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Both methylerythritol phosphate and mevalonate pathways contribute to biosynthesis of each of the major isoprenoid classes in young cotton seedlings

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ABSTRACT

In higher plants, both the methylerythritol phosphate (MEP) and mevalonate (MVA) pathways contribute to the biosynthesis of isoprenoids. However, despite a significant amount of research on the activity of these pathways under different conditions, the relative contribution of each to the biosynthesis of diverse isoprenoids remains unclear. In this work, we examined the formation of several classes of isoprenoids in cotton (*Gossypium hirsutum* L.). After feeding [5,5-$^2H_2$]-1-deoxy-D-xylulose ([5,5-$^2H_2$]DOX) and [2-^{13}C]MVA to intact cotton seedlings hydroponically, incorporation into isoprenoids was analyzed by MS and NMR. The predominant pattern of incorporation followed the classical scheme in which C_5 units from the MEP pathway were used to form monoterpenes (C_{10}), phytol side chains (C_{20}) and carotenoids (C_{40}) while C_5 units from the MVA pathway were used to form sesquiterpenes (C_{15}), terpenoid aldehydes (C_{15} and C_{25}) and steroids/triterpenoids (C_{30}). However, both pathways contributed to all classes of terpenoids, sometimes substantially. For example, the MEP pathway provided up to 20% of the substrate for sterols and the MVA pathway provided as much as 50% of the substrate for phytol side chains and carotenoids. Incorporation of C_5 units from the MEP pathway was highest in cotyledons, compared to true leaves, and not observed at all in the roots. Incorporation of C_5 units from the MVA pathway was highest in the roots (into sterols) and more prominent in the first true leaves than in other above-ground organs. The relative accumulation of label in intermediates vs. end products of phytosterol metabolism confirmed previous identification of slow steps in this pathway.

Introduction

Isoprenoids constitute the largest group of natural products in plants with nearly 30,000 compounds having been described (Buckingham, 1994). These substances possess an astounding variety of chemical structures and their functions in plants are also very diverse, including roles in both primary (sterols, carotenoids, various hormones) and secondary (monoterpenes, sesquiterpenes, diterpenes) metabolism. All isoprenoids are formed from a pair of isomeric C_5 diphosphates, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP).

In plants, two independent pathways located in separate intracellular compartments are involved in the biosynthesis of IPP and DMAPP (Rohmer, 1999). The 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway, localized in the plastids, is also found in protozoa and eubacteria, while the mevalonate (MVA) pathway, present in the cytosol (or possibly the peroxisomes (Sapir-Mir et al., 2008)), is also characteristic of animals, fungi and archaebacteria. The classical picture is that the MEP pathway supplies C_5 isoprenoid units for making C_{10} (monoterpene), C_{20} (diterpene) and C_{40} (carotenoid) compounds, while the MVA pathway provides C_5 units for the synthesis of C_{15} (sesquiterpene) and C_{30} (triterpene and sterol) compounds (Rodriguez-Concepcion, 2006). However, many exceptions are known (Bartram et al., 2006; Hemmerlin et al., 2003; Schuhr et al., 2003). For example, in snapdragon flowers, the MEP pathway supplies substrate for making both monoterpenes and sesquiterpenes (Dudareva et al., 2005). Dolichols in *Coluria geoides* hairy root cultures contain units from both the MEP and MVA pathways (Skorupinska-Tudek et al., 2008). The exchange of intermediates between plastid and cytosolic pathways, frequently referred to as cross-talk, is well-documented (Bick and Lange, 2003; Jux et al.,

Abbreviations: MEP, methylerythritol phosphate; MVA, mevalonate; DXS, deoxyxylulose-5-phosphate synthase; HMGR, hydroxymethylglutaryl coenzyme A reductase; DOX, 1-deoxy-D-xylulose; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate.

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2001). The overall pattern of how the two pathways contribute to the biosynthesis of diverse classes of plant isoprenoids is still poorly understood, except for the recent generalization that isoprenoid secondary metabolites (especially monoterpenes and sesquiterpenes) are more likely to be supplied from both pathways than isoprenoid primary metabolites, such as sterols and carotenoids (Hemmerlin et al., 2012).

One of the problems in obtaining a unified view of the relative roles of the MEP and MVA pathways is the use of many different methods in studying them, including incorporation of labeled precursors, inhibitor experiments and analyses of transgenic lines and mutants. Most information regarding the utilization of the two pathways is derived from the incorporation of stable isotope-labeled precursors, such as glucose or pyruvate (McCaskill and Croteau, 1995; Schuhr et al., 2003). However, early steps in both the MEP pathway, 1-deoxyxylulose-1-phosphate synthase (DXS), and the MVA pathway, hydroxylmethylglutaryl-CoA reductase (HMGR), can limit the metabolism of glucose or pyruvate. Hence, isotopically labeled intermediates that occur after these potentially rate-limiting steps, the MEP intermediate, deoxyxylulose phosphate (fed as deoxyxylulose, DOX) and the MVA intermediate mevalonic acid (fed as mevalonolactone) are often used as probes to assess what pathway produces which isoprenoids. However, an overview of studies with DOX or MVA in higher plants (Table 1) shows that labeling results vary considerably even when identical compounds or compound groups were administered (Arigoni et al., 1997; Bartram et al., 2006; Dudareva et al., 2005; Fukusaki et al., 2004; Hampel et al., 2006, 2005, 2007; Hemmerlin et al., 2003; Kasahara et al., 2002; Piel et al., 1998; Schuhr et al., 2003; Schwender et al., 1997). For example, incorporation of DOX into monoterpenes varies from 0–90% and labeling into sterols varies from 0–100%. Part of the problem is that many workers used detached plant organs, tissue pieces or cell cultures instead of intact plants. And, in many cases experiments using DOX or MVA traced their fate into only a few isoprenoids without looking at a broader range of products and their biosynthetic relationships. For instance, incorporation of DOX into major isoprenoid groups, such as the phytol side chain of chlorophyll or carotenoids, has rarely been measured.

