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The role of nitric oxide in plant biology: current insights and future perspectives

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Nitric oxide (NO) is a redox active gaseous signal uniformly present in eukaryotes, but its formation, signalling and effects are specific within the plant kingdom in several aspects. NO synthesis in algae proceeds by mechanisms similar to that in mammals, but there are different pathways in higher plants. Beyond synthesis, the regulatory processes to maintain steady-state NO levels are also integral for the projection of NO function. As a key redox molecule, NO exhibits a number of pivotal molecular interactions, for example, with reactive oxygen species, hydrogen sulphide and calcium, with these molecular interplays largely underpinning NO bioactivity. In this context, NO has emerged as a key regulator in plant growth, development and environmental interactions. In this special issue, a collection of reviews discuss the current state-of-the-art and possible future directions related to the biology and chemistry of plant NO function.

In the past 40 years of plant nitric oxide (NO) research, we have come closer to better understanding the behaviour of this multifunctional signal molecule. Several reductive and oxidative, enzymatic and non-enzymatic pathways involved in the synthesis of endogenous NO have been explored, and it has been determined that the transfer of NO bioactivity is achieved primarily by posttranslational modifications (PTMs). NO's role in promoting growth and development, supporting plant immunity and enhancing abiotic stress tolerance has also been demonstrated in several plant systems. Consequently, the accruing information has future potential for application within plant biotechnology and crop breeding, highlighting the importance of plant NO research.

The “8th International Plant NO Meeting” in 2021 will be an excellent online forum to both review and generate a future road map for the continued development of plant NO research. Consequently, this special issue focuses on the “hot topics” of this research field, with reviews discussing the control of NO metabolism, NO signalling and NO’s involvement in plant interactions with the environment.

Control of NO metabolism

The most burning issue of plant NO science in the last 20 years has been the understanding of the mechanisms leading to endogenous NO synthesis in land plants. It was a breakthrough when nitric oxide synthase (NOS) showing structural and functional homology to animal NOS was characterized in *Ostreococcus tauri* (Foresi *et al.,* 2010), and since then more attention has been paid to the study of algal NO synthesis as well as to signal transduction. **Astier *et al.,* (2020)** discuss the recent results regarding oxidative and reductive pathways of NO production in algae, and based on the data the authors suggest that a classical, animal-type NO signalling pathway is missing from algae. *S*-nitrosation may be an important signalling mechanism also in algae, but the algal *S*-nitrosome is much less explored compared to that of land plants. Furthermore, the authors encourage consideration of algae as a model for understanding the evolution of NO signalling.

It is known that NO formation and signalling is associated to organelles such as chloroplast, peroxisome and mitochondrion (Kolbert *et al.,* 2019). The involvement of uncoupled mitochondrial respiration in regulating the levels of reactive oxygen species (ROS) and NO as well as inducing signalling events is discussed by **Popov *et al.,* (2020)**. The mechanisms of the regulation of the NDA, NDB and NDC type non-coupled NADH and NADPH dehydrogenases, the alternative oxidase, and the uncoupling protein involved in non-coupled respiration is also examined by the authors in detail, and it is suggested that the uncoupling of respiration in plant mitochondria is involved in abiotic stress adaptation *via* the tight regulation of ROS and NO levels.

NO signalling and interactions

A key route for NO bioactivity is through *S*-nitrosation/*S*-nitrosylation and this redox-based, PTM can modify protein function (Yu *et al*., 2014; Astier and Lindermayr, 2012).

An important new theme emerging in NO research is the NO-mediated transcriptional control of gene expression. Within this area, NO has been shown to directly modulate the function of a number of transcription factors and histone deacetylases within the plant nucleus (Lindermayr *et al*., 2010; Cui *et al*., 2018; Mengel *et al*., 2017; Cui *et al*., 2020). Thus, the review of **Wurm and Lindermayr, 2020** is especially timely, here these authors discuss the recent developments integral to the function of NO signalling in the plant nucleus. In addition, they identify the significant knowledge gaps within this developing area deepening our appreciation of NO activity within the physiology of plants.

NO does not act alone, but in close cooperation with other reactive molecules such as ROS and reactive sulphur species (RSS) formed simultaneously in space and time (Hancock and Whiteman, 2016). As emphasized by **Hancock and Veal, (2020)** in their thought-provoking review, redox cellular environment affects NO metabolism and also the severity and longevity of NO signalling. The over-reduction of the cellular milieu due to the accumulation of NADH and NADPH or to changes in the redox state of glutathione can cause reductive stress (Torreggiani *et al.,* 2009), which is a poorly understood process in plants, although it can have a significant effect on the molecular interactions of NO and associated signalling.

An example for the cooperation of NO and hydrogen sulphide (H_2S) is their regulatory effect on NADP-dependent dehydrogenases, such as glyceraldehyde-3-phosphate dehydrogenase, glucose-6-phosphate dehydrogenase or NADP-isocitrate dehydrogenase as discussed by **Corpas *et al.,* (2020).** Both signalling molecules act through PTMs mainly Tyrosine (Tyr)-nitration, *S*-nitrosation and persulfidation and in this way might modulate NADP-dependent dehydrogenase activity and consequently affect the cellular redox status. However, the exact NO and H_2S dependent mechanistic processes regulating the NADPH/$NADP^+$ pool in a cellular/subcellular environment require future clarification.

Regarding the role of NO signalling in ripening of tomato, novel results were provided by the comprehensive research of **Zuccarelli *et al.,* (2020)**. Using holistic approaches, it was determined that NO downregulates ripening-associated genes at multiple levels leading to a reduction in ethylene content and sensitivity of the fruit tissues to this phytohormone. Additionally, NO triggers nitro-oxidative stress due to the inactivation of antioxidant enzymes and at the same time causes the accumulation of ascorbate and flavonoids. The amount of compounds associated with fruit taste and aroma were slightly affected by NO. These results explain the effect of NO on ripening at the molecular level, which supports the use of gaseous NO as an effective way of fruit ripening delay.

