1, this predicted decline in active FCA-FY due to elevated ABA could lead to an increase in FLC transcripts and therefore a delay in flowering. However, the level of ABA under long day grown vtc1 has not been determined so invoking an involvement of ABA would be pure supposition. However, recent experiments support a regulatory role of SA in flowering. Some authors also suggest that SA may regulate flowering phenotype both under short and long days. Unlike short-day conditions, SA may regulate flowering time in a photoperiod-independent but FLC-dependent pathway. Given these intriguing results, one might speculate that the high levels of SA present in vtc1 grown under long days[22], promotes early flowering via the photoperiod yet co-independent pathway regulated by SA. Whether or not SA affects the delayed flowering phenotype of vtc1 under short day conditions is not clear. Thus, AA may regulate the floral induction via a network of phytohormone signaling pathways.

Senescence

Senescence is an impairment of physiological functions associated with the loss of antioxidant capacity and consequently with an increase in ROS[35]. 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 Photo system-I (PS-I) and the overall transfer of electrons from water to molecular oxygen by which the plant is able to dissipate excess reducing power under conditions when CO_2 is limited[36]. According to the free radical theory of ageing, accumulated damage caused by oxygen radicals in cells and eventually, organs to stop functioning. As a result, damage of the photosynthetic apparatus would be increased, leading to a faster decline in photosynthetic activity in AA-deficient tissue and thus accelerate senescence. According to programmed senescence theory, ageing is the result of the sequential switching on and off of certain genes, with senescence being defined as the time when age-associated deficits are manifested.

A possible role of ROS in the induction of senescence raises another important question that is still a matter of debate. Can senescence be considered a form of apoptosis or PCD[35]? If ROS are mainly involved in the induction of cell death, but not in developmental senescence, then that could possibly explain the reported phenotype by Pavet et al (2005)[9]. Leaf senescence is triggered by a decline in photosynthetic process.

However, alterations in photosynthetic activity, antioxidant capacity and the amount of ROS produced have been reported neither under long nor under short-day conditions. Hence, it appears that the flowering and senescence prototypes linked to the endogenous AA content are not connected to changes in photosynthetic capacity. It is possible that AA deficiency causes very subtle and gradual changes in these processes that may be difficult to detect. Moreover, 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. Furthermore, 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.

In addition, during senescence, cell undergoes highly co-ordinate changes in cell structure, metabolism, and gene expression. In the early stages of senescence, chlorophyll degrades and photosynthetic activity decreases due to a decrease in the expression of, for example, the small subunit of Rubisco and chlorophyll a/b-binding (CAB) genes. These genes are referred to as senescence-down-regulated genes. Thus, senescence is characterized by a decrease in chlorophyll as well as in the content of total RNA and protein. The early phase of senescence is followed by an up-regulation of senescence-associated genes (SAGs) that aid in the remobilization of biomolecules. 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[22], 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 program. At the terminal phase of senescence, plants lose antioxidant capacity and the release of ROS increases[35]. At this final stage of senescence, cells undergo lipid peroxidaton, DNA degradation and the nuclei, mitochondria, membranes and vacuoles distingrates.

Environmental stresses: Abiotic and biotic

(a) Osmotic stress

While the complex field conditions with its heterogeneity conditions, abiotic stresses combinations and global climatic changes are but a few of the challenges facing modern agriculture. Abiotic stress conditions such as drought heat or salinity induced dehydration constitute direct osmotic stress and thus, causes extensive losses to agricultural production worldwide. Whereas chilling and hypoxia can indirectly cause osmotic stress via effects on water uptake and loss. Plants respond to dehydration and low temperature with a number of physiological and developmental changes. Plants have evolved a high capacity to synthesize and accumulate non-toxic solutes (osmoprotectants). For examples, proline, glycinebetaine, mannitol and trehalose (figure 3). Drought and more salinity cause plants to produce high levels of ABA, exogenous application of ABA also induces a number of genes that respond to dehydration and cold stress. Nevertheless, the role of ABA in low temperature-responsive gene expression is not clear. Several reports have described genes that are induced by dehydration and low temperature. It is likely, therefore, that both ABA-independent and ABA-dependent signal transduction cascades exist[37]. Progress in generating transgenic crops with enhanced tolerance to abiotic stresses has nevertheless been slow.

