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Antioxidant defense mechanisms enhance oxidative stress tolerance in plants. A review

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ABSTRACT

Plants are mostly challenged by different abiotic stresses such as salinity, drought, temperature extremes, heavy metal toxicity, nutrient deficiency, UV-B radiation, ozone, etc. which cause damage to plants and losses in the quantity and quality of a yield. A key sign of such stresses is the over production of reactive oxygen species (ROS) include singlet ($O^{\bullet}_2$), superoxide (O2•−), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$). They cause cellular/tissue damage when they attack protein, DNA and Lipids, etc. Leading to irreparable metabolic dysfunctions and ultimately cell death. To overcome these free radicals and counter their effects as prooxidants another category of compounds called antioxidants (Enzymatic and Non-enzymatic) are produced either endogenously or received from exogenous sources. The enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POX), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), glutathione *S*-transferase (GST) and glutathione peroxidase (GPx). Non-enzymatic antioxidants include vitamins like vitamin C and E, minerals like Se, and Zn, carotenoids, glutathione (GSH), osmoregulators, phenolic and flavonoids, etc. Generally, plants have the ability to keep ROS within the limits generated under normal metabolic conditions whereas under prolonged stress, plants with higher antioxidative potential and higher levels of defense have well developed ROS-scavenging pathways or detoxification mechanisms to mitigate the adverse impact of oxidative stress and induce tolerance of plants.

Keywords: Stress, Free radicals, Lipid peroxidation, Antioxidant enzymes, ROS-scavenging, Tolerance.

Introduction

Plants are frequently exposed to a plethora of unfavorable or even adverse environmental conditions. These environmental stresses such as salinity, drought, chilling, metal toxicity nutrient deficiency, intense light, herbicides and UV-B radiation as well as pathogens attack (Mekki and Orabi, 2007; Miller *et al.,* 2009 Suzuki *et al.,* 2012), the all have been demonstrated to increase the enhancement of ROS generation in plants. Different types of ROS at high concentrations are very harmful to all cells and tissues and the cell here is said to be in a state of oxidative stress when the level of ROS exceeds the level of defense mechanisms. When the existence of biotic or abiotic stresses the enhanced production of ROS can pose a threat to cell when causes lipid peroxidation, Electrolyte leakage, beside oxidation of proteins, damage of nucleic acids, enzyme inhibition, activation of programmed cell death pathway and finally leading to poorly development or death of the cells (Meriga *et al*, 2004; Maheshwari and Dubey, 2009) As a result of The multifunctional roles of ROS (act as damaging or signaling molecule depending on their concentration) the cells have to tightly control the level of ROS to avoid any oxidative injury. Various workers have reported increased antioxidant enzymes activities of the defense system in plants to overcome oxidative damages induced by different abiotic stresses (Orabi and Mekki, 2008; Ahmed *et al.,* 2009, 2010; Hussein *et al.,* 2009; Kassab *et al.,* 2012; Orabi *et al.,* 2018a,b.c)

Molecular oxygen is relatively non-reactive in its normal configuration. During normal metabolic activity and as a consequence of environmental variations, O_2 can generate reactive excited states such as free radicals and derivatives: the singlet oxygen (1O2), superoxide radical (O2•−), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH•), the latter is the most potent oxidant known. Under stress ROS attack biomolecules causing DNA mutation, protein denaturation and

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membrane lipid peroxidation; these oxidations disturb normal cellular metabolism and cause molecular damage, and if sufficiently severe can result in cell death. In plants, the two major sources of ROS are the photosynthetic and respiratory electron transport chains. The main source of ROS in the light is chloroplasts and peroxisomes, while in darkness the mitochondria seem to be the main ROS producers. ROS are detected in virtually all intracellular organelles, as well as at the plasma membrane and, extra-cellular, in the apoplast. In acute or prolonged treatments, the ROS generated can overwhelm the constitutive systems necessitating additional defenses where oxidation is one of the destructive processes, it breaks down and damages various molecules. Thus, ironically, oxygen that it is essential for aerobic life-in its reduced forms in the same time it is one of the most toxic substances with which all organisms must cope.

