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Research article

Metabolic cross-talk between pathways of terpenoid backbone biosynthesis in spike lavender

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ABSTRACT

The metabolic cross-talk between the mevalonate (MVA) and the methylerythritol phosphate (MEP) pathways in developing spike lavender (*Lavandula latifolia* Med) was analyzed using specific inhibitors and on the basis of ^{13}C-labeling experiments. The presence of mevinolin (MEV), an inhibitor of the MVA pathway, at concentrations higher than 0.5 μM significantly reduced plant development, but not the synthesis of chlorophylls and carotenoids. On the other hand, fosmidomycin (FSM), an inhibitor of the MEP pathway, at concentrations higher than 20 μM blocked the synthesis of chlorophyll, carotenoids and essential oils, and significantly reduced stem development. Notably, 1.2 mM MVA could recover the phenotype of MEV-treated plants, including the normal growth and development of roots, and could partially restore the biosynthesis of photosynthetic pigments and, to a lesser extent, of the essential oils in plantlets treated with FSM. Spike lavender shoot apices were also used in ^{13}C-labeling experiments, where the plantlets were grown in the presence of [U−$^{13}C_6$]glucose. GC-MS-analysis of 1,8-cineole and camphor indicated that the C_5-precursors, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) of both monoterpenes are predominantly biosynthesized via the methylerythritol phosphate (MEP) pathway. However, on the basis of the isotopologue profiles, a minor contribution of the MVA pathway was evident that was increased in transgenic spike lavender plants overexpressing the 3-hydroxy-3-methylglutaryl CoA reductase (HMGR), the first enzyme of the MVA pathway. Together, these findings provide evidence for a transport of MVA-derived precursors from the cytosol to the plastids in leaves of spike lavender.

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1. Introduction

Plant terpenoids are the most abundant and structurally most versatile group of natural products, with about 30.000 compounds described (Buckingham, 1994), encompassing both primary metabolites (carotenoids, the prenyl chains of chlorophyll, sterols and some hormones) and secondary metabolites such as essential oils (Baldwin at al., 2006; Dudareva et al., 2004; Enfissi et al., 2005; Ruiz-Sola and Rodríguez-Concepción, 2012; Tholl, 2006). The latter are mainly involved in defense against herbivores and pathogens, allelopathic interactions and pollination (Baldwin at al., 2006; Dudareva et al., 2004; Tholl, 2006). Terpene secondary metabolites have also a significant commercial importance due to their uses in the food, perfume, cosmetic and pharmaceutical industries (Verlet, 1993) and for being a rich pool for exploring new drugs and lead compounds (Cheng et al., 2007).

In plants, the biosynthesis of the C_5 universal terpene precursors, isopentenyl diphosphate (IPP) and its isomer, dimethylallyl diphosphate (DMAPP), proceeds via two alternative pathways, the cytosolic MVA and the plastidial methylerythritol-4-phosphate (MEP) pathways (Fig. 1) (Lange and Ahkami, 2013; Eisenreich et al., 2004; Rodríguez-Concepción and Boronat, 2002). Traditionally, it is believed that the MEP pathway supplies C_5 precursors for the production of monoterpenes, plastoquinones, carotenoids, the phytol tail of chlorophylls, phytohormones (e.g. cytokinins, gibberellins, abscisic acid), while the MVA pathway provides precursors for the synthesis of sesquiterpenes, sterols including brassinosteroid hormones, and triterpenes (Rodriguez-Concepcion,

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2006). However, metabolic cross-talk between the two pathways of terpenoid backbone biosynthesis is well documented (Bartram et al., 2006; Opitz et al., 2014; Peña-Rodríguez et al., 2014; Rodriguez- Concepción and Boronat, 2015; Skorupinska-Tudek et al., 2008) (Fig. 1). Therefore, the relative contribution of each pathway to the biosynthesis of the various classes of terpenes or even of a specific plant terpenoid remains uncertain and could depend on the given plant species or environmental conditions (Vranová et al., 2013).

Lavandula latifolia Medicus (spike lavender) is an aromatic shrub with economic interest due to its essential oil, mainly consisting of the monoterpenes linalool, 1,8-cineole and camphor (Muñoz-Bertomeu, 2007b). Up-regulation of either the DXS enzyme from *Arabidopsis thaliana*, which catalyzes the first step of the MEP pathway, or the HMGR enzyme from *A. thaliana* (HMG1) which catalyzes the first committed step of the MVA pathway, increased the yield of essential oil in spike lavender (Muñoz-Bertomeu et al., 2006 and 2007a, respectively). Although essential oil yield was always higher in those transgenic spike lavender plants overexpressing the *DXS* gene, suggesting that the MEP pathway is the principal donor of C_5 precursors for monoterpene biosynthesis, these results also supported the view that the MVA pathway could also be involved in the biosynthesis of monoterpenes in spike lavender. To experimentally validate whether the MVA pathway can provide C5 units for monoterpene biosynthesis, both chemical inhibitor and labeling experiments were employed in developing *L. latifolia*. Specifically, we perturbed the metabolic fluxes of the two pathways by using mevinolin (MEV) or fosmidomycin (FSM), specific inhibitors of the MVA and MEP pathways, respectively (Fig. 1). MEV competitively inhibits the HMGR enzyme, whereas FSM is an inhibitor of the DXR (IspC) enzyme that catalyzes the formation of DXP into MEP; both compounds have been successfully used in earlier studies dealing with terpene metabolism (Bach and Lichtenthaler, 1983; Re et al., 1995; Rodríguez-Concepción, 2006; Shigi, 1989). We also used ^{13}C-labeling experiments using [U−$^{13}C_6$]glucose as tracer, in an attempt to directly measure the relative contributions of the MVA and MEP pathways in monoterpene biosynthesis of wild-type and transgenic plants overexpressing HMGR.

