

Review Article

Non-Enzymatic Antioxidant Defence Mechanism in Plants

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Abstract

Reactive Oxygen Species (ROS) are a natural consequence of the aerobic metabolism, and plants have mechanisms to deal with them in normal conditions, controlling the formation and removal rates. ROS affect many biochemical processes by nucleic acids damage, proteins oxidation and lipid peroxidation (LPO). Under stress conditions, cell homeostasis is disrupted and ROS production put a heavy burden on the antioxidative mechanisms, in order to eliminate the excess ROS. These mechanisms usually include non-enzymatic substances like ascorbate, glutathione, phenolic compounds, proline, tocopherols and carotenoids, with reported antioxidant properties. Future study, with advanced analytical techniques, of biochemical networks and compounds involved in cellular responses to oxidative stress will be helpful in producing plants with in-built capacity of enhanced levels of tolerance to ROS using biotechnological approach.

Keywords: Reactive oxygen species, Stress, Antioxidative enzymes, Non-enzymatic substances

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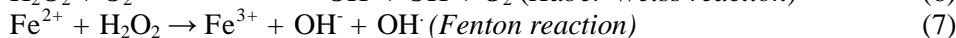
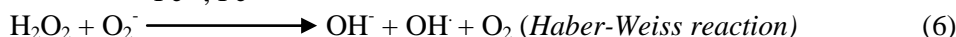
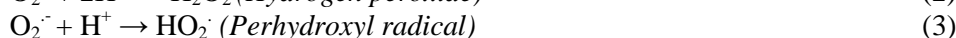
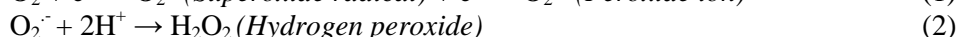
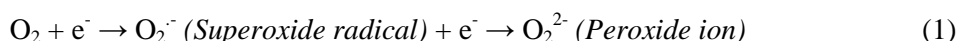
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Introduction

Plants are subjected to a number of abiotic stresses throughout their life and accumulation of reactive oxygen species as a result of various environmental stresses is a major cause of loss of crop productivity worldwide [1]. ROS affect many biochemical processes by nucleic acids damage, proteins oxidation and lipid peroxidation (LPO). The delicate equilibrium between ROS production and scavenging at the proper site and time decides whether ROS will act as damaging, protective or signaling factors [2, 3].

ROS Production

A highly oxidizing metabolic activity and intense rate of electron flow occurs in chloroplast, mitochondria or peroxisomes, major sources of ROS in plant cells [4]. It has been estimated that 1-2% of oxygen consumed by plants is side tracked to produce ROS in various subcellular loci [5, 6]. The single electron reduction of oxygen results in the generation of the $O_2^{\cdot-}$. At low pH, dismutation of $O_2^{\cdot-}$ is unavoidable, with one $O_2^{\cdot-}$ giving up its added electron to another $O_2^{\cdot-}$, and then with protonation resulting in the generation of H_2O_2 . Furthermore, $O_2^{\cdot-}$ can be protonated to form the HO_2^{\cdot} . Haber-Weiss reaction involves $O_2^{\cdot-}$, H_2O_2 and iron that rapidly generates OH^{\cdot} , whereas Fenton's reaction involves the oxidation of Fe^{2+} by H_2O_2 . The peroxyxynitrite also forms when $O_2^{\cdot-}$ react with signaling free radical species, NO^{\cdot} . ROS production through various reactions is listed below.

**Effects of ROS**

In biological systems, the reactions of activated oxygen are more complicated and the nature and extent of reactions with oxygen differ within a single cell and at various sites. The nature of the oxidative injury that causes cell death is not always obvious. ROS is capable of inducing damage to almost all cellular macromolecules.

Lipid Peroxidation (LPO)

Under aerobic conditions, lipid peroxidation is a natural metabolic process and the most investigated consequences of ROS. Polyunsaturated fatty acids are susceptible to peroxidation and products like small hydrocarbon fragments such as ketones, malondialdehyde (MDA), etc are formed [7]. Above threshold ROS levels effect membrane structure and function through production of lipid-derived radicals thereby aggravating oxidative stress [8].

Initiation, progression and termination are the three stages of LPO. Initiation step involves transition metal complexes (Fe and Cu). However, $O_2^{\cdot -}$ and H_2O_2 are capable of initiating the reaction. OH^{\cdot} is sufficiently reactive and initiate the LPO production by the abstraction of a hydrogen atom of a polyunsaturated fatty acid (PUFA) residue. The oxygen (in aerobic environment) will add to the fatty acid at the carbon-centered lipid radical to give rise to a ROO^{\cdot} and can propagate the peroxidation chain reaction by abstracting a hydrogen atom from adjacent PUFA side chains. The resulting lipid hydroperoxide decompose into several reactive species including malondialdehyde, lipid epoxides etc [9]. The overall effects of LPO: decrease membrane fluidity; easier phospholipids exchange between the two halves of the bilayer; increase in membrane leakiness, damage membrane proteins, inactivating receptors, enzymes and ion channels [10].