ROS are detoxified by antioxidant defense systems including enzymatic and non-enzymatic protectants. Attenuating the imbalance between generation and scavenging of ROS is fundamental for healthy plants. The equilibrium between both sides is a central element in maintaining steady state conditions [(redox homeostasis), Foyer and Noctor, 2005]. A network of scavenging enzymes. Which include superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR) and catalase (CAT) (Mittler, 2002). Non enzymatic antioxidants of which the protective function of carotenoids and tocopherols is rooted in their high antioxidative potential. Carotenoids (e.g. carotenes and xanthophylls) are indispensable for plant photo protection and their role has been comprehensively reviewed (Baroli and Niyogi, 2000; Demmig-Adams and Adams, 2002). They are very efficient quenchers and scavengers of reactive oxygen species (ROS), and, in particular, of $^1O_2$. In higher plants, a dynamic protection mechanism, the xanthophyll cycle, enhances plant photoprotection (Demmig *et al*., 1987).

Besides carotenoids and tocopherols plant protection involves the water-soluble low molecular weight molecules ascorbate, vitamin C (Smirnoff, 2000) and glutathione (Noctor and Foyer, 1998), the main antioxidants in chloroplasts. Ascorbate and glutathione are connected via the ascorbate–glutathione cycle. There is also a functional relationship between these water soluble and lipophilic antioxidants. Ascorbate and glutathione are required for the regeneration of tocopherol, whereas ascorbate is a co-factor for the enzyme violaxanthin de-epoxidase (Eskling *et al*., 1997; Smirnoff, 2000b). Moreover, osmoregulators have protective role under osmotic stress, whereas phenolic compounds and flavonoids are more best than others in many stressfull conditions.

This article aims to introduce an overview on the different plant tools for significant up-regulation of the enzymatic and non-enzymatic antioxidant mechanisms for the best enhancement of oxidative stress tolerance. However gene transfer technology and biologists sciences of biotechnology, physiology and molecular biology, the all are needed for plant protection under stresses.

Free radicals

Free radicals are chemical species of atoms or molecules that possess an unpaired electron on their orbit. And it can exist independently, as it contains an odd number of electrons and that make it unstable, short lived and highly reactive, so it can react quickly with other compound to capture the needed electron to gain stability. In general, free radical attacks the nearest stable molecule, to steal its electron and attacked molecule to become a free radical itself, beginning a chain reaction cascade resulting in disruption of a living cell. These free radicals are highly unstable and can, therefore, react with other molecules by giving out or accepting single electrons (Crastes, 1990). ROS include both non-radicals and radicals. Most biological molecules are non-radicals containing two electrons per orbital, which is a stable configuration in a molecule. A free radical is a molecule that can exist independently and contains one or more unpaired electrons. An unpaired electron means that there is only one electron in an orbital (shown in red), which is an unstable configuration and makes free radicals highly reactive.

Reactive oxygen species

ROS, the most important free radicals in biological system, they are considered to be radical derivatives of oxygen. Oxygen is required to transfer various substances to release of the energy and detoxify xenobiotics. Where oxygen acts as terminal electron acceptor and is eventually converted to more stable compound, water. Where the occurrence of reduction of one molecule of O_2 via the cytochrome oxidase system of respiratory chain requires electrons (McCord and Fridovich, 1969) . That reduction is known as transvalent reduction of oxygen to water (Mayes, 2000). Thus, where this reduction takes place by a series of incomplete univalent reductions each of which needs a single electron, such type of reductions can produce a series of reactive radicals and non-radicals which are collectively known as ROS and act as oxidants in the living tissues (Hochstein and Atallah, 1988). ROS has the following known types:

Superoxide free radical anion(O^{-}_2)

It is the most common ROS. Which most existed by leakage of electrons and also it can produce direct and also indirect toxic effects by producing other ROS.

