Review

An overview of the non-mevalonate pathway for terpenoid biosynthesis in plants

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Terpenoids are known to have many important biological and physiological functions. Some of them are also known for their pharmaceutical significance. In the late nineties after the discovery of a novel non-mevalonate (non-MVA) pathway, the whole concept of terpenoid biosynthesis has changed. In higher plants, the conventional acetate-mevalonate (Ac-MVA) pathway operates mainly in the cytoplasm and mitochondria and synthesizes sterols, sesquiterpenes and ubiquinones predominantly. The plastidic non-MVA pathway however synthesizes hemi-, mono-, sesqui- and di-terpenes, along with carotenoids and phytol chain of chlorophyll. In this paper, recent developments on terpenoids biosynthesis are reviewed with respect to the non-MVA pathway.

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1. Introduction

Terpenoids constitute the largest family of natural plant products with over 30,000 members (Sacchettini and Poulter 1997; Dewick 2002). Terpenoids are classified by the homologous series of number of five carbon isoprene units in their structure: hemiterpenes C_5 (1 isoprene unit), monoterpenes C_{10} (2 isoprene units), sesquiterpenes C_{15} (3 isoprene units), diterpenes C_{20} (4 isoprene units), triterpenes C_{30} (6 isoprene units), tetraterpenes C_{40} (8 isoprene units), polyterpenes $(C_5)_n$ where 'n' may be 9–30,000 (McGarvey and Croteau 1995).

Terpenoid biosynthesis involves mostly head to tail addition of isopentenyl diphosphate (IPP, the active C_5 isoprene unit), to its isomer dimethylallyl diphosphate (DMAPP) synthesizing geranyl diphosphate (GPP, C_{10}). Further, condensation of enzyme-bound geranyl diphosphate with additional IPP units forms successively larger prenyl diphosphates e.g. farnesyl diphosphate (FPP, C_{15}), geranylgeranyl diphosphate (GGPP, C_{20}), that might undergo cyclization, coupling and/or rearrangement to produce the parent carbon skeleton of sesquiterpenes and diterpenes (figure 1; Singh *et al* 1989; McGarvey and Croteau 1995; Luthra *et al* 1999b). GPP and FPP yield monoter-

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Abbreviations used: ATP, adenosine triphosphate; CDP, cytidine diphosphate; CDP-ME, 4-diphosphocytidyl-2-C-methyl-D-erythritol; CDP-ME2P, 4-diphosphocytidyl-2-C-methyl-D-erythritol-2-phosphate; CTP, cytidine triphosphate; DMAPP, dimethylallyl diphosphate; DOXP, 1-deoxy-D-xylulose-5-phosphate; DXS, DOXP synthase; DXR, DOXP reductoisomerase; FPP, farnesyl diphosphate; GAP, glyceraldehyde-3-phosphate; GGPP, geranylgeranyl diphosphate; GPP, geranyl diphosphate; HMBPP, 1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase; IPP, isopentenyl diphosphate; MECP, 2C-methyl-D-erythritol 2, 4-cyclodiphosphate; MEP, 2-C-methyl-D-erythritol-4-phosphate; MVA, mevalonate; P, phosphate; PP, diphosphate; TPP, thiamin diphosphate.

pene and sesquiterpene skeletons, respectively. Furthermore FPP and GGPP dimerize to produce parental precursors to synthesize triterpenes and tetraterpenes, respectively. These parental precursors are subjected to structural modification through oxidation, reduction, isomerization, hydration, conjugation and/or other transformations to give rise to a variety of terpenoids (McGarvey and Croteau 1995). Terpenoids play multifunctional roles in plants, human health and commerce. Monoterpenes (C_{10}) and sesquiterpenes (C_{15}), the constituents of essential oils, are important flavouring and fragrance agents in foods, beverages, cosmetics, perfumes, soaps and exhibit specific biological, pharmaceutical and therapeutical activities as well (Singh *et al* 1989; Dubey 1999; Mahmoud and Croteau 2002).

In plants, terpenoids are synthesized via two IPP generating pathways i.e. acetate-mevalonate (Ac-MVA) and non-mevalonate (non-MVA) pathways (figure 2; Lichtenthaler 1999; Rohmer 1999). The present review is an update in the area of plant terpenoid biosynthesis with reference to recent developments reported on the non-MVA pathway.

