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REACTIVE OXYGEN SPECIES (ROS) GENERATION AND OXIDATIVE DAMAGE IN PLANTS UNDER STRESSFUL CONDITIONS

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Reactive oxygen species (ROS) is a general term to describe variety of oxygen containing radicals for instance, "hydroxyl radical ($HO^{\cdot}$), superoxide anion ($O_2^{\cdot-}$), nitric oxide ($^{\cdot}NO$), perhydroxyl radical ($HOO^{\cdot}$) and non-radical species such as hydrogen peroxide (H_2O_2) and hypochlorous acid HClO". These are mainly formed as a byproduct during normal cellular metabolism but also produced as a cellular response to various environmental stresses such as biotic, abiotic, etc.

Generation of ROS in Plants

Biotic and abiotic stresses are directly correlated with enhanced accumulation of ROS. The balance between production and elimination of ROS can be disrupted through various biotic and abiotic factors, which increase the intracellular concentration of ROS. When the level of ROS is exceeded and is not countered by protection mechanisms, the cell suffers from the oxidative stress. However, high concentrations of ROS are rather very dangerous to plants, causing irreversible damage to the cells and ultimately cell death. Consequently, defense mechanisms against oxidative damage are activated during stress to regulate toxic levels of ROS.

A common consequence of various biotic and abiotic stresses is the production of ROS *viz*. superoxide anion radical ($O_2^{\bullet-}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$) that could cause extreme oxidative damage to plant tissues. The production of ROS is the unavoidable byproduct of aerobic life. In higher plants, as an example, lower levels of ROS have been found to regulate differentiation, redox homeostasis,

stress signaling, interactions with other organisms, systemic responses, and cell death however, high levels have the ability to harm cellular components *via* lipid peroxidation, protein damage and membrane destruction. Thus, these are very deadly and substantially affect normal cellular functioning. In this regard, plants perceive stress through their roots and send signals to alter their metabolism for the activation/synthesis of defensive genes in some plant parts.

Sites of ROS Production

Under optimal growth conditions, ROS are in particular produced at a low concentration in organelles like chloroplasts, mitochondria, endoplasmic reticulum, plasma membrane, apoplast and peroxisomes. However, during stressful conditions, their rate of production is dramatically elevated and the chloroplasts become the principal site for ROS generation. The photosynthetic fixation of CO_2 can regulate the generation of ROS. Limited CO_2 fixation accompanies reduced ATP and NADPH consumption, resulting in an excess of NADPH, especially under strong light. The reduced utilization of NADPH resulted in reduced level of $NADP^+$. Since $NADP^+$ is a major electron acceptor in photosystem I, depletion of $NADP^+$ speeds up the transfer of electrons from photosystem I to molecular oxygen resulting in the generation of H_2O_2 through $O_2^{\bullet-}$. The increased level of ROS inhibits the repair of damaged photosystem II and ends in photoinhibition.

Mitochondria are a chief source of cellular ROS production. Eleven sites related with the production of superoxide ($O_2^{\bullet-}$) and/or hydrogen peroxide (H_2O_2) associated with substrate metabolism, electron transport and oxidative phosphorylation had been identified in mammalian mitochondria. The mitochondrial ETC harbours electrons with sufficient free energy to reduce O_2 that is considered as a primary supply of ROS generation, an essential accompaniment to aerobic respiration. Pathways of O_2 intake undoubtedly exist in isolated plant mitochondria and submitochondrial debris; specifically, O_2 intake through cytochrome oxidase to produce H_2O. However, this O_2 can be reduced directly to $O_2^{\bullet-}$ within the flavoprotein region of the NADH dehydrogenase portion of the respiratory chain. In mitochondria at complexes, I and III, the ubisemiquinone intermediate is formed, which is the main electron donor to oxygen, although other complex I sites are also potential donors. Production of ROS will increase if the rate of electrons leaving the ETC *via* the terminal oxidases is slowed down and the rate of electron transport increases in excess of the ability of the two respiratory pathways to process the electrons, leading to an over-reduced ubiquinone

pool. The superoxide anion formed at complex I and III in turn is reduced through dismutation to H_2O_2 which can react with reduced Fe^{2+} and Cu^{+} ions to produce distinctly toxic $^{\cdot}OH$ which being uncharged can penetrate membranes and go away from the mitochondrion.

