

AN OVERVIEW OF ASCORBIC ACID DEFENCE EFFECT IN PLANTS AGAINST DISEASES-RELATED PATHOGENS AND POLLUTANTS

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ABSTRACT

Ascorbic acid (AA) is a powerful antioxidant found in high concentrations in many plants. AA acts as the initial protective barrier against potentially harmful reactive oxygen species (ROS), shielding plant cells from various external variables that might cause oxidative stress. Some of these stressors include pathogen infections, wounding, ozone exposure, and excessive salinity. Yet, vitamin C is more than just an antioxidant; it is also a cofactor for many enzymes involved in plants' metabolic processes. The production of AA in plants as an intermediate defence against invading pathogens at diverse places in plants and its interconnected network involves alterations in the nuclear expression of genes through retrograde signalling pathways, as well as systemic responses, which are all related to disease resistance. On another side, ascorbic acid was found to significantly impact hazardous environmental pollutants by inhibiting the formation of reactive oxygen species (ROS). This event is responsible for oxidative stress in cells. This analysis shows that there has been significant recent development on the potential role of AAs in plants' tolerance to pathogenic infection and pollutants.

KEYWORDS: Ascorbic acid, ROS, defence response, pathogen resistance.

INTRODUCTION

There is a vast diversity of disease microbes that plants can be exposed to (viruses, bacteria, fungi, and insects). They adapted intricate molecular pathways and strategic plans to combat and fend off this invaders.^[1] When plants are attacked by pathogens, they respond by expressing a large number of genes involved in protection, both in the plant's innate defence mechanisms and its induced resistance mechanisms. Rapidly generating reactive oxygen species (ROS) is one such response.^[2] Antioxidant mechanisms in plants have adapted to counteract the damaging effects of ROS. Ascorbic acid and other water-soluble antioxidants are included in this category.^[3]

Vitamin C (L-ascorbic acid) appears to be a crucial molecule in all known eukaryotic organisms.^[4] It helps prevent cell damage, is essential for enzymatic activity, and is required to produce oxalate and tartrate. In addition to playing a role

in photosynthesis, photoprotection, cell wall development, and cell elongation, tolerance to changes in the environment, it also plays a role in a number of other activities like ethylene, gibberellin, abscisic acid (ABA), anthocyanin, and hydroxyproline biosynthesis.^[5] Additionally, it has been proposed that endogenous ascorbic acid (AA) plays a role in controlling developmental senescence and disease resistance in plants.^[6] Moreover, The antioxidant activity of AA is linked to protection against oxidative stress and increased life span in animals and plants alike.^[7] It has been found that AA is involved in a wide variety of enzymatic processes within the plant cell. The enzyme violaxanthin dehydrogenase (VDE) uses it as a cofactor in the process of converting violaxanthin to zeaxanthin in response to light, which in turn acidifies the thylakoid lumen.^[8]

Still, it is unclear how ascorbate is metabolized in plants, despite the fact that it is considered the primary provider of vitamin C in the food consumed by individuals (who have no ability to produce it). Lately, a metabolic pathway has been described, which provides new insight into the metabolic activities and function of ascorbate in plants.^[9]

1. Pathogenesis-related interaction between ascorbic acid and plants

Based on molecular and genetic investigations, it has been suggested that AA communicates with several plant hormones by shifting the expression of genes involved in the biosynthesis of hormones and signalling pathways. Many mutants with varying amounts of AA have been developed better to understand their function in plants' defensive mechanisms.^[10] During pathogen attack, it has been hypothesized that a rise in AA levels will cause a change in the oxidative state of the AA pool.^[11] According to some studies, AA is a valuable stress indicator in cellular stress settings since it can be altered in response to both microbial pathogens and therapeutics using disease resistance inducers.^[12]

Several defence strategies of ascorbic acid are listed below:

A. Ascorbic acid-Related Redox Status

The redox state associated with AA may affect the expression of crucial defence components following pathogen attacks. Among them, the regulation of defence-hormone signalling pathways and the stimulation of pathogenesis-related (PR) genes after an alteration in the nonexpressor of Pathogenesis-Related protein 1 (NPR1) regulatory transcription factor configuration transitional form (S-S to SH-SH).^[13]