Moreover, despite the high turnover of AA[38] intracellular levels of AA are in the mili molar (mM) range. A combination of *de novo* synthesis and efficient AA recycling via reductases are obviously important in maintenance of this high AA concentration. Realization of the importance of this *de novo* synthesis led to the recent elucidation of the major AA biosynthetic pathway in plants[11]. This pathway includes the intermediates GDP-mannose and galactose and has been supported in part by the identification of the Arabidopsis thaliana vtc1 gene which encodes a GDP-mannose pyrophosphorylase.

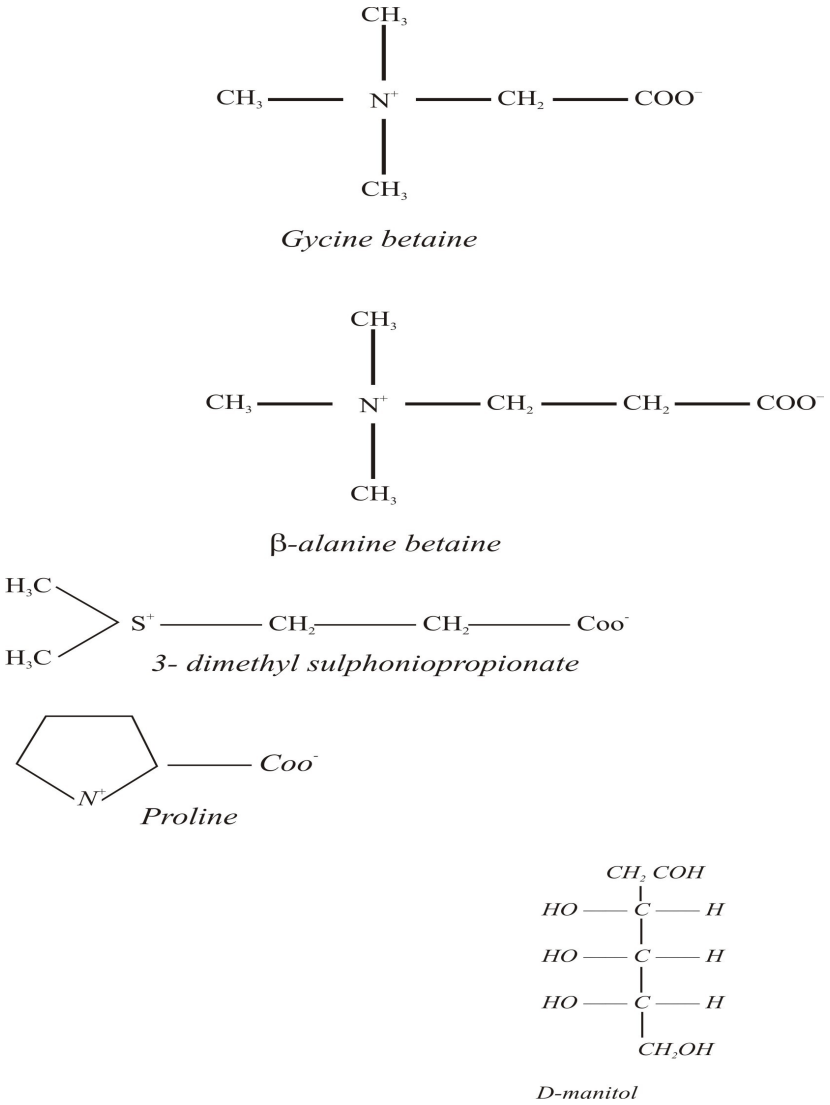

Figure 3. Molecular structures of various osmoprotectants formed during osmotic stress in plants

Imamul-Haq and Larher (1983)[39] observed that leaves of plants subjected to water stress often show a decrease in starch, which is generally accepted by an increase in reducing sugar content. When NaCl is provided in low concentrations (40 and 80mM), more soluble proteins are synthesized in shoots and roots of wheat plants than when the plants are treated with higher concentrations (160mM) of NaCl. Moreover, soaking of grains in AA exhibited a favourable effect on the accumulation of soluble proteins and ameliorated the inhibitory effects of high salinity concentrations on soluble proteins accumulation in shoots. In roots, AA application inhibits production of soluble proteins. The results of Al-Hakimi and Hamada (2001)[40], and those obtained by other[41] are indicative of the extent to which sugar and nitrogen metabolism in plants cells is affected by salinity stress. The remarkable responsiveness in the biosynthesis of sugars and proteins to the presence of AA may be taken as a further evidence of the role played by the AA in adaptation mechanisms of plants. It is generally assumed that stress-induced proteins play a role in stress tolerance, which may be essential for the survival of plants under extreme stress conditions[42].

