



Reactive oxygen species in plant stress responses

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Abstract: Plant cellular metabolism regularly produces reactive oxygen species (ROS). Multiple environmental pressures cause an excessive amount of ROS to be produced, which progresses oxidative damage and finally results in cell death. They are well-described second messengers in a number of cellular functions, including conferring resistance to diverse environmental stimuli, despite their destructive activity. The delicate balance between ROS generation and their scavenging determines whether ROS would act as signalling molecules or may harm tissues through oxidative stress. The activity of several enzymatic and nonenzymatic antioxidants present in the tissues is necessary for the efficient scavenging of ROS generated during diverse environmental stressors.

Introduction

Salinity, drought, very high temperatures, toxic metals/metalloids, flooding/waterlogging (WL), etc. are examples of environmental stressors that are increasingly commonplace as a result of a severe and abrupt climate change (Pereira *et al.*, 2016). Sustainable crop production is now seriously threatened by the escalating effects of such a wide variety of abiotic stressors. The overabundance of reactive oxygen species (ROS), which includes free radicals like the superoxide anion (O_2^\bullet), hydroperoxyl radical (HO_2^\bullet), alkoxy radical (RO^\bullet), and hydroxyl radical (OH^\bullet), as well as nonradical molecules like hydrogen peroxide (H_2O_2) and singlet oxygen (O_2^\bullet), causes oxidative stress in addition to other harmful effects (Hasanuzzaman *et al.*, 2019). The partly reduced or activated forms of molecular oxygen discussed above are produced from atmospheric oxygen (O_2) by high-energy initiation or electron transfer processes (Choudhury *et al.*, 2017). Chloroplasts, mitochondria, peroxisomes, apoplasts, and plasma membranes are where most cellular ROS are produced (Singh *et al.*, 2019).

Plants mainly deal with oxidative stress via defensive mechanism consisting of different enzymatic such as Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), dehydro-ascorbate reductase (DHAR), glutathione peroxidase (GPX), guaiacol peroxidase (GOPX) while non- enzymatic such as ascorbic acid, AsA; glutathione, GSH; phenolic acids; alkaloids; flavonoids; carotenoids; α -tocopherol; nonprotein amino acids; etc. The antioxidant defence mechanism and ROS buildup maintain a steady-state equilibrium in plant cells. The management of various crucial activities for plants, including growth and development, depends on maintaining an ideal ROS level in the cell (Mittler *et al.*, 2017). The harmony between ROS scavenging and production keeps this intermediate level in place. However, under stressful circumstances, excessive ROS production destroys the balance and results in cellular damage, which lowers plant yield and induces programmed cell death (PCD) (Raja *et al.*, 2017).

In addition to their destructive properties, ROS are well known as secondary messengers or signalling molecules that carry the signal to the nucleus through redox reactions using the mitogen-activated protein kinase (MAPK) pathway in various cellular mechanisms to increase tolerance against various abiotic stresses (Singh *et al.*, 2019). Major molecules involved in the adaptation of plants to environmental stressors are reactive oxygen species. They primarily function as signal transduction molecules that regulate several pathways during the plant's acclimatisation to stress situations (Antoniou *et al.*, 2016).

Numerous studies have demonstrated that ROS are necessary for a variety of basic natural processes, such as cellular proliferation and differentiation. Furthermore, agricultural plants including rice (Sohag *et al.*, 2020), wheat, maize, mung bean, soybean, cucumber, sour orange, strawberry, basil, and rapeseed



also depend on H_2O_2 to regulate their stress responses. Additionally, it is known that reactive carbonyl species (RCS), reactive sulphur species (RSS), and reactive nitrogen species (RNS) all play important signalling roles and collaborate to increase plant tolerance to abiotic stress. As a result, ROS play a vital and dual function in plant biology, constituting an exciting field of study for plant biologists.