Peroxisomes are probably important sites of intercellular H_2O_2 production and occur in almost all eukaryotic cells. H_2O_2 is normally generated inside the peroxisomal respiration pathway *via* unique flavin oxidases. Like chloroplasts and mitochondria, plant peroxisomes additionally produce $O_2^{\bullet-}$ as byproduct of their normal metabolism. Production of $O_2^{\cdot -}$ was attributed to the matrix-localized enzyme, xanthine oxidase (XOD), which catalyses the oxidation of xanthine or hypoxanthine to uric acid, together with $^{-}O_2^{\bullet}$ and H_2O_2. The small ETC of peroxisome consists of a flavoprotein NADH: ferricyanide reductase of approximately 32 kDa and a cytochrome b.

Production of ROS in the ER stimulates Ca^{2+} transfer at the ER-mitochondria surface. Further, H_2O_2 is released out of the ER and attacks the neighboring mitochondrial membrane, bypassing the protection given by mitochondrial SOD which is present inside the mitochondrial matrix. The apoplast is a vital site for H_2O_2 production in response to abscisic acid (ABA) and environmental situations, like drought and salinity. AtRbohD and AtRbohF encode fundamental NADPH oxidases which are expressed in guard and mesophyll cells in *Arabidopsis*. They have been proven to be responsible for the apoplastic ROS production that is required for ABA-induced stomatal closure.

Impact of ROS in Plant Cells- Severe Oxidative Damage

The ROS have both beneficial as well as harmful effects on plant species. They affect the lipids, proteins as well as DNA. During stressful conditions, lipid peroxidation (LPO) increases drastically through the formation of lipid radicals. The polyunsaturated fatty acids (PUFA) like linoleic and linolenic acids are most susceptible to attack by ROS like singlet oxygen and hydroxyl radicals. Overall, LPO products increase fluidity of the membrane, inhibit the membrane receptors, various enzymes that are present in the membrane as well as ion channels. The ROS also cause oxidation and modifications of the protein that can be both direct as well as indirect. Direct changes include nitrosylation, carboxylation, formation of disulfide bonds, and glutathionylation. Interaction of LPO products with protein may also lead to indirect changes. Various amino acids like proline, lysine, threonine, arginine, methionine, and cysteine are highly susceptible to ROS attack.

ROS also cause damage to DNA at multiple sites that include changes in nitrogenous bases, breakage of DNA strands, oxidation of deoxyribose sugar etc. The hydroxyl radical interacts with double bonds of nitrogenous bases. The cross linking between DNA and protein is very harmful and deadly to the plant if it is not repaired in time.

Conclusion

ROS are unavoidable by the normal cellular metabolism and are considered as necessary evil in them. They are mainly generated from ETC of chloroplast, mitochondria, peroxisomes, apoplast and plasma membrane as a byproduct of numerous metabolic processes present in various cellular compartments. During absence of any external stress and normal metabolic conditions lower concentration of ROS works as signaling molecule for various biological processes inside the cell. However, abiotic and biotic stress related conditions leads to the overproduction of ROS that damages the lipids, proteins and DNA thereby, disrupt the cellular homeostasis. Balance between production of ROS and antioxidative defensive machinery to eliminate the ROS is necessary for normal cellular growth and development. Higher plants possess both enzymatic and non-enzymatic antioxidative defense machinery to combat deleterious effects of ROS. Although rapid progress has been made in recent years, there are many uncertainties and gaps in our knowledge of ROS formation and their effect on plants mainly due to short half-life and high reactivity of ROS. Furthermore, progression in the field of genomics, proteomics, ionomics and metabolomics will help in clear understanding of biochemical networks involved in cellular responses to oxidative stress. An improved understanding of these will be helpful in producing plants with in-built capacity of enhanced levels of tolerance to ROS using a biotechnological approach.

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