In order to shed new light on the division of labor between the pathways, we chose to study a species that produces a large variety of isoprenoids belonging to different classes of both primary and secondary metabolites. Cotton (*Gossypium hirsutum* L.) produces several classes of secondary isoprenoids, including monoterpenes (C_{10}), sesquiterpenes (C_{15}) and C_{25} terpenoid aldehydes (Liu et al., 1999; Opitz et al., 2008). Intact cotton plants were fed via the roots with ^{2}H-labeled DOX or ^{13}C-labeled MVA. By analyzing the incorporation of these precursors, we determined the contribution of the MEP and MVA pathways to the biosynthesis of monoterpenes, sesquiterpenes, terpenoid aldehydes (Liu et al., 1999; Opitz et al., 2008), the phytol side chains of chlorophyll, sterols, and carotenoids in different organs of the plant at the same stage of development. The flux of precursors into different isoprenoid classes may not always be clear in short-term feeding experiments if only end products are measured due to the presence of intermediate biosynthetic steps occurring at low rates. For example, the enzymes that catalyze methylation reactions appear to be rate-limiting steps in sterol biosynthesis, and so might prevent high incorporation into end products such as sitosterol (Neelakandan et al., 2012, 2010; Nes, 2000). Thus we also analyzed the levels of labeled intermediates in the biosynthesis of sterols and terpenoid aldehydes. The results reveal new information about pathway flux and relative contribution of the two isoprenoid pathways.

Results

Accumulation of isoprenoids in true leaves

To understand the contribution of the methylerythritol phosphate (MEP) and mevalonate (MVA) pathways to the formation of various types of isoprenoids in cotton, we first determined the amounts of six structural classes of isoprenoids in the first true leaves of cotton seedlings (Table 2). For each class, the compounds measured (Table 3) represented 75–95% of the total amounts of that class per leaf. The lowest amounts were found for monoterpenes and sesquiterpenes and the highest for terpenoid aldehydes. To compare the biosynthetic importance of each class, we calculated the levels of C_5 units (in nmol) needed to form the amount present in the first true leaves. The highest proportions of C_5 units are needed to form the chlorophyll side chains followed closely by terpenoid aldehydes; the lowest proportion is needed to form the monoterpenes. The plants in this study thus expended comparable amounts of C_5 units to produce primary (sterols, chlorophyll side

Table 1
Maximum incorporation (in %) of labeled stable isotopes of 1-deoxy-D-xylulose (DOX) and mevalonolactone (MVA) into various isoprenoids of higher plants as observed in previous studies. The table lists the different structural classes that were studied in the present work. (-), indicates feeding studies on these isoprenoids with the given precursors were not performed.

Species	Monoterpenes		Sesquiterpenes		Steroids		Phytol		Carotenoids		Citations
	DOX	MVA	DOX	MVA	DOX	MVA	DOX	MVA	DOX	MVA	
Phaseolus lunatus (Fabaceae)	90	22	67	85	-	-	-	-	-	-	Bartram et al. (2006)
Phaseolus lunatus	90	<20	-	-	-	-	-	-	-	-	Piel et al. (1998)
Hedera helix (Araliaceae)	83	89	-	-	-	-	-	-	-	-	Piel et al. (1998)
Eucalyptus globulus (Myrtaceae)	83	-	94	-	-	-	-	-	-	-	Piel et al. (1998)
Clematis vitisalba (Ranunculaceae)	77	-	70	-	-	-	-	-	-	-	Piel et al. (1998)
Passiflora caerula (Passifloraceae)	83	-	81	-	-	-	-	-	-	-	Piel et al. (1998)
Callicarpa japonica (Lamiaceae)	74	-	-	-	-	-	-	-	-	-	Piel et al. (1998)
Antirrhinum majus (Scrophulariaceae)	70	0	70	95	-	-	-	-	-	-	Dudareva et al. (2005)
Arabidopsis thaliana DXS mutant (Brassicaceae)	-	-	-	-	27	98	-	-	-	-	Kasahara et al. (2002)
Lemna gibba (Araceae)	-	-	-	-	1	10	10	1	-	-	Schwender et al. (1997)
Populus nigra (Salicaceae)	-	-	-	-	0	-	0	-	-	-	Schwender et al. (1997)
Nicotiana tabacum (Solanaceae)	-	-	-	-	0	59	-	-	-	-	Fukusaki et al. (2004)
Nicotiana tabacum bright yellow cells	-	-	-	-	100	100	-	-	-	-	Hemmerlin et al. (2003)
Vitis vinifera (Vitaceae)	74	>1	31	12	-	-	-	-	-	-	Hampel et al. (2005)
Fragaria, sp. (Rosaceae)	2	2	>1	87	-	-	-	-	-	-	Hampel et al. (2006)
Rubus idaeus (Rosaceae)	0	61	-	-	-	-	-	-	-	-	Hampel et al. (2007)
Catharanthus roseus Cell cultures (Apocynaceae)	-	-	-	-	2	-	17	-	17	-	Arigoni et al. (1997)
Catharanthus roseus Cell cultures	-	-	-	-	-	48	-	-	-	7	Schuhr et al. (2003)