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Oxidative Stress and Antioxidant Defense System in Plants under Drought Stress

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ABSTRACT

At the moment, climate change is thought to be the biggest environmental concern. Due of its significant impact on agriculture, it is currently receiving a lot of attention from farmers, researchers, and politicians. Global climate change, environmental unpredictability, and the environment's increasing complexity are all contributing factors to the situation's growing severity. Drought has a severe negative impact on crop plant growth and development, making it one of the most severe environmental pressures now affecting agriculture. During a number of processes linked to drought stress, plant cells generate oxygen radicals and their byproducts, known as reactive oxygen species (ROS). The four major active oxygen species includes superoxide anion (Radicals) ($O_2^{\bullet-}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$). One of the most crucial consequences of abiotic stress is the disturbance of the equilibrium between the generation of ROS and antioxidant defense systems triggering the excessive accumulation of ROS and inducing oxidative stress in plants. ROS are highly reactive in the absence of any protective mechanism and can seriously disrupt normal metabolism through oxidative damage to membrane lipids, proteins, carbohydrates, pigments and nucleic acids, which ultimately results in the cell death. The major potential sources of ROS in plants are chloroplasts, mitochondria and peroxisomes. Enzymatic and nonenzymatic antioxidant defence systems maintain the equilibrium between the detoxification and generation of ROS under water deficit conditions. One of the most important effects of water stress is the disruption of the balance between the production of reactive oxygen species (ROS) and antioxidant defence systems, which leads to an excessive build-up of ROS and oxidative stress in plants. ROS are highly reactive in the absence of any protective mechanism and can seriously disrupt normal metabolism through oxidative damage to membrane lipids, proteins, carbohydrates, pigments and nucleic acids, which ultimately results in the cell death. Chloroplasts, mitochondria, and peroxisomes are the main possible sources of ROS in plants. Under conditions of water stress, the balance between ROS formation and detoxification is maintained by both enzymatic and nonenzymatic antioxidant defence systems. The literature is reviewed in terms of ROS production and its antioxidative defence mechanism during drought.

Key words : Drought stress, ROS, Antioxidant defense, Oxidative stress

1. Introduction

Drought, salt, heavy metals, nutrient deficiencies, light intensity, pesticide contamination, and severe temperatures are just a few of the abiotic challenges that plants face since they are sessile. In the upcoming decades, it is anticipated that abiotic and biotic stresses will become much more common, leading to significant declines in crop plant growth and quality [1,2,3]. Among the various abiotic stresses, drought stress is the most important factor limiting crop productivity throughout the world and has been the focus of much research [4]. As an unfortunate consequence of aerobic life, reactive oxygen species are formed by partial reduction of molecular oxygen. The cellular ROS molecules can be of two major forms: free radicals including superoxide anions ($O_2^{\bullet-}$), hydroxyl radicals ($OH^{\bullet}$) and hydroperoxyl radicals ($HO_2^{\bullet-}$), where hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) are known to be nonradical molecules [5]. It is well established that chloroplasts, mitochondria, peroxisomes, apoplast, and plasma membranes are the primary sites of cellular ROS generation but chloroplasts are the leading sites for ROS production [6], and plants have mechanisms to deal with them in normal conditions, controlling the formation and removal rates. Under water stress conditions, ROS production can exceed the plant's defense mechanism, an imbalance in intracellular ROS content is established and this results in an oxidative stress [7]. ROS can pose a threat to cells by causing peroxidation of lipids, oxidation of proteins, damage to nucleic acids which ultimately leads in cell death [8]. On the one hand, plants need to control the levels of these oxidants because of their harmful nature, but on the other hand, they also use ROS as signaling molecules especially in response to various stress or threats to the plant integrity, as pathogen attacks, or non-optimal growth conditions [9]. Because of the multifunctional roles of ROS, it is necessary for the cells to control the level of ROS tightly to avoid any oxidative injury and not to eliminate them completely. Plants possess very efficient enzymatic and non-enzymatic antioxidants defense systems that can protect cells from the oxidative damage. The enzymatic antioxidants includes superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPOX), enzymes of ascorbate-glutahione (AA-GSH) cycle such as ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) [5,10]. Ascorbate (AA), glutathione (GSH), carotenoids, tocopherols and phenolics serve as potent non-enzymatic antioxidants within the cell [8]. It has also been reported that plants with high levels of antioxidants, whether constitutive or induced by drought stress, have a greater resistance to oxidative damage [7,10]. Keeping this in mind, this chapter will focus on oxidative stress and antioxidant defense in plants induced by drought.