Hydrogen peroxide (H_2O_2)

It is not a radical and it has no unpaired electrons. Meanwhile it is an oxidising agent to the cells, it is able to diffuse easily through cellular membranes, it participates in the formation of the very active species hydroxyl radical, by the fenton reaction in the presence of iron which catalyze the decomposition of oxygen peroxide, that leads to damage to DNA and it is associated indirectly with mutation and carcinogenesis through OH radical formation (Oshima *et al*., 1996).

Hydroxyl free racical ($OH^{\cdot}$)

It is considered to be one of the most reactive free radical in the living cells (Bedwell *et al.,* 1989). It is formed from H_2O_2. It can cause damage to DNA beside it is the source of various deleterious lipid peroxidation products occurred in microsomal mitochondria and cell membrane (Halliwell and Gutteridge, 1989).

Singlet oxygen($^1O_2$)

It is not a true radical and it is most reactive oxidant though it causes damage to living tissues. Singlet oxygen formation occurs when spin reversal of electron in outer orbital of oxygen molecule occurred. It is reported to be an important ROS in reactions related to ultraviolet exposition. It was suggested that singlet O_2 may be formed during the degradation of lipid peroxides and the presence of metals contributes to increase its production

Nitric oxide ($NO^{\cdot}$)

It is produced in many types of cells and in vascular endothelium (O'Donnell and Freeman, 2001). With oxygen it reacts to form nitrogen dioxide ($NO_2^{\cdot}$) and it can react with $NO^{\cdot}$ to yield nitrogen trioxide (N_2O_3).

Ozone (O3)

Ozone is formed by photochemical oxidation of primary pollutants such as nitrogen oxides, hydrocarbons and carbon monoxide (Lelieveld and Crutzen 1990) Atmospheric ozone enhances the formation of highly reactive intermediates such as peroxyl, superoxide radicals, H_2O_2, etc. In plant tissues that occurs very fast in an ozone enriched environment, so ROS- scavenging system by a successful plant should respond quickly before extensive damage can occur (Gupta *et al*., 1991).

Oxidative stress and Lipid peroxidation

ROS homeostasis in cells is reached at the balance between ROS production and ROS scavenging by antioxidant compounds and enzymes. Under normal growth conditions, the production of ROS in cells is low (240 μM s−1 O2− and a steady state level of 0.5 H_2O_2 μM in chloroplast) (Polle, 2001) and ROS are normally in balance with antioxidant molecules. Non-enzymatic defenses include compounds of intrinsic antioxidant properties, water soluble such as ascorbate (vitamin C), glutathione, phenolic compounds and flavonoids, and lipid-soluble metabolites such as carotenoids and a-tocopherols (vitamin E). Antioxidants act as electron donors, reducing ROS to less or no harmful molecules; the enzymatic defenses or scavengers are mainly superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POX) ascorbate peroxidase (APX), glutathione reductase (GR) and glutathione peroxidase, (GPX), which protect cells by directly scavenging superoxide radicals and hydrogen peroxide, converting them to less reactive species. Homeostatic defenses can be overwhelmed in stress conditions, which increase ROS production (240–720 µM s−1 O2− and a steady state level of 5–15 H_2O_2 µM) (Polle, 2001). Aerobic organisms up-regulate both enzymatic and non enzymatic antioxidant defenses (Apel and Hirt, 2004) to decrease injuries resulted by ROS

