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Regulatory role of nitric oxide in lipopolysaccharides-triggered plant innate immunity

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Recent studies have suggested that lipopolysaccharides (LPS) induce nitric oxide (NO) production and defense gene expression in plants. Our current work investigated the signaling mechanism of NO and the role of NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1 (NPR1) in LPS-induced innate immunity of Arabidopsis (*Arabidopsis thaliana*). We have provided evidence that LPS-elicited NO generation as well as increased antioxidant enzyme activities capable of maintaining the redox state could be important to protect plants against oxidative damage from pathogen attack. In addition, LPS-activated defense responses, including callose deposition and defense-related gene expression, are regulated through an NPR1-dependent signaling pathway. Our results contribute to elucidation of the signaling mechanism of NO and highlight an important role of NPR1 in modulating LPS-triggered innate immunity in plants. However, further research is necessary to clarify the cross-talk between mitochondria and NO on activating LPS-induced defense responses, and the regulatory mechanism of NO in LPS-induced innate immunity needs further improvement.

Keywords: nitric oxide, lipopolysaccharides, NPR1, nitric oxide synthase, plant innate immunity, priming

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Emerging evidence suggests that pathogen-associated molecular patterns (PAMPs) are potent elicitors that could induce defense mechanisms against bacterial, fungal and viral pathogens.[1] Bacterial Lipopolysaccharides as the prototypical PAMP can induce various plant defense-related responses, including the oxidative burst, nitric oxide (NO) generation, cell-wall alteration and pathogenesis-related (PR) gene expression.[2-4] NO is emerging as an important multifunctional signaling molecule in plants.[5,6] During defense-related responses, NO production is an important hallmark of innate immunity elicited by LPS.[7] Two key enzyme pathways are responsible for plant NO synthesis: oxidation of arginine to citrulline by a nitric oxide synthase (NOS)-like enzyme and reduction of nitrite to NO by a nitrate reductase (NR).[8] Although no NOS gene has yet been identified in plants, NOS activity as well as inhibition of NO synthesis by animal NOS inhibitors has been reported in plants.[9,10] NO mediates *Tobacco mosaic virus* (TMV)-induced AOX induction that triggers systemic basal defense against viral pathogen, and as a redox regulator of the NPR1/TGA1 system promotes the nuclear translocation of NPR1.[11] NPR1 plays important roles in activating defense gene and mediating plant defense response.[12] These findings prompt us to further investigate the signaling mechanism of NO and the important role of NPR1 in LPS-induced innate immunity.

Accumulating experimental evidence support that LPS as a plant defense activator can lead to the production of NO and play a key role in plant disease resistance.[7,13] Our current work has shown that LPS as a typical PAMP could directly induce defense-related responses including *PR1* gene induction and callose deposition.[14] However, some biological agents and synthetic compounds cannot induce *PR1* expression and callose deposition per se until pathogen infection; these induced reactions are frequently associated with

a primed state in which the plants are able to "recall" previous infection, root colonisation or chemical treatment.[15-17] In consequence, primed plants respond more rapidly and/or effectively to subsequent attack. It has been suggested that priming comes at much lower costs than direct-defense induction.[16] However, unlike direct defense induction, which could be determined directly by measuring various defense markers, priming is mostly analyzed by examining the speed and intensity of defense responses after disease exposure. To assess the mechanism and significant roles of priming will be a challenge for the future.

Our genetic and pharmacological analysis also provides evidence that arginine-dependent NOS-like enzyme is likely responsible for the biosynthetic mechanism involved in LPS-induced NO generation. Interestingly, the NR activity was found to be slightly inhibited by LPS treatment. The generated NO is not stable and would format the stable degradation of products, nitrate and nitrite. It has been proposed that post-translational regulation of NR takes place in response to various treatments, and nitrite accumulation is likely to be eliminated by post-translational inactivation of NR.[18] We speculate that the inhibited NR activity might result from the feedback repression by nitrite, and it may be modulated by a post-translational modification as the transcript levels of NR genes, *NIA1* and *NIA2* do not change during LPS induction. Recently, generation of NO can be observed in mitochondria induced by bacterial protein harpin, which support the model of cross talk between NO and mitochondria in the activation of stress-related responses in plants.[19] NO is required for TMV-induced mitochondrial AOX induction and promotes systemic basal defense against TMV.[20] AOX helps to maintain the electron flux and to reduce mitochondrial ROS levels,[21,22] which is often induced by various treatment.[23,24] Our data have determined the involvement of LPS-elicited NO in mediating the expression of the AOX gene and the upregulation of antioxidant enzyme activities, which might then modulate mitochondrial (or cellular) redox. Thus, it is important to clarify the function of mitochondria cross-talk with NO on LPS-induced defense activation, and requires further attention. In addition, the translocation of NPR1 into the nucleus is promoted by NO during LPS induction, which is consistent with previous study that the nuclear translocation of NPR1 is induced by GSNO/NO.[10] Although GSNO/NO-mediated S-nitrosylation of NPR1 facilitates its oligomerization, it is suggested that this S-nitrosylation-mediated oligomerization is not seen as an inhibitory effect of NPR1 signaling but rather as a step prior to monomer accumulation.[10] We did not show evidence how the NPR1 activity was regulated by NO. Further studies to elucidate the more comprehensive mechanism are warranted.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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