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A SNAPSHOT ON ANTIOXIDATIVE DEFENSE MACHINERY OF PLANTS UNDER STRESS CONDITIONS

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All the crops suffer significant yield losses from various biotic/abiotic stresses and stressed plants usually stimulate the production of reactive oxygen species (ROS), which include superoxide anion radical ($O^{\bullet-}_2$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$) which can cause severe oxidative damage to plant tissues. High levels of these ROS have the capability to damage different cellular components by lipid peroxidation (stimulating membrane permeability and fluidity), protein damage (by inactivating the enzymes, proteolytic degradation, and breakage of peptide linkage) and DNA destruction (by breaking of the DNA strands, depurination and depyrimidation). These are very lethal and extensively affect normal cellular functioning. Many factors like high temperature, high salt concentration, heavy metals, infection of pathogens etc. alter the subtle balance between ROS production and scavenging.

Oxidative stress tolerance is not a single step phenomenon but it is an integrated mechanism accompanied by changes in antioxidative/defensive enzymes, nonenzymatic antioxidants, free radical scavenging activities, signaling molecules, and osmolytes. Induced defense can be facilitated *via* defensive enzymes such as peroxidases, catalase, superoxide dismutase (SOD), polyphenol oxidases (PPO), ascorbate peroxidase (APX), phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL) along with secondary metabolites such as phenols, flavonoids, carotenoids and condensed tannins and also through the utilization of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA).

Enzymatic Antioxidative Defense System of Plants

Following are the major defense related enzymes that play role during severe oxidative stress

a. Superoxide dismutase (SOD)

Itbelongs to the family of metalloenzymes present in all organisms. During various environmental stresses or pathogenic attack, SOD forms the first line of defense against ROS induced damages. The major function of SOD is to catalyze the removal of $O^{\bullet}_{2}{}^{-}$*via*, dismutating it into O_2 and H_2O_2. SODs are categorized into 3 types on the basis of binding of metal ion: Mn-SOD (localized in mitochondria), Fe-SOD (present in chloroplasts), and Cu/Zn-SOD (residing in cytosol, peroxisomes, and chloroplasts). It has been reported that H_2O_2 during contact with a transition metal ion like Iron and Copper can produce hydroxyl radicals ($OH^{\cdot}$) through Fenton's reaction.

$$O^{\bullet}_{2}{}^{-} + O^{\bullet}_{2}{}^{-} + 2H^{+} \xrightarrow{SOD} 2H_2O_2 + O_2$$

b. Catalase (CAT)

It is a tetrameric heme-containing enzyme which causes dismutation of H_2O_2 into H_2O and O_2. It has high affinity for H_2O_2 and has a completely high turnover rate (6×10^6 molecules of H_2O_2 to H_2O and O_2 in one minute). It is highly important among other antioxidant enzymes since it does not require a reducing equivalent. Various isoforms of CAT are present in plant species.

$$H_2O_2 \xrightarrow{CAT} H_2O + (^1/2)O_2$$

c. Peroxidase (POX)

It is also a heme containing protein that consumes H_2O_2, thereby responsible for decreasing levels of H_2O_2 in the cell under normal as well as stressful conditions. Peroxidases which participate in lignin biosynthesis may create a physical barrier against heavy metal toxicity or pathogen attack. The opposite organization of peroxidases scavenges H_2O_2 in the cell and utilizes glutathione, Cyt c, pyridine nucleotide and ascorbate as electron donors *in vitro.*

Ascorbate peroxidase (APX) belongs to class I superfamily of heme peroxidases. This enzyme also exists in diverse isoforms *viz.* cytosolic, stromal, thylakoidal, mitochondrial and

peroxisomal. Ascorbate peroxidase reduces H_2O_2 concentration in chloroplast and cytosol of plant cells. It utilizes ascorbate as H-donor to breakdown H_2O_2 and release water and monodehydroascorbate (MDHA). Ascorbate peroxidase is one of the vital peroxidases, of ubiquitous occurrence in plants. Thus, it is regarded as an accepted housekeeping protein inside the cytosol and chloroplasts of plant cells.

$$H_2O_2 + AA \xrightarrow{APX} 2H_2O + DHA$$

d. Glutathione reductase (GR)

It is a flavoprotein, an oxidoreductase located in both eukaryotes and prokaryotes. It catalyzes reduction of GSSG in NADPH dependent manner and thus is critical in maintaining GSH pool. It is an important enzyme of ascorbate-glutathione cycle and maintains a high cellular GSH/GSSG ratio. Mainly, it is present in chloroplasts with little amounts present inside the mitochondria and cytosol. Glutathione is a low molecular weight thiol tripeptide (γ-glutamyl-cysteinyl-glycine) frequently present in nearly all of the cellular compartments. This versatility of GSH is due to its excessive reductive capability.

$$GSSG + NADPH \xrightarrow{GR} 2GSH + NADP$$

e. Phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL)