Protein Oxidation

ROS induces irreversible covalent modification reactions of a proteins whereas, a few reactions involving sulfur-containing amino acids are reversible [11]. The proteins site-specific amino acid modifications, fragmentation of the peptide chain, altered electrical charge and increased proteolysis are the results of oxidative attack. Primary, secondary, and tertiary protein structures alter the relative susceptibility of certain amino acids. The oxidation of a number of protein amino acids may inhibit or alter their activities [12].

ROS are likely to target proteins that contain sulphur-containing amino acids and thiol groups as these are quite reactive especially with 1O_2 and OH^{\cdot} irrespective of location [13]. The metal cofactors that binds to a divalent cation binding site on the protein and reacts with hydrogen peroxide to form hydroxyl radical (oxidize an amino acid at or near the cation binding site of the protein) increases the oxidative degradation of protein [14].

DNA Damage

Reactive metabolites produced due to abiotic and biotic factors or are produced endogenously results DNA damage and therefore exerts genotoxic stress. ROS damage to DNA includes base degradation, base deletion, pyrimidine dimers, cross-links, strand breaks and base modification [15, 16]. The principle cause of single strand breaks is oxidation of the sugar moiety by the hydroxyl radical and through Fenton reactions with a metal catalyst by hydrogen peroxide and superoxide [17].

DNA damage can result either in arrest or induction of transcription, induction of signal transduction pathways, replication errors, cell membrane destruction and genomic instability which affect the growth and development of the whole organism [18]. DNA can tolerate less damage as compared to other macromolecules. As a consequence, the cell has a number of DNA repair enzymes [19].

Non-Enzymatic Compounds

There is a whole range of other non-enzymatic substances that have been reported to be involved in antioxidative defence in plants. ROS are normally scavenged immediately at the site of their production by the locally present antioxidants.

Ascorbic Acid (Vitamin C)

Ascorbic acid is the most abundant, powerful and water soluble antioxidant that acts to prevent or minimize the damage caused by ROS in plants [20]. It occurs in all plant tissues and is maximum in mature leaves with fully developed chloroplast and highest chlorophyll. It has been reported that ascorbate mostly remain available in reduced form in leaves and chloroplast under normal physiological conditions [21].

In plants, mitochondria play central role in the metabolism of ascorbate. The indirect role of ascorbate as an antioxidant is to regenerate membrane-bound antioxidants, such as α -tocopherol, that scavenge peroxy radicals and singlet oxygen, respectively. Plant mitochondria not only synthesize ascorbate by L-galactono- γ -lactone dehydrogenase but also take part in the regeneration of ascorbate from its oxidized forms [22]. Ascorbate is considered as a most powerful ROS scavenger because of its ability to donate electrons in a number of enzymatic and non-enzymatic reactions. As an antioxidant, ascorbate reacts with superoxide, hydrogen peroxide or the tocopheroxyl

radical to form monodehydroascorbic acid and/or dehydroascorbic acid. The reduced forms are recycled back to ascorbic acid by monodehydroascorbate reductase and dehydroascorbate reductase using reducing equivalents from NAD(P)H or glutathione, respectively. In addition to the importance of ascorbate in the ascorbate-glutathione cycle, it also plays an important role in preserving the activities of enzymes that contain prosthetic transition metal ions [21].

Glutathione (GSH)

A tripeptide glutathione (γ -glutamylcysteinylglycine, GSH) is an abundant compound in plant tissues. It is virtually found in all cell compartments: cytosol, endoplasmic reticulum, vacuole and mitochondria in reduced form [23]. GSH is the main storage form of sulfur and is a potent detoxifier of xenobiotics through GSH-conjugation. It can serve as a precursor of phytochelatins [10]. It also plays a central role in signal transduction [24] and the expression of stress-responsive genes [25].

Together with its oxidized form (GSSG) glutathione maintains a redox balance in the cellular compartments. GSH reduce dehydroascorbate to ascorbate via ASH-GSH cycle [26] or reduce the disulphide bonds of proteins. The synthesis of glutathione occurs in two ATP-dependent steps. First, glutamate-cysteine ligase (GCL) catalyzes formation of γ -glutamylcysteine from cysteine and glutamate which is thought to be the rate limiting step of the pathway. Second, glutathione synthetase (GS) adds glycine to γ -glutamylcysteine to yield GSH. It had been reported that with increase in the intensity of a stress, GSH concentrations usually declined and redox state became more oxidized, leading to deterioration of the system [27]. The role of GSH in the antioxidant defence system provides a strong basis for its use as a stress marker [11].

