

Mini-Review

Discovery of oxidative burst in the field of plant immunity

Looking back at the early pioneering works and towards the future development

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This article is introductory to the series of works presented in this special issue on the homeostasis and the signaling roles of reactive oxygen species (ROS) in plants. Upper half of this article briefly describes the history of the ROS study in the field of plant immunity research initiated by the observation that the attacks by pathogenic microorganisms possibly stimulate the burst of ROS production in the plant tissues. The topics covered in the series of works presented here include the plants' responses to abiotic oxidative stress (atmospheric ozone), regulation of seed germination, chemical interaction between parasitic and host plants and the draught tolerance, all controlled through homeostasis of ROS at biochemical and molecular biological levels. Lastly a discussion forum was proposed to further deepen our understanding of ROS behaviors in plants.

Introduction

In this issue, we are offering a series of works (mostly review papers) focusing on the signaling roles and synthesis of reactive oxygen species (ROS) in plants. The topics covered in this issue include the responses to atmospheric ozone through regulation of hormonal signaling cascades, the regulation of seed germination via scavenging and generation of ROS, the chemical interaction between parasitic and host plants involving ROS as the key signals, and the draught tolerance determined by ROS.

Dating Back to Early Days

Our original intention to plan the special issue focusing on the roles of ROS in plant (proposed by T.K., later assisted by H.Y. and F.B.), was to memorize the event for wining the recognition of the ROS plant biology by the Japanese public. In November, 2006, from the hands of his majesty Akihito (the 125th emperor of Japan), the Medal of Honor with Purple Ribbon was handed to Prof. Noriyuki Doke of Nagoya University for honoring his pioneering works

(carried out since early 1980's) leading to the discovery that members of ROS (especially superoxide anion, O₂⁻) could be produced from the plant tissues challenged by the pathogenic microorganisms. We understand that the honor was also to the area of plant ROS biology that has emerged out and developed in the last two decades. In the below section, we would like to briefly look through the historical phase of the ROS studies in plants by focusing on the plant-microbe interactions.

The production of ROS (mostly O₂⁻, H₂O₂, HO·) at the cell surface well known as the "oxidative burst" is one of the earliest events detectable during the incompatible interactions between plants and pathogens. To date, multiple roles of ROS have been proposed in direct microbicidal actions, strengthening of cell wall through oxidative cross-linking of glycoproteins, induction of intracellular signaling pathway such as the synthesis of salicylic acid (SA) and activation of mitogen-activated protein kinase (MAPK) cascade, or activation of systemic acquired resistance (SAR) associated with systemic propagation of the oxidative burst.

In 1983, Dr. Noriyuki Doke, a plant pathologist in Nagoya University (retired in 2006) in Japan reported that the infection by *Phytophthora infestans* (late blight pathogen of potato) into potato tubers causes the generation of O₂⁻ at the host cells' plasma membrane, only in the incompatible interactions.¹ His work demonstrated that the members of ROS possibly function as the chemical signals required for induction of hypersensitive responses (HR) as typified by host cell death. Apart from photosynthetic or photochemical reactions, this was the first report on the ROS generating activity in plants which is specifically responsive to the attacks by pathogenic microorganisms. Doke further reported that the treatment of potato tuber protoplasts with the cell wall preparation from *P. infestans* readily induces the ROS production, suggesting that chemical components derived from pathogenic microorganisms (elicitors) trigger the burst of ROS production in order to stimulate the plant defense mechanisms.²

In 1985, Doke and his coworkers have discovered that the membrane fractions isolated from the potato tubers inoculated with *P. infestans* produce the O₂⁻ in an NADPH-dependent manner, and thus suggested that the enzyme for the ROS production is the NADPH oxidase, closely resembling those known to be operated in activated neutrophils.³⁻⁵ Further report from this group provided information that inoculation with an avirulent isolate of *P. infestans* onto potato leaves causes biphasic oxidative bursts,

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consisted of earlier phase peaking at initial 3 h followed by a massive continuous phase of ROS production.⁶ In the same year, the data correlating the systemic induction of resistance to *P. infestans* and the systemic regulation of ROS, i.e., activation of both the pro-oxidative (O_2^- generating reaction) and anti-oxidative mechanisms (superoxide dismutase and peroxidases) in potato plants were reported.^{7,8} Later, Park et al.^{9,10} provided a similar set of evidence in support of the resistance-mediating function of the oxidative burst in the distant area of potato tubers in the absence of phytoalexin accumulation. Above works suggested that the oxidative burst induced by pathogen-derived signals may play a key role in systemic immunity development by activation of defense responses throughout the plants.¹¹

