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Review

Plant volatile terpenoid metabolism: Biosynthetic genes, transcriptional regulation and subcellular compartmentation

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ABSTRACT

Volatile terpenoids released from different plant parts play crucial roles in pollinator attraction, plant defense, and interaction with the surrounding environment. Two distinct pathways localized in different subcellular compartments are responsible for the biosynthesis of these compounds. Recent advances in the characterization of genes and enzymes responsible for substrate and end product biosynthesis as well as efforts in metabolic engineering have revealed new aspects of volatile terpenoid biosynthesis. This review summarizes recent progress in the characterization of volatile terpenoid biosynthetic genes, their spatio-temporal expression patterns and subcellular localization of corresponding proteins. In addition, recent information obtained from metabolic engineering is discussed.

1. Introduction

Volatile terpenoids represented by mainly isoprene (C_5), monoterpenes (C_{10}) and sesquiterpenes (C_{15}) constitute the largest class of plant volatile compounds. They play important roles in direct and indirect plant defense against herbivores and pathogens, in reproduction by attraction of pollinators and seed disseminators, and in plant thermotolerance [1]. Apart from their importance in plant physiology and ecology, volatile terpenoids are also used as natural flavor and aroma compounds and have beneficial impact on humans as health promoting compounds [2]. All terpenoids are synthesized from the universal five carbon precursors, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), which are derived from two alternate biosynthetic pathways localized in different subcellular compartments (Fig. 1). While DMAPP formed in plastids is used by isoprene synthase (ISPS) to form isoprene in some plants, IPP and DMAPP precursors are further condensed by prenyl diphosphate synthases in the respective compartments to form prenyl diphosphate intermediates serving as substrates for a large group of terpene synthase (TPS) enzymes, resulting in the final terpenoid compounds [1]. In the past several years there has been a significant progress in identification and characterization of volatile terpenoid biosynthetic genes and enzymes, determination of their spatio-temporal expression and compartmentalization, and metabolic engineering. This has led to several new insights resulting in better understanding of volatile terpenoid biosynthesis.

2. Formation of IPP and DMAPP

In plants two biosynthetic pathways are responsible for the synthesis of IPP and DMAPP, the universal precursors of all terpenoids. The classical cytosolic mevalonic-acid (MVA) pathway gives rise to IPP from acetyl-CoA [3], whereas the plastidial 2-*C*-methyl-erythritol 4-phosphate (MEP) pathway [4] described during the 1990s leads to the formation of IPP and DMAPP from pyruvate and glyceraldehyde-3-phosphate (Fig. 1). Although the subcellular compartmentalization of two pathways allows them to operate independently, metabolic cross-talk between the two pathways has been reported [5,6].

The MVA pathway starts with the condensation of two molecules of acetyl-CoA into acetoacetyl (AcAc)-CoA by the enzyme AcAc-CoA thiolase (AACT) (Fig. 1). The first plant AACT was cloned from radish (*Raphanus sativus*) by functional complementation of a yeast mutant defective for AACT [7]. Recent characterization of *Arabidopsis* genes encoding AACT1 and AACT2 and analysis of T-DNA insertion mutants for both genes indicated that only AACT2 is involved in the MVA pathway, whereas AACT1 could possibly be associated in the last step of fatty acid degradation [8,9]. In the second step, HMG-CoA is formed by the condensation of one molecule of acetyl-CoA with one molecule of AcAc-CoA. It was

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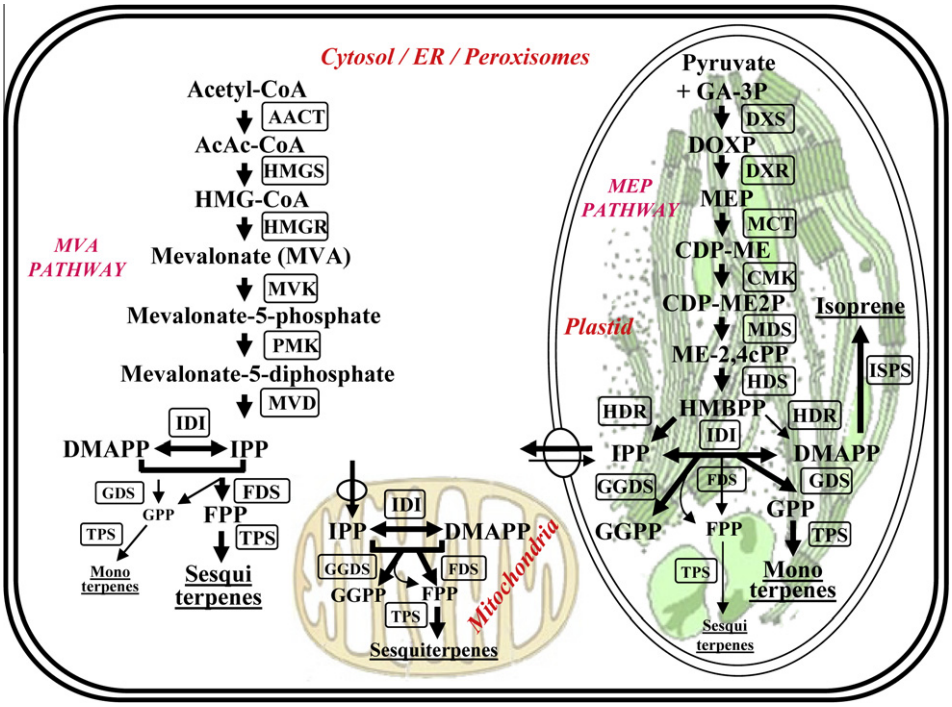

