N-hydroxyippecolic acid: a general and conserved activator of systemic plant immunity

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This article comments on: Schnake A, Hartmann M, Schreiber S, Malik J, Brahmam L, Yildiz I, von Dahlen J, Rose LE, Schaffrath U, Zeier J. 2020. Inducible biosynthesis and immune function of the systemic acquired resistance inducer N-hydroxyippecolic acid in monocotyledonous and dicotyledonous plants. Journal of Experimental Botany 71, 6444–6459.

Long-lasting and broad-spectrum disease resistance throughout plants is an ever-important objective in basic and applied plant and crop research. While the recent identification of N-hydroxyippecolic acid (NHP) and its central role in systemic plant immunity in the model Arabidopsis thaliana provides a conceptual framework toward this goal, Schnake et al. (2020) quantify levels of NHP and its direct precursor in six mono- and dicotyledonous plant species subsequent to attacks by their natural pathogens, thereby implicating (phloem-mobile) NHP as a general and conserved activator of disease resistance.

N-hydroxyippecolic acid in systemic acquired resistance

Systemic acquired resistance (SAR) denotes the establishment of an immune memory in plant parts distal from the local site of pathogen attack. The perception of pathogens in a primary local infection event induces immune signalling that activates local defences and is propagated to distal plant parts to prepare for future threats. In these so-called ‘primed’ leaves, a faster and stronger immune response is mounted when exposed to a subsequent pathogen attack, and leaf infection damage is reduced to a minimum (Navarova et al., 2012; Hake and Romeis, 2019).

The nature of the systemic signalling molecules enabling the onset of priming and SAR is a long-standing matter of debate. Several metabolites have been discussed, which may propagate the information as a defence signal throughout the entire plant (Fu and Dong, 2013; Shah and Zeier, 2013). A recent hallmark study in Arabidopsis identified N-hydroxyippecolic acid (NHP) as a key molecule, which not only is induced by pathogen attack and accumulates at 24 h post-local infection in distal leaves, but is additionally sufficient to trigger (systemic) defence responses and disease resistance (Chen et al., 2018; Hartmann et al., 2018). NHP biosynthesis occurs in a three-step metabolic sequence from l-Lys through enzymes ALD1 (AGD2-LIKE DEFENSE RESPONSE PROTEIN), SARD4 (SAR-DEFICIENT4), and FMO1 (FLAVIN-DEPENDENT-MONOXYGENASE1). In particular, the last N-hydroxylation step catalysed by FMO1 has received a lot of attention, in which the precursor substrate piperolic acid (Pip), demonstrated to be an immune modulator by itself (Navarova et al., 2012), is converted to NHP. FMO1 has independently been identified and characterized in Arabidopsis as required and sufficient to induce pathogen resistance (Bartsch et al., 2006; Koch et al., 2006; Mishina and Zeier, 2006). The ectopic expression of the FMO1 gene resulted in reduced pathogen growth, and external application of NHP to leaves reduced systemic pathogen growth and potentiated SAR to bacterial and oomycete infections (Hartmann and Zeier, 2019). However, the complementary analysis of biochemical quantification of Pip and NHP metabolite levels and their dynamic kinetics during SAR induction was lagging behind for a long time because of the technical challenges.

NHP biosynthesis and resistance function of NHP in mono- and dicotyledonous plants

Schnake et al. (2020) established six different natural plant pathosystems encompassing members of the dicotyledonous plant families Cucurbitaceae [cucumber (Cucumis sativus)–Pseudomonas syringae pv. lachrymans], Solanaceae [tomato (Solanum lycopersicum)–Phytophthora infestans or P. syringae pv. tomato]; tobacco (Nicotiana tabacum)–P. syringae pv. tabaci], and Fabaceae [soybean (Glycine max)–P. savastanoi pv. glycinea],...
Box 1. Plant immunity through systemic acquired resistance (SAR)

Plant immunity consists of several layers of defence that guarantee an interaction-specific blocking of respective intruders.

