



## THE ROLE OF ENZYMATIC AND NON-ENZYMATIC ANTIOXIDANTS IN FACING THE ENVIRONMENTAL STRESSES ON PLANT : A REVIEW

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### Abstract

Plants are subjected to a variety of biotic and non-biological stresses, which cause oxidative stress within plant cells due to the high leakage of the electrons toward molecular oxygen ( $O_2$ ) during the metabolic processes of photosynthesis and respiration. As a result, rising the values of Reactive oxygen species (ROS) including the radicals of hydrogen peroxide ( $H_2O_2$ ), super oxide ( $O_2^-$ ), single oxygen ( $^1O_2$ ), and hydroxyl ( $^1O_2$ ). These free radicals can directly attack cell components such as cell membranes, nucleic acids, proteins, lipids, and plant dyes and subject the cell for death. In order to resist or tolerate high levels of ROS, the plants have developed a defense system that protects the cells and limits the oxidative effects of the ROS group. This system includes enzymatic antioxidants and non- enzymatic antioxidants.

**Keywords** : Antioxidant, Environmental Stress, oxygen species

### Introduction

Stress, according to biology is defined as the inappropriate effect caused by any environmental (external) factor leading to preventing the organism from performing the original function appropriately. The term, stress, has been used to express the unsuitable conditions that the organism lives in. Stresses facing plant are divided into two types: first, the Biotic stresses which are caused by pathogens such bacteria, viruses, or fungi (Alvarez and Lamb, 1997) and second, the abiotic stresses including many types of such as temperature, salinity, herbicides, drought, ultra violet, or ozone (Mohamed *et al.*, 2003; Von Wiren, 2006; O'Rourke, 2007) whose studies indicate that subjecting plant to one or more of these stresses raise the values and concentrations of Reactive Oxygen Species (ROS) (Reddy *et al.*, 2004; Genc *et al.*, 2007).

### Free Radicals Production

Reactive Oxygen Species (ROS) refer to a group of ions or radicals that oxygen contributes in their formation; as they are unstable ions or radicals, they are distinguished by the high capability of oxidation. ROS include a huge group such as super oxide ( $O_2^-$ ), hydroxyl ( $OH^*$ ), peroxide ( $O_2^{2-}$ ), hydrogen peroxide ( $H_2O_2$ ), and ozone ( $O_3$ ). All aerobic organisms, including plants, produce ROS naturally (Lindquist *et al.*, 1991). This occurs by binding an electron of electron pathway in mitochondria and chloroplast with an Oxygen molecule ( $O_2$ ) in absence of other electron receivers. Under the natural conditions, there is a

balance between the destroyed ROS and the produced ones, so they are really important as they act as defense messengers against the pathogens, as well as, super oxide ( $O_2^-$ ) and hydrogen peroxide ( $H_2O_2$ ) play an important role in forming lignin (Gratao *et al.*, 2005). When plants are subjected to an environmental stress, the ROS production greatly increased in way affecting the plant growth; furthermore, these types of oxygen are so highly oxidized that can interact with (oxidize) proteins, nucleic acids, and lipids causing significant damages to the living cell. Super oxide ( $O_2^-$ ) can act as an oxidative or reductive factor. It can oxidize many compounds such as NADPH at the same time, it can reduce Cytochrome c and mineral ions. Chloroplasts, mitochondria, plasma membrane and cellular wall are the most locations producing ROS (Gill and Tuteja, 2010), while the main location for producing super oxide is mitochondria where it is produced as a result of oxidizing the compounds reduced during the respiratory chain (Dat *et al.*, 2000).

### Oxidative Damage to Biological Molecules

The biotic and abiotic stresses cause what is called the oxidative damage in plant as a result of the free radicals, or ROS. It is proven that all stresses lead to oxidative stress and the plant's ability to control oxidation level is related to stress tolerance (Cheeseman, 2007) and increasing environmental stress subject the organic molecules, such as lipids, proteins, nucleic acids, are strongly damaged or destroyed in way affecting the plant growth and crop production (Aly, 2012).

### Lipid oxidation

Lipids are hydrophobic molecules such as fats and oils, but they are dissolved in organic solvents and not dissolved in water liquids. Lipid oxidation process increases the oxidative stress by producing free radicals derived from lipids able to react with proteins and DNA and destroy them (Sharma *et al.*, 2010). Lipid oxidation consists of three stages: start, progress, and finish. It starts when one of the free radicals, for instance  $\cdot\text{O}_2$ ,  $\cdot\text{OH}$ , or  $\text{H}_2\text{O}_2$  attacks the polyunsaturated fatty acids, where  $\cdot\text{OH}$  activity is more efficient than others at the beginning of lipid oxidation (Gupta, 2011). Lipid oxidation in plant cells starts by the enzyme lipoxygenase (LOX) which is activated during the senility stage where the polyunsaturated fatty acids (PUFAs) are oxidized to lipid hydroperoxides (LOOH) (Gupta, 2011). The resultant compounds are called the primary compounds and there are also secondary compounds produced from lipid oxidations including MDA, common compounds used for measuring the lipid oxidation as they are the oxidative product of polyunsaturated fatty acids (PUFA) of the bio membranes (Cheng, 2011).

