Chapter

Mechanistic Role of Reactive Oxygen Species and Its Regulation *via* the Antioxidant System under Environmental Stress

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Abstract

The antioxidant potential is referred to as compounds that are capable of protecting the biological system against the deleterious effect of reactions involving reactive oxygen species (ROS). ROS are toxic byproducts of oxygen metabolism. ROS have a significant role in plant signaling, growth, development, and majorly in response to environmental fluctuations. The ROS family plays a double role under various environmental stress conditions. In various key physiological phenomena, they act as secondary messengers and induce oxidative damage. ROS led to cellular damages that manifest themselves in degradation of biomolecules, which eventually amalgamate to cellular death in plants. To assure survival, plants have developed efficient antioxidant machinery having two branches, that is, an enzymatic and a nonenzymatic antioxidant. This chapter will emphasize the various types of ROS, their sites of cellular production, targets, and scavenging mechanisms mediated by antioxidants in abiotic stress. Such profound knowledge will let us build strategies against environmental stress.

Keywords: ROS, abiotic stress, antioxidant, free radical

1. Introduction

Plants are continually susceptible to environmental changes, prompting them to regulate their metabolism in such a way as to maintain a constant balance between the generation of energy and its consumption. This delicate balance is majorly dependent on a network signaling that mainly coordinates among the key operations in plant life including dark respiration, photorespiration, and photosynthesis, all of these activities are linked by reductants, substrate, energy, and electron transfer [1, 2]. Plant organelle metabolic pathways are sensitive to climate change and metabolic inequities in cells that can cause oxidative stress by boosting the oxidation of cellular components, production as well as accumulation of reactive oxygen species (ROS), impeding metabolic activities, and affecting organelle integrity [3, 4].

Environmental abiotic stresses, such as chilling, salinity, harsh temperature, drought, toxic metals or metalloids, flooding/waterlogging, and ultraviolet (UV) radiation, have become more common as a result of abrupt and extreme climate change [5]. The escalation of various abiotic stresses emerged as a key threat to agricultural crop production. Furthermore, an excess of ROS such as free radical alkoxy radical ($RO^{\bullet}$); hydroperoxyl radical ($HO_2^{\bullet}$); hydroxyl radical ($OH^{\bullet}$); superoxide anion ($O_2^{\bullet}$); and nonradical molecules (singlet oxygen, $^1O_2$, and hydrogen peroxide, H_2O_2) causes plant oxidative stress [6]. The principal sites of cellular ROS formation are mitochondria, chloroplast, apoplast, plasma membranes, and peroxisome [7]. Although as a component of regular cellular metabolism ROS are produced in plants, their overabundance due to several stresses severely destroys essential cellular constituents such as DNA, proteins, carbohydrates, lipids, and so on due to their highly reactive nature [8]. Nonenzymatic, low-molecular substances such as ascorbic acid (AA), reduced glutathione (GSH), carotenoids, tocopherol, proline, phenols, and flavonoids as well as guaiacol peroxidase (GPX), peroxidase (APX), superoxide dismutase (SOD), catalase (CAT), and glutathione-S-transferase (GST). The pervasiveness of both of the antioxidant machinery's arms supports the necessity for ROS detoxification for cellular viability [9].

1.1 Types of ROS

ROS are generally a molecular O_2 that has been incompletely activated or reduced or the principal product or the O_2-containing molecule byproduct that has an elevated reactivity than ambient O_2. ROS are produced by the electrons from the O_2 molecule or transfer of energy. The most prevalent cellular ROS in plants are $OH^{\bullet}$, $O_2^{\bullet}$, $^1O_2$, and H_2O_2. Cells generate both nonradical and free radical ROS. Free radicals include $OH^{\bullet}$, $O_2^{\bullet}$, $ROO^{\bullet}$, and $RO^{\bullet}$, while the nonradicals include $^1O_2$ and H_2O_2. The other ROS nonradicals found in plants include excited carbonyl (RO^{*}) and hypochlorous acid (HOCl). Furthermore, few acidic molecules such as hypoiodous acid (HOI), hypobromous acid (HOBr), and hypochlorous acid (HOCl) and radical compound ($CO_3^{\bullet}$) are incorporated into ROS. In addition, biological systems may contain alkoxyl radical ($LO^{\bullet}$), peroxyl radical ($LOO^{\bullet}$), hydroperoxyl radical ($HO_2^{\bullet}$), peroxynitrite (HNO_3), trichloromethyl peroxyl radical ($Cl_3COO^{\bullet}$), and ozone (O_3).

1.1.1 Superoxide radical ($O_2^{\bullet-}$)

In the chloroplasts, ROS are constantly produced as a result of partial O_2 reduction or energy transfer to O_2. During the noncyclic electron transport chain (ETC), $O_2^{\bullet-}$ is generated mostly in the thylakoid localized PSI and other cellular compartments. When cytochrome c oxidase reacts with O_2, H_2O is normally produced. Occasionally, O_2 reacts with the various ETC components to produce $O_2^{\bullet}$. It is typically the first ROS to develop. $O_2^{\bullet-}$ can potentially undergo a further reaction, resulting in the formation of other ROS [10].