The generation of ROS such as the superoxide anion ($\ddot{O}_2$) and hydrogen peroxide (H_2O_2) are a common event associated with normal plant biochemical processes (Zhou *et al.,* 2004). ROS attack membrane lipids and proteins are leading to lipid peroxidation and oxidative damage of proteins, to impair membrane structure and hence permeability (Gill and Tuteja 2010, Avery, 2011). Lipids and proteins are the two constituents of biomembranes and it is documented that membrane lipids are responsible for determining major biological properties of the membranes (Uitert *et al.,* 2010). When lipid peroxidation increases plasma membrane permeability might be that lipid peroxidation products are responsible for ordering phospholipids into gel phase which destabilizes the membrane structure (Thompson *et al.,* 1983, Russell 1989). In the same time oxidative damage of membrane proteins induced destabilization of their configuration (Gill and Tuteja 2010). Energy is produced from lipids in the body when lipids are β-oxidized and oxygen is reduced to water in the respiratory chain by mitochondria in different tissues (Murray *et al.,* 2000). When lipids are oxidized without release of energy, unsaturated lipids go rancid due to oxidative deterioration when they react directly with oxygen molecule (Moore and Roberts, 1998; de Zwart *et al.,* 1999; Halliwell and Gutteridge, 1999) This process is called lipid peroxidation (LPO) and the insertion of an oxygen molecule is catalyzed by free radicals (non-enzymatic lipid peroxidation) or enzymes (enzymatic lipid peroxidation) (Gutteridge, 1995). LPO induces disturbance of fine structures, alteration of integrity, fluidity, and permeability, and functional loss of biomembranes, modifies low density lipoprotein (LDL) to proatherogenic and prion flammatory forms, thus produces potentially toxic products (Greenberg *et al.,* 2008). Finally lipid peroxidation causes disturbance of fine structures, alteration of integrity, fluidity, and permeability, and functional loss of biomembranes and it is used as an indicator of oxidative stress in cells and tissues

Antioxidant defense mechanisms (Enzymatic and non Enzymatic)

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. Antioxidative system including spontaneously reactive antioxidants and enzymatically catalyzed reaction aids the cells in displacing the harmful reactive oxygen species (ROS). When this neutral equilibrium is disrupted (the accumulation of ROS exceeds the capacity of defense mechanisms) due to multiple abiotic or biotic stress factors, the cell is then called under oxidative stress. ROS production and accumulation of damage is greatly affected by the associated conditions such as light intensity and temperatures (Caverzan *et al.*, 2012). Duration and severity of stress, as well as the ability of the tissue to withstand or to acclimate to the energy imbalance and restore cellular homeostasis, the all are closely interlinked to this situation (Miller *et al.*, 2009). However, oxidative stress or damage occurred when the production of ROS exceeded the capacity of antioxidative system.

Defense mechanisms

Non-enzymatic antioxidants

Ascorbate

Ascorbate fulfills many key functions in plant biology. As well as being the most abundant low molecular weight antioxidant in the plant cell, it participates in the regulation of mitosis and cell expansion (Noctor and Foyer, 1998). Ascorbate is also a substrate for key enzymatic reactions, for example in the production of ethylene (McGarvey and Christoffersen, 1992). Orabi and Mekki (2008) mentioned that application of ASA on Suger beet plant grown under salinity resulted in obvious increments in all growth characters, root yield beside enhancement the activities of APX & GR to ascertain its role in ASA-GSH cycle. In plant cells, the most important reducing substrate for the removal of H_2O_2 is AsA (Del Río *et al*., 2006; Wu *et al*., 2007) it could react with H_2O_2, O2•− and 1O2 and it maintains the reduced state of the α-tocopherol. AsA may be involved in the synthesis of zeaxanthin, which dissipates excess light energy in the thylakoid membranes to prevent oxidative damage in plants (Conklin *et al*., 1996).

Glutathione

Glutathione (GSH) is very abundant in plants, it is the major non-protein thiol in plants and performs a multiplicity of important functions include scavenging of reactive oxygen species, heavy metal detoxification, transport and storage of sulfur, control of cell redox status, progression of the cell cycle, and protection of protein thiol groups, detoxification of xenobiotics and plays a role in gene activation and in the protection from oxidative stress (May et a!., 1998; Noctor and Foyer, 1998). In the leaves the synthesis of GSH is thought to take place in the chloroplasts and cytosol (Noctor and Foyer, 1998). GSH is a substrate for glutathione reductase and glutathione peroxidase (GSH-PX). Orabi *et al.,* (2017a) found that glutathione treatment enhanced increments in glutathione reductase enzyme activity in Zea mays plants under salinity stress.

