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Review Article

A REVIEW OF REACTIVE OXYGEN SPECIES (ROS) IN PLANTS

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ABSTRACT

Reactive oxygen species (ROS) are highly reactive intermediates of oxygen formed during successive reduction of oxygen. Most common ROS include singlet oxygen $^1O_2$, Superoxide ($O_2^{\bullet -}$), Hydrogen Peroxide (H_2O_2), and Hydroxyl radical ($^{\bullet}OH$). ROS are produced as by-products of normal aerobic metabolism in plants. However, during biotic or abiotic stress conditions ROS greatly increase in number causing oxidative stress. The highly reactive nature of these species poses a problem for cellular metabolism. Oxidative damage caused by ROS can even lead to cell death. As the production of ROS is inevitable, plants have evolved means to eliminate ROS and maintain a reducing environment in the cell. These strategies involve antioxidant enzymes as well as non-enzymatic antioxidant compounds. Two systems work together to neutralise reactive species and protect the cell from oxidative injury. A useful aspect of ROS has also been observed in that they act as second messengers in signalling. Ultimately, it is important to maintain equilibrium between the production and removal of ROS for a healthy cellular environment. This review deals with mechanisms of production of ROS and strategies of a plant cell to scavenge them.

Keywords: Reactive Oxygen Species, Oxidative Stress, Free Radicals, Antioxidant.

1. INTRODUCTION

Today, earth has nearly 21% of oxygen in its atmosphere, essential for the survival of most living organisms. Most elements or molecules occur naturally in a singlet ground state. In most cases, the triplet state is an excited state of molecules when they absorb energy. In comparison, molecular oxygen has quite an odd ground state configuration with two unpaired electrons in its outermost orbital [1]. Oxygen in the atmosphere is in molecular form with two atoms bonded by a double bond. The electron configuration of oxygen is $1S^2$ $2S^2$ $2P^4$, so the 2P orbital is partially filled. Two unpaired electrons of 2P orbital have the same spin quantum number, the state is denoted as the triplet ground state of oxygen. As two electrons occupying molecular orbital must have opposite spins, according to Pauli's exclusion principle, dioxygen needs two electrons of parallel spin to complete its octet. This explains the reactivity of oxygen towards molecules with a doublet state (with one unpaired electron) rather than a singlet state (with no unpaired electron) [2].

In aerobic organisms, molecular oxygen in its ground state accepts the first electron with great difficulty and forms a superoxide anion ($O_2^{\bullet -}$). However, after it forms superoxide it becomes highly reactive and readily accepts more electrons to form peroxide ion, hydrogen peroxide, and most reactive hydroxyl radical successively. Production of ROS is a natural process in aerobic organisms and therefore they have developed methods to eliminate excess ROS. Environmental stress conditions, however, disturb this delicate balance between production and elimination which leads to oxidative stress. ROS at high concentrations can cause serious damage to biomolecules such as nucleic acids, proteins, and lipids. Many of these changes are irreversible and permanent [3].

2. TYPES OF ROS

Reactive oxygen species are highly reactive free radicals or ions derived from oxygen. The most common ROS are Singlet oxygen $^1O_2$, Superoxide ($O_2^{\bullet -}$), Hydrogen Peroxide (H_2O_2), and Hydroxyl radical ($^{\bullet}OH$). The triplet molecular oxygen in its ground state is harmless. It can be activated by two methods: first by receiving sufficient energy to reverse the spin of unpaired electrons in its outer orbitals and second by single electron reductions. Singlet oxygen is formed by the first method while stepwise reduction of O_2 forms $O_2^{\bullet-}$, H_2O_2, and $^{\bullet}OH$ successively [4].

2.1. Singlet Oxygen ($^1O_2$)

When triplet ground state molecular oxygen receives energy such as UV-irradiation, it gets elevated to an excited singlet state ($^1O_2$) [5]. Singlet oxygen is less stable and thus much more reactive than triplet oxygen. In plants, photosystem II (PSII) in particular, produces singlet oxygen during light reactions of photosynthesis. The PSII reaction center appears to be the site of the production of singlet oxygen [6]. However, carotenoids present in the antenna complex serve as efficient $^1O_2$ quenchers. They convert singlet oxygen back to a triplet state and dissipate energy as heat. This is called physical quenching [7].

