



The Role of a Glucosinolate-Derived Nitrile in Plant Immune Responses

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Specialty section:

This article was submitted to
Plant Metabolism
and Chemodiversity,
a section of the journal
Frontiers in Plant Science

Received: 31 October 2019

Accepted: 19 February 2020

Published: 10 March 2020

Citation:

Ting H-M, Cheah BH, Chen Y-C,
Yeh P-M, Cheng C-P, Yeo FKS,
Vie AK, Rohloff J, Winge P, Bones AM
and Kissen R (2020) The Role of a
Glucosinolate-Derived Nitrile in Plant
Immune Responses.
Front. Plant Sci. 11:257.
doi: 10.3389/fpls.2020.00257

Glucosinolates are defense-related secondary metabolites found in Brassicaceae. When Brassicaceae come under attack, glucosinolates are hydrolyzed into different forms of glucosinolate hydrolysis products (GHPs). Among the GHPs, isothiocyanates are the most comprehensively characterized defensive compounds, whereas the functional study of nitriles, another group of GHP, is still limited. Therefore, this study investigates whether 3-butenenitrile (3BN), a nitrile, can trigger the signaling pathways involved in the regulation of defense responses in *Arabidopsis thaliana* against biotic stresses. Briefly, the methodology is divided into three stages, (i) evaluate the physiological and biochemical effects of exogenous 3BN treatment on *Arabidopsis*, (ii) determine the metabolites involved in 3BN-mediated defense responses in *Arabidopsis*, and (iii) assess whether a 3BN treatment can enhance the disease tolerance of *Arabidopsis* against necrotrophic pathogens. As a result, a 2.5 mM 3BN treatment caused lesion formation in *Arabidopsis* Columbia (Col-0) plants, a process found to be modulated by nitric oxide (NO). Metabolite profiling revealed an increased production of soluble sugars, Krebs cycle associated carboxylic acids and amino acids in *Arabidopsis* upon a 2.5 mM 3BN treatment, presumably via NO action. Primary metabolites such as sugars and amino acids are known to be crucial components in modulating plant defense responses. Furthermore, exposure to 2.0 mM 3BN treatment began to increase the production of salicylic acid (SA) and jasmonic acid (JA) phytohormones in *Arabidopsis* Col-0 plants in the absence of lesion formation. The production of SA and JA in nitrate reductase loss-of function mutant (*nia1 nia2*) plants was also induced by the 3BN treatments, with a greater induction for JA. The SA concentration in *nia1 nia2* plants was lower than in Col-0 plants, confirming the previously reported role of NO in controlling SA production in *Arabidopsis*. A 2.0 mM 3BN treatment prior to pathogen assays effectively alleviated the leaf lesion symptom of *Arabidopsis* Col-0 plants caused by *Pectobacterium carotovorum* ssp. *carotovorum* and *Botrytis cinerea* and reduced the pathogen growth on leaves. The findings of this study demonstrate that 3BN can elicit defense response pathways in *Arabidopsis*, which potentially involves a coordinated crosstalk between NO and phytohormone signaling.

Keywords: secondary metabolites, glucosinolates, nitriles, metabolomics, transcriptomics, plant innate immunity