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Non-enzymatic antioxidant defense system in plants

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Abstract
The defense system in plants have efficient complex enzymatic and non-enzymatic antioxidant defense systems to avoid the toxic effects of free radicals. Chloroplasts and mitochondria are the two main powerhouses and sites of reactive oxygen species (ROS) generation within plant cells AA is the most abundant and the most extensively studied antioxidant compound. It is can donate electrons to a wide range of enzymatic and non-enzymatic reactions. Majority of AA in plant cells is the result of Smirnoff-Wheeler pathway, catalyzed by L-galactano-γ-lactone dehydrogenase in the plant mitochondria, with the remaining being generated from D-galacturonic acid. Vitamin E (α-tocopherol), is an efficient lipid soluble antioxidant that functions as a 'chain breaker' during lipid peroxidation in cell membranes and various lipid particles including low-density lipoprotein (LDL). It functions to intercept lipid peroxyl radicals (LOO) and to terminate the lipid peroxidation chain.

Keywords: Non-enzymatic, antioxidant, defense system, plants

Introduction
Plants have an innate ability to biosynthesize a wide range of non-enzymatic antioxidants capable of attenuating ROS (reactive oxygen species) induced oxidative damage. The defense system in plants have efficient complex enzymatic and non-enzymatic antioxidant defense systems to avoid the toxic effects of free radicals. Enzymatic systems include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione reductase (GR), while non-enzymatic systems consist of low molecular weight antioxidants (ascorbic acid, glutathione, carotenoids, phenolic acids, flavonoids, etc.) and high molecular weight secondary metabolites such as tannins.

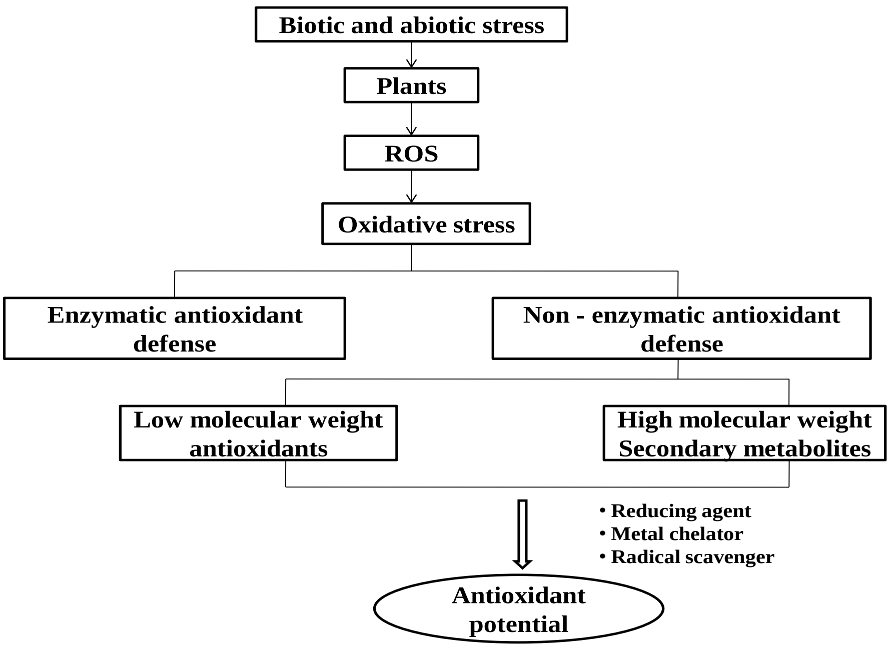

Fig 1: Antioxidant system in plants.

Site of ROS production and types of ROS
Chloroplasts and mitochondria are the two main powerhouses and sites of reactive oxygen species (ROS) generation within plant cells. These materials are also involved in maintenance of a fine balance between energy linked functions and control of ROS production.

Peroxisomes, single membrane-bound subcellular organelles, are a third important site of production of ROS such as hydrogen peroxide (H2O2), superoxide (O2●-) and nitric oxide (NO●) within plant cells. Peroxisomes contain basic enzymatic constituents such as catalase (CAT), as well as hydrogen peroxide (H2O2) producing flavin oxidases. Within the plant cell, ROS generation occurs at photosystem I and II (PS I and PS II) of the chloroplasts, membrane and matrix of the peroxisome, and complex I, ubiquinone and complex III of the mitochondrial electron transport chain (ETC). Under normal physiological conditions, there is electron slippage from PS I and PS II of the chloroplasts, membrane of mitochondrial ETC and peroxisome. These electrons later react with molecular oxygen to produce superoxide radical (O2-●). The superoxide radical is subsequently converted to hydroperoxyl radical (HO2●) and finally to H2O2 (del rio *et al.*, 2006) [5]. Similar to ROS, reactive nitrogen species (RNS) such as the nitric oxide radical (NO•) and peroxinitrite (ONOO-) are also formed in various compartments of the cell including the chloroplasts, mitochondria and peroxisomes (Carocho and Ferreira, 2013) [3].