2. Cytoplasmic Ac-MVA pathway for IPP synthesis and its restricted role in the biosynthesis of terpenoids

Earlier studies suggested mevalonate (MVA), a well-known intermediate of Ac-MVA pathway, to be the key

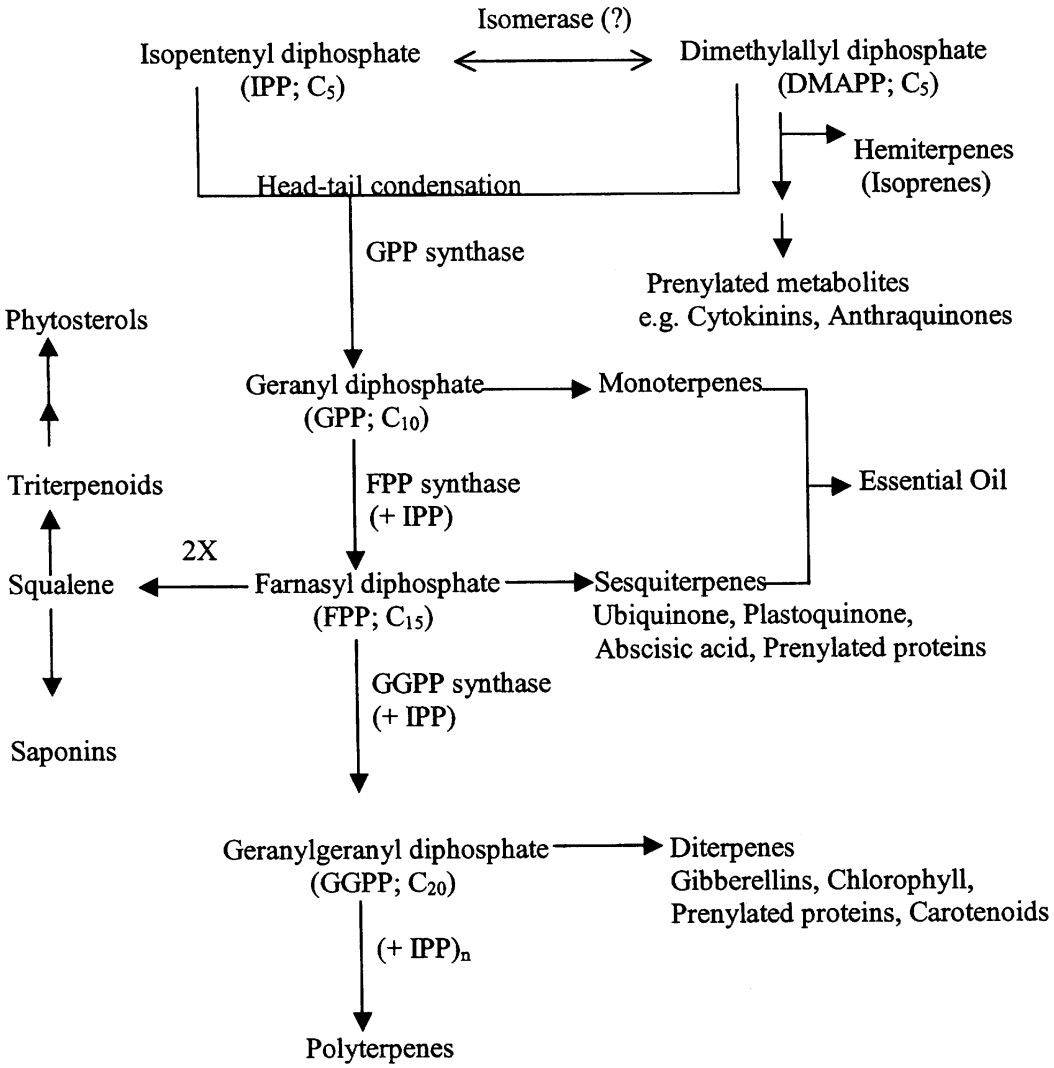

Figure 1. Synthesis of various classes of terpenoids in plants. The question mark (?) indicates the controversial role of isomerase via non-MVA route in which both IPP and DMAPP are reported to be synthesized independently.