2. ROS and Drought

In plants, drought stress triggers a number of reactions that result in oxidative stress. Stomatal closure is one of the first reactions to dryness, which can reduce photosynthesis by limiting plant transpiration and CO_2 uptake [11]. Decreased availability of CO_2 stimulates ribulose–1,5–bisphosphate oxygenation and, thus, photorespiratory hydrogen peroxide (H_2O_2) production in the peroxisomes. Insufficient availability of the electron acceptor CO_2 slows down the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) in the Calvin–Benson cycle. Lack of $NADP^+$ causes a backlog of electrons and over-reduction of the photosynthetic electron transport which in turn increases the reduction rate of oxygen as alternative electron acceptor in the Mehler reaction at photosystem I (PSI) and enhanced release of superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) [5,8,10]. Additionally, ROS can damage the lipids in thylakoid membranes through lipid peroxidation, leading to membrane destabilization and impaired photosynthetic efficiency. Hence, chloroplasts are primary targets of excess light and CO_2 starvation in drought. In addition, photorespiration produces NADH in the mitochondrion. Mitochondrial electron transport chain generates $O_2^{\bullet-}$ at complex I and III. $O_2^{\bullet-}$ can also be produced by NADPH oxidase in the plasma membrane and xanthine oxidase in perioxisomes [8]. Beyond the photosystems, the oxidative stress induced by drought also affects nucleic acids, leading to mutations, strand breaks, and the formation of adducts. These damages can interfere with the replication and transcription processes, affecting the overall cellular metabolism and stress response mechanisms. Moreover, the accumulation of ROS under drought conditions can lead to the activation of programmed cell death (PCD) pathways, resulting in the loss of cells and tissues critical for plant growth and development [9]. Lipid peroxidation is commonly taken as an indicator of oxidative stress in response to drought [12] and ROS-mediated membrane damage has been demonstrated to be a major cause of the cellular toxicity by water stress [13]. One of the more susceptible targets in proteins are thiol groups the oxidation of which can lead to protein denaturation and loss of functional conformation. Oxidative attack on DNA results in deoxyribose oxidation, strand breakage, removal of nucleotides, variety of modifications in the organic bases of the nucleotides, and DNA-protein

crosslinks. The drastic increase in lipid peroxidation due to drought stress was reported by many researchers [7,9].

It is well established that as a result of drought stress the generated harmful ROS damage the cell structures, proteins, lipids, carbohydrates, and nucleic acids, and disrupt cellular homeostasis, in severe cases leading to cell death. However, in spite of the detrimental effects of ROS, act as a signaling molecule, secondary messenger, mediating the acquisition of tolerance to drought which have been studied in several reports [5,8]. ROS is also modulate the activities of many components in signaling, such as protein phosphatases, protein kinases, and transcription factors and communicate with other signal molecules and the pathway forming part of the signaling network that controls response downstream of ROS [14].