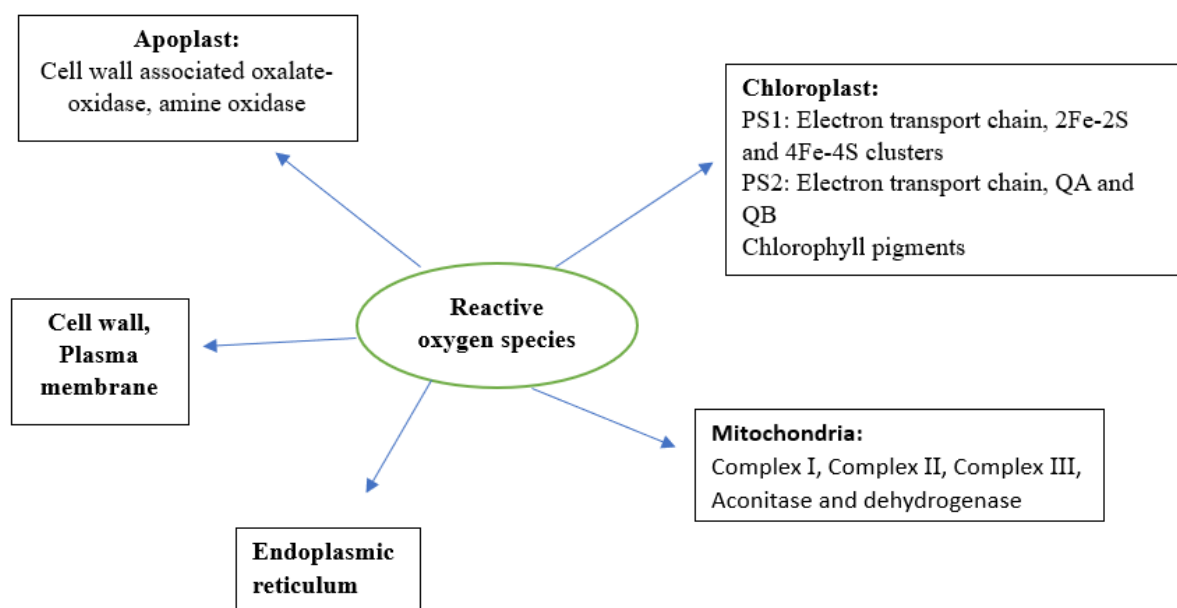
Chemistry of Reactive Oxygen Species

Atmospheric O_2 is a free molecule that occurs in the ground state (triplet oxygen, 3O_2) and has two identically numbered unpaired parallel spin electrons that reduce the reactivity of the molecule. Although some biological activities, electron transport chains (ETC), and other sources of extra energy Ionising radiation and ultraviolet-B rays help 3O_2 overcome the spin limitation and transform into ROS (Mailloux *et al.*, 2016).

Sites of Production of ROS

In both healthy and stressed cells, ROS are created in a number of places, including the endoplasmic reticulum, chloroplasts, mitochondria, plasma membranes, peroxisomes, apoplast, and plasma membranes (Figure 1). ROS are always produced as a consequence of several metabolic processes that are located in various cellular compartments or as a result of the unavoidable leaking of electrons onto O_2 from the electron transport functions of chloroplasts, mitochondria, and plasma membranes.

Fig 1: Sites of Production of ROS



Role of ROS as Messengers

In intracellular signalling cascades that mediate several plant responses in plant cells, such as stomatal closure (Yan *et al.*, 2007), programmed cell death, gravitropism, and acquisition of tolerance to both biotic and abiotic stresses, ROS have been implicated as second messengers at low/moderate concentrations. With the aid of certain redox-sensitive proteins, calcium mobilisation, protein phosphorylation, and gene expression, plants are able to sense, transduce, and transform ROS signals into suitable physiological responses. Key signalling proteins like a tyrosine phosphatase can sense ROS directly by oxidising conserved cysteine residues. Additionally, ROS may connect with other signal molecules and the pathway that is a part of the signalling network that regulates response downstream of ROS. These components of signalling include protein phosphatases, protein kinases, and transcription factors. The balance between the synthesis of oxidants and their removal by antioxidants determines the potency, longevity, and size of the ROS signalling pool. Miller and colleagues (Miller *et al.*, 2008) found a signalling pathway that is triggered in cells in response to ROS



buildup using mutants defective in important ROS-scavenging enzymes. It's interesting to note that many of the important players in this pathway, including as various zinc finger proteins and WRKY transcription factors, are also critical regulators of abiotic stress responses related to temperature, salt, and osmotic stressors.

ROS and oxidative damage to biomolecules

To prevent oxidative stress, production and elimination of ROS must be tightly managed. The term "oxidative stress" refers to the condition in which a cell is in when the quantity of ROS surpasses the capacity of its defences. However, under a number of stressful situations, such as salinity, drought, bright light, toxicity owing to metals, viruses, and so forth, the balance between the generation and scavenging of ROS is disturbed. Biomolecules including lipids, proteins, and DNA may get damaged when ROS levels are elevated. Cell death may come from these events that change inherent membrane characteristics including fluidity, ion transport, loss of enzyme function, protein cross-linking, suppression of protein synthesis, DNA damage, and so on.

Overproduction of ROS under stressful conditions

Low levels of ROS are produced by plants during typical growing circumstances. However, plants' ROS are dramatically enhanced in response to diverse environmental challenges, disrupting the usual equilibrium of O₂, OOH, and H₂O₂ in the intracellular environment (Sharma *et al.*, 2010). Following is a discussion of how several environmental conditions, such as pathogen assault, metal toxicity, UV-B radiation, and drought, salt, and cold, affect the formation of ROS.