Carotenoids

It is a lipophilic antioxidant involved in the protection of the photosynthetic apparatus against photo inhibitory damage by singlet oxygen (102) which is produced by the excited triplet state of chlorophyll. Carotenoids protect the photo systems when reacts with lipid peroxidation to terminate chain reactions (Burton and Ingold, 1984) it can directly deactivate 102 and can also quench the excited triplet state of chlorophyll, to indirectly reducing the formation of 102 species (Foyer and Harbinson, 1994). It was found that zeaxanthin is involved in the de-excitation of excess energy via nonradioactive dissipation in the pigment bed (DemmigAdams and Adams, 1996). There was a primarily physical mechanism that Carotenoids "quench" singlet oxygen, in which the excess energy of singlet oxygen is transferred to the carotenoid's electron-rich structure. The carotenoid is excited by this added energy into a "triplet" state (3Car*), and then relaxes into its ground state (1Car) by losing the extra energy as heat. Orabi and Abdelhamid (2016) found increments in the carotenoids content of faba bean plants under salt stress.

Tocopherols

It is a lipophilic antioxidant, tocopherols are a group of lipid soluble antioxidants. In plants, tocopherols (vitamin E) are synthesized and localized in plastids and accumulate to varying degrees in all tissues, with seed generally containing the highest levels (Sheppard *et al*., 1993). Tocopherols also function as recyclable chain reaction terminators of polyunsaturated fatty acid (PUFA) radicals generated by lipid oxidation (Girotti, 1998). Tocopherols scavenge lipid peroxy radicals and yield a tocopheroxyl radical that can be recycled back to the corresponding tocopherol by reacting with ascorbate or other antioxidants (Liebler, 1993).

Tocopherol levels increase in photosynthetic plant tissues and have protective role in response to a variety of abiotic stresses (Munne-Bosch and Alegre, 2002; Orabi and Abdelhamid, 2016; Orabi *et al.,* 2014, 2017b).

Polyamines

Polyamines (PAs) are organic polycations exist in almost all cells and free-living microbes, they have stimulatory roles in plant metabolic processes, they are regarded as plant biostimulants. In higher plants, the most common PAs are spermidine (Spd), spermine (Spm), and their diamine precursor putrescine (Put). In plant cells, PAs are found in the vacuole, cytoplasm, plastids, mitochondria, and in the cell wall, but Spm is also present in the nucleus (Alcázar *et al.,* 2010). They are able to form linkages to phenolic acids, especially hydroxycinnamic acid and other compounds with low molecular weight or to nucleic acids and proteins. PAs are able to form electrostatic linkages with negatively charged molecules, causing conformational stabilization/destabilization of DNA, RNA, chromatin, and proteins (Alcázar *et al*., 2010). Polyamines as a new type of plant growth biostimulant play vital roles in a range of developmental and physiological processes such as gene expression, protein and DNA synthesis, and cell division and differentiation; in growth and developmental processes such as somatic embryogenesis, organogenesis, and dormancy breaking of tubers; and in seed germination, development of flowers and fruits, and senescence (Shi and Chan 2014). polyamines (PAs)were involved in the defense reaction of plants to various environmental stresses including water shortage (Tang *et al*., 2004). Treatment with polyamines e,g putrescine or spermine counteracted the harmful effect of abiotic stresse by decreasing the lipid peroxidation as compared with the corresponding controls(Orabi *et al.,* 2015, 2016, 2017[c]).