B. Supporting Cell Walls

Many wall-associated defensive mechanisms are expressed in plants when pathogens seek to penetrate plant barriers for colonization. The three cell wall-related protective mechanisms that are the most well-known are: the suppression of fungal enzymes that break down cell walls, the release of fungi toxic peptides, and the hardening of the cell wall through the lignification mechanism.^[14] Lignification of cell walls is a multistep concept that only higher plants go through; it primarily reinforces the plant's vascular system.^[15] Based on previous research, Huckelhoven (2007) concluded that the apoplastic AA pool played a significant role in lignification following pathogen recognition.^[14]

C. Defense-Hormone Signaling Pathway Modulation

Hormones play an essential role in how plants react to environmental challenges. Complicated hormonal signalling pathways regulate plant defence responses.^[16] Plants use four hormones, Salicylic acid (SA), jasmonic acid (JA), ethylene (Et), and abscisic acid (ABA), to respond under biotic stress.^[17] Typically, SA controlled defence-signalling to numerous biotrophs, while JA and Et were engaged in response to necrotrophs. It has been suggested that AA plays a significant part in producing many plant hormones, such as (Et) and (GA), as well as cell wall glycoproteins and

secondary compounds that include antimicrobial characteristics. Also, AA's availability and redox status may affect JA signaling.^[18]

D. Protective Effects Against Diseases

For various pathosystems, AA was discovered to be a disease-resistance-inducing factor.^[19] Researchers found that AA was effective against a wide variety of infections, not just viruses. As an illustration, the use of AA on the rice blast fungus *Magnaporthe oryzae* led to a reduction in the proportion of typically forming appressoria.^[20] According to research published in 2016, citrus plants exposed to 600 μM of AA applied exogenously were resistant to Huanglongbing (HLB), the most destructive disease generally in greenhouse and field environments.^[21] Botanga et al. (2012) reported that using AA directly influenced the hyphal growth of *Alternaria brassicicola*, suggesting that AA may have effective antifungal properties.^[22]

1.1 Applications of ascorbic acid and its derivatives against diseases-related pathogen

A. Ascorbic Acid Rescues Soybean Plant Development from Pathogen-Induced Oxidative Stress

Macrophomina phaseolina (Tassi) Goid, a soil-borne fungus responsible for a wide variety of plant diseases, is also responsible for the devastating charcoal rot of soybeans (*Glycine max* (L.) Merr.).^[23] Symptoms of wilting are brought on by *M. phaseolina* because this pathogen clogs the vascular pathways, triggers the synthesis of plant degradative enzymes, and ultimately leads to phytotoxin-mediated necrosis.^[24] Since *M. phaseolina* is a hemibiotroph, the biotrophic phase is essential for *M. phaseolina*'s primary invasion and growth, and the necrotrophic stage is necessary for the pathogen's continued preservation.^[25] Plants produce reactive oxygen species (ROS) as a defensive mechanism responding to abiotic and biotic stressors.^[26]

Recent studies suggest that ascorbic acid, an H_2O_2 scavenger, a type of reactive oxygen molecule, can reduce pathogen-mediated senescence in soybeans by protecting them from oxidative stress.^[27] The oxidized dehydroascorbic acid (DHAA) and reduced (LAA) forms of ascorbic acid were investigated as ROS scavengers to evaluate their ability to decrease ROS-induced impacts in planta during an investigation into pathogen-induced ROS-mediated senescence in soybean.^[22] The results of their research showed that the growth of the fungus was hindered and limited. It is possible to draw the conclusion that more significant amounts of ascorbic acid are both poisonous and inhibiting to the growth of fungi.^[22] It has been demonstrated that ascorbic acid may effectively scavenge reactive oxygen species (ROS) that are produced in response to abiotic stress in plant systems.^[26] For instance, applying exogenous ascorbic acid effectively preserved both proteins and lipids from the effects of dehydration and salt damage.^[28] In addition to vitamin E, ascorbate, a potent antioxidant molecule, has demonstrated promise ROS neutralizing properties via both its immediate and subsequent activities (enzyme catalysis).^[29]

Furthermore, researchers have reported that transgenic plants with elevated amounts of ascorbic acid had lower rates of senescence due to *P. infestans* infection because the gene expression of gibberellic acid and abscisic acid hormones, involved in plant defence, were better regulated.^[30]

