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Nitric oxide biosynthesis in *Arabidopsis*: Amine oxidases are potential candidates

R. Wimalasekera

Department of Botany, Faculty of Applied Sciences, University of Sri Jayewardenepura, Sri Lanka
rinukshiw@gmail.com

Nitric oxide (NO) regulates an array of developmental and physiological processes in plants including germination, flowering, and senescence. NO plays an important role as a signaling molecule in abiotic and biotic stresses. Polyamines (PAs) which are essential growth regulators in plants are oxidatively catabolized by copper amine oxidases (CuAOs) and flavin adenine dinucleotide (FAD)-dependent amine oxidases (PAOs). These two classes of enzymes differ in their substrate specificity, catalytic mechanism and subcellular localization. In *Arabidopsis*, eight genes coding for putative functional CuAOs and five PAOs are identified. CuAOs and PAOs have similar functions in almost all aspects of plant growth and development and in defense responses against stresses such as drought, heat and salinity and pathogen invasion. This study was aimed on finding the possible involvement of CuAOs and PAOs in NO production in response to abscisic acid (ABA)-mediated stress responses. Homozygous T-DNA insertional knockout seedlings of *CuAO1*, *CuAO2*, *CuAO5*, *PAO2*, *PAO3* and *PAO6* were employed along with wild type (WT) to investigate the ABA-induced NO production. NO detection was observed by fluorometry and fluorescence microscopy using NO-specific dye DAF-2A and DAF-2A-DA. When treated with 5 µM and 50 µM of ABA, WT and knockouts showed an increase in NO release above ABA-untreated endogenous levels. However, increase in NO level was lower in all knockouts compared to WT. ABA-induced NO production was enhanced in the seedlings over-expressing *PAO2* (PAO2OX) and *PAO3* (PAO3OX). Fluorescence microscopic analysis showed that ABA-induced NO production in the root tips and shoots of the knockouts was comparably lower than in WT. PAO2OX and PAO3OX exhibited stronger florescence in root tips. Mean differential NO production in the knockouts and over-expressers suggests a regulatory function of CuAOs and PAOs in ABA-mediated NO biosynthesis (n=6). Thus, amine oxidases can be suggested as new players in NO biosynthesis in ABA signaling. It can be hypothesized that PA catabolism by amine oxidases is a direct pathway leading to NO biosynthesis and/or NO biosynthesis is mediated through an indirect pathway through H_2O_2 which is a product of PA catabolism. Further studies are needed to elucidate the mechanism of PAs and amine oxidases induced ABA-mediated NO biosynthesis.

Keywords: ABA, amine oxidases, nitric oxide, polyamines

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