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ORIGINAL PAPER

Evidence for Methylerythritol Pathway (MEP) Contributions to Zerumbone Biosynthesis as Revealed by Expression Analysis of Regulatory Genes and Metabolic Inhibitors Studies

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Abstract

Sesquiterpenoid Zerumbone, the principal secondary metabolite in *Zingiber zerumbet* Smith, has been identified as the putative molecule conferring resistance against soft-rot causative, *Pythium myriotylum*. Metabolic precursors for sesquiterpenoid biosynthesis namely, isopentenyl diphosphate (IPP) and DMAPP, are generated either from cytosolic mevalonic acid (MVA) and/or the plastidal methylerythritol phosphate (MEP) pathway. Evaluation of expression pattern of regulatory genes of MEP and MVA pathway following *P. myriotylum* infection revealed that while transcripts of MVA regulatory gene, 3-hydroxy-3-methylglutaryl-coenzyme A reductase (*HMGR*), did not show significant changes, biphasic expression pattern was observed for MEP pathway regulatory genes, viz., 1-deoxy-D-xylulose-5-phosphate reductase (*DXR*) and 1-deoxy-D-xylulose-5-phosphate synthase (*DXS*) as well for *ZzTPS* (terpene synthase) and Farnesyl phosphate synthase (*FPS*) compared with uninfected control. Contribution of the two pathways in zerumbone biosynthesis was evaluated in axenic tissue culture raised *Z. zerumbet* plantlets using MVA and MEP pathway specific inhibitors, mevinolin (MEV) and fosmidomycin (FOS) respectively. Results generated by regulatory gene and metabolite analysis are informative with respect to the role of plastidial IPP pool generated via MEP pathway in zerumbone biosynthesis and the possible redirection of flux in the event of metabolic perturbations mediated by chemical inhibitors.

Keywords Zerumbone · MVA · MEP · Mevinolin · Fosmidomycin · *HMGR* · *DXR* · *DXS*

Key Message

- Biphasic expression observed for methylerythritol (MEP) pathway regulatory genes in *Zingiber zerumbet* following *Pythium myriotylum* infection.
- Metabolite analysis of in vitro plantlets treated with mevinolin (MEV) and fosmidomycin (FOS) reveals 54-fold decrease in zerumbone following FOS treatment.
- Identified plastidial IPP flux contributing more towards zerumbone biosynthesis.

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Introduction

Z. zerumbet (Family Zingiberaceae) also called pine cone or shampoo ginger is a perennial rhizomatous plant found either in cultivated, wild, or naturalized states (CABI 2014). Taxon has a Southeast Asian origin with center of diversity in the Indo-Malayan region and possibly spread to the Oceania (Yob et al. 2011) by humans. Rhizomes of *Z. zerumbet* find a wide range of ethnomedicinal uses (Sulaiman et al. 2010; Yob et al. 2011; Koga et al. 2016) as documented in Ayurvedic and Chinese Pharmacopoeias (Yob et al. 2011). Characterization of essential oil from *Z. zerumbet* rhizomes has identified zerumbone (37%), α-humulene (14.4%), and camphene (13.8%) as major metabolites (Ming et al. 2011). The principal bioactive sesquiterpenoid compound, zerumbone (1,2,6,9-humulatrien-8-one, or 2,6,9,9-tetramethyl-[2E,6E,10E]-cycloundeca-2,6,10-trien-1-one), has been widely investigated for its medicinal properties. Our earlier studies have identified zerumbone to play a crucial role in imparting *Pythium* resistance to the wild congener (Keerthi et al. 2014). *Z. zerumbet* being rich in terpenoids and phenylpropanoid

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