1.1.2 Singlet oxygen ($^1O_2$)

It is an unusual ROS that is produced *via* the chlorophyll reaction (in the antenna system, in the triplet state) rather than electron transport to O_2. Heavy metals, salinity, and drought all cause stomatal closure, resulting in lack of intracellular CO_2 concentration. Facilitating the production of $^1O_2$ and causing significant damage to both the photosystems (PSI and PSII) put the whole photosynthetic apparatus in

danger, causing damage to a wide spectrum of the target. These substances include pigments, proteins, lipids, and nucleic acids and are the primary ROS responsible for light-induced loss of PSII function, resulting in cellular death [11].

1.1.3 Hydrogen peroxide (H_2O_2)

It is created in plant cells under normal conditions as well as in response to oxidative stresses (drought, cold, UV radiation, bright light, pathogen infection, and wounding). $O_2^{\bullet-}$ undergoes both univalent protonation and reduction, and a moderately reactive H_2O_2 is produced. The ER, mitochondria, ETC in the chloroplast, oxidation of fatty acids, photorespiration, and cell membrane are the key sources of H_2O_2 generation in plant cells. In plants, H_2O_2 is useful at low quantities but harmful at greater amounts. It operates as a regulatory signal for critical physiological processes such as senescence, stomatal movement, photosynthesis and photorespiration, growth as well as development at low intracellular concentrations [12].

1.1.4 Hydroxyl radical ($OH^{\bullet}$)

$OH^{\bullet}$ is the most reactive and destructive ROS. At neutral pH, it is produced *via* the Fenton reaction between H_2O_2 and $O_2^{\bullet-}$ catalyzed by transition metals such as Fe (Fe^{2+}, Fe^{3+}). $H_2O_2 + O_2^{\bullet-} \rightarrow OH^- + O_2 + OH^{\bullet}$. It is capable of causing harm to several biological components through lipid peroxidation (LPO), protein degradation, and membrane disintegration. Because no enzymatic system exists to scavenge this deadly radical, excessive $OH^{\bullet}$ buildup causes cellular death [13].

1.1.5 Peroxyl radical ($ROO^{\bullet}$)

The key chain-propagating step in lipid peroxidation and non-lipid systems is the formation of $RO_2^{\bullet}$ and $RO^{\bullet}$ radicals that can be generated by the decomposition of protein and lipid peroxides when heated or by the addition of transition metal ions. It is easy to produce peroxyl radicals by combining O_2 with carbon-centered radical $>C^{\bullet} + O_2 > C^-OO^{\bullet}$. The peroxyl radical plays an important role in the oxidation of lipids, DNA damage, changes in the protein backbone, and the degradation of food.

1.1.6 Alkoxy radical ($RO^{\bullet}$)

When lipids are oxidatively degraded or peroxidized without the help of enzymes, alkoxyl radicals are generated by the Fenton reaction, by electron reductions, or by combining two peroxyl radicals. Apoptosis and DNA alterations may result from alkyl radical oxidation. DNA damage and apoptosis can be caused by alkoxyl radicals, which are very oxidizing.

2. Sites of ROS production/generation in plant cells

It is proven that ROS can be produced in multiple places in the mitochondria, chloroplasts, plasma membranes, peroxisomes, endoplasmic reticulum, and cell wall both under normal and stressful conditions. ROS are produced primarily by peroxisomes and chloroplasts when light is present, while the mitochondria generate ROS when light is not present.

2.1 Chloroplast

Chlorophyll (chl) and light interact with each other to produce ROS in the chloroplast, which is the most important site where ROS are produced. In this case, ROS are principally generated by triplet chl and electron transport chains (ETC), including PSI and PSII. SOD converts $O_2^{\bullet-}$ into H_2O_2 under PSI in the Mehler reaction [14]. The $O_2^{\bullet-}$ and H_2O_2 generate more highly reactive $HO^{\bullet}$ when combined with metal ions such as Fe^{2+}. Various ecological stressors cause stomatal closure, resulting in a decrease in CO_2 levels, thereby causing the generation of chloroplastic ROS [3, 15].

2.2 Mitochondria

Although on a smaller scale, mitochondria are also responsible for damaging ROS production, including H_2O_2 and $O_2^{\bullet-}$. This is due to the mitochondrial ETC (mt ETC) containing enough energetic electrons to reduce O_2 and produce ROS. The two main mt ETC components responsible for the production of ROS are complexes I and III [16]. Additionally, the mitochondrial matrix contains numerous enzymes that produce ROS. Mn-SOD and APX reduce O_2 into H_2O_2 under the influence of oxygen, despite its abundance in the mitochondria. When mitochondria are under abiotic stress, ROS production increases dramatically [17].

2.3 Apoplast

The diffusible region around the plant cell membrane appears to be in charge of converting incoming CO_2 into a soluble, diffusible form that can be transported into the cytoplasm to carry out photosynthesis. During the harsh environmental situation, stress signals combined with abscisic acid make the apoplast a significant location for H_2O_2 production. Several other substances, such as pH-dependent peroxidases (POXs), polyamine oxidases, and cell-wall-linked oxidases, also generate ROS [18].