Osmoregulators

Compatible solutes as osmoregulators are highly soluble compounds with low molecular weight. They are confined mainly to the sytosol, chloroplasts, and other cytoplasmic compartments to protect cellular components from dehydraltion injury resulted by osmotic stress , they include amino acids (e.g. proline), quaternary ammonium compounds (e.g. glycinebetaine) and a variety of sugars and suger alcohols (e.g. mannitol and trehalose). During the osmotic stress there are an increase in external osmolarity results in an efflux of water from the interior, leading to a reduction in the turgor pressure in the cell and reduction in the cytoplasmic volume and that elevates the concentration of various intracellular ions, which are toxic to the cell. Osmoregulators act to maintain membrane integrity, protect macromolecular structure, enhance antioxidant enzymes and stress proteins for scavenging of reactive oxygen species (Ashraf and Foolad, 2007; Hayat *et al.,* 2012). Application of glycinebetaine, proline and trehalose had a significant role on plant growth, seed yield and stress tolerance as observed in some crops e.g., wheat (Raza *et al.,* 2014), faba bean (Orabi and El-Noemani, 2015) and arabidopsis seedlings (Yang *et al.,* 2014)

Phenolic Compounds and Flavonoids

Phenolics have at least one aromatic ring (C6) bearing one or more hydroxyl groups. They are mainly synthetized from cinnamic acid. L phenylalanine ammonia-lyase PAL (EC 4.3.1.5), act to produce cinnamic acid which form phenolic compounds. Phenolics are considered to be diverse secondary metabolites (flavonoids, tannins, hydroxycinnamate esters and lignin) exist in plant tissues (Grace and Logan, 2000). Flavonoids are secondary metabolites of plants with polyphenolic structure. They are formed by the polypropanoid pathway and the startup component is phenylalanine molecule. Phenolics and flavonoids are more effective than Vitamin C, E and carotenoids (Dai and Mumper, 2010) The antioxidant properties of phenolic and flavonoid compounds are mediated by the following mechanisms: (1) scavenging radical species such as ROS/ reactive nitrogen species (RNS); (2) suppressing ROS/RNS formation by inhibiting some enzymes or chelating trace metals involved in free radical production; (3) up regulating or protecting antioxidant defense (Cotelle, 2001). Their activity depends on the number of free hydroxyl groups in the molecular structure, which would be strengthened by steric hindrance (Rice-Evans *et al.,* 1996).

Defense mechanisms

Antioxidant enzymes

A sequence of ROS- scavenging or detoxification steps is often required to avoid the conversion of one reactive species into a second, more harmful one Superoxide dismutase converts the superoxide radicals to H_2O_2 . The latter is scavenged by catalase and/or guaiacol peroxidase in peroxisomes and ascorbate peroxidase in the chloroplast and cytosol (Perl-Treves and Perl, 2002). Glutathione reductase helps in regeneration of ascorbate through the ascorbate- glutathione cycle.

Superoxide dismutase

Superoxide dismutase (SOD, 1.15.1.1) is known to catalyse the dismutation of superoxide to hydrogen peroxide and oxygen. Therefore, the activity of this enzyme determines the relative proportions of the two constituents of the Haber-Weiss reaction which generates hydroxyl radicals. SOD exists in all aerobic organisms and most or all subcellular compartments that generate activated oxygen, therefore SOD is assumed to have a central role in the defense against oxidative stress (Scandalias, 1993). There are three distinct types of SOD classified on the basis of the metal cofactor: the copper/zinc (Cu/Zn-SOD), the manganese (Mn-SOD) and the iron (Fe-SOD) isozymes. Howevere, SOD is considered to be the first line of defense $O2^-$.

