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Role of Reactive Oxygen Species in Plant Development and Its Detection Assays

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Abstract

Reactive oxygen species (ROS) are chemically active compound consist of singlet oxygen and alpha-oxygen. Examples comprise of radicals (superoxide, hydroxyl radical, nitric oxide) and non-radical species (peroxides, peroxy nitrate). In almost all cells ROS are the by-products of different metabolic pathways. They are well-identified as a secondary messenger and pathological mediators. However, the recent study has exposed their importance in several life cycle processes of the plant such as seed development and germination, through to root, shoot and flower development. Here, we provide an overview of ROS production and signaling in the context of plant growth and development.

Introduction

For increasing crop yield and productivity it needed a proper understanding of the coordinated growth of plant tissues and organs. Both intrinsic genetic programmes and external environmental factors controlled the plant morphogenesis processes. The by-products of plant metabolic process, Reactive oxygen species (ROS) are generated in numerous cellular compartments like chloroplasts, mitochondria and peroxisomes. ROS are responsible for irreversible DNA damage and cell death. Rather than this they also play a major role in cell signaling that control normal plant growth, and responses to stress.

ROS in Plant Development

In plants, ROS are formed during basal metabolism at diverse subcellular sites such as during respiration at the mitochondrial site, during photosynthesis in chloroplasts, endomembrane system (cyclooxygenase CO_x, lipooxygenase) in peroxisome confined to a small area that is photorespiratory reactions, and by apoplastic NADPH oxidases [such as the respiratory burst oxidase homologs (RBOHs)] and other oxidases. This compartmentalization of ROS production and oxidation-reduction (redox)-associated as secondary messenger reactions establish the further control of ROS levels and allows redox signaling between organelles and the nucleus (Mignolet-Spruyt *et al.,* 2016).

Phytohormone and ROS

ROS have major interaction with other metabolites, including phytohormones as like gibberellic acid (GA), ethylene (ET), salicylic acid (SA), jasmonic acid (JA) and abscisic acid (ABA). ROS plays a major role in case of SA and JA signaling towards stress. Moreover, interaction between ROS and growth as well as development-associated hormones, such as auxin and cytokinin, has been reported, although some parts and questions are still hazy (Tognetti *et al.*, 2017).

Effect of ROS on Stress

Since long back ROS have been recognized for their roles in operating abiotic and biotic stress conditions. Firstly under the biotic stress effect, different pathways induce the increased formation of ROS, which will lead to pathogen infections activate specific enzymes (i.e. NADPH oxidase, cell wall peroxidases), which results in amassing of cellular or intercellular ROS, such as superoxide or hydrogen peroxide. Secondly, abiotic stresses, cause elevated ROS production principally through mutilation of photosynthetic and respiratory electron transport reactions. As a result of these two types of stresses have devastating effects on the antioxidant system of the plant.

Role of ROS on Germination

Hydrogen peroxide (H_2O_2) amplified the germination percentage of pea seeds, as well as boosts the growth of seedlings. Seed treatment with 10 mM ABA leads to reduce the effects of H_2O_2. In addition to that, H_2O_2-pretreatment will enhance in ascorbate peroxidase (APX), peroxidase (POX) and ascorbate oxidase (AAO) level. The raise in these ascorbate-oxidizing enzymes linked with the increase in the augmentation of the pea seedlings as well as with reduce in the redox state of ascorbate. Moreover, the boost in APX activity was due to a rise in the transcript levels of cytosolic and stromal APX (cytAPX, stAPX). The proteomic analysis showed that H_2O_2 related to plant signaling and growth. A strong association between the effect of H_2O_2 on plant growth and the decreases in ABA and zeatinriboside was observed (Figure1).

ROS Detection Assays in Plants

For the detection and visualization of ROS in plant tissues and organs now a day's a range of methods have been used. However, some points need to be well thought-out before making dense conclusions on ROS measurements in plants when using these approaches; detailed guidelines are presented in Noctor *et al*. (2016).

Biochemical Assays

As for other redox metabolites, ROS should not be extracted in water or neutral buffers due to the presence of contaminating antioxidant enzymes. There are different methods for assay for ROS which are described in the following table.

Table 1: Different Biochemical Assay of ROS

Methods and Probe used	Description	Advantage and limitation
Chemiluminescence (Luminal as probe)	Chemiluminescence is light emission with restricted release of heat.	*Advantage* high sensitivity real-time detection for detecting H_2O_2 formation. *Limitation* low selectivity and display high background levels.
Histo-chemical methods (diaminobenzidine (DAB) and nitro blue tetrazolium (NBT) as probe)	H_2O_2 oxidized DAB in the presence of peroxidases and form a reddish brown precipitate. NBT reacts with O2- to form a dark blue insoluble formazan compound.	*Advantage* Widely used method for detection of ROS. *Limitation* Both dyes are not specific or direct measurements of ROS.
Dichloro fluorescein (DCF)-derived fluorescent dyes (fluorescein dye as probe)	The method determine the capacity of compounds to prevent the creation of DCF by 2,2'-Azobis(2-amidinopropane) dihydrochloride (ABAP)-generated peroxyl radicals in cells. By itself, dichlorofluorescin (DCFH) also quantifies intracellular hydrogen peroxide and oxidative stress.	*Advantage* Probe is trapped and oxidized to fluorescent within cells dichlorofluorescein (DCF) with no trouble. *Limitation* The presence of endogenous auto fluorescent compounds makes ROS imaging complicated. Permeability of the dye and its stability over time.
Genetically encoded probes [green fluorescent protein (GFP) and its homologs]	Genetically encoded probes can be incorporated into living cells or organisms in the format of DNA, and next, proteins will be expressed by intracellular machineries.	*Advantage* More suitable for ROS imaging due to non-invasive, flexible and more stable over time nature. *Limitation* Limited availability of Encoded probes and controls of pH needed simultaneously.

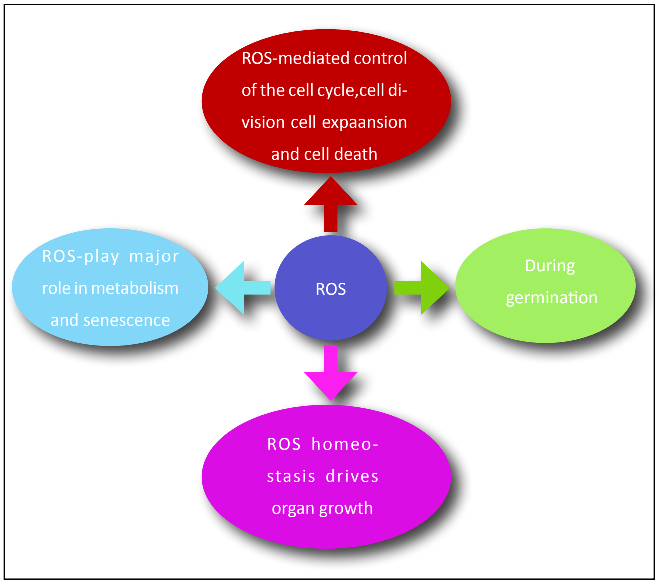

Figure 1: Role of Reactive Oxygen species in Growth and Development of Plant

Conclusion

For the growth and development of the plant Reactive Oxygen Species (ROS) are playing a crucial role. It will be vital to investigate the cross-talk between ROS and epigenetic modifications, which had major roles in plant development and stress responses, and biotic and abiotic stresses that significantly affected plant development and redox states. Therefore now it is alarming time for selective and sensitive detection of each redox signaling molecule to assist the elucidation of biological execution of these signaling molecules in plant physiology and pathology.

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