2.4 Plasma membranes

Plant cells are surrounded by a plasma membrane, which is constantly interacting with changing external conditions, thereby providing essential information for their survival.

During electrons transferring from cytosolic NADPH to O_2, either e^- dismutates spontaneously to H_2O_2 or is catalyzed by NADPH oxidase; SOD forms $O^{\bullet}_2$. The importance of NADPH oxidase in plant defense against pathogenic infection and abiotic stress conditions has been well established [19].

2.5 Cell walls

The cell wall-localized lipoxygenase (LOX) creates polyunsaturated fatty acid (PUFA) hydroperoxidation, making it an active generator of ROS such as $O_2^{\bullet}$, $OH^{\bullet}$, $^1O_2$, and H_2O_2. Using diamines or polyamines, cell wall-localized diamine oxidases produce ROS in the cell wall. During the pathogenic attack, lignin precursors undergo considerable cross-linking *via* H_2O_2-mediated pathways, resulting in the formation of recombinant lignin [20].

2.6 Endoplasmic reticulum (ER)

Cyt P450, which is located in the ER, is used to create $O_2^{\bullet-}$ *via* NADPH-mediated electron transport. RH (an organic substrate) interacts with CytP450, which is formerly reduced by a flavoprotein to form a free-radical intermediate (Cyt P450 R). This intermediate forms an oxygenated complex when it reacts with triplet oxygen ($^3O_2$) (Cyt P450-ROO). When the complex decomposes to Cyt P450-Rh, $O_2^{\bullet}$ is produced as a byproduct [21].

3. Role of ROS as messengers

ROS have been identified as the second messenger in intracellular signaling cascades that mediate a variety of plant responses, including programmed cell death, stomatal closure, gravitropism, and abiotic and biotic stress-tolerance acquisition. ROS can also affect the activity of numerous signaling components, including protein phosphatases, transcription factors, and protein kinases as well as communicate with other signal molecules and the pathway that administers response downstream of ROS. The balance between oxidant formation and antioxidant removal determines the life span, strength, and size of the ROS signaling pool [19, 20].

4. ROS and oxidative damage to biomolecules

The creation and removal of reactive oxygen species must be appropriately regulated to avoid oxidative stress. When the number of reactive oxygen species exceeds the cell's defensive systems, the cell is said to be in "oxidative stress." The balance between ROS formation and scavenging is upset in several stressful circumstances, such as salt, thirst, metal toxicity, intense light, viruses, and so on. High quantities of ROS can harm biomolecules, such as lipids, proteins, and DNA. These processes can alter inherent membrane properties including fluidity, enzyme activity loss, ion transport, protein synthesis suppression, protein cross-linking, DNA damage, etc., resulting in cell death. Redox homeostasis develops in plant cells as a result of equilibrium between the creations of ROS and the functioning of antioxidant enzymes, where a well-functioning defense system in plants maintains the right balance between ROS generation and its removal.

For proper redox signaling in the cell, it is, therefore, necessary to maintain an amount of ROS above or below the cytotoxic concentration, which is achieved by maintaining the equilibrium between ROS production and scavenging. As a result, a constant equilibrium between ROS formation and scavenging systems is maintained by cooperating with cellular redox-sensitive components to precisely adapt the downstream signaling procedures in a context-specific and cell-specific manner. Under varied abiotic stress conditions, any disruption in the equilibrium between ROS formation and scavenging by antioxidants leads to ROS excess buildup, culminating in oxidative stress [9]. Oxidative stress results in damaging the nucleic acid and protein and lipid peroxidation, thereby altering the carbohydrate metabolism and thus leading to cellular death and its dysfunction.

4.1 Lipids

Increasing ROS levels trigger lipid peroxidation in membranes of cells and organelles, affecting normal cellular activity. Oxidative stress is exacerbated by lipid peroxidation by producing radicals from lipids, which affect proteins and DNA. In

stressed cells, lipid peroxidation can be used as a biomarker of ROS-mediated membrane damage. Environmental challenges have been shown to cause increased lipid peroxidation and its degradation in several plants [22].

4.2 Proteins

Besides direct changes, ROS can indirectly affect proteins. Direct modification is the process of modifying a protein's function by disulfide bond formation, nitrosylation, carbonylation, and glutathionylation. Through the breakdown of fatty acid peroxidation products, proteins are indirectly altered. The increased ROS production alters amino acid sites, fragments peptide chains, aggregates cross-linked reactions, changes the charge, and increases the proteolytic activity of proteins. The amount of carbonylated proteins in oxidatively damaged tissues is higher, a measurement of protein oxidation. Plants are reported to modify proteins in response to diverse stressors. There is a wide range of ROS attacks that can be performed on amino acids in peptides [23, 24].