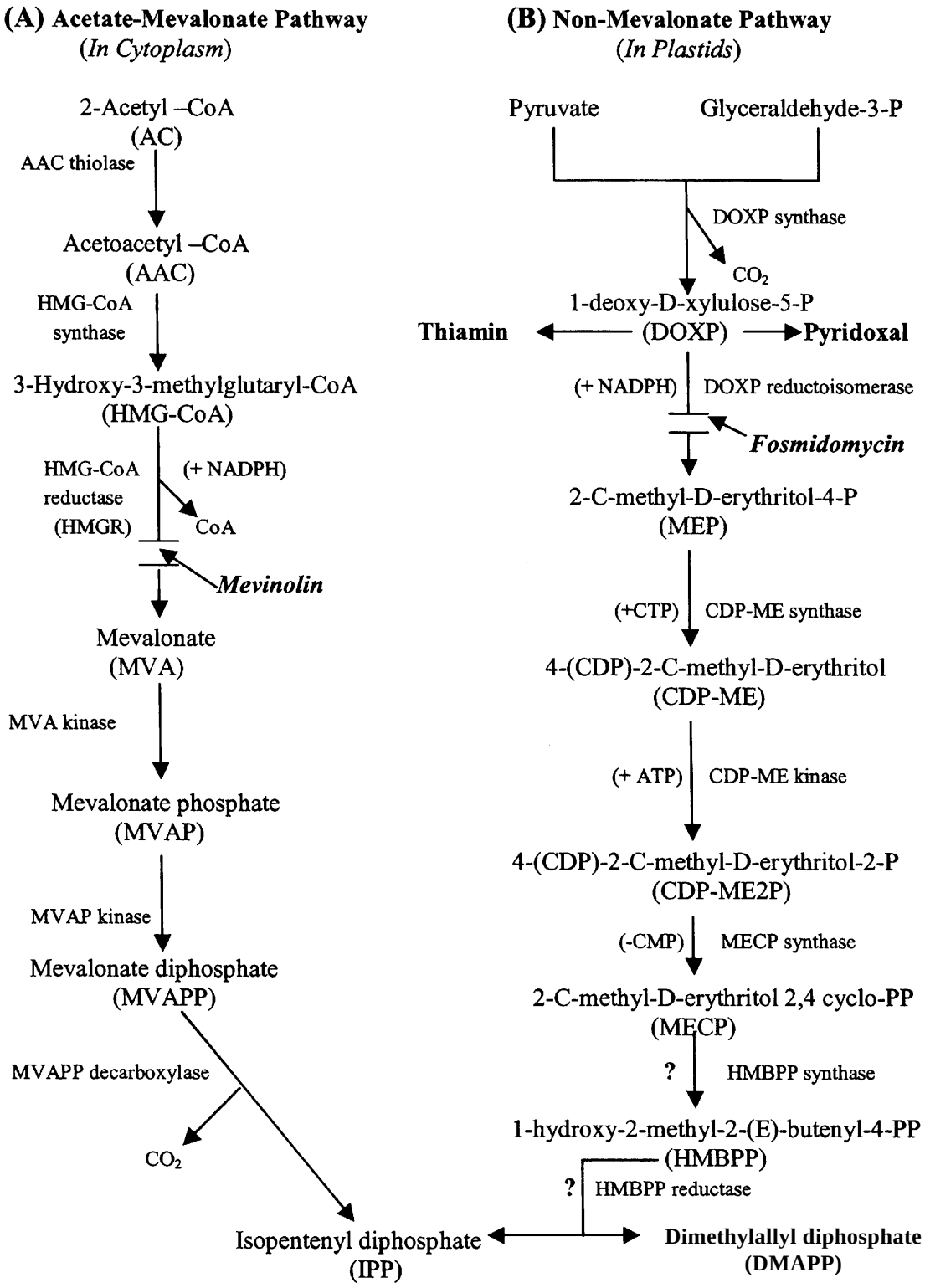

Figure 2. Two independent pathways for biosynthesis of IPP and DMAPP in plants. The terminal steps [from MECP to IPP/DMAPP (marked by '?')] are not well characterized in plants. However, the steps are shown on the basis of related studies done in *E. coli* and demonstration of orthologous genes in various plants, encoding such enzymes that catalyze the same reaction. The role of DOXP in biosynthesis of thiamin (vitamin B1) and pyridoxal (vitamin B6) and the known inhibitors (mevinolin and fosmidomycin) for each pathway are also shown. P, Phosphate; PP, diphosphate, and CMP, CDP, CTP and ATP are mono-, di- and triphosphates of cytidine and adenosine, respectively.

precursor of plant terpenoids (Croteau 1987; Lalitha and Ramasarma 1987; Bach 1995; Chappell 1995). The classical Ac-MVA pathway involves condensation of three units of acetyl CoA to form 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA), which after reduction yields MVA. MVA is subsequently transformed to IPP via three sequential steps involving phosphorylation and decarboxylation (figure 2A). The reduction of HMG-CoA to MVA is catalyzed by HMG-CoA reductase (HMGR), a key regulatory enzyme of this pathway that has been extensively studied (Rodwell *et al* 2000).