3. ROS scavenging antioxidative system in plant cells

Plants have developed several antioxidation strategies by an array of enzymatic and non-enzymatic antioxidants, that can protect cells from oxidative damage and scavenge toxic ROS that are produced in excess of those normally required for various metabolic reactions. The well-documented antioxidant enzymes in plants are superoxide dismutase (SOD), catalase, ascorbate peroxidase, glutathione reductase, monodehydroascorbate reductase, dehydroascorbate reductase, glutathione peroxidas, guaicol peroxidase, glutathione-S-transferase, and so on [8,15]. Among the nonenzymatic antioxidant components, ascorbic acid, glutathione (GSH), phenolic compounds, alkaloids, non-protein amino acids, and a-tocopherols are commonly found in plants [15]. Enhancing the antioxidant defence system is thought to be an effective strategy to help plants acquire adaptive traits and tolerance to drought stress. Several studies on plants show a positive correlation between increased antioxidant enzyme activity and a plant's ability to withstand drought stress [16]. Tolerant species, varieties, and genotypes typically exhibit higher levels of antioxidant enzyme activity than non-tolerant plants, supporting their crucial function in drought tolerance [1,16]. SOD, the family of metalo enzymes are the first line of defense against injury caused by ROS, catalyzing the dismutation of $O_2^{-\cdot}$ to H_2O_2 and O_2 [7,8]. SOD removes superoxide, hence decreases the risk of hydroxyl radical formation from superoxide via the metal-catalyzed Haber-Weiss-type reaction. SOD has a metal cofactor and depending on the metal can be classified in three different groups (isoforms), localized in different cell compartments: FeSOD (chloroplasts, cytosol and mitochondria), MnSOD (mitochondria and peroxisomes), Cu/ZnSOD (chloroplast, peroxisomes, cytosol and apoplast) [8]. SOD activity has been reported to increase under drought stress, and exhibited tolerance towards oxidative damage [10,16]. There are various conflicting reports about the activities of SOD enzyme. A decline in SOD activity has been reported under drought stress in sunflower [17]. Catalase is predominantly found in leaf peroxisomes where it functions chiefly on H_2O_2, generated during photorespiration and/or β-oxidation of fatty acids in glyoxysomes, converts to oxygen and water [8]. CAT catalyses the hydrogen peroxide breakdown to water. CAT comprises a family of isoenzymes CAT-1, CAT-2 and CAT-3 that differed in their molecular, biochemical properties, cell or organ specific and temporal patterns of expression and also known to be differentially affected by environmental factors [18]. A decline in catalase activity under water deficits in tobacco and mungbean was reported [19]. Peroxidases comprise a family of enzymes with different characteristics which play a crucial role in the detoxification of cellular H_2O_2, the toxic product of superoxide dis-mutation. It scavenges excess amount of hydrogen peroxide formed in plant cells under normal and stress conditions. Induction in peroxidase has been documented under various oxidative stress factors [18], and it has been linked with protection from oxidative damage, lignification and cross linking of cell wall to prevent from such adverse conditions. High POX activity and isoforms under stress conditions has been correlated with reduction in plant growth, and this reduction has been attributed to POX catalysis of feruloylation of hemicelluloses and insolubilization of hydroxyproline-rich glycoproteins causing cell-wall stiffening [20]. Unlike catalase, peroxidase require a substrate (R) for catalysis and it represents different electron donors in their family of enzymes, guaiacol peroxidase uses mainly phenolic donors, ascorbate peroxidase uses ascorbic acid and glutathione peroxidase uses glutathione. Apart from scavenging of H_20_2, GPX also serve to detoxify products of lipid peroxidation formed due to activity of ROS. GPX decomposes peroxides to water (or alcohol) while simultaneously oxidizing GSH. GPX competes with CAT for H_20_2 as a substrate and is the major source of protection against low levels of oxidative stress. APX is involved in scavenging of H_20_2 in water-water and ascorbate-glutathione cycles and the APX family consists of at least five different iso-forms including thylakoid and microsomal membrane bound forms, as well as soluble stromal, cytosolic and apoplastic enzymes. The water-water cycle occurs in chloroplasts and is a fundamental mechanism to avoid photooxidative damage [21], using SOD and APX to scavenge the superoxide radical and hydrogen peroxide in the location where they are produced avoiding the deleterious effects of their reactivity

with other cellular components [8]. The ascorbate-glutathione cycle is an important group of reactions involved in ROS detoxification, as it converts hydrogen peroxide (formed as a consequence of an induced stress or via SOD action) and occurs in several cell compartments, like chloroplasts, cytosol, mitochondria, peroxisomes and apoplast. It uses APX and GPX as well as other enzymes like monodehydroascorbate reductase and dehydroascorbate reductase that have a role in the regeneration of the reduced form of ascorbate. Glutathione reductase, is a potential enzyme of ASH-GSH cycle and plays essential role in defense system against ROS by sustaining the reduced status of GSH. It is found in chloroplasts as well as in mitochondria and cytoplasm. Increased cellular glutathione and glutathione reductase have been reported in plants which are tolerant to abiotic stresses [22]. Increased activity of glutathione reductase has been reported under water stress in several plants [7,16]. It has been shown that O_2^- and H_2O_2 generated in the leaves during water stress might be responsible for the induction of GR [16]. Glutathione S-transferases are a large and diverse group of enzymes which catalyses the conjugation of electrophilic xenobiotic substrates or endogenous organic compounds with reduced glutathione tripeptide. The enhancement of GST enzyme activity has been considered as a marker for plant response to multiple environmental stress [23]. GSTs are believed to play a role in antioxidant metabolism by mechanisms that probably aid in the reduction of secondary noxious products resulting from exposure to stress-induced reactive oxygen species [24]. Polyphenol oxidases catalyze the O_2-dependent oxidation of mono- and o-diphenols to o-diquinones, highly reactive intermediates whose secondary reactions are believed to be responsible for the oxidative browning which accompanies plant senescence, wounding, and responses to pathogens. PPO may have a role in the development of plant water stress and potential for photoinhibition and photooxidative damage that may be unrelated to any effects on the Mehler reaction [5]. The general increase in membrane lipid peroxidation is proportional to the severity of drought stress and may result from the spontaneous interactions of ROS with organic molecules found in the membranes [25]. The accumulation of osmolytes like amino acids, GB, proline, sugars, and polyamines seems to have positive correlation in preventing oxidative damages triggered by drought through their capacity to scavenge reactive oxygen species, protecting important cellular macromolecules from oxidative deterioration [26,27]. The nonenzymatic antioxidants such as AsA, GSH, α-tocopherol, phenolic compounds (PhOH), flavonoids, alkaloids, and nonprotein amino acids work in a coordinated fashion with antioxidant enzymes such as SOD, CAT, POX, polyphenol oxidase (PPO), APX, MDHAR, DHAR, GR, GPX, GST, TRX, and PRX to inhibit overproduction of ROS [5,8].