4.3 Damage to nucleotides and DNA

The hydroxyl radical ($OH^{\bullet}$) is one of the damaging factors for polynucleic acids, because it changes the pyrimidine and purine structure by liberating H^{+} from the C–H bonds of methyl and 2-deoxyribose group, generating deoxyribose radical, thymine glycol, hydroxyl methyl urea, and other compounds, thereby breaking double-stranded DNA into single-stranded DNA. DNA damage is caused by oxidative stress. By oxidizing deoxyribose sugar, changing nucleotide bases, cross-linking DNA, proteins, and abstracting nucleotides, ROS cause DNA nucleotide damage. Plant growth, as well as development, is influenced by DNA damage through a variety of physiological mechanisms, including abnormal protein synthesis and damage to photosynthetic proteins, among others. It also can prevent replication mistakes, signal transduction, transcription, and overall genomic instability. In addition, DNA bases are damaged and irreversible not only by direct oxidation but also by reactive intermediates (associated with ROS attack) reacting with macromolecules [25, 26].

5. Oxidative stress under abiotic stress

There are different types of abiotic stresses as depicted in **Figure 1**.

5.1 Oxidative stress under salinity

Among all above-mentioned abiotic stresses, salinity or salt stress is regarded as one of the most damaging, reducing land area and agricultural productivity. Soil salinity is a global issue that affects around 20% of irrigated land and severely lowers agricultural production [27]. Salinity has a negative impact on crop germination, yield, and vigor. Osmotic stress, nutritional ionic specificity, hormonal problems, altered physiological and metabolic processes, and, finally, oxidative damage occur when plants are exposed to high salt concentration. Some of the most typical effects of salt stress in plants are photosynthetic reduction, nutritional unavailability, cellular membrane disruption, the creation of several toxic metabolites, and eventual plant death.

Salinity stress causes overproduction of ROS that affects the plants leading to ion toxicity, nutritional inadequacy, osmotic stress, and genotoxicity that causes oxidative stress [28, 29].

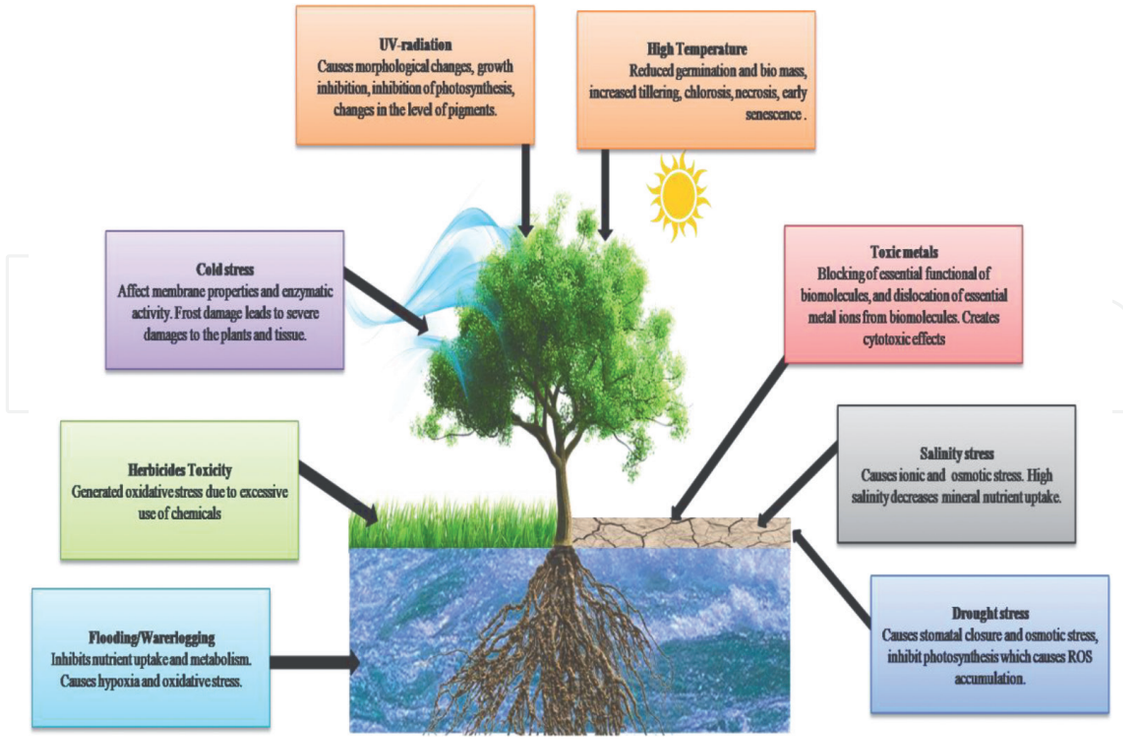

Figure 1.
Different types of abiotic stresses for plants.

5.2 Drought

Drought causes stomatal closure and decreases CO_2 fixation in plant leaves. As a result, this stress causes an imbalance between light capture and utilization, lowering the photosynthetic rate [30]. During drought, the photochemistry of chloroplasts changes, and an imbalance between electron acceptance and release results in an increase in the formation of ROS from surplus light energy in the photosystems. In reality, ROS are produced by absorbed light energy, which cannot be converted to CO_2 fixation. Photorespiration directly causes drought-induced H_2O_2 production. Furthermore, under drought stress, if chloroplasts are exposed to excessive light energy, ferredoxin levels plummet; $NADP^+$ regeneration is hampered, interfering with electron uptake, resulting in lower ETC and increased electron leakage, both of which contribute to ROS overproduction. Drought-induced LPO, malondialdehyde (MDA), and H_2O_2 accumulation cause malfunction of several physiological and cellular processes including membrane functions, water-use efficiency, stomatal conductance, carboxylation efficiency, transpiration, respiration, and photosynthesis. Drought stress increases MDA and H_2O_2 levels in several plant species, including maize, chili, rapeseed, alfalfa, soybean, and others, which, along with other harmful ROS, cause oxidative damage [31].