HMGR, a highly conserved enzyme occurring commonly in eukaryotes, catalyzes the rate-limiting step of IPP biosynthesis in animals and possibly also in the cytosolic terpene biosynthesis in plants. In higher plants, HMGR is encoded in the nuclear genome by a multigene family. The characterization of HMGR from different plant species revealed developmental and organ-specific expression of HMGR isoforms (Rodwell *et al* 2000). Furthermore, HMGR activity responds to a variety of environmental and physiological signals including light, plant growth regulators, inhibitors, phosphorylation, metabolic feed back, wounding and plant pathogens (Chappell *et al* 1995; Luthra *et al* 1999a). However, correlation between cytosolic HMGR activity and the biosynthesis of plastid-bound prenyllipids like chlorophyll or carotenoids is not fully understood, and HMGR isoforms with typical leader peptides to target these into the chloroplasts have not been reported (Rodwell *et al* 2000). There are some indications of existence of a plastidic HMGR, however, whether HMGR plays any role in the biosynthesis of plastidic terpenoids is a moot point (McCaskill and Croteau 1998). In transgenic tobacco plants, the level of total sterols was found to be limited by the HMGR activity, whereas the levels of other terpenoids such as carotenoids, phytol chain of chlorophyll and sesquiterpene phytoalexins remained relatively unaltered (Chappell *et al* 1995). Furthermore, mevinolin (a highly specific inhibitor of HMGR; figure 2A) strongly inhibits sterol biosynthesis in higher plants, but the biosynthesis of chlorophyll, carotenoids and plastoquinone remains unaffected (Chappell *et al* 1995). The lack of inhibition of plastidic terpenoids (chlorophylls, carotenoids) by mevinolin, added doubts on the involvement of Ac-MVA pathway in biosynthesis of plastidic-terpenoids (Chappell *et al* 1995; Luthra *et al* 1999a).

3. Elucidation of plastidic non-MVA pathway for IPP synthesis

The existence of the alternative pathway for IPP biosynthesis was first demonstrated by Rohmer and his coworkers in different bacterial species (see Rohmer 1999). Earliest fore-runner data on plants include [^{13}C]-labelled glucose incorporation into ginkgolides in *Ginkgo biloba* as a labelling pattern inconsistent with the Ac-MVA route (Schwarz 1994). The [^{13}C]-NMR data interpretation of labelling patterns in various terpenoids, later suggested that C-3, C-2 and C-1 of a triose could supply C-1, C-2 and C-4 of IPP, and that C-2 and C-3 of pyruvate could supply C-3 and C-5 of IPP via the non-MVA pathway (Lichtenthaler 1999; Rohmer 1999). Accordingly, a biochemical scheme was proposed with a head-to-head condensation of D-glyceraldehyde 3-phosphate (GAP, a triose) and 'activated acetaldehyde' derived from pyruvate, resulting in the formation of 1-deoxy-D-xylulose-5-phosphate (DOXP) as the first precursor of this novel biosynthetic pathway (step 1 in figure 2B). DOXP was already known as a precursor for the biosynthesis of thiamin (vitamin B1) and pyridoxal (vitamin B6) in plants (Julliard and Douce 1991; Julliard 1992).

In the non-MVA pathway, addition of a C_2 unit (derived from pyruvate decarboxylation) to a C_3 unit (a triose phosphate or its derivative) involved a transketolase type reaction, as well as a rearrangement similar to that observed in the biosynthesis of branched-chain amino acids such as valine (cited in Luthra *et al* 1999b). Glyceraldehyde-3-phosphate (GAP) and pyruvate were later identified as the direct precursors of IPP (Rohmer 1999). The subsequent steps of this pathway in plants have been demonstrated by various radio-tracer studies (Fellermeier *et al* 1998; Lichtenthaler 1999; McCaskill and Croteau 1999), and various enzymes and the genes involved have also been identified (Lichtenthaler 2000; Eisenreich *et al* 2001; Rohdich *et al* 2001). The existence of non-MVA pathway to synthesize a variety of terpenoids has been shown in several plant species (table 1).

4. Cross-talk between two independent IPP generating pathways in plants

The literature search till date indicates that, in higher plants, the Ac-MVA pathway operates in the cytoplasm and mitochondria to synthesize mainly sterols, however in the plastids terpenes are formed exclusively via non-MVA pathway (Lichtenthaler 1999; Rohmer 1999; Eisenreich *et al* 2001). Various tracer studies have shown that the compartmental separation of the two IPP biosynthetic pathways is not always absolute, because in some cases at least one metabolite can be exchanged between pathways in two separate compartments (Arigoni *et al* 1999; Theil and Adam 2002). In higher plants, both the IPP biosynthetic pathways operate simultaneously. Biosynthetic studies on *Matricaria recutita* (Adam and Zapp 1998) and *G. biloba* (Schwarz 1994) have indicated that the two pathways of IPP synthesis can cooperate to

provide isoprenoid units for sesquiterpene and diterpene synthesis, respectively. The [^{13}C]-labelling patterns of ginkgolides in *G. biloba* indicated that three isoprenoid units of the ginkgolide carried the [^{13}C]-labelling characteristics of Ac-MVA pathway, whereas the fourth isoprenoid unit was labelled via the non-MVA route (Schwarz 1994). A mixed labelling of isoprene units of phytol and other diterpenes from both pathways was also observed in liverwort (*