

## Reactive Oxygen Species (ROS) and Antioxidant Machinery of the Plant System

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### Abstract

Environmental fluctuation leads to overproduction of ROS, which are extremely toxic and damages cellular biomolecules i.e. nucleic acid, DNA, lipid, and protein. However, the delicate balance between ROS production and antioxidant machinery of the plant is always maintained under normal condition but during stress, this balance comes to a halt. To ensure the plant survival under stressful environment, plants have adopted well equipped antioxidant defense machinery having two forms: (i) Enzymatic such as Superoxide Dismutase (SOD), Ascorbate Peroxidase (APX), Catalase (CAT), Dehydroascorbate Reductase (DHAR), Monodehydroascorbate Reductase (MDHAR), Glutathione Reductase (GR) and Guaiacol Peroxidase (GPX); and (ii) Non-enzymatic such as reduced glutathione (GSH), Ascorbic Acid (AA), Carotenoids,  $\alpha$ -tocopherol, proline and flavonoids. In this review, a summarized form of ROS action and their regulation via antioxidant machinery of plant cells have been discussed.

**Keywords:** Antioxidants, Oxidative stress, Reactive Oxygen species, Redox status.

### Introduction

About 2.7 billion years ago, molecular oxygen ( $O_2$ ) was introduced into the atmosphere by  $O_2$  evolving photosynthetic organisms, which lead to the evolution of undesirable by-products i.e. reactive oxygen species (ROS) which mainly includes  $^1O_2$ ,  $O_2^{\bullet-}$ ,  $H_2O_2$  and  $OH^{\bullet}$  (Halliwell, 2006). Under the favourable condition, the primary ROS production (various cellular compartments i.e. chloroplast, mitochondria, apoplast and peroxisomes) is constantly maintained to regulate the cellular metabolism; however, stressful environment (pathogen infection, drought, salinity, heavy metals, high temperatures, pollution etc.) disturb the delicate balance between ROS production and their scavenging by antioxidant defense system of plants (Foyer and Noctor, 2005). These ROS are lethal and cause damage to DNA, lipids

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and proteins, thereby disturbing cell functioning (Foyer and Noctor, 2005). The redox homeostasis is maintained by two types of antioxidants machinery- (i) Enzymatic antioxidants involving Superoxide Dismutase (SOD), Peroxidase (POD), Catalase (CAT), Glutathione-S-transferase (GST), Ascorbate Peroxidase (APX), Dehydroascorbate Reductase (DHAR) and Glutathione Reductase (GR) and, (ii) Non-enzymatic Antioxidants (low molecular) involving Ascorbate (AA), Reduced Glutathione (GSH), Cysteine (Cys), Proline (Pro), Non-Protein Thiols (NPTs), Carotenoids,  $\alpha$ -tocopherol, flavonoids and Phenolics (Gill and Tuteja, 2010;

Miller et al., 2010; Gill et al., 2011, Singh, 2019). In this review, a deeper insight into ROS toxicity and their detoxification by well-equipped antioxidant machinery of plants have been discussed.

### ROS defense machinery

The ROS defense machinery has been categorized into enzymatic and non-enzymatic antioxidants.

### Enzymatic antioxidants

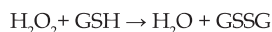
#### *Superoxide dismutase (SOD; E.C 1.15.1.1)*

The metalloenzyme SOD is considered as the first line of defense, which catalyzes the dismutation of  $O_2^{\bullet-}$  into  $O_2$  and  $H_2O_2$ , thereby eliminating the opportunity of  $OH^{\bullet}$  formation by Haber-Weiss reaction. Based on metal ion binding, there are three isoenzymes of SODs; Fe-SOD (confined in chloroplasts), Mn-SOD (confined in mitochondria) and Cu/Zn-SOD (confined in the cytosol, chloroplasts, and peroxisomes) (Mittler, 2002).



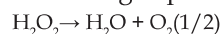
#### *Peroxidase (POD; E.C.1.11.1.7)*

The heme-containing POD enzyme removes excess  $H_2O_2$  in both healthy and stressful environment. It is essential in lignin biosynthesis and removes  $H_2O_2$  from the cell by using guaiacol and pyrogallol as electron donors, and degrading indole acetic acid (IAA) against stressful condition (Asada, 1999). It functions both intracellularly (cytosol, vacuole), in the cell wall and extracellularly, with higher  $K_m$  value.



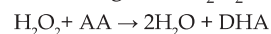
#### *Catalase (CAT; E.C.1.11.1.6)*

The tetrameric heme-containing CAT enzyme catalyzes the dismutation of  $H_2O_2$  into  $H_2O$  and  $O_2$  in peroxisome, cytosol, mitochondria and chloroplast. CAT is unique among the antioxidants; with a high turnover rate. It does not require a reducing equivalent (Mittler, 2002). CAT has three genes; CAT1 which is restricted in the cytosol and peroxisome and express in seeds and pollens; CAT2 is restricted in peroxisome and cytosol and express in root, seed and photosynthetic tissues and CAT 3 is restricted in mitochondria and express in leaves and vascular tissues of angiosperms.



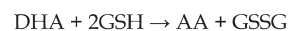
#### *Ascorbate peroxidase (APX; E.C.1.1.1.11)*

Being the immense part of AsA-GSH cycle, APX predominantly scavenges  $H_2O_2$  in chloroplast and cytosol, while CAT performs the same function in peroxisomes. APX reduces  $H_2O_2$  to  $H_2O$  and dehydroascorbate (DHA) using AA as an electron donor (reducing agent). APX have five isoforms located in cytosol, peroxisomes, mitochondria, and chloroplast (Sharma and Dubey, 2004). Due to its higher affinity for  $H_2O_2$  and wide distribution, it is a more efficient scavenger of  $H_2O_2$  than CAT.