B. Tomatoes resistant to Fusarium wilt after seed bioprimering with ascorbic acid.

As a parasitic fungus, *Fusarium wilt* poses a significant challenge to tomato yields and product safety. Tomatoes afflicted with *Fusarium wilt* have drastically reduced germination and growth rates.^[31] This may have been caused by a fungal pathogen's aggressive invasion on the plant, which interfered with its physiological system. Tomato plants that

have been challenged by the fusarium wilt pathogen have been shown to experience a decrease in development and productivity in a number of previous investigations.^[33,32] A developing method to improve seed germination in adverse environments is seed biopriming.^[34] However, some research shows that under both conventional and pathogen stress circumstances, the frequency of implantation, shoot and root length, fresh and dry weight, and photosynthetic pigments were all raised after combined treatment (*Trichoderma asperellum* BHU P-1) and (*Ochrobactrum* sp. BHU PB-1) with ascorbic acid.^[35] Gallie (2013) claims that ascorbic acid and other plant growth regulators play a crucial role in resistance mechanisms during the plant infection stage and stressful environments through a process known as "seed priming".^[36]

C. Ascorbic acid acts as a protection against the turnip mosaic virus.

Arabidopsis thaliana and the economically significant members of the genus *Brassica* are susceptible to infection by turnip mosaic virus (TuMV; family Potyvirus).^[37] Several development changes have been recognized in virtually all plant organs affected by Turnip mosaic virus (TuMV) infections.^[38] The inflorescence's primary stem's growth and erectness, as well as the inflorescence's pattern of branching, were significantly modified at varying rates.^[39] Virus infection is one example of biotic stress that might raise total ascorbic acid (TAA) levels.^[12]

It is interesting to notice that TAA functions like two distinct categories of antimicrobial molecules: a phytoanticipin, which builds up to a particular level during normal circumstances, and a phytoalexin, which is generated in reaction to pathogen infection.^[40] On the other hand, an excessive level of endogenous ascorbic acid (AS) may prevent the development of defence responses regulated by H₂O₂, which results in plants being more vulnerable to attack by pathogens. Because of this, many studies believe that the endogenous levels of TAA need to be closely managed for plants to be able to activate their defence mechanisms appropriately based on the sort of the pathogen.^[40]

D. Septoria Tritici Blotch (STB) control by ascorbic acid

Wheat is highly susceptible to septoria tritici blotch (STB), a devastating foliar infection caused by *Mycosphaerella graminicola*.^[41] It has a severe impact on wheat yields in areas with excessive relative humidity and cooler climates. The principal technique for controlling this disease over the past decades has been the utilization of fungicides (azoles and strobilurins).^[42] Still, since no totally tolerant wheat cultivar is now present, tolerance to these fungicides has emerged and spread rapidly. Among the most common wheat infections, STB has been shown in a previous study to be affected by AA by blocking the action of fungal CWDEs like -1,4-endoxylanase, -1,3-endoglucanase, and protease; and reducing the rate the invasion and lowers the aggressiveness of the disease. Wheat, regardless of species, benefits from AA's ability to act as a defensive inducer. There was a more significant response to based-AA treatment for PR2 and Chi4 precursor gene expression, as well as PO, CAT, and LOX functions. They hypothesized that the examined product's stimulatory impact could be related to AA, a predefined ROS metabolism inducer.^[43]

2. Ascorbic acid's impact on plants in polluted environments

Pollutants with oxidizing properties, such as NO_x, SO₂, and O₃, induce oxidative stress in plants and generate ROS, both of which have deleterious effects on plant metabolism.^[44] Plants can be either highly sensitive or highly resistant to gaseous contaminants. Deformities in plant physiology and morphology can be attributed to exposure to specific environmental contaminants. Such abnormalities will be prevalent in sensitive plants but uncommonly intolerant ones. There is value in sensitive species as an emergency alert of pollution and intolerant species as a means to reduce pollution. Four biochemical measures, including leaves extract pH, ascorbic acid, chlorophyll concentrations, and

percentage of water, have been proposed as a means of calculating the Air Pollution Tolerance Index (APTI).^[45,46] Ascorbic acid is the frontline defender against oxidative stress caused by environmental contaminants.^[47] Ascorbic acid is widely recognized as an antioxidant and cellular reductant that plays a significant part in the susceptibility of plants to ozone exposure.^[48] Hence, a plant's tolerance is determined by the level of ascorbate contained in its cellular components, which gives plants resistance to the harmful effects of oxidative contaminants.^[44]