α -Tocopherols (Vitamin E)

Tocopherol concentrations vary among plant tissues and has been found in all higher plants, in both photosynthetic and non-photosynthetic tissues [28]. Tocopherols, a lipid soluble antioxidant are considered as potential scavengers of ROS and lipid radicals [29]. Out of four isomers of tocopherols (α -, β -, γ -, δ -) found in plants, α -tocopherol has the highest antioxidative activity due to the presence of three methyl groups in its molecular structure. α -Tocopherol synthesis occurs in plastids with the aromatic ring formed by the shikimic acid pathway and the phytyl chain synthesized from geranylgeranyl pyrophosphate through the terpenoid pathway [30]. Chloroplast membranes of higher plants contain α -tocopherol as the predominant tocopherol isomer, and are hence well protective against photo-oxidative damage [10].

Tocopherols had been shown to prevent the chain propagation step in lipid autooxidation which makes it an effective free radical trap. It had been estimated that one molecule of α -tocopherol can scavenge upto 120 singlet oxygen molecules by resonance energy transfer [31]. Oxidative stress activated the expression of genes responsible for the synthesis of tocopherols in higher plants. Increase in tocopherol during water stress in plants had also been reported [28].

Proline (PRO)

PRO is a potent nonenzymatic antioxidant and potential inhibitor of programme cell death (PCD). PRO is required by the microbes, animals and plants to mitigate the adverse effects of ROS [32]. The synthesis of L-PRO from L-glutamic acid via Δ^1 -pyrroline-5-carboxylate is catalyzed by the activities of the enzymes Δ^1 -pyrroline-5-carboxylate synthetase and Δ^1 -pyrroline-5-carboxylate reductase in plants [33]. On the other hand, mitochondrial enzymes PRO dehydrogenase and Δ^1 -pyrroline-5-carboxylate dehydrogenase metabolize L-PRO into L-glutamate via Δ^1 -pyrroline-5-carboxylate [34].

Salt, drought and metal stress caused accumulation of PRO which may be due to increased synthesis or decreased degradation. Free PRO had been proposed to act as an osmoprotectant, a potent stabilizer, a metal chelator, an inhibitor of LPO and hydroxyl radical and singlet oxygen scavenger [35]. Enhanced synthesis of PRO under drought or salt stress has been implicated as a mechanism to alleviate cytoplasmic acidosis and maintain NADP⁺: NADPH at values compatible with metabolism. An additional advantage of the refilling of NADP⁺ supply by PRO synthesis may be to support redox cycling, which is especially important in plant antioxidant defence mechanisms during stress. Several lines of evidence suggested the important role for PRO synthesis in potentiating pentose-phosphate pathway activity because this pathway is an important component of antioxidant defence mechanisms, which need NADPH to maintain GSH and ascorbate in the reduced state [36].

Phenolic Compounds

Phenolics are diverse secondary metabolites (flavonoids, tannins, lignin) abundant in plant tissues [37]. Polyphenols had ideal structural chemistry for free radical scavenging activity and they had been shown to be more effective antioxidants *in vitro* than tocopherols and ascorbate. Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors, and from the ability of the polyphenol-derived radical to stabilize and delocalize the unpaired electron (chain-breaking function) and to chelate transition metal ions (termination of the Fenton reaction) [10]. Another mechanism underlying the antioxidative properties of phenolics is the ability of flavonoids to alter peroxidation kinetics by modification of the lipid packing order and to decrease fluidity of the membranes. These changes could sterically hinder diffusion of free radicals and restrict peroxidative reactions [38].

Phenolic compounds could also be involved in the hydrogen peroxide scavenging cascade in plant cells [10]. Many flavonoid biosynthetic genes are induced under stress conditions. It has been found that there is considerable increase in flavonoid levels following biotic and abiotic stresses [11].

Sugars

Carbohydrates serve as a source of energy and also act as signaling molecules in the regulation of various processes associated with plant growth and development under normal and stressed conditions [39]. When different abiotic stresses affect plant functionality, alterations in photosynthesis and carbon partitioning are common features that take place at organ level as well as in whole plant [40]. Soluble sugars do not only function as metabolic resources and structural constituents of cells [41].

Sucrose and hexoses both play dual functions in gene regulation as exemplified by the upregulation of growth-related genes and downregulation of stress-related genes [42]. Sugar signalling pathways interact with stress pathways creating into a complex network to modulate metabolic plant responses [43]. Accumulation of simple sugars such as glucose and fructose following an increase in the invertase activity and oligosaccharides such as raffinose and galactinol were among the sugars synthesized in stress and function as osmoprotectants rather than providing osmotic adjustment [44].

Conclusion

Future progress in genomics, metabolomics, and proteomics will help in clear understanding of biochemical networks and compounds involved in cellular responses to oxidative stress. Improved understanding of these will be helpful in producing plants with in-built capacity of enhanced levels of tolerance to ROS using biotechnological approach.

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