### Protein Denaturation

Proteins play an important role in osmosis regulation. Plants store proteins in a nitrogen form when they are subjected to stress, since proteins provide stability for the membranes, and it would be reutilized later, after the elimination of stress effects (Mehr and Bahabadi, 2013). Attacking proteins by ROS affects them negatively either directly or indirectly. These effects include modifying the amino acids, breaking down the peptide chain, changing electrical charge, increasing protein undergoing to proteolysis, increasing oxidative amino acids, and increasing the concentration of carbonized proteins (increasing the carbonyl groups) which are used as a sign for protein oxidation (Sharma *et al.*, 2012). During the oxidation process, the proteins can be fragmented or lose some amino acids, such thing can destroy the amino acids (Finaud *et al.*, 2006). It has become known that the dissolved protein content is an important indicator of the physiological plant status, as well as that protein synthesis is one of the mechanisms that are affected by environmental stresses.

### DNA oxidation

ROS cause fragmenting the DNA chains and destroy nucleotides. If any part of the DNA strand is subjected to an attack by free radicals it may lead to an exchange in the nucleotide loci and then modifying the DNA strand (Finaud *et al.*, 2006). The oxidative attack on DNA resulted in oxidizing the Deoxy ribose. When ROS attacks the DNA, it withdraws the tetra hydrogen atom from deoxy ribose and thus leads to producing

Deoxy ribose radical that react to produce fragmented DNA (Sharma *et al.*, 2012).

### Enzymatic antioxidant scavenging ROS

**Superoxide dismutase enzyme (SOD) :** SOD belongs to the family of protein enzymes classified under the world classification, EC 1.15.1.1, SOD. It is characterized by the ability of converting the super oxide radical ( $\text{O}_2^-$ ) into hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and oxygen in most aerobic organisms in addition to some non-aerobic organisms (Wang *et al.*, 2009). Because of the super oxide radical is the first free radical produced as a result of reducing oxygen formed in many biological systems, SOD is considered the first defense line against the poisoning of oxidative stresses in plants and reducing them.

**Catalase enzyme (CAT) :** Catalase enzyme (CAT) is found almost in all plant and animal cells as well as bacteria. It is an antioxidant enzyme that has the systematic nomination (EC, 1.11.1.6). It prevents harmful hydrogen peroxide from aggregation, so it converts the  $\text{H}_2\text{O}_2$  into  $\text{O}_2$  necessary for removing poisonous ROS under the stress conditions (Gargand Manchanda, 2009). The enzyme is characterized that it is one of enzymes known of their high conversion rate. It can convert about 6,000,000 molecules of  $\text{H}_2\text{O}_2$  to  $\text{H}_2\text{O}$  and  $\text{O}_2$  in one minute, therefore, it is essential to remove the hydrogen peroxide, generated from peroxidation by oxidizing the fatty acids, light respiratory, and purine demolition (Gill and Tuteja, 2010).

**Peroxidase enzyme (POD) :** This enzyme is one of Oxidoreductase and it is common in plant and animal, addition to microorganism (Dey *et al.*, 1997). It has the systematic nomination (EC, 1.11.1.7). It stimulates oxidizing many Hydrogen donor substances such as phenol compounds, aromatic amines, hydroquinone, hydroquinone amines, and gasoline derivatives in the presence of hydrogen peroxide  $\text{H}_2\text{O}_2$  as a hydrogen receiver substance, where hydrogen is moved from the donor substance to the receiver substance (Fagain, 1994). Peroxidase enzyme is characterized by its stimulation capability to convert hydrogen peroxide into water, so it is one of the plant defense enzymes against the oxidative stresses (Kawano, 2003).

### Non- enzymatic anti-oxidants

**Vitamin C :** Ascorbic acid is one of the non- enzymatic anti- oxidants. It has the capacity for reducing or minimizing the harmful effect of ROS in plants (Athar *et al.*, 2008). It can donate electrons for many enzymatic and non-enzymatic reactions and protect the membranes by reducing super oxides and hydroxyl and oxygen radicals directly, as well as reducing hydrogen peroxide into water by the reaction of ascorbate peroxidase (Noctor and Foyer, 1998) in addition to regenerating

vitamin E from tocopheroxyl radical. The study of Conklin and Barth (2004) refers to reducing stress and plant tolerance in addition to the synthesis of some compounds such as Phenylpropanoid glycosides (PPGS) using vitamin C as a source (Mungaiy – Lopez, 2011).

**Carotenoids** : Carotenoids are classified as organic, natural soluble lipid dyes and they also as Isoprenoid or Terpenoids compounds. They are found in plants and microorganisms (Fraser and Bramley, 2004; Cazzonelli, 2011). Carotenoids protect photosynthesis system by inhibiting tri chlorophyll (Chl<sup>3</sup>) and ROS especially the single oxygen (<sup>1</sup>O<sub>2</sub>) produced naturally during the photosynthesis process, thus the Carotenoids act as antioxidants.

**Proline** : Proline is one of amino acids participate in protein synthesis. Its quantity in plant tissues is proportional to the amount and duration of stress that plant subjected to. Proline is accumulated due to the plant's inability to synthesize protein furthermore its demolition process. It plays an important role in membrane stability and inhibiting free radicals as well as regulating the oxidation and reduction effort of the cell (Ashraf and Foolad, 2007); moreover, it is an antioxidant since it is characterized by the ability to reduce the radical of each of hydroxyl (OH•) and single oxygen (1O<sub>2</sub>) as well as inhibit lipid oxidation (Trovato *et al.*, 2008).

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