



Hydrogen peroxide mediates the expression of ascorbate-related genes in response to methanol stimulation in *Oncidium*

Chin-Hui Shen, Kai-Wun Yeh ^{*}

Institute of Plant Biology, National Taiwan University, No. 1, Sec. 4, Roosevelt Road, Taipei 10607, Taiwan

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ABSTRACT

We investigated the signaling role of hydrogen peroxide (H_2O_2) in regulating the ascorbate (AsA) level after exogenous methanol (MeOH) application. The endogenous H_2O_2 and AsA levels as well as the expression of related genes were monitored after MeOH treatment of cultures of *Oncidium* pseudonobile (PUB). A high MeOH concentration was detrimental and caused irreversible consumption of endogenous AsA. However, a low MeOH concentration (20 mM) triggered the synthesis of H_2O_2 and was effective in enhancing the expression of AsA biosynthetic genes at the transcript, protein and subcellular (Cytosol) pathways. The increased expression of three genes could be blocked by the addition of hydroxytyrosine, an inhibitor of ascorbic oxidase (AO) 1.1.5.1.1, and dihydroxyacetone (DHP), an inhibitor of NADPH oxidase (EC: 1.6.3.1). Thus, the H_2O_2 generated by MeOH application is a product of MeOH detoxification through alcohol oxidase and NADPH oxidase activation. In this chain, H_2O_2 acts as a secondary messenger for the activation of AsA-related genes. Our results reveal the signaling function of H_2O_2 and confirm AsA homeostasis in *Oncidium* culture in response to MeOH stimulation. A mechanism for the MeOH effect on AsA production is suggested.

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Introduction

Methanol (MeOH) is a volatile organic product, originating from the demethylation of pectin by pectin methylesterase (PME; EC: 3.1.1.11) for softening of the cell wall, especially throughout the early stage of leaf expansion (Fell and Bennett, 1996). Some MeOH emissions have also been observed during changes in cell wall construction during the development of roots and fruits (Fell and Bennett, 1996). Additionally, MeOH might be produced and emitted in large quantities by methanolic ascending or under various stresses (Pothol and Dordy, 1998; Pothol et al., 2000; van Dael et al., 2000; Pothol et al., 2007). Methanol accumulates in the intercellular air space or in the liquid pool at night, when the stomata close, and is rapidly converted to formaldehyde,

formic acid and CO₂ to prevent damage by alcohol oxidase (Coud et al., 2000). Although the metabolism of MeOH is not completely understood in plants, its contribution to plant physiology is highlighted by its use in C₄ plants for photosynthetic productivity (Nelson and Bennett, 1992). Methanol influences C₄ plant growth under foliar spray or irrigation (Ramirez et al., 2006), but has no effect on C₃ plants. Foliar application of MeOH causes an increase of fresh and dry weight in *Arabidopsis* and tobacco, whereas MeOH irrigation significantly delays the growth of *Arabidopsis*, tobacco and tomato (Ramirez et al., 2006). The growth promotion by foliar application was attributed to the increased carbon fixation due to detoxification from photorespiration. Radiotracer ¹⁴C and ¹³C NMR studies revealed that MeOH is metabolized by alcohol oxidase to formaldehyde and formic acid, which are further converted to acetic, malic, succinic, oxalic and glyoxylic (Coud et al., 2000). The CO₂ produced from the metabolism of MeOH is utilized within the C₄ cycle for glucose metabolism (Mansueti and Buge, 1981).

Recently, a global gene expression profile resulting from 100 MeOH stimulations in *Arabidopsis* leaves was reported (Dionisi et al., 2004). Most of the genes induced by MeOH function in detoxification and stress responses. After 1 h of MeOH treatment, the genes with the highest up-regulation are associated with methylation, cell communication/signaling transduction processes, defense, and RNA processing, but none are involved in photosynthesis. At 24- and 72-h MeOH treatments, the genes with

^{*} Corresponding author. Tel.: +886 2 23692734; fax: +886 2 23692735.
E-mail addresses: YehKW2004@ntu.edu.tw (C.-H. Shen), yehkw@ntu.edu.tw (K.-W. Yeh).

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