2.2. Superoxide ($O_2^{\bullet-}$)

The monovalent reduction of molecular oxygen gives $O_2^{\bullet-}$, and $O_2^{\bullet-}$ is considered both a radical and an anion with the radical sign (•) and a charge of −1. The name 'superoxide' suggests that it is a very reactive and strong oxidant [8]. The prominent site of superoxide production in the chloroplast is photosystem I under normal conditions. Mitochondrial electron transfer reactions are also responsible for generating superoxide anions.

If superoxide anions fail to react with Superoxide Dismutase, they may interact with other oxidants in a cell to form hydrogen peroxide [1].

$$O_2^{\bullet-} + AH_2 + H^+ \xrightarrow{\text{Superoxide Dismutase}} H_2O_2 + AH$$

2.3. Hydrogen Peroxide (H_2O_2)

H_2O_2 is moderately reactive and has a relatively long half-life than other oxygen-free radicals. $O_2^{\bullet-}$ accepts one electron and two protons to form H_2O_2. It is formed either non-enzymatically or by a reaction catalysed by SOD [9].

$$2O_2^{-} + 2H^+ \xrightarrow{\text{Superoxide Dismutase}} H_2O_2 + O_2$$

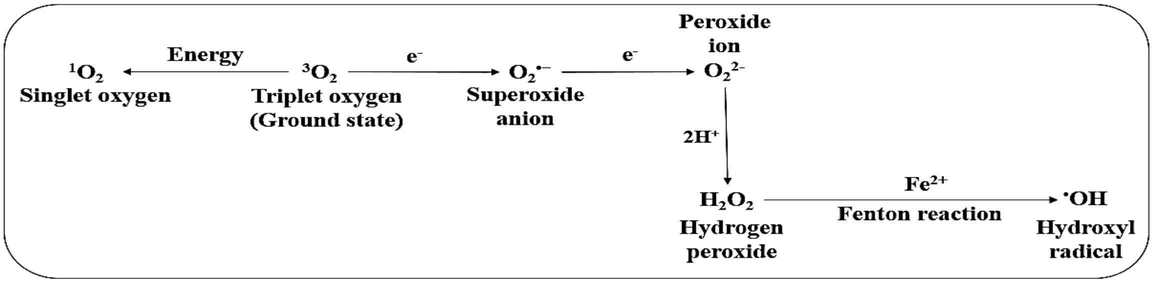

Fig. 1: Modes of generation of ROS

H_2O_2 has no unpaired electron and it can diffuse through biological membranes, thus can cause oxidative damage far from its site of formation. Also, it has gained the attention of scientists, owing to its relative stability, as a signalling molecule in stress-induced responses. It is beneficial only at low concentrations while damaging at high concentrations.

Oxidative damage by H_2O_2 becomes evident when it gets converted to more reactive species such as hydroxyl radical ($^{\bullet}OH$). Catalases are the enzymes that degrade hydrogen peroxide to oxygen and water [4].

$$H_2O_2 \xrightarrow{\text{Catalase}} H_2O + O_2$$

2.4. Hydroxyl Radical ($^{\bullet}OH$)

It is the most reactive ROS and has a very short half-life. The formation of $^{\bullet}OH$ depends on both $O_2^{\bullet-}$ and H_2O_2.

The Haber-Weiss reaction generates $^{\bullet}OH$ (hydroxyl radicals) from H_2O_2 (hydrogen peroxide) and superoxide ($O_2^{\bullet-}$) catalysed by iron ion [10].

$$Fe^{3+} + O_2^{\bullet-} \longrightarrow Fe^{2+} + O_2$$

The second step is the Fenton reaction.

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + {}^{\bullet}OH$$

Net reaction:

$$^{\bullet}O_2^{-} + H_2O_2 \longrightarrow {}^{\bullet}OH + OH^- + O_2$$

3. GENERATION OF ROS

Reactive oxygen species are produced as a result of normal metabolism as well as during stress conditions. Biotic agents such as pathogens and extreme environmental conditions can lead to a generation of stress in plants. Major sites of production of ROS in plants are chloroplast, mitochondria, and peroxisomes.