NO in biotic interactions

For sessile plants it is crucial to respond quickly and efficiently to environmental signals. In these complex plant responses, NO has been emerged as a major regulator.

One of the attacks on plants from the living environment is the colonization and disease causing effect of biotrophic and necrotrophic fungi and fungi-like oomycetes (Doehlemann *et al.,* 2017). In a comprehensive review, **Jedelska *et al.,* (2020)** evaluate the role of NO formation in the colonization of filamentous pathogens as well as in pathogen recognition and defence processes. The authors emphasize that NO interacts with ROS to regulate colonization, cell death, and resistance processes, and highlight the different roles of NO in various plant-pathogenic fungal interactions.

Recent advances associated with protein *S*-nitrosation during plant immunity are highlighted by **Lubega *et al.,* (2020)**. Protein *S*-nitrosation is not only an important signalling mechanism to activate transcriptional reprogramming during the defence response, but also to inactivate pathogen derived effector proteins and consequently, disarming a key pathogen infection strategy. Moreover, they discuss the role of *S*-nitrosation in promoting autophagy and provide insight in the regulation of SUMOylation by *S*-nitrosation during the plant immune response.

While a key role for NO in plant immunity is now well established, the emerging data is also beginning to highlight a central function for this signalling molecule in symbiotic interactions with Rhizobia. In the review of **Berger *et al.,* (2020),** the disparate sources underpinning NO production and its subsequent metabolism during the symbiotic process from nodule organogenesis to senescence are documented. Within this continuum, these authors discuss how NO has been shown to regulate symbiosis-related gene expression and associated enzymatic activity, which are especially subject to change following the transition from normoxia to hypoxia during nodule development.

NO in abiotic interactions

NO is implicated in most of environmental abiotic stress responses, since it is essential for freezing, heat, salinity, drought and heavy metal tolerance (Nabi *et al.,* 2019; Sánchez-Vicente *et al.,* 2019).

Light is an environmental factor, which influences plant development and photosynthesis (Liu *et al.,* 2020). The review paper of **Lopes-Oliveira *et al.,* (2020)** points out that the relationship between light and NO is bidirectional since light regulates NO synthesis through affecting nitrate reductase activity and the NO produced in photosynthetically active tissues targets photosynthetic electron transport and stomatal movements at multiple sites. Furthermore, NO interacts with the hormonal and signalling cascade regulating photomorphogenesis as well as light stress responses.

Manrique-Gil *et al.,* (2020) describe the response of plants to hypoxia through a complex reprogramming of their molecular activities with the aim of reducing the impact of stress on their physiological and cellular homeostasis. They focus on the regulatory interplay of oxygen, ethylene and NO and compile those molecular mechanisms mediated by phytoglobins and by the N-degron proteolytic pathway.

Recently, nitro-fatty acids, such as nitro-linolenic acid and nitro-oleic acid, have been proposed to act as mediators of cell signalling in plant development and abiotic stress response. **Begara-Morales *et al.,* (2020)** highlight that nitro-fatty acids activate the antioxidative system and transcription of many abiotic stress-related genes. Furthermore, they present an overview of the mode of action of these molecules, which can act as both, protein modifiers and NO donors.

Nanomaterials released into the environment have emerged as new stressors for plants (Sardoiwala *et al.,* 2018). Numerous types of nanomaterials (e.g. chitosan, metal oxide nanoparticles and carbon nanotubes) have been shown to alter endogenous NO metabolism and signal transduction in various plant species, as well as the NP stress-ameliorating effects of chemical NO donor treatments has been characterized. The related literature is summarized and discussed by **Kolbert *et al.,* (2020)** who also highlight the fact that NO-releasing nanoparticles and NP-based nanosensors may solve the methodological problems of NO detection and administration in plants.

Conclusions and future perspectives

In the past few decades of plant research, NO has undoubtedly emerged as a multifunctional signal molecule. In higher plants, endogenous NO synthesis differs from that which operates in animals and algae, thus the study of algae as a model system for improving our understanding of the evolution of NO synthesis and signalling, may be a promising future strategy. NO metabolism is regulated by the cells' redox state and, in cooperation with other redox molecules (ROS and RSS), NO itself regulates the redox processes of the cell. Therefore, this viewpoint needs to be expanded and future studies have to examine NO in association with other redox molecules and with the redox state of the cell. In the absence of a specific receptor in plant cells, the perception and transfer of NO bioactivity is mediated primarily by PTMs; however, the role of NO-regulated transcriptional gene regulation and the possible signalling role of nitrolipids are gaining more attention and will be interesting areas to examine in the future.

Traditional research topics of practical relevance examine the role of NO during fruit ripening, biotic (pathogenic and symbiotic) and abiotic interactions of plants. Exploring novel roles of *S*-nitrosation in regulating other PTMs during immune response is an exciting new area of plant NO research. Future studies should reveal molecular details regarding the role of NO in plant responses to fungal pathogens as well as to nitrogen-fixing bacteria. Understanding NO metabolism and signalling at the molecular level should be an important focus of future research also in case of global environmental stressors such as changes in temperature and light conditions, varying water supply, or phytotoxicity of nanomaterials. Additionally, the advances in nanotechnology may provide a solution to the current methodological challenges of NO research in the near future. Based on these highlights and the rapid development of plant NO science to date, suggests that we are quickly moving towards an exciting and productive future for this multifunctional plant signal.

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