These are the members of a enzyme family having a lyase activity that forms α, β–unsaturated acids from amino acids by removal of ammonia or a mutase activity that forms β-amino acids. PAL is one of the most entensively studied enzyme of plant secondary metabolism, consisting of tetramer with each subunit of 77-83 kDa. It is the primary enzyme in the phenylpropanoid metabolism and performs an important role in the synthesis of numerous secondary metabolites which include phenols and lignin. It catalyzes the deamination of phenylalanine; a carbon-carbon double bond is formed at some stage with the release of ammonia, yielding trans-cinnamic acid. In some plants, tyrosine is transformed to 4-hydroxycinnamic acid in an analogous way with the aid of TAL. This enzyme has been employed for production of biochemicals, aromatic compounds, or for the synthesis of stilbenes together with resveratrol, flavonoids including naringenin, or plastic precursors consisting of p-hydroxystyrene.

f. Polyphenol oxidase (PPO)

It has three domains which include N-terminal plastid transit peptide, a highly conserved type-3 copper centre, and a C-terminal part. The family of PPO enzymes catalyzes the oxidation of monophenols and/or o-diphenols to o-quinones. PPOs are broadly distributed in bacteria, animals, plants, and fungi. The decrease in PPO activity because of stress conditions is related to improved antioxidant potential. There had been several reports which depicted that PPO should directly affect photosynthesis, along with it functioning as an oxygen buffer or water–water cycle to facilitate reactive oxygen scavenging.

Non-enzymatic Antioxidants and Cell Membrane Damage Indicators

a. Malondialdehyde (MDA)

Pathogen attack prompts production of ROS that results in lipid peroxidation and membrane damage, which in turn can be a key element of pathogenesis. The level of lipid peroxidation has been used as a marker for ROS induced damage to cellular membrane under stress conditions. As the membrane balance of the plant cells gets disrupted, crucial solutes leak out from the organelles as well as from the cell and results in membrane damage and metabolic imbalances. Malondialdehyde (MDA) that is produced during lipid peroxidation, is a valuable indicator of cell membrane damage due to various stresses like fungal infection. It is the final product of the decomposition of polyunsaturated fatty acids malondialdehyde. It has been generally used as affordable biomarkers for lipid peroxidation and for membrane permeability when crops are exposed to extreme environments.

b. Phenolic acids

Phenolic compounds are produced in response to pathogen attack and their production is considered as a part of active defense response. Accumulation of low molecular weight phenolics together with benzoic acid and the phenylpropanoids in response to infection results in slower growth of pathogen and permits activation of numerous phytoalexins. Eight phenolic acids trans-cinnamic, salicylic, ferulic, chlorogenic, p-hydroxy benzoic, protocatechuic, coumaric and vanillic were identified in barley grains. These compounds are bioactive, non-nutrient secondary metabolites present in culms, vegetables, and cereals. They guard the plant against pathogen attack or ultraviolet radiation. As antioxidants, phenolic compounds prevent oxidative damage to cellular organelles, organic molecules which include proteins, membrane lipids, DNA, and RNA. Phenolic compounds can also contribute in

strengthening host cell components with the aid of lignin and suberin biosynthesis that are involved in the formation of physical boundaries which can block the spread of pathogens. Additionally, they act as reducing agents, hydrogen donors, and singlet oxygen quenchers.

c. Flavonoids

Flavonoids are abundantly present in the kingdom *Plantae*occurring typically within the leaves and floral organs. On the basis of their shape, they can be categorized into four types i.e.flavonols, flavones, isoflavones, and anthocyanins. They play various roles in imparting pigmentation to flowers, fruits and seeds and are also involved in providing protection from pathogens. Flavonoids can be considered as a secondary ROS scavenging system in plants experiencing damage to the photosynthetic pigments, due to the extra excitation power. Apart from this, they have major role in scavenging $^1O_2$ and alleviating the damages caused to the outer envelope of the chloroplastic membrane.

d. Carotenoids

Carotenoids are yellow, orange and red pigments, C_{40} isoprenoids with a long conjugated polyene chain which is responsible for their color and biological activities. The core structural element of carotenoids is a polyene backbone together with a chain of conjugated C=C bonds. This unique characteristic in most cases is responsible for both their pigmentation properties and the potential of a large number of compounds to interact with free radicals and singlet oxygen and consequently act as powerful antioxidants. Carotenoids show their antioxidative potential by protecting the photosynthetic machinery in following ways, (a) reacting with lipid peroxidation products to terminate the chain reactions, (b) scavenging $^1O_2$ and dissipating heat, (c) preventing the formation of $^1O_2$ by reacting with $^3Chl^*$ and excited chlorophyll (Chl^*).

Conclusion and Future Directions

We know that plants, being sessile in nature, encounter numerous unfavourable environmental conditions that lead to reduced productivity and yields. Although plants have developed a variety of mechanisms to protect themselves against environmental stresses, still some stressful situations such as drought results in considerable yield losses. If we look at the Indian scenario, many of our states are facing serious threats of drought stress.It is suggested that plants remember what they experience/adopt to cope with environmental stress. Such potential of plant is known “stress memory” and it is referred as imprint, training, priming,

acclimatization, which result in positive effects like better response of the plant to subsequent stressful conditions. Plants utilize antioxidant defensive strategies, accumulation of protective metabolites, expression of stress-associated genes, and epigenetic alterations as stress-protective mechanisms, which enable plant for a quicker and efficient response to the stress on next exposure to the stress. Though involvement of biochemical factors like proline, phenolics, antioxidants, MDA, defense enzymes etc. in stress tolerance has been well-established, their role in stress memory has not yet been reported. Various studies are been conducted to interrogate the role of biochemical factors in imparting stress memory.

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