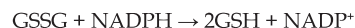
#### *Dehydroascorbatereductase (DHAR; M.C.1.8.5.1)*

DHAR enzyme reduces DHA into AA using GSH as an electron donor, which means regenerates AA pool in both apoplast and symplast; therefore, it maintains the redox status of the cell (Eltayeb et al., 2007). It is predominantly found in root, shoot and seeds.



#### *Glutathione reductase (GR; E.C.1.6.4.2)*

The flavoprotein oxidoreductase enzyme GR, reduce oxidised glutathione (GSSG) to GSH using NADPH as an electron donor. GR catalyzes the formation of disulfide bond in GSSG and maintains the high GSH/GSSG ratio, mainly in the chloroplast. Its small amount is also present in cytosol and mitochondria.



### Nonenzymatic antioxidants

#### *Ascorbate (AA)*

AA is a powerful electron donor, mainly comes from L-galactano- $\gamma$ -lactone dehydrogenase catalysed Smirnoff-Wheeler pathway of mitochondria and D-galacturonic acid. It is oxidized into succeeding steps, starting with oxidation into MDHA, which if not immediately reduced into AA, disproportionate to AA and DHA. AA protects the membrane by directly scavenging  $O_2^{\bullet-}$  and  $OH^{\bullet}$  and regenerating  $\alpha$ -tocopherol from tocopheroxyl radical (Shao et al., 2005). In its reduced state, AA acts as a cofactor of violaxanthin de-epoxidase and maintains the dissipation of excess excitation energy (Smirnoff, 2000), prevents the photo-oxidation of PS II and its down-regulation is associated with zeaxanthin formation.

#### *Glutathione (GSH)*

The low molecular weight thioltripeptide

( $\gamma$ -glutamyl-cysteinyl-glycine) GSH is found in almost all the cellular compartments and participates in a wide range of processes (Mullineaux and Rausch, 2005), as it has high reductive potential due to centrally located Cys residues with nucleophilic character. GSH protects various biomolecules by scavenging ( $^1\text{O}_2$ ,  $\text{O}_2^{\bullet-}$ ),  $\text{H}_2\text{O}_2$  and  $\text{OH}^\bullet$  and forms GSSG as by-product. It is also essential in (i) regenerating AA and forms GSSG, which with the help of GR enzyme converted back in to GSH and (ii) Synthesizing PCs by PC synthase, which is necessary for heavy metals chelation and ROS scavenging (Roy Choudhury et al., 2012). Thus, the delicate balance between GSH and GSSG is essential for maintaining the redox status of the cells.

#### **Proline (Pro)**

The osmolyte Pro is a powerful antioxidant, which efficiently scavenges  $^1\text{O}_2$  and  $\text{OH}^\bullet$  and can prevent the damage due to LPO. Pro is synthesized via pyrroline 5-carboxylate (P5C) using glutamic acid as a substrate; the step in plants completed by two enzymes:  $\delta$ 1-pyrroline-5-carboxylate synthetase (P5CS) and pyrroline-5-carboxylate reductase (P5CR). During stress, Pro accumulation could either be due to enhanced synthesis or reduced degradation (Verbruggen and Hermans, 2008).

#### **$\alpha$ -Tocopherol**

The  $\alpha$ -tocopherol is the active member of the lipophilic antioxidants' family, which is presents in green tissues of the plants because it is synthesized only by the photosynthetic organisms. They protect lipid and other elements of chloroplasts by reacting with  $\text{O}_2$  and quenching its excess energy, thus protects PSII, both structurally and functionally (Kiffin et al., 2006). Tocopherol traps free radical by hampering chain proliferation of the LPO cycle. The  $\alpha$ -tocopherol reacts with lipid radicals i.e.,  $\text{ROO}^\bullet$ ,  $\text{RO}^\bullet$  and  $\text{RO}$ , by reducing them and itself converted into  $\text{TOH}^\bullet$ , which then recycled to get its reduced form by interacting with AA and GSH (Igamberdiev et al., 2004).

#### **Carotenoids (Car)**

The Car also belongs to lipophilic antioxidants family and localized into plastid of plant tissues, which absorbs 450–570nm wavelength of light and transfers energy to chlorophyll (Chl) molecules. Car reveals their antioxidative property by protecting the photosynthetic machinery by (i) Scavenging  $^1\text{O}_2$  and generating heat as a by-product, (ii) reacting with LPO products to end the chain reactions,

(iii) dissipating excess excitation energy via xanthophyll cycle, and (iv) preventing  $^1\text{O}_2$  formation by reacting with  $3\text{Chl}^*$  and excited  $\text{Chl}^*$ .

#### **Flavonoids**

Flavonoids mainly found in the floral organs, leaves, and pollen grains in four forms i.e. flavones, isoflavones, flavonols, and anthocyanins and provides pigmentation to seed, fruit, and flowers and defend plants against stress. These are mainly secondary ROS scavenging compounds and function when photosynthetic apparatus experiences damage due to excess excitation energy and protects outer envelope of chloroplast membrane by scavenging  $^1\text{O}_2$  (Fini et al., 2011; Agati et al., 2012).

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