5.3 Metal toxicity

Toxicity from metals or metalloids not only disrupts morphophysiological features but also causes increased oxidative stress due to an imbalance between the antioxidant defense system and ROS generation. In a study, it was found that nickel (100 M) stressed *Pisum sativum* L. seedlings accumulated more LPO and H_2O_2. Such increases in oxidative stress indicators were even greater in the same crop with the same Cd content. Meanwhile, cadmium stress increases the H_2O_2 and MDA production in

numerous crops. Aside from H_2O_2 and MDA, the rate of $O_2^{\bullet}$ generation in *Vigna radiata* L. was also higher [5, 32].

5.4 Flooding or waterlogging

In the natural environment, unexpected extreme climate change occurs, such as flooding, which can disrupt the natural distribution of plants and even lead to extinction. Flooding or waterlogging (WL) can cause anoxia as well as hypoxia by impeding respiration and producing toxic chemicals that impede metabolic processes. The main reasons for yield reduction are decreased growth and production of biomass, disruption in light interception, limitation of stomatal conductance and CO_2 assimilation, reduced respiration and photosynthesis, and altered accumulation of secondary metabolites. Under WL conditions, such metabolic process deficiencies result in the production of ROS and cause oxidative damage to the cell [33].

5.5 Chilling

Temperatures (1–10°C) in the chilling-sensitive plant's cells cause multiple physiological abnormalities, resulting in chilling injury and mortality in many tropical and subtropical plant species, including many vegetable species. According to a recent study, exposing chilling-sensitive plants to low temperatures disrupts all physiological functions, including water regime, mineral feeding, photosynthesis, respiration, and metabolism [34]. The reported inactivation of metabolism during chilling of chilling-sensitive plants is a complicated function of both temperature and exposure period. Plants' responses to low temperature are connected with changes in the rate of gene transcription of a variety of low-molecular-weight proteins.

Chilling stress denotes low-temperature harm without the development of ice crystals, whereas freezing stress denotes injury caused by ice formation within plant tissues. Plant species are dependable in their tolerance to chilling or freezing conditions. Chilling temperature decreases crop productivity by interfering with various components of plant growth and development. At the vegetative stage, freezing temperatures restrict seedling growth and create aberrant phenotypes, such as stem discoloration, leaf yellowing or whitening, white patches/spots, wilting, and diminished tillering. Mung bean seedling development and dry weight reduced when exposed to a chilling temperature of 6°C. These seedlings also have aberrant phenotypic characteristics. Cold (11°C) damage symptoms were detected in rice [35, 36]. In chill-affected rice plants, stunted development, leaf chlorosis, an uneven number of tillers, and deformed and discolored grain symptoms were common. Lower temperatures affected soybean growth time, biomass accumulation, harvested index, seed number, and seed weight [37].

5.6 Extremely high temperature

Rising global temperatures over the previous few decades have resulted in major crop losses in a variety of regions throughout the world. By the year 2100, global temperature is anticipated to rise by up to 2.5–5.4°C. Temperature intensity, duration, and rate of increase are all critical factors in causing plant damage. Some frequent damaging consequences of high temperature include reduced germination and biomass, increased tillering, chlorosis, necrosis, early senescence of floral buds, premature mortality, and fruit senescence. Heat stress during seed development causes structural disintegration and physiological problems, reducing germination and vigor, emergence, and seedling

establishment even further. High temperatures hampered rice seed germination by lowering the levels of a collection of proteins involved in methionine metabolism, amino acid biosynthesis, energy metabolism, reserve degradation, and protein folding [38, 39].

5.7 Ultraviolet radiation

UV is a type of radiation that is produced by the sun. Solar radiation provides essential energy for plant growth and development through photosynthesis, but high light and, in particular, its integral ultraviolet (UV) fraction cause stress, potentially resulting in severe injury to plant cellular components such as DNA and protein.

The regular discharge of chlorofluorocarbons and other pollutants as a result of human activity increases the amount of UV radiation on the earth's surface, which is the primary cause of stratospheric ozone layer depletion. Plants and animals are harmed as a result of stratospheric ozone depletion and increased solar UV radiation. Because sunlight is required for photosynthesis in terrestrial plants, they are most vulnerable to UV radiation [14, 40].

5.8 Herbicide toxicity

Herbicides are frequently used to control weeds in cultivated agricultural plants. Nonetheless, unintentional pesticide usage may cause oxidative stress in plants. Herbicides cause oxidative stress by producing too much ROS, which degrades plant photosynthetic pigments, lipids, cell membranes, and enzyme activity, affecting plant growth and production. Glyphosate, an herbicide, generated this stress in plants by limiting the shikimate pathway, resulting in excessive production of ROS and disruption of redox homeostasis. Glyphosate strongly hindered the growth of *Hordeum vulgare* L. (Barley) in response to larger accumulations of H_2O_2 and O_2, which raised LPO [41, 42].