Plants memorize previous local attacks and prepare themselves against subsequent microbial infections either by the same or by different pathogens. This so-called systemic acquired resistance (SAR) requires the generation and propagation of a defence signal from the initial infected site to distal plant parts.

While the signalling cascades involved in local perception and downstream induction of defence responses are well studied, components mediating systemic signalling and their distal receptors are largely unknown.

N-Hydroxy-pipecolic acid (NHP) has recently been identified to be crucial for the induction and maintenance of SAR, and it is a matter of debate whether NHP may even serve as the key systemic signal itself. This concept is supported by research included herein showing that NHP is bioactive in resistance priming in mono- and dicotyledonous plants to infection by (hemi-) biotrophic bacterial, oomycete, and fungal pathogens.

Plant-specific conversion kinetics of Pip to NHP?

Detailed metabolite quantification in Schnake et al. (2020) reveals that in most investigated systems, NHP synthesis was paralleled by an increase in its direct precursor Pip, whereas the absolute levels of Pip to NHP vary between different species and pathosystems. In Solanaceae, Pip and NHP levels increase in parallel upon pathogen exposure, with final concentrations of Pip and NHP reaching either about the same level [tobacco ~6 μg g⁻¹ FW at 72 h post-infection (hpi)] or with Pip exceeding NHP levels by ~8-fold (tomato). In Brachypodium and barley, fungal pathogen-induced Pip levels are up to 20- to 40-fold higher (barley 30 μg g⁻¹ FW at 5 dpi) than those of NHP (0.1 μg g⁻¹ FW), indicating that only small proportions of Pip are converted to NHP in these monocotyledonous plants. In contrast, cucumber displays constitutively high amounts of Pip already in both unstressed leaves (10 μg g⁻¹ FW) and phloem sap. Pseudomonas syringae pv. lachrymans infection does not yield any further increase of Pip, whereas it nevertheless triggers accumulation of NHP (1.5 μg g⁻¹ FW at 5 dpi). These data indicate that the mode and rate of the conversion from Pip to NHP are subject to a plant species-specific control. Whereas precursor Pip is present and stored in high amounts ready for its conversion to NHP in some species, the precursor will have to be synthesized de novo on demand in others.

Despite acknowledging distinct infection procedures [(spray-) inoculation, infiltration, or incubation], which may affect the total values measured in different pathosystems, an overall conserved pattern of pathogen-induced accumulation of (free) NHP seems to exist in different plant species. These data hint toward NHP as a general dose-dependent information mediator that triggers systemic signalling cascades to mount plant immunity.

In cucumber, with constitutive Pip levels measured, NHP biosynthesis may only underlie a strict control through the converting FMO1 enzyme as gatekeeper. In tobacco or tomato, in addition, Pip synthesis is likewise pathogen induced and depends on the activities of the biosynthesis enzymes ALD1 and SARD4, and on an export step out of the chloroplast organelle. These data validate once more the crucial function of the FMO1 enzyme. Intensive phylogenetic studies highlight the conservation of the FMO1 gene throughout the plant kingdom (Hartmann et al., 2018; Holmes et al., 2019). Ectopically expressed selected homologues of Arabidopsis FMO1 from different plant species catalyse the conversion from Pip to NHP, and metabolic engineering of NHP synthesis based on the Arabidopsis enzymes triggered SAR in tomato (Holmes et al., 2019). Taken together
with results from Schnake et al. (2020), these data not only provide the proof for FMO1 as a valuable biotechnological target to engineer plant health and crop disease resistance by speeding up SAR signalling, but they also lead the way to investigate FMO1 enzymes from plant species other than Arabidopsis. It will be exciting to uncover their individual biochemical properties and enzyme kinetic parameters that help fulfil their function in the context of gatekeepers for NHP synthesis.

**Keywords:** N-Hydroxy-pipecolic acid, pipecolic acid, systemic acquired resistance, systemic immune signalling

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