Catalase

Catalase (CAT, 1.11.1.6) is a heme-containing enzyme that catalyses the dismutation of hydrogen peroxide into water and oxygen.CAT enzyme is important in the removal of hydrogen peroxide generated in peroxisomes by oxidases involved in ß-oxidation of fatty acids, the glyoxylate cycle (photorespiration) and purine catabolism. All forms of the enzyme are tetramers in excess of 220,000 molecular weights. Cat-1 and cat-2 are localized in peroxisomes and the cytosol, whereas cat-3 in mitochondria. Stress conditions which decrease protein turnover rate, such as salinity, heat extremes lead to the depletion of catalase activity (Hertwig *et al.,* 1992). CAT activity may be significant in the plant's ability to protect plants and tolerate the oxidative components of adverse environmental stresses.

Guaiacol peroxidas*e*

Many isoenzymes of (GPOX, 1.11.1.7) exist in plant tissues localized in vacuoles, the cell wall, and the cytosol (Asada, 1992). GPX is participated in many important biosynthetic processes, such as lignification of cell wall, degradation of IAA, biosynthesis of ethylene, wound healing, and defense against abiotic and biotic stresses (Kobayashi *et al.,*1996). GPX is effective quencher of reactive intermediary forms of O2 and peroxy radicals under stressed conditions (Vangronsveld and Clijsters, 1994). Orabi and Abdelhamide (2016) concluded that greater protection of salt-sensitive faba bean plants from salt-induced oxidative damage and reduction in lipid peroxidation were parallel to the increase in the guaiacol peroxidase (POX) activity

AsA-GSH Cycle Enzymes

The AsA–GSH cycle has a major defense system against ROS under normal or stress conditions exists in chloroplasts, cytosol, mitochondria, peroxisomes and apoplasts. It involves four enzymes (APX, MDHAR, DHAR and GR) as well as AsA, GSH and NADPH working to detoxify the ROS (H_2O_2) through many reactions to regenerate AsA and GSH. APX catalyses the reduction of

H_2O_2 to H_2O with the simultaneous production of monodehydroascorbate (MDHA), which is converted to AsA by the action of NADPH-dependent MDHAR or disproportionates nonenzymatically to AsA and dehydroascorbate (DHA) (Asada 1992). In this case DHA undergoes irreversible hydrolysis to 2, 3-diketogulonic acid or form AsA by DHAR, the role of GSH is to act as the reductant (Chen *et al.,* 2003). This lead to the generation of GSSG, which is regenerated to GSH by GR.

Ascorbate Peroxidase

Ascorbate Peroxidase (APX, 1.11.1.11) it is considerd to be the first H_2O_2 scavenging in the ASA-GSH cycle and protect cells in higher plants (Asada 1994). APXs enzymes involved in scavenging H_2O_2 in water-water and AsA-GSH cycles using AsA as the substrate, to catalyz the transfer of electrons from AsA to H 2O2, to produc DHA and water (Pang and Wang 2010) .The enhancement of APX activity in plants in response to different abiotic stress conditions will be occured (Orabi, 2004; Abd Elmotty and Orabi, 2013; Orabi *et al.,* 2010, 2013; Hasanuzzaman and Fujita, 2011).

Glutathione reductase (GR)

Glutathione reductase (GR, 1.6.4.2) is considered to be a potential enzyme in the AsA-GSH cycle to protect plants against stress. GR activity confers oxidative stress tolerance where it alters the redox state of important components of the ETC. It catalyses the NADPH-dependent reduction of disulphide bond of GSSG and to be very important for maintaining the GSH pool (Chalapathi Rao and Reddy, 2008). Thus GR also maintains a high ratio of GSH/GSSG in plant cells, through catalyzing the reduction of GSH. GR has a crucial role to maintain the tolerance of a plant under deferent stresses by maintaining the antioxidant machinery of the cell, and stress tolerance (Hossain *et al.,* 2011; Hasanuzzaman *et al.,* 2011) .

In conclusion, all organisms in aerobic environments including plant cells have a variety of antioxidant defense mechanisms (enzymatic and non-enzymatic compounds) to overcome the over production of reactive oxygen species (ROS) produced under stress. These antioxidant systems in successful plants prevent oxidation of cellular components which could lead to cell death.

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