6. ROS defense machinery

This defense mechanism is comprised of antioxidant machinery, which aids in the mitigation of the above-mentioned oxidative stress-induced harms. The antioxidant machinery is composed of two arms: enzymatic components and nonenzymatic antioxidants (**Figure 2**) [43].

6.1 Enzymatic antioxidants

Within the cellular and subcellular compartments, the antioxidant machinery is composed of several antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POD), catalase (CAT), dehydroascorbate reductase (DHAR), guaiacol peroxidase (GPX), glutathione reductase (GR), and monodehydroascorbate reductase (MDHAR).

6.1.1 Superoxide dismutase (SOD)

All aerobic organisms contain the metalloenzyme SOD. This is the initial line of defense against ROS-induced damage under environmental stress. SOD catalyzes $O_2^{\bullet-}$ elimination by dismutating it into H_2O_2 and O_2. This eliminates $OH^{\bullet}$ generation as a result of the Haber-Weiss reaction. The SODs can be classified into three groups based

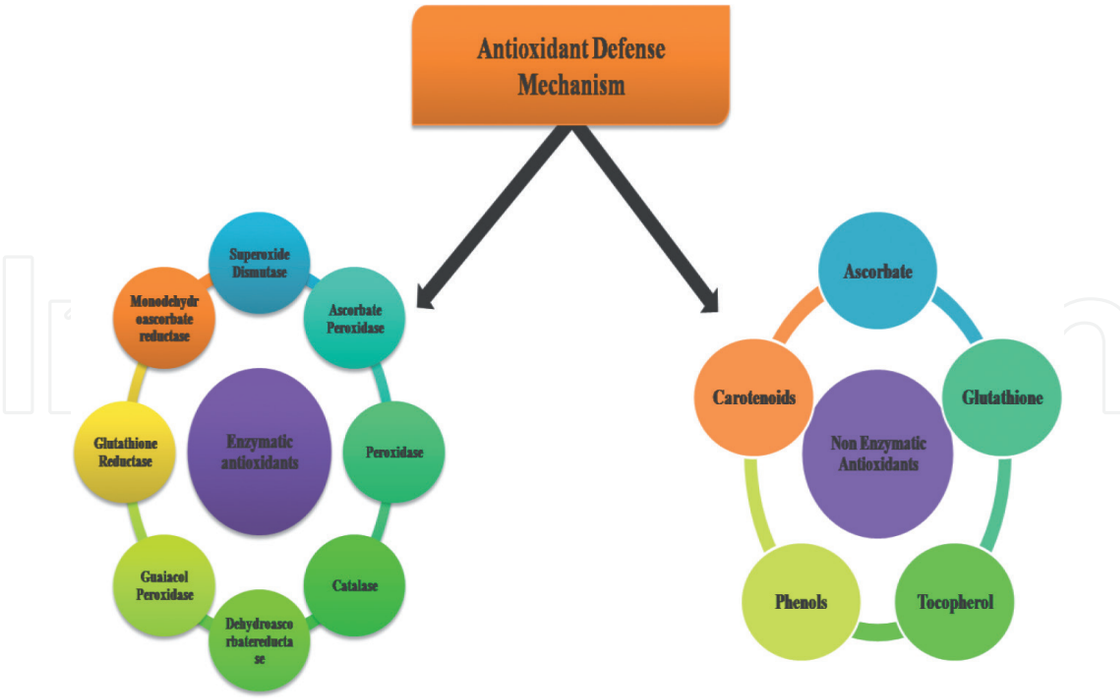

Figure 2.
Components of antioxidant defense mechanism.

on the metal ion they bind: Mn-SOD located in mitochondria, Cu/Zn-SOD located in cytosol, (chloroplasts and peroxisomes), and Fe-SOD localized in the chloroplasts. Under abiotic stress (including salinity), the SOD levels get increased, as plants tend to defend themselves from oxidative stress [21].

$$O_2^{\bullet -} + O_2^{\bullet -} + 2H^- \rightarrow 2H_2O_2 + O_2 \quad (1)$$

6.1.2 Catalase (CAT)

It is a tetrameric heme enzyme that catalyzes the dismutation of H_2O_2 into O_2 and H_2O. It has a strong affinity for H_2O_2, but a weaker attraction for organic peroxides. It has a very rapid turnover rate and is unique among antioxidant enzymes in that it does not require a reducing counterpart. Peroxisomes are hotspots for H_2O_2 generation due to photorespiration, purine catabolism, fatty acid oxidation, and oxidative stress. Recent studies reveal that CAT is also located in other subcellular compartments including chloroplast, mitochondria, and cytosol; however, considerable CAT activity has yet to be observed. CAT eliminates H_2O_2 in an energy-efficient manner [21, 44]:

$$2H_2O_2 \rightarrow H_2O + {}_{(1/2)}O_2 \quad (2)$$

6.1.3 Ascorbate peroxidase (APX)

The ascorbate glutathione cycle (ASC-GSH) is driven by ascorbate peroxidase (APX). While CAT primarily scavenges H_2O_2 in the peroxisomes, APX does the same in the cytosol and chloroplast. The APX uses ascorbic acid (AA) as a reducing agent to convert H_2O_2 to H_2O and DHA.