3.1. Chloroplast

Chloroplasts have a highly ordered system of electron carriers on their thylakoid membranes. Abiotic stress factors like salinity, drought, and high temperature in addition to excess light lead to the formation of superoxide anion within photosystem I via the Mehler reaction [11]. PSII acts as a major site for the production of singlet oxygen. When plants experience stress conditions such as intense light, PSII gets over-reduced. This leads to the inactivation of PS II and inhibition of photosynthesis. This is called photoinhibition [12].

3.2. Mitochondria

Mitochondria are responsible for the production of harmful ROS such as H_2O_2 and $O_2^{\bullet-}$. Like chloroplast, mitochondria also have a series of electron carrier protein complexes in their membranes. Complex I and III of the electron transport chain where superoxide anion is formed and further converted to hydrogen peroxide by dismutation [11].

3.3. Peroxisomes

Peroxisomes are major sites of production of H_2O_2. In addition, superoxide anion ($O_2^{\bullet-}$) is also formed by their oxidative metabolism. Xanthine oxidase oxidises xanthine or hypoxanthine and uses NAD^+ as the usual electron acceptor [13]. During stress conditions, lack of NAD^+ causes a reduction of $O_2$to $O_2^{\bullet-}$ by enzyme activity. The main cause of H_2O_2 generation in peroxisomes is photorespiration. It is the light-dependent process in which O_2 is taken in and CO_2 is released. At low CO_2 concentrations, the enzyme RuBisCO accepts O_2 in place of CO_2 which leads to the formation of glycolate. Glycolate is transported to peroxisomes where it gets oxidised to glyoxylate by the enzyme glycolate oxidase. The enzyme simultaneously reduces O_2 to H_2O_2 [14].

4. OXIDATIVE STRESS

Energy transfer and monovalent reductions produce oxygen radicals within living organisms. Also, electron transfer systems in chloroplast and mitochondria generate ROS as by-products. Under a normal physiological state, antioxidant mechanisms efficiently remove ROS from the system. This equilibrium of generation and scavenging of ROS get disturbed during stress conditions. Oxidising enzymes are activated in the process and ROS concentration rapidly increases. This is called oxidative burst. When the level of ROS within the cell goes beyond the capacity of defence mechanisms, the cell is said to be under oxidative stress. High concentrations of ROS can be highly damaging to critical cellular components and can lead to cell death [3].

5. NON-ENZYMATIC ROS SCAVENGING STRATEGIES

Plants have developed mechanisms to get rid of excess ROS produced and also control the processes that lead to their generation. Nonenzymatic antioxidants include ascorbate and glutathione (GSH), as well as tocopherol, flavonoids, and carotenoids [12].

5.1. Ascorbic Acid (AsA)

Ascorbic acid or vitamin C is a water-soluble micronutrient and powerful reducing agent. It acts as a predominant free-radical scavenger in a cell. Ascorbic acid plays an important role during abiotic stress by protecting against damage due to ROS generated [15].

It is a part of the ascorbate-glutathione cycle and acts as a substrate for the anti-oxidative enzymes of the cycle. It gets oxidised to monodehydroascorbate which is further disproportionate into ascorbate and DHA. The reaction is catalysed by ascorbate peroxidase and harmful ROS get converted into comparatively benign products. The reduced form is regenerated by monodehydroascorbate reductase enzyme. It is localised in all subcellular compartments including the apoplast [16].

5.2. Reduced Glutathione (GSH)

Reduced glutathione is a vital antioxidant in eliminating reactive oxygen species in the cell. It is a part of both enzymatic as well as non-enzymatic antioxidant systems in the plant. Structurally, it is a tripeptide consisting of Glutamic acid - Cysteine-Glycine with gamma (γ) peptide linkage. GSH takes part in the ascorbate-glutathione cycle and glutathione-peroxide cycle.

Two molecules of reduced glutathione lose hydrogen of the thiol group to form disulphide bond in oxidised glutathione [17].

$$2GSH \longleftrightarrow GSSG + 2H^+ + 2e^-$$

Ascorbate is converted back to its reduced form by using GSH as a reducing agent, thereby replenishing the cellular ascorbate pool. GSSG made in this process is reduced to GSH by the enzyme glutathione reductase using NAD(P)H as an electron donor.