Table 1. Major enzymes involved in ROS scavenging with their location and catalyzed reaction

Antioxidants	Reaction catalyzed	Enzyme code	Location
SOD	$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \rightarrow 2H_2O_2 + O_2$	1.15.1.1	Cyt, Chl, Per, Mit
CAT	$2H_2O_2 \rightarrow O_2 + 2H_2O$	1.11.1.6	Per, Gly Mit
APX	$H_2O_2 + AA \rightarrow 2H_2O + DHA$	1.11.1.11	Cyt,Per,Chl, Mit
GPX	$H_2O_2 + 2GSH \rightarrow 2H_2O + GSSG$	1.11.1.7	Chl, Cyt, Mit, ER
MDHAR	$MDHA + NAD(P)H \rightarrow AA + NAD(P)^+$	1.6.5.4	Chl, Mit, Cyt
DHAR	$DHA + 2GSH \rightarrow AA + GSSG$	1.8.5.1	Chl, Mit, Cyt
GR	$GSSG + NAD(P)H \rightarrow 2GSH + NAD(P)^{+`}$	1.6.4.2	Cyt, Chl, Mit
PPO	$PhOH + O_2 \rightarrow Cat$	1.14.18.1	Cyt, Chl, Vac

4. Conclusions and future perspectives

It is widely accepted that a variety of abiotic stressors cause plants to produce more dangerous and reactive reactive oxygen species (ROS), which in turn causes oxidative stress. Severe drought stress increases ROS in plants' mitochondria, peroxisomes, and chloroplasts, harming cellular constituents including proteins and lipids and ultimately causing cell death. However, it is now widely known that ROS are also effective signalling molecules that are involved in triggering acclimatory responses to stress stimuli and controlling plant growth and development, despite their ability to cause damaging oxidations. Therefore, it's critical that cells strictly regulate the amount of ROS they produce, without totally getting rid of them. Plants mitigate drought-induced ROS-based oxidative damage through the enzymatic and non-enzymatic antioxidant defense mechanisms. For agricultural output to remain sustainable in the face of the harsh climatic conditions brought on by climate change, it is crucial to develop plant varieties that can effectively scavenge and/or regulate the

amount of cellular ROS. Even though oxidative stress has advanced quickly in recent years, there are still a lot of unknowns and gaps in the regulatory networks that govern the generation and scavenging of ROS as well as the coordination of antioxidants and related compounds with plant growth and development in a dynamic environment. Hence, identifying the genes and comprehending their mechanisms in the regulation of ROS signalling pathways is also urgently needed.

Abbreviations: AA, ascorbic acid; ABA, abscisic acid; Apo, apoplast; APX, ascorbate peroxidase; CAT, catalase; Chl, chloroplast; CW, cell wall; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; DNA, deoxyribonucleic acid; ET, electron transport; FA, Fatty acid; GSH, reduced glutathione; GSSG, oxidized glutathione; GPOX, guaiacol peroxidase; GPX, glutathione peroxidase; GR, glutathione reductase; GST, glutathione-s-transferase; HO, heme oxygenase; H_2O_2, hydrogen peroxide; $OH^{\bullet}$,hydroxyl radical; MDA, malondialdehyde; MDHA, monodehydroascorbate; MDHAR, monodehydroascorbate reductase; Mit, mitochondria; NAD, nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; PSI, Photosystem: PCD, programmed cell death; Per, peroxisome; $OONO^{-}$, peroxynitrate; $HO_2^{\bullet}$, perhydroxyl radical; PM, plasma membrane; POX, peroxidase; PUFAs, polyunsaturated fatty acids; ROS, reactive oxygen species; $O_2^{\bullet -}$, superoxide radical; $^1O_2$, singlet oxygen; SA, salicyclic acid: Cat, Catechol; Vac, Vacuole; GB, Glycine betaine.

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