$$H_2O_2 + AA \rightarrow 2H_2O + DHA \quad (3)$$

The APX family is divided into five distinct isoforms depending on amino acid sequences and location, including chloroplastid (thylakoidal and stromal), mitochondrial, peroxisomal, and cytosolic. Because APX is more extensively distributed and has a higher affinity toward H_2O_2 than CAT, it is a more effective H_2O_2 scavenger during stress [45].

6.1.4 Monodehydroascorbate reductase (MDHAR)

It is in charge of regenerating AA from the short-lived MDHA by employing NADPH as a reducing agent and thus refilling the cellular AA pool. Because it regenerates AA, it colocalizes with APX in mitochondria and peroxisomes where APX scavenges H_2O_2 and oxidizes AA. MDHAR has several isozymes that are found in mitochondria, peroxisomes, chloroplasts, cytosol, and glyoxysomes [2, 46]:

$$MDHA + NADPH \rightarrow AA + NADP^+ \quad (4)$$

6.1.5 Dehydroascorbate reductase (DHAR)

It uses reduced glutathione (GSH) as an e^- donor to convert dehydroascorbate (DHA) to AA. This makes it, in addition to MDHAR, another drug that regenerates the cellular AA pool. It is essential for regulating the AA pool size in both apoplast and symplast, hence maintaining the plant cell's redox status. DHAR can be detected in high concentrations in seeds, roots, and both green and etiolated shoots [47, 48].

$$DHA + 2GSH \rightarrow AA + GSSG \quad (5)$$

6.1.6 Glutathione reductase (GR)

It is a flavoprotein oxidoreductase that reduces GSSG to GSH using NADPH as a reductant. Reduced glutathione (GSH) is depleted when it is utilized to produce AA from DHA and MDHA, and it is thus transformed to its oxidized state (GSSG). To maintain a high cellular GSH/GSSG ratio, GR, a critical enzyme of the ASC-GSH cycle, catalyzes the creation of a disulfide bond in glutathione disulfide. It is mostly found in chloroplasts, with trace amounts being detected in cytoplasm and mitochondria. GSH is a low-molecular-weight molecule that acts as a reductant, preventing thiol groups from being oxidized and reacting with harmful ROS members [49, 50].

$$GSSG + NADPH \rightarrow 2GSH + NADP^+ \quad (6)$$

6.2 Nonenzymatic components of antioxidative defense system

The primary cellular redox buffers glutathione, ascorbate, phenols, carotenoids, and tocopherol that are nonenzymatic components of the antioxidative defense mechanism. Plant growth and development are influenced by numerous antioxidants that modulate several processes ranging from cell elongation and mitosis to senescence and cellular death. They not only protect various cell components from harm, but also

play a significant role in plant growth, maturation, and development by modifying cellular processes such as mitosis, senescence, cell elongation, and cell death.

6.2.1 Ascorbate (AsA)

AsA is the most prevalent, low-molecular-weight antioxidant, and it plays an important role in the defense against oxidative stress induced by increased ROS levels. Because of its potential to donate e^- in a variety of enzymatic and nonenzymatic processes, AsA is regarded as a potent antioxidant. AsA has been demonstrated to be involved in several physiological processes in plants. The majority of AsA, about 90%, is found in the cytoplasm, but unlike other soluble antioxidants, a significant fraction is transported to the apoplast. The first line of defense in apoplastic AsA is thought to be against potentially harmful exogenous oxidants. AsA shields important macromolecules from oxidative degradation. Under normal physiological conditions, AsA is usually found in a reduced state in chloroplasts. It protects the membrane by directly interacting with H_2O_2, $O_2^{\bullet}$, producing tocopherol from tocopheroxyl radicals, and preserving the activities of enzymes containing prosthetic transition metal ions. AsA plays a beneficial role in the elimination of H_2O_2 *via* the AsAGSH cycle [51, 52].

6.2.2 Glutathione

Glutathione tripeptide (−glutamylcysteinyl-glycine, GSH) is a critical low-molecular-weight nonprotein thiol that plays a significant role in intracellular defense action against ROS-induced oxidative damage. It has been documented in almost every cell compartment, including the chloroplasts, cytosol, vacuoles, endoplasmic reticulum, and mitochondria. GSH is produced in plant cells, chloroplasts, and cytosol, by compartment-specific isoforms of glutathione synthetase and glutamylcysteinyl synthetase. The balance of glutathione disulfide (GSSG) and GSH is essential for sustaining cellular redox state. A variety of biological processes depend on GSH's reducing abilities, including cell growth/division, sulfate transport regulation, metabolite conjugation, signal transduction, enzymatic regulation, nucleic acid and protein synthesis, xenobiotic detoxification, synthesis of phytochelatins, and stress-responsive gene expression. GSH acts as an antioxidant in several ways. It can chemically react with $OH^{\bullet}$, $O_2^{\bullet -}$, and H_2O_2 and hence work directly as a free radical scavenger. GSH can protect macromolecules (proteins, lipids, and DNA) by directly forming adducts with reactive electrophiles [53].