In the glutathione-peroxide cycle, GSH acts as a substrate for the glutathione peroxidase enzyme which neutralizes hydrogen peroxide and generates GSSG. GSH is regenerated by a similar mechanism as the ascorbate-glutathione cycle discussed above [18].

5.3. Tocopherols

Vitamin E family is composed of tocopherols and tocotrienols. All members of the family have a chromanol head group and prenyl side chain. They are amphipathic molecules normally found in cell membranes. Their hydrophobic prenyl tails can join with membrane lipids and the hydrophilic head group remains on the cell surface. Tocopherol and tocotrienol differ only in their level of saturation.

Tocopherols are found in photosynthetic bacteria, fungi, algae, plants, and animals though later cannot synthesize them. In plants, tocopherols are found primarily in α-tocopherol form, along with its other isomers. Study reveals that most of the tocopherol in plants is located in plastids. Tocopherols are synthesized by the shikimic acid pathway by its precursor molecule homogentisic acid. The antioxidant activity of tocopherols depends on their ability to donate phenolic hydrogen to lipid free radicals. It has been observed in vivo that the order of their antioxidant ability against lipid peroxidation is $\alpha > \beta > \gamma > \delta$[19]. Tocopherols are known to scavenge free radicals like lipid peroxy radicals and singlet oxygen. Their main role however is to quench lipid peroxy radicals which cause lipid peroxidation. In due process, α-tocopherol which gets oxidised to α-tocopheroxyl radical is recycled by the ascorbic acid-glutathione cycle. Ascorbic acid forms monodehydroascorbate and is converted back to ascorbic acid by using either NAD(P)H or glutathione as an electron donor or both. Each of these methods uses specific enzymes and reductants [4].

5.4. Carotenoids

Carotenoids are lipophilic antioxidants present in the membranes of plant cells. Chemically, carotenoids are tetraterpenes made up of eight isoprene units containing a 40-carbon basal structure. Carotenoids are synthesized by two pathways: the mevalonic acid pathway and the methylerythritol phosphate (MEP) pathway. They are classified into two groups, hydrocarbon carotenoids containing only carbon and hydrogen and xanthophylls having at least one oxygen atom [20].

Carotenoids are efficient in quenching singlet oxygen and peroxyl radicals. The energy from singlet oxygen is transferred to the carotenoid forming an excited carotenoid molecule and ground-state oxygen. The excess energy of the carotenoid is dissipated to the surroundings bringing it to the ground state. Carotenoids also scavenge peroxyl radicals thereby preventing cell membranes from the harmful effects of lipid peroxidation. They work in association with other antioxidants such as vitamin E(tocopherol) and vitamin C (ascorbic acid) to protect against oxidative stress [21].

5.5. Flavonoids

Plants synthesize various chemical compounds which are not directly involved in growth and development, called secondary metabolites. Secondary metabolites can be classified into three major classes: Terpenes, phenolics, and nitrogen-containing compounds. Flavonoids consist of one of the largest classes of plant phenolics.

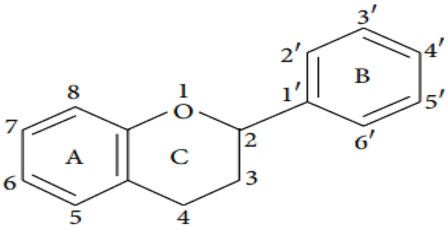

Fig. 2: Basic structure of a flavonoid

The basic structure of flavonoids contains 15 carbons arranged in two benzene rings linked by heterocyclic pyran ring. This structure is synthesized by two separate pathways: The shikimic acid pathway and the malonic acid pathway. Flavonoids are classified into different subgroups based on the degree of unsaturation and oxidation of the C ring (fig.1). These are flavones, isoflavones, flavonols, flavanones, and anthocyanins [22].

Nearly every group of flavonoids can act as antioxidants. Antioxidant activity depends on the arrangement of functional groups, the total number of hydroxyl groups, and also number and position of sugar moieties. The mechanisms of action may include a). Direct scavenging of ROS b). Inhibition of oxidising enzymes or chelating metal cofactors required for ROS generation. The B ring hydroxyl groups usually donate hydrogen and electron for neutralising free radicals directly and forms comparatively stable flavonoid radical [23].