2.1 Applications of ascorbic acid and its derivatives against pollutants

A. Ascorbic acid as possible ozone tolerance indicators

When sensitive plant species take in tropospheric ozone (O₃), it slows their photosynthesis, stunts their growth, and causes other phenological changes.^[49] As a result, plants have developed a variety of defence mechanisms to deal with high levels of ROS, including the production of antioxidants like ascorbic acid and polyphenolic compounds, as well as enzymes like catalase, superoxide dismutase, and peroxidases.^[50] The AsA parameter has been found to help check soybean cultivars through studies evaluating their responses to O₃ exposure.^[51] Moreover, the more minor apoplast AA pool function as the leaf's initial detoxifying system, where the contaminant reaches the leaf. In addition to acting as a mediator in many processes and playing various roles in crop nutrition, growth, blooming time, seed survivability, and implantation, AA is also responsible for regulating the central metabolism through the levels of dehydroascorbate (DHA).^[52] According to a study done by^[53], there is a substantial correlation between the levels of AA and thiols at the beginning of development and the ultimate yield response. This finding suggests that these parameters are valuable in measuring the production responses of grain cultivars to O₃.

B. Cadmium toxic effects reduced by exogenous ascorbic acid

Among the most critical risks to the long-term viability of agricultural production worldwide is heavy metal poisoning of plants and soils.^[53] The highly poisonous and non-essential element cadmium (Cd) poses a concern to public health. It may easily penetrate the consumable portion of plants and get enriched in biological systems through nutrient cycling.^[55] In addition to disrupting membrane function, Cd also induces the generation of ROS in plant cells, including superoxide anion (O₂•) and hydroxyl radicals (OH•).^[56] Several studies investigate the crucial role of exogenous ascorbic acid in reducing the toxic effect of cadmium within different crops. Zhang et al. research recommended that AA decreased Cd absorption in wheat by 10.3-12.3% and, when applied as a foliar, has shown promise to lower Cd poisoning in maize and promote healthy photosynthesis, osmoregulation and antioxidant defence.^[57] Similarly, a different study demonstrated that exogenous AA treatment reduced Malondialdehyde (MDA) buildup and increased growth, total proteins, photosynthesis, and antioxidant defence mechanisms in wheat plants, mitigating Cd damage.^[58]

C. Tolerating chromium (VI) poisoning in tomato roots by ascorbic acid

Contamination caused by chromium (Cr) is a major scientific issue because of its negative impact on crop yields around the world.^[59] The release of chromium into the ecosystem can be attributed to either environmental factors (such as the weathering of rocks) or artificial operations, such as the employment of chromium in several different sectors (chrome plating, alloys, paintings, etc.), as well as the use of inappropriate fertilizers.^[60] There is evidence that chromium (VI) can impair photosynthesis by disrupting the electron carriers mechanism of plants. Increased production of ROS occurs in conjunction with chromium (VI)-mediated suppression of photosynthesis, which damages lipids and polypeptides due to abnormalities in the antioxidant defence mechanism.^[61] According to a study by Al-Huqail et al., endogenous AA plays a critical role in the development of Cr(VI) poisoning resistance in tomato roots. When ascorbic

acid was applied, cell damage was decreased in response to Cr(VI) exposure in tomato roots. This was associated with a decrease in cellular Cr(VI) concentration and oxidative stress as a result of increased antioxidant activity ascorbate peroxidase (APX) and glutathione-S-transferase (GST) and an enhanced pool of AA.^[62]

CONCLUSION

Ascorbic acid (AA) was revealed to modulate crucial host-defence processes in addition to its function in ROS neutralization after pathogen infections. The modulation of AA after Pathogen invasion causes changes in the redox system of the cell surroundings, which in turn triggers a variety of defence responses, including activation of PR genes via the NPR1 pathway, cell wall hardening, and modification of defensive system signalling networks. In addition, AA was discovered to play a role in inducing disease defence strategies across a range of pathosystems. Conversely, Without metabolic and morphological information, ascorbic acid can be used as a biomarker for assessing responses to pollutants.

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