Temperature stress

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 photosynthesis 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 by AA/DHA and GSH/GSSG ratio may protect the thio-modulated enzymes of the Benson-Calvin cycle (C-3) from oxidation by H_2O_2 and allow photosynthesis to proceed at relatively high rates even during oxidative stress. Moreover, it has recently been argued that DHA detected in extracts is artefactual and that *in vivo* levels are much lower or negligible[43]. This connection is principally supported by the observation that inclusion of DHA in enzyme assays, at concentration, thought to exist in the chloroplast *in vivo*, led to oxidation inactivation of the two enzymes known to be regulated by the thioredoxin system[43]. The authors therefore, concluded that significant formation of DHA *in vivo* must be avoided[43]. It is well established that the soluble stromal enzymes regulated by the thioredoxin system require ongoing reduction to remain active[44]. Data obtained by addition of oxidants to enzymes removed from the light and membrane dependent thioredoxin reduction system lack any relevance whatsoever to *in vivo* condition, where the activation state of thiol-regulated enzymes will reflect the differences between reductive and oxidative fluxes (figure 2).

A plethora of evidence indicates that ROS are involved in the DNA damage, cell death and signal transduction[45]. ROS generated under UV stress especially $OH^{\cdot}$, $^1O_2$, react with sugars, purines and pyrimidines[46]. In addition, studies of Kumari et al. (2010)[47] 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. Yamane et al. (2011)[48] 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. He and Hader (2002)[49] detected a significant decrease in dsDNA levels under UV-stress and their studies also indicate the elevated formation of ROS and oxidative stress from UV-B treatment. Under moderate UV-B radiation, blue green algae (BGA) thrived to survive and the damage caused which did not result in lethal effects on all of the organisms, although a significant loss in survival occurred. The protective effect of AA indicates that survival of the irradiated organisms by UV-B is associated with the extent of DNA damage[49]. Previously, DNA damage is considered to be closely related to or a result of membrane lipid peroxidation[50]. However, no direct evidence is observed in study of He and Hader (2002)[49] in respect to lipid peroxidaton and DNA damage.

Furthermore, the exogenous application of AA is confirmed to demonstrate the protective effects against the UV-B irradiation[51]. The addition of AA decreased the ROS levels detected by DCF fluorescence. In particular, AA did not demonstrate a significant higher ROS scavenging efficiency than other antioxidants such as NAC during the starts of the irradiation while there was a significant higher antioxidant effect from AA than that of NAC after 24h of irradiation[49]. AA is involved in the *in vivo* ROS scavenging action in multiple ways, i.e. the direct scavenging or via the ascorbate-glutathione cycle[52]. Also, the exogenous added AA was taken up significantly and no efflux of AA occurred during the uptake of AA as demonstrated by [^{14}C]-labelling in potato leaves[53]. Studies of Hu and Shih (1997)[54] suggests that AA inhibits lipid per-oxidation but enhanced DNA damage in rat liver nuclei incubated with Fe but, studies of He and Hader (2002)[49] demonstrated that AA was capable of inhibiting both the lipid per-oxidation and DNA strand breaks significantly. The UV-B induced damage to photosynthesis also inhibited significantly by the addition of AA (1mM).

Hebicide and weedicides stress

Pesticide may be a chemical substance or biological agent (such as a virus or bacteria) used against pests including insects, plant pathogens, weeds, molluscs, birds, mammals, fish, nematodes and microbes that compete with humans for food, destroy property, spread disease or are a nuisance. Modern pesticides are designed to ensure the effectiveness against target organism. Many pesticides have been developed to target specific biochemical reactions within their target organism. However, it is of great concern to understand their effects on non-targeted crops they are meant to protect. In addition, Pesticides are widely used in agriculture to protect seeds and crops. As their persistence in the geosphere may cause problems in the biosphere, their presence in soil and water is continuously monitored.