6. ENZYMATIC ROS SCAVENGING STRATEGIES

Antioxidant enzymes in plants include Superoxide Dismutase (SOD), Catalase (CAT), Ascorbate

Peroxidase (APX), Monodehydroascorbate Reductase (MDAR), Dehydroascorbate Reductase (DHAR) Glutathione Peroxidase (GPX) and Glutathione Reductase (GR).

6.1. Superoxide Dismutase (SOD)

SODs (E.C.1.15.1.1) are the first enzymes to get activated causing the dismutation of superoxide anion to form hydrogen peroxide and molecular oxygen. As $O_2^{\bullet -}$ is unable to cross membranes, it is important to have SODs localized at the source of ROS formation. There are different types of SODs present in the plant cell, depending on the type of metal cofactor attached to them. Fe SOD the most ancient group occurs in chloroplasts. Mn- containing SOD is present in mitochondria and peroxisomes. Cu-Zn SODs are found in chloroplasts, cytosol, and even extracellular space [24].

$$O_2^{\bullet -} + O_2^{\bullet -} + 2H^+ \xrightarrow{\text{Superoxide Dismutase}} 2H_2O_2 + O_2$$

6.2. Catalase (CAT)

Catalase (E.C.1.11.1.6) was the first antioxidant enzyme to be discovered. It occurs in nearly all aerobic organisms. The enzyme catalyses the decomposition of two H_2O_2 molecules into $H_2O$and O_2. It does not require any reducing agent to carry out the reaction. Catalase occurs in peroxisomes where H_2O_2 is produced due to photorespiration and also by the action of the enzyme SOD. SOD converts $O_2^{\bullet -}$ to H_2O_2 and O_2. H_2O_2 gets further acted upon by catalase [25].

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

7. ENZYMES OF THE ASCORBATE-GLUTATHIONE CYCLE

7.1. Ascorbate Peroxidase (APX)

Ascorbate peroxidase (E.C.1.11.1.11) is an enzyme of the ascorbate-glutathione cycle which is an important ROS scavenging mechanism. APX converts H_2O_2 to H_2O by using ascorbate as a reductant. Ascorbate gets converted to monodehydroascorbate which spontaneously decomposes to dehydroascorbate. APX has more affinity for H_2O_2 than catalase. Catalase is localised in peroxisomes while APX is found in cytosol and chloroplast [4].

$$H_2O_2 + \text{Ascorbate} \xrightarrow{\text{Ascorbate Peroxidase}} 2H_2O + \text{DHA}$$