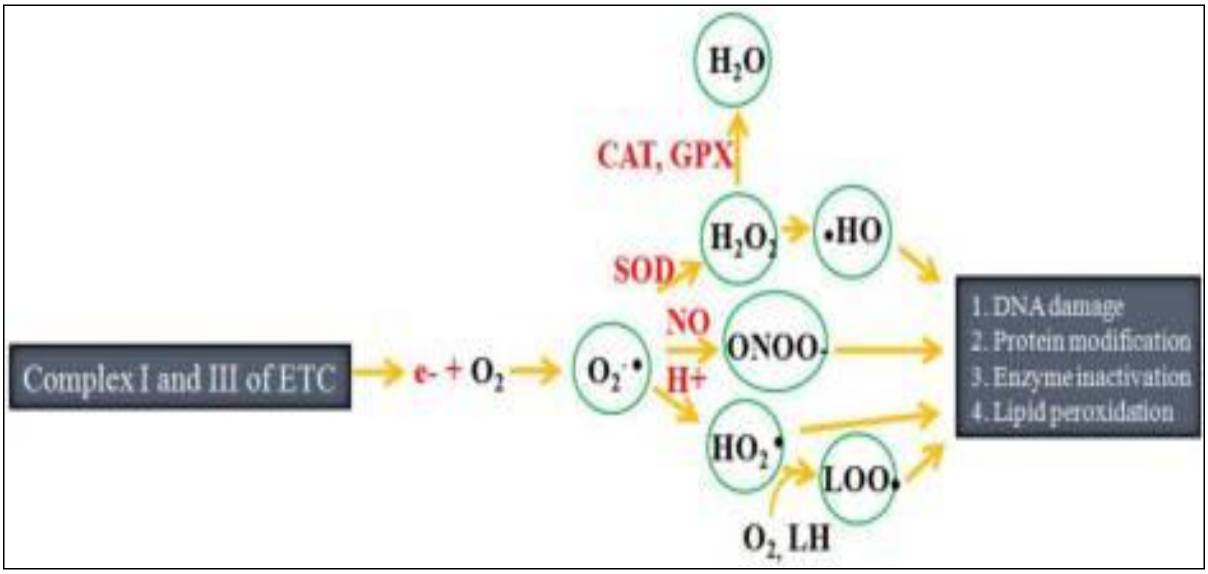

Fig 2: Free radical production in plants (Adapted from Carocho and Ferreira, 2013 and Lu *et al.*, 2010) [3, 8]

The third type of free radical, reactive sulfur species (RSS), are reportedly formed from thiols by reaction with ROS (Lu *et al.*, 2010) [8]. The overall process of free radicals generation is summarized in Fig.2. These free radicals are constantly produced in the subcellular organelles of living cells. Most of the time, the production of free radicals is genetically planned, since they function as signaling molecules. However, overproduction of free radicals can also sometimes damage biomolecules such as DNA, proteins and lipids.

Non-enzymatic antioxidant defense system

Ascorbic Acid (AA)

AA is the most abundant and the most extensively studied antioxidant compound. It is can donate electrons to a wide range of enzymatic and non-enzymatic reactions. Majority of AA in plant cells is the result of Smirnoff-Wheeler pathway, catalyzed by L-galactano-γ-lactone dehydrogenase in the plant mitochondria, with the remaining being generated from D-galacturonic acid. Ninety per cent of the AA pool is concentrated not only in the cytosol, but also substantially in apoplast, thus making it the first line of defense against ROS attack (Barnes *et al.*, 2002) [2]. AA is oxidized in two successive steps, starting with oxidation into MDHA (monodehydroascorbate), which if not reduced immediately to ascorbate, disproportionates to AA and DHA (dehydroascorbate). It reacts with H_2O_2, OH●, O2●-, and regenerates α-tocopherol from tocopheroxyl radical, thereby protecting the membranes from oxidative damage. It also protects and preserves the activities of metal-binding enzymes. AA in its reduced state acts as the cofactor of violaxanthine de-epoxidase and maintains the dissipation of the excess excitation energy (Smirnoff, 2000) [13]. AA has also been reported to be involved in preventing photo-oxidation by pH-mediated modulation of PSII activity and its down regulation, associated with zeaxanthine formation.

Glutathione

Glutathione is a redoxactive molecule that can be present in a reduced form (GSH) or an oxidized glutathione disulfide form (GSSG) and plays important roles in biosynthetic pathways, detoxification, antioxidant biochemistry and redox homeostasis (Noctor *et al.*, 2012) [11]. GSSG is reduced to GSH by the enzyme glutathione reductase, which requires NADPH as the reducing power. GSH acts as an anti-oxidant by quenching reactive oxygen species and is involved in the ascorbate-glutathione cycle, which eliminates damaging peroxides.