According to EU regulations, concentration limits in drinking water are 0.1µg/l for a single pesticides and 0.5µg/l for mixtures. Current standard procedures for pesticides determination in soil and water samples (HPLC and GC/MS) are accurate but rather time-consuming and beyond the analytical capacities of smaller water works as they require expensive instrumentation. Consequently, immunological methods are being widely investigated as an alternative analytical procedure and in fact, some cases have already been successfully adopted to environmental analyses[55]. Enzyme-linked immunosorbent assay (ELISA) kits are now commercially available for a wide range of environmentally important compounds such as pesticides, polyaromatic hydrocarbons (PAH), halogenated aromatic compounds and explosives[56].

AA is reported to be effective in reducing damage caused by pesticides. AA along with other two chemicals viz., thiourea and thiamine capable of potentiating-SH turnover and improve growth and productivity of maize[57]. It is further noted that foliar spray of thiourea (1000ppm) and AA (100 ppm) significantly increased leaf area index (LAI), number of green leaves plant^{-1} and biological yield ha^{-1} in maize. This improvement appeared to have resulted from increased biosynthetic efficiency and conopy photosynthesis on account of the biological activity of -SH group[57]. Phytotoxic effect on cucumber leaves of nine pesticides, including three herbicides (Paraquat, fluzifop-pbutyl, and Haloxyfop), three fungicides (flusilazole, cuproxat and cyazofamid) and three insecticides (imidacloprid, chlorpyrifos, and abamectin) has been examined. Plants treated with paraquat showed the severest phytotoxic symptoms with the highest reduction in net photosynthetic rate (Pn), while other pesticides except flusilazole inhibited Pn to various degrees. The inhibition of Pn by cuproxat is accompanied by declines both in stomatal conductance (gs) and intercellular CO_2 concentration whereas decreased Pn for the cyazofamid is associated with increased intracellular CO_2 concentration is increased or unaffected. Interestingly, inhibitions of Pn are alleviated by AA pre-treatment, as for the pesticides except paraquat and flusilazole. Chlorophyll a (Chl) level, chl a/chl b ratio, photosynthetic rate (Pn) and transpiration rate (Tr) significantly increase, whereas chl b and stomatal conductance (gs) have no obvious change after exogenous AA treatment.

In addition, pesticides application impairs the photosynthesis of cucumber seedlings, while AA pre-treatment can increase the resistance of plants to pesticides, which might be mediated by enhanced activities of CO_2 assimilation. Organophosphate enhanced the rate AA of oxidation in aqueous solution and shows antioxidant activity. Results of Rehfeld and Pratt (1969)[58] indicates that the phosphate moiety of malathione (an organophosphate) is responsible for antioxidant activity and, it might be due to the phosphate's ability to chelate metabolism (Cu^{2+}). The physiological role of the pyridine nucleotide monodehydroascorbate acid reductase (MDHAR) and of the ascorbic acid-monodehydroascorbate system in photosynthesis is indicated by the findings that the illumination of chloroplast suspensions supplemented with AA and an AA oxidising agent markedly slow down the role of AA oxidation and thus, supporting the hypothesis of a participation of AA, as an electron carrier in the oxidative-phosphorylative chain functioning in the illuminated chloroplast[59].

Moreover, some herbicides, commonly called weed killers, are a type of pesticides used to kill unwanted plants. Selective herbicides kill specific targets while leaving the desired crop relatively unharmed. Some of these act by interfering with the growth of the weed and are often synthetic "imitations" of plant hormones. They are widely used in agriculture and in landscape turf mangement. In the U.S., they account for about 70% of all agricultural pesticides use. Plants have developed resistance to Atragine and to ALS-inhibitors and more recently, to glyphosate herbicides. Marestail is one weed that has developed glycophosphate resistance. Also, herbicides application to potato fields limits harmful effects of weeds. However, they can have some effects on changes in the chemical composition of potato tubers[60]. From the aspect of consumption, AA is one of the most important constituents of a potato tuber. Therefore, it make table potatoes as the cheapest and commonest source of AA[61]. Studies of Zarzecka and Gugala (2003)[62] suggest that the application of herbicides to potato fields caused an increase in the content of AA in comparison with the control. The statistical analysis showed a significant effect of potato cultivars on AA concentrations.

Furthermore, a significantly higher concentration of AA is determined after application of some herbicides as sencor 70WG, sencor 70WG+ Fusilade super and Basagram600 SL+Focus ultra. Some authors[63] reported positive effects of pesticides on the content of AA.