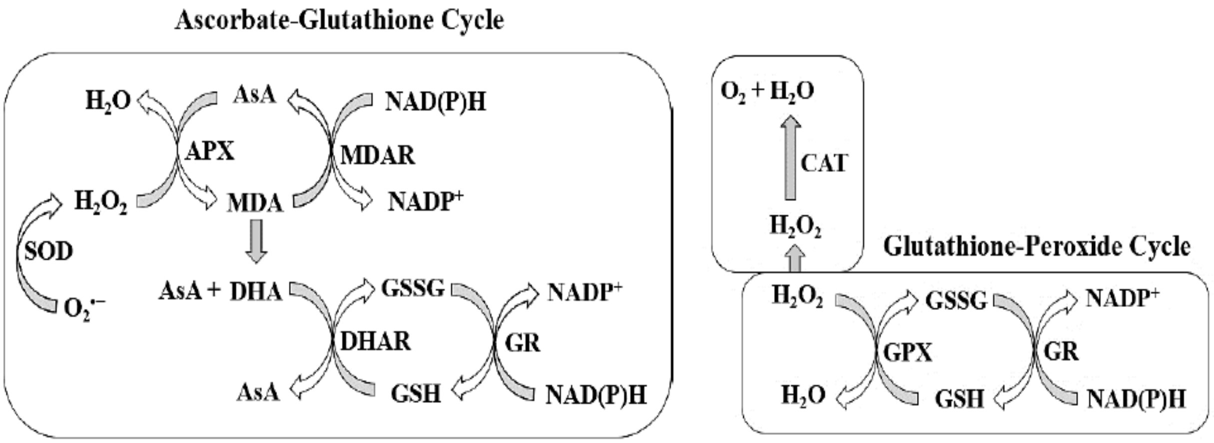

Fig. 3: Ascorbate-Glutathione Cycle and Glutathione-peroxide Cycle

SOD - Superoxide Dismutase, AsA - Ascorbate, APX - Ascorbate Peroxidase, MDA - Mono-dehydroascorbate, MDAR - Mono - dehydroascorbate Reductase, DHA - Dehydroascorbate, DHAR - Dehydroascorbate Reductase, GSH - Glutathione, GSSG - Oxidised Glutathione, GR - Glutathione Reductase, CAT - Catalase, GPX - Glutathione Peroxidase [26].

7.2. Monodehydroascorbate Reductase (MDAR)

MDAR (E.C.1.6.5.4) acts on the product formed by APX. It reduces monodehydroascorbate to ascorbate by using NAD(P)H as an electron donor. Thereby it regenerates the ascorbate pool, an important non-enzymatic antioxidant agent in the cell. The enzyme activity is important to restore ascorbate level for the continuity of the cycle. MDAR co-exists with APX in cytosol and chloroplast. Also, its activity has been observed in mitochondria, peroxisomes, and glyoxysomes [26].

$$\text{MDHA} + \text{NADPH} \xrightarrow{\text{Monodehydroascorbate Reductase}} \text{Ascorbate} + \text{NADP}^+$$

7.3. Dehydroascorbate Reductase (DHAR)

DHAR (E.C.1.8.5.1) also known as Glutathione Dehydrogenase (ascorbate) is another enzyme that re-establishes ascorbate concentration. It reduces dehydroascorbate to ascorbate by using glutathione as a reductant. Reduced glutathione (GSH) gets converted into oxidised glutathione (GSSG). As the enzyme replenishes the ascorbate concentration, it helps in maintaining a healthy ratio of reduced to oxidised ascorbate [11].

$$\text{DHA} + 2\text{GSH} \xrightarrow{\text{Dehydroascorbate Reductase}} \text{Ascorbate} + \text{GSSG}$$

7.4. Glutathione Reductase (GR)

Glutathione Reductase (E.C.1.8.1.7) is a flavoenzyme that reduces GSSG to GSH with the help of NAD(P)H as a reducing agent. It is a crucial enzyme of the ascorbate-glutathione cycle as it regenerates GSH and maintains a reducing environment in the cell. A high GSH/GSSG ratio is an indicator of the healthy status of the cell. It occurs in cytosol, chloroplast, mitochondria, and peroxisomes.

$$\text{GSSG} + \text{NADPH} \overset{\text{Glutathione Reductase}}{\longleftrightarrow} 2\text{GSH} + \text{NADP}^+$$

7.5. Glutathione Peroxidase (GPX)

Glutathione Peroxidase (E.C.1.11.1.9) refers to the enzyme family having peroxidase activity which usually breaks peroxides. Selenium and its derivatives form an indispensable part of the enzyme structure. GPX converts H_2O_2 directly to H_2O by oxidising GSH to GSSG. There are several isoforms of the enzyme present coded by different genes [14].

$$H_2O_2 + \text{GSH} \xrightarrow{\text{Glutathione Peroxidase}} 2H_2O + \text{GSSG}$$

8. CONCLUSION

The peculiar arrangement of electrons in molecular oxygen allows the formation of reactive oxygen species. ROS are produced during the electron transport system in chloroplast and mitochondria and also by reactions occurring in peroxisomes. At low concentrations, these reactive species help in signalling in plants but at high concentrations cause damage to DNA, proteins, and cellular integrity leading to cell death. Particularly during stress conditions, rapid increase in oxygen free radicals generates oxidative stress. However, in due course of time plants have developed various antioxidative strategies to quench ROS and maintain equilibrium between their production and elimination. Also, plants have evolved means to use ROS for their advantage, as messengers in signalling pathways. This review states that production of ROS is inevitable and at basal level they are beneficial to cell.

Conflicts of interest

Authors declare that no conflicts of interest exist.

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