In 1988, a possible role for the O_2^- generating system in induction of cell death (formation of necrotic lesions) in tobacco leaves infected with tobacco mosaic virus was first implied.¹² In 1995, feature of the O_2^- generating enzyme was investigated using isolated plasma membrane fractions⁵ or by pharmacological studies on the potato tubers.^{13,14} Following the finding that plants possess calcium-binding proteins and other related signaling molecules,¹⁵ ROS plant biology also went into the world of signal transduction studies.¹⁶ Some reports from Doke's group also contributed to the development of ROS biology involving signal transduction, by providing the data on the required influx of Ca^{2+} into cytoplasm, and the regulation of NADPH oxidases, which are now known as *RBOHs* (respiratory burst oxidase homologs).¹⁷ The defense-related *RBOHs*, which play a pivotal role in ROS production in response to pathogen signals, are transcriptionally regulated by MAPKs.¹⁸ The group also indicated that a calcium-dependent protein kinase (CDPK) activates the *RBOHs* by direct phosphorylation of the N-terminal regions.¹⁹ A recent review article on the action of salicylic acid¹⁶ described that the ROS propagating pathway proposed by Doke and his colleagues involving *RBOHs*, MAPK cascade and calcium was described as one of the bridging paths between the early and late plant responses to salicylic acid in which oxidative burst may be amplified via dual cycles of (1) early signals \rightarrow *rboh* activation \rightarrow ROS increase \rightarrow activation of MAPK cascade + SIPK \rightarrow *rboh* activation \rightarrow ROS increase, and (2) early signals \rightarrow *rboh* activation \rightarrow ROS increase \rightarrow increase in cytosolic Ca^{2+} \rightarrow Ca^{2+} binding to *rboh* \rightarrow enhanced ROS production.

The predicted signaling events for the oxidative burst and the following diverse defense responses are comprehensively summarized to date provided the opportunities for the researchers to challenge the hidden immune systems in higher plants through molecular identification of the components required for generation of ROS and its regulations [17–20, etc.], by building up the foundation for modern plant pathology.

Future Development of the ROS Plant Biology

By aiming to further enhance the study in the area, here we intended to gather a series of articles in this issue, from several teams internationally collaborating and/or stimulating each other, focusing on the mechanisms how ROS members are produced and how they regulates the cellular activity and gene expressions through fine tuning of the signaling processes. Nowadays, a number of teams working on plant ROS biology are distributed worldwide and their studies concern numerous aspects of the plant physiology throughout the plants' life cycle. ROS production is actually recognized as common

denominator not only to biotic stress but also abiotic environmental stressful conditions such as high salinity, drought, high incident light and low or high temperature stresses that cause major crop losses worldwide. ROS are in fact, inevitably produced by-products emerging as a consequence of normal metabolic reactions including mitochondrial respiration, photosynthetic processes and fatty acid metabolism (21–23, etc.). A common property of all ROS types is that they can cause oxidative damage to cellular components such as proteins, DNA, and lipids.²⁴ However, they have the potential to be beneficial to living organisms in addition to their harmful action against cellular metabolism, depending on the conditions.²⁵ The specificity of the biological response of living plant cells to ROS depends on the chemical identity of ROS, intensity of the signal, sites of production and developmental stages.²⁶

Exposures to environmental stresses increase intra- and intercellular levels of H_2O_2 by modulating the finely elaborated ROS-detoxification and regeneration networks, composed of ROS-producing enzymes, antioxidant enzymes, and biosynthetic pathways for low molecular antioxidants all responsible for maintaining the homeostasis of ROS levels under tight control.^{18,26–29} This allows ROS to serve as signaling molecules in regulation of plant metabolism and cellular signaling in response to environmental stresses (30, etc.). Emerging evidence suggests that hormonal signaling pathways are regulated through ROS production as observed for SA, abscisic acid, jasmonic acid and ethylene. Such ROS-mediated hormonal regulations play key roles in the crosstalk between biotic and abiotic stress signaling.^{27,31,32} Although many components of the ROS signaling networks have recently been identified, the mechanisms for orchestrated controls of the diversified ROS production mechanisms at different cellular sites through fine tuning of ROS feedback control to meet the physiological requirements such as plant growth, development, stress adaptation and programmed cell death largely remain to be further elucidated.

A Forum for ROS Studies Planned

Here, in this issue, we have access to five good articles on plant stress biology involving ROS, including two original articles (Ishibashi et al. and El-Maarouf-Bouteau et al.) and three review articles (Tamaoki; de Carvalho; El-Maarouf-Bouteau and Bailly). These works altogether contribute to enhance and deepen the discussion in the field.

To further bring more meaningful and intense discussion and to share the knowledge among the researchers in the international community, we are planning to have a ROS-relate forum at the Plant Neurobiology 2008, the 4th International Symposium on Plant Neurobiology (PNB2008), to report and discuss the up-to-date experimental data on the ROS-mediated signaling in plant cells in June 2008, in Fukuoka, Japan. We are expecting to have a lively symposium with intensive discussion on hot topics in a friendly atmosphere there (for details, please refer to following URL:

<http://www.env.kitakyu-u.ac.jp/ja/PNB2008/>).

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