α-tocopherol

Vitamin E (α-tocopherol), is an efficient lipid soluble antioxidant that functions as a 'chain breaker' during lipid peroxidation in cell membranes and various lipid particles including low-density lipoprotein (LDL). It functions to intercept lipid peroxyl radicals (LOO) and to terminate the lipid peroxidation chain. The α-tocopherol has the highest antioxidant capability among the four isomers (α-, β-, γ-, δ-). The tocopherols are synthesized only by photosynthetic organisms and thus only present in green tissues of plants. The α-tocopherol is synthesized from γ-tocopherol by γ-tocopherol-methyl-transferase (γ-TMT encoded by *VTE4*). Tocopherols are known for their ability to protect lipids and other membrane constituents of the chloroplasts by reacting with O_2 and quenching its excess energy, thus protecting the PSII, both structurally and functionally. Tocopherol also serves as an effective free radical trap by halting the chain propagation step of the LPO (lipid peroxidation) cycle (Igamberdiev *et al*., 2004) [7].

$$LOO^{\cdot} + \alpha\text{-tocopherol–OH} \rightarrow LOOH + \alpha\text{-tocopherol–O}^{\cdot}$$

The resultant tocopheroxyl radical is relatively stable and in normal circumstances, insufficiently reactive to initiate lipid peroxidation itself, which is an essential criterion of a good antioxidant.

Carotenoids

Carotenoids are among the most common lipid soluble phytonutrients biosynthezised by phytoene. Lycopene and β-carotene are the prominent carotenoids among other 600 different compounds. Carotenoids belong to family of lipophilic antioxidants which are localized in the plastids of both photosynthetic and non-photosynthetic plant tissues. They are found not only in plants, but also in micro-organisms. They are the accessory pigments which absorbs light in the 450–570 nm and transfers the energy to the chlorophyll molecule. Carotenoids exhibit their antioxidative activity by protecting the photosynthetic machinery in four ways, (a) reacting with LPO products to end the chain reactions, (b) scavenging $^1O_2$ and generating heat as a by-product, (c) preventing the formation of $^1O_2$ by reacting with excited chlorophyll (Chl*), and (d) dissipating the excess excitation energy, via the xanthophyll cycle (Namitha & Negi, 2010) [10].

Flavanoids

Flavonoids can be classified into four classes on the basis of their structure, flavonols, flavones, isoflavones, and anthocyanins (low molecular weight), and tannins (high molecular weight). They serve vital roles in plant structural integrity, UV photoprotection, reproduction, and internal regulation of plant cell physiology and signaling. They also act as key chemical modulators of plant communication with insects and microbes, either as attractants or repellants, as phytoalexins against pathogens and herbivores, and as attractants to pollinators via flower color. They also induce root nodulation when excreted by symbiotic nitrogen-fixing rhizobia.

Flavonoids and phenolic acids, the largest classes of plant phenolics, are biosynthetically derived from the acetate and shikimate pathways, as well as the shikimate pathway from phenylalanine or tyrosine (Dewick, 2009) [6]. Moreover, they are known to interact with other physiological antioxidants such as ascorbate or tocopherol and to synergistically amplify their biological effects (Croft, 1998) [4]. Flavonoids and phenylopropanoids are also oxidized by peroxidase, and act as H_2O_2 scavengers (Morgan *et al.,* 1997) [9]. Under experimental conditions, the antioxidant potential of plant phenolics is always linked to their electron donation, reducing power and metal ion chelating ability (Sakihama *et al.,* 2002) [12]

The photosynthetic electron transport system in plant is the major source of active oxygen species. Chloroplasts have evolved a highly developed ascorbate-glutathione cycle i.e. detoxification system to avoid oxygen-mediated toxicity. Flavonoids may scavenge photo produced active oxygen species in the chloroplast. Super oxide anion radical can not readily diffuse into vacuoles where flavonoids are localized from chloroplast but H2O2 can diffuse across membranes. Efficient scavenging of ROS by phenolic compounds has been found to reduce ultraviolet radiation stress. Flavonoid-Peroxidase reaction may act as a mechanism for H2O2 scavenging and thus flavonoids acts as detoxifying agent (Amalesh *et al*., 2011) [1]. Flavonoids accumulated under the elicitation of toxic metals not only able to detoxify the ROS but also the toxic metals by chelating depending on the diversity of molecular structures.

Conclusion

Plants synthesize and accumulate several non-enzymatic antioxidants such as ascorbic acid, glutathione and phenolics. Reactive oxygen species (ROS) were initially recognized as toxic by-products of aerobic metabolism. In recent years, it has become apparent that ROS plays an important signaling role in plants, controlling processes such as growth, development and especially response to biotic and abiotic environmental stimuli. The major members of the ROS family include free radicals like $O^{\bullet -}_2$, $OH^{\bullet}$ and non-radicals like H_2O_2 and $^1O_2$. The ROS production in plants is mainly localized in the chloroplast, mitochondria and peroxisomes. To ensure survival, plants have developed efficient antioxidant machinery (i) enzymatic antioxidants; (ii) non-enzymatic antioxidants These two components work hand in hand to scavenge ROS. In this review, emphasis is on the different types of ROS, their cellular production sites, and their scavenging mechanism highlighting the potential role of non-enzymatic antioxidants in plants.

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