2. Material and methods

2.1. Plant material, culture media and conditions

Shoot-apices (1–2 cm in length) grown *in vitro* from wild-type (WT) and transgenic HMGR5 spike lavender lines (Muñoz-Bertomeu et al., 2007a) were used as initial plant material. Wild-type lines were obtained from seeds provided by Intersemillas SA (Valencia, Spain) and germinated *in vitro* as described by Calvo and Segura (1988). Germinated seeds were placed in glass tubes (25 × 15 mm) covered with polypropylene closures (Wellco, Vineland, NJ, USA) containing 25 ml of basal medium (BM). This medium consisted of half-strength MS salts and vitamins (Murashige and Skoog, 1962), 3% sucrose, 0.8% of agar (Pronadisa, Spain) and a pH of 5.7. Medium was sterilized by autoclaving (20 min at 120 °C, 10^5 Pa). Transgenic HMGR5 lines overexpressing the *HMG1* gene of *A. thaliana* were previously generated (Muñoz-Bertomeu et al., 2007a) and maintained by shoot-tip culture on BM medium.

Cultures were kept in growth chambers at 25 ± 2 °C and a 16 h photoperiod provided by Sylvania (GTE gro-lux, F36W/GRO, Erlangen, Germany) cool-white fluorescent tubes (60 μmol m^{-2} s^{-1}

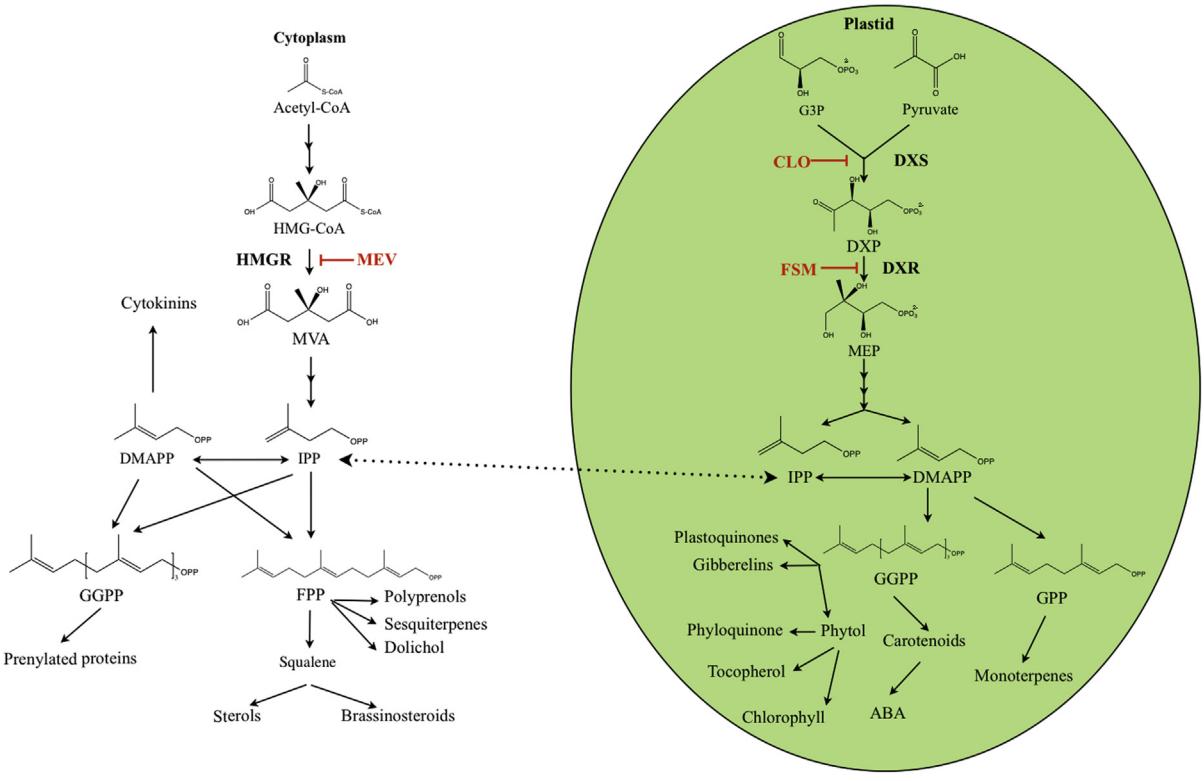

Fig. 1. General scheme of the terpene synthesis pathways in plants and their inhibitors. MVA: mevalonate. HMG-CoA: 3-hydroxy-3-methylglutaryl-coenzyme A. HMGR: HMG-CoA reductase. MEP: 2C-methyl-D-erytritol-4-phosphate. IPP: isopentenyl diphosphate. DMAPP: dimethylallyl diphosphate. FPP: farnesyl diphosphate. GGPP: geranylgeranyl diphosphate. G3P: D-glyceraldehyde-3-phosphate. DXP: 1-deoxy-D-xylulose-5-phosphate. DXS: DXP synthase. DXR: DXP reductoisomerase. MEV: mevinolin. CLO: clomazone. FSM: fosmidomycin.

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