Fig. 1. Biosynthetic pathways and their compartmentalization leading to volatile terpenoids in plants. AACT, acetoacetyl-CoA thiolase; AcAc-CoA, acetoacetyl-CoA; CDP-ME, 4-(cytidine 5′-diphospho)-2-*C*-methyl-D-erythritol; CDP-ME2P, 4-(cytidine 5′-diphospho)-2-*C*-methyl-D-erythritol phosphate; CMK, CDP-ME kinase; DMAPP, dimethylallyl diphosphate; DOXP, 1-deoxy-D-xylulose 5-phosphate; DXR, DOXP reductoisomerase; DXS, DOXP synthase; FDS, farnesyl diphosphate synthase; FPP, farnesyl diphosphate; GA-3P, glyceraldehyde-3-phosphate; GDS, geranyl diphosphate synthase; GGDS, geranyl geranyl diphosphate synthase; GGPP, geranyl geranyl diphosphate; GPP, geranyl diphosphate; HDR, (*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate reductase; HDS, (*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate synthase; HMBPP, (*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; HMGR, HMG-CoA reductase; HMGS, HMG-CoA synthase; IDI, isopentenyl diphosphate isomerase; IPP, isopentenyl diphosphate; ISPS, isoprene synthase; MCT, 2-*C*-methyl-D-erythritol 4-phosphate cytidylyltransferase; MDS, 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate synthase; ME-2,4cPP, 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate; MEP, 2-*C*-methyl-D-erythritol 4-phosphate; MVD, mevalonate diphosphate decarboxylase; MVK, mevalonate kinase; PMK, phosphomevalonate kinase; TPS, terpene synthase. Names of the enzymes are boxed and volatile terpenoids are underlined.

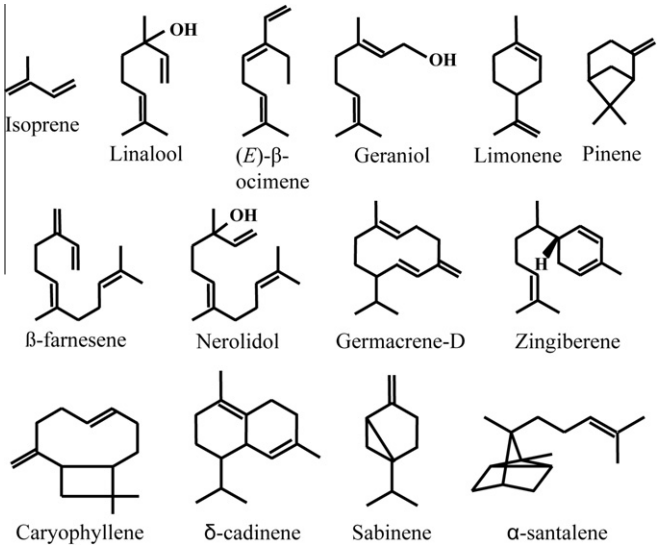

Fig. 2. Structures of representative volatile terpenoid compounds.

earlier proposed that the conversion from acetyl-CoA to HMG-CoA involves two enzymatic steps carried out by a single enzyme [10]. However, HMG-CoA synthase (HMGS) from *Arabidopsis* was reported to functionally complement yeast mutants defective for HMGS [11]. Further biochemical characterization of HMGSs from *Brassica juncea* [12] and *Hevea brasiliensis* [13] that catalyze the conversion of acetyl-CoA and AcAc-CoA to HMG-CoA indicated that the conversion from acetyl-CoA to HMG-CoA involves two independent enzymes; AACT and HMGS. In the next step, HMG-CoA reductase (HMGR), a NADPH-dependent enzyme that catalyzes a double reduction reaction involving four electron transfers, catalyzes the biosynthesis of mevalonate from HMG-CoA [14]. HMGR has been extensively studied in some plant species. For example, *Arabidopsis* contains two differentially expressed HMGR genes which encode three isoforms [15], whereas in snapdragon (*Antirrhinum majus*) three genes encoding HMGR have been identified [16]. The downstream steps from mevalonate to IPP involve two phosphorylations and a decarboxylation event carried out by mevalonate kinase (MVK), phosphomevalonate kinase (PMK), and mevalonate diphosphate decarboxylase (MVD) enzymes, respectively. Although the enzymes involved in these steps are well studied in yeast and mammalian systems, there is surprisingly little information available in terms of their biochemical characterization in plants. IPP derived from the MVA pathway in the cytosol, is further acted upon by isopentenyl diphosphate isomerase (IDI), a divalent, metal ion-requiring enzyme, to form dimethylallyl diphosphate (DMAPP) [17].

The mevalonate-independent pathway, named the MEP pathway was first elucidated in *Escherichia coli* and subsequently plant homologues have been characterized using a combination of biochemical and genomic approaches [18]. The MEP pathway consists of seven enzymatic steps involved in the formation of IPP and DMAPP from pyruvate and D-glyceraldehyde 3-phosphate (GAP) (Fig. 1). The first step in this pathway is the condensation of