6.2.3 Tocopherols

Tocopherols are a class of lipophilic antioxidants that scavenge oxygen-free radicals, lipid peroxy radicals, and $^1O_2$. The relative antioxidant activities of the tocopherol isomers α-, β-, γ-, and δ- are related to the number of methyl groups and methylation pattern that are connected to the phenolic ring of the polar head structure. As a result, tocopherol has the highest antioxidant activity due to its three methyl substituents. Tocopherols are only generated by photosynthetic organisms and are found only in the green portions of plants. As precursors, the tocopherol biosynthesis pathway uses two compounds: homogentisic acid (HGA) and phytyl diphosphate (PDP). Tocopherols inhibit the chain propagation stage in lipid autoxidation, making them an efficient free radical trap. In redox interactions with $^1O_2$, tocopherol's fully substituted benzoquinone ring and fully reduced phytyl chain operate as antioxidants [54].

6.2.4 Carotenoids

Carotenoids are lipophilic antioxidants capable of detoxifying several kinds of ROS. Carotenoids can be found in both plants and microbes. During the visible range between 400 and 550 nm, carotenoids absorb light and transmit it to the chloroplast. They act as an antioxidant by scavenging $^1O_2$ to avoid oxidative damage and quenching triplet sensitizer (3Chl) and excited chlorophyll (Chl) molecules to prevent the creation of $^1O_2$ and therefore protect the photosynthetic system. Carotenoids are also the precursors of several signaling molecules, which regulate various biotic/abiotic stress responses and plant development. Carotenoids' ability to scavenge, inhibit, or reduce the formation of triplet chlorophyll may be explained by their chemical specificity. Carotenoids comprise a chain of isoprene residues with many conjugated double bonds, allowing for simple energy intake from excited molecules and heat dissipation [55].

6.2.5 Phenolic compounds

These are the group of secondary metabolites with antioxidant capabilities that include tannins, flavonoids, lignin, and hydroxycinnamate esters. They are abundant in plant tissues. Polyphenols have an aromatic ring with –OH or OCH_3 substituents that contribute to their biological activity, which includes an antioxidant activity. In addition to chelating transition metal ions, polyphenols absorb molecular species of active oxygen and inhibit lipid peroxidation by scavenging lipid alkoxyl radicals. They also change the lipid packing order and reduce membrane fluidity. These modifications may severely hamper free radical transport and limit peroxidative processes. Furthermore, it has been demonstrated that flavonoids and phenylpropanoids, in particular, are oxidized by peroxidase and act in an H_2O_2-scavenging, phenolic system [56].

6.2.6 Proline

Proline, an osmolyte, is also considered a potent antioxidant. It is frequently employed as a nonenzymatic antioxidant across various kingdoms to combat the detrimental effects of various ROS members. This is produced from glutamic acid *via* a pyrroline 5-carboxylate intermediate. This route is mediated in plants by two enzymes, pyrroline-5-carboxylate reductase and 1-pyrroline-5-carboxylate synthetase. It is an effective $OH^{\bullet}$ and $^1O_2$ scavenger and can prevent LPO damage. Proline accumulates in huge concentrations in plants during stress, either due to increased synthesis or due to decreased breakdown [57].

7. Conclusion

ROS have long been known to play an important role in controlling plant responses to both biotic and abiotic stressors. They are unavoidable harmful metabolic byproducts that serve as signaling molecules under stress situations. Although ROS appear to be damage agents in plants, their importance in boosting the stress signaling component to prevent future losses is also noteworthy. Despite the constant increase in stress-related publications, there is no novelty in the content. ROS has a dual purpose: it is an unavoidable byproduct of aerobic metabolism, on the one hand, and it serves as a marker during stressful conditions, on the other hand. They not only act as damage agents in plants but also activate stress-signaling components to prevent future harm. ROS synthesis

is extensive, with production sites found both intracellularly and extracellularly. ROS causes severe damage, and its targets include all biomolecules such as DNA, proteins, and lipids compromising the cell's integrity and ultimately leading to death. This chapter explains how both components of the antioxidant machinery, antioxidant enzymes and non-antioxidant metabolites work together to mitigate the harmful effects of ROS and build a tolerance to diverse environmental stress situations. Despite substantial advances in recent years, there are still uncertainties and gaps in our knowledge of ROS production and how they influence plants, owing to their short half-life and highly reactive nature. Although the highly compartmentalized nature of antioxidants is well understood, the sensing and response mechanisms, as well as the regulation of the delicate balance between production and scavenging, require more investigation. In the future, molecular research might lead to a better understanding of ROS metabolism. Advanced functional genomics, in conjunction with proteomics and metabolomics, will provide extensive insights into the ROS network and its associated reactions.

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Conflict of interest

The authors declare no conflict of interest.

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