Hamouz et al (1996b)[64] did not find any significant influence of cultivation methods on AA concentration. Mostly herbicides kill plants (weeds) by blocking PS-II of photosynthesis like paraquat. As AA has a critical role in photosynthesis, as the high concentration in stroma of chloroplast (10-25mM)[65] 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 H_2O_2 that are found during photosynthetic activity[66] and against photo-inactivation; Since it is a cofactor of carotenoid-de-epoxidation[67]. Also, AA in photosynthesis works in its three biochemical modes. Firstly, it acts as an antioxidant by removing H_2O_2 formed by oxygen photoreduction in PS-I (Mehler reaction), catalysed by APX, and some of which is bound to thylakoids where it can scavenge H_2O_2 as it forms. Secondly, MDA, formed by APX can act as a direct electron acceptor to PS-I. Thirdly, it is a cofactor for violaxanthin de-epoxidase. The de-epoxidase, which is bound to the lumen side of the thylakoid membrane, is dependent on ascorbate as a cofactor[68]. Ascorbate thus contributes to electron flow and to formation of zeazanthin, which act as a photoprotectant (figure 2).

Pathogenic stress

AA acting simply as an antioxidant in the apoplastic space, but however it is to be involved in a complex phytohormone mediated signaling network that ties ozone and pathogen responses and influences the onset of senescence. Now it has become increasingly clear that AA function is intertwined in a complex network that meshes the plant response to pathogens and the onset of senescence. Acute exposure to ozone (250 p.p.b.) for a short time period cause necrotic lesion on leaves and induce plant reaction that resemble the hypersensitive response (HR), suggesting similarities between ozone and pathogen induce responses[69]. HR is a form of PCD in plant and is considered a part of a complex defense response to microbial pathogens in which death of host cells at the site of a virulent pathogen entry occurs within a few hours of pathogen attack (incompatible interaction i.e. plant is resistant to the invading pathogen)[70]. Pathogen-induced HR is a rapid oxidative burst at the site of microbial infection. ROS production during HR (and recognition of the invading pathogen), are mediated by a NADPH oxidase localized in the plasma membrane[71]. Results of Dias et al. (2011)[72] confirmed that AA is the main precursor of oxalic acid in susceptible and resistant cocao (*Theobroma cocao* 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)[73] 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)[74] 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)[75] 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.

Generally, ROS formed during HR[76] activate ET, SA and JA signaling pathways, serve to induce defense gene expression to counteract the invading pathogen and to minimize lesion formation in plants exposed to oxidative stress like O_3-ET synthesis and emission increase in plants exposed to O_3[77]. ET triggers PCD in the accelerated cell death mutant (acd1)[78] and is involved in regulating PCD in plant pathogen interaction[79]. SA signaling is regulated for O_3-induced cell death responses[80], induction of pathogens related proteins (PR)[81] and SAR (SAR increased to subsequent infection by a broad range of pathogens)[82]. JA biosynthesis is induced in O_3 treated Arabidopsis and hybrid poplar plants within several hour of treatment[83]. Treatment with JA has been shown to reduce the extent of cell death in tobacco[84]. Shan et al. (2011)[85] 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*. Arabidopsis mutants constitutively expressing JA, such as CEV1 are mere resistant to powdery mildew[86].

These plant hormones do not act independently in response to O_3 and/or pathogens but rather in a complex signaling network.

Moreover, studies of Conklin and Barth (2004)[8] evidence that AA involved as a cofactor in the synthesis of ABA, GA, ET and AA-dependent dioxygenases are involved in ABA biosynthesis. Specifically, NCED, a dioxygenases catalyzing the formation of Xanthoxin, the precursor of ABA, can be activated before addition of AA and Fe^{3+} [87]. ABA has been demonstrated to induce PR genes in several other plant species, such as in rice[88] and in Lithospermum[89]. AA is also strictly required by some enzymes that are involved in GA biosynthesis. A role of GA in pathogen defense has been suggested, for example, in tomato and in arbuscular mycorrhizia plants of *linum Usitatissimum* when infected by fungal pathogens[90]. ET like SA, ABA and GA also plays a role in the pathogen response and specifically in the induction of PR genes[91]. In ET biosynthesis, AA is required for 1-aminocyclopropane-1-carboxylate (ACC) oxidase that forms ET[92] (figure 2).

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 during developmental programmes and interaction with abiotic and biotic type of environmental stresses. 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 phenomenons. 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 biotic and 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 like senescence and bud induction and flowering.

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