Drought

The production of ROS is improved in a number of ways under drought stress. Through the chloroplast Mehler reaction, ROS are produced more quickly when carbon dioxide (CO₂) absorption is inhibited under drought stress along with alterations in photosystem activity and photosynthetic transport capacity. Stomatal closure during drought stress limits CO₂ fixation, which in turn reduces NADP⁺ regeneration throughout the Calvin cycle. Lack of an electron acceptor causes overreduction of the photosynthetic ETC, which increases the amount of electrons that leak to O₂ during the Mehler reaction.

Salinity

An excessive amount of ROS is produced as a result of salinity stress. By impairing cellular electron transport within various subcellular compartments including chloroplasts and mitochondria, as well as through the activation of metabolic pathways like photorespiration, high salt concentrations cause an overproduction of the ROS- O₂ •, •OH, H₂O₂, and ¹O₂. Salt stress can cause stomatal closure, which lowers the amount of CO₂ that is available to the leaves and prevents carbon fixation. As a result, the chloroplasts are exposed to too much excitation energy and the photosynthetic electron transport system is overly reduced, which increases the production of ROS and causes oxidative stress. Low chloroplastic CO₂/O₂ ratios are also more favourable to photorespiration, which increases the formation of ROS such H₂O₂ (Hernandez *et al.*, 2001).

Chilling

A significant environmental element restricting agricultural plants development and production is cold stress. By suppressing Calvin-Benson cycle activity, increasing photosynthetic electron flow to O₂, and inducing an overreduction of respiratory ETC, cooling exacerbates the imbalance between light absorption and light usage, which in turn increases the formation of ROS. Significant decreases in *rbcL* and *rbcS* transcripts, RUBISCO content, and early RUBISCO activity are also brought on by cooling stress, which increases the flow of electrons into O₂. The early RUBISCO activity and photosynthetic rate were adversely linked with the buildup of H₂O₂ in chloroplasts. An important contributing component to chilling damage in plants is chilling-induced oxidative stress, which is demonstrated by an increase in ROS generation, including H₂O₂ and O₂ •, lipid peroxidation, and protein oxidation.

Metal toxicity



Metal contamination levels in the environment are rising, which has a negative impact on plant development and metabolism and, as a result, severely reduces agricultural yields. The creation of ROS, which may be triggered directly or indirectly by the metals and subsequently leading to oxidative damage to various cell components, is one of the effects of the presence of hazardous metals inside plant tissues. Net photosynthesis (Phn) declines under metal stress conditions because photosynthetic metabolism, including photosynthetic electron transport (Phet), is damaged.

UV-B radiation

Due to the danger to productivity in global agriculture, plant biologists are currently very concerned about the effects of UV-B radiation on plants. Increased UV-B severely reduces net photosynthetic rate. The light saturated rate of CO₂ assimilation is observed to decrease after UV-B treatment, along with the carboxylation velocity, RUBISCO content, and activity.

Pathogens

Following effective pathogen detection, oxidative burst, which produces ROS, is one of the first cellular responses. O₂• or its dismutation product H₂O₂ is produced in the apoplast in response to the detection of certain pathogens (Grant *et al.*, 2000). H₂O₂ and MDA concentrations were shown to be greater in bean yellow mosaic virus-infected *Vicia faba* leaves than in the matching controls by Radwan and colleagues.

Conclusion:

Unavoidable by-products of regular cell metabolism include ROS. The production of ROS results from the electron transport functions of the chloroplast, mitochondria, and plasma membrane as well as from a number of metabolic pathways that are located in various cellular compartments. The generation of ROS in many cell compartments is minimal during normal development conditions. The cellular homeostasis is disrupted and the formation of ROS is increased by a number of environmental stimuli, including as drought, salt, cold, metal toxicity, and UV-B, if they are sustained for an extended period of time. In plants, ROS serve two distinct roles. In low concentrations, they function as signalling molecules that drive a variety of plant responses, including responses to stressors, but in high concentrations, they exacerbate damage to cellular components. An increased quantity of ROS results in oxidative damage to DNA, proteins, and lipids, altering intrinsic membrane features including fluidity, ion transport, enzyme function loss, protein crosslinking, and protein synthesis suppression, all of which eventually cause cell death. Higher plants have a sophisticated antioxidative defence mechanism with both enzymatic and nonenzymatic components to prevent oxidative damage. The short half-life and high reactivity of ROS, together with recent fast advancements, have led to several questions and information gaps regarding ROS generation and their impact on plants. Advanced analytical methods used in the study of ROS generation and destiny will aid in the development of a more comprehensive understanding of the function of ROS in plants. Clear knowledge of the biochemical networks involved in cellular responses to oxidative stress will be made possible by future advancements in genomes, metabolomics, and proteomics. A better knowledge of these will be useful for developing biotechnologically-based plants that have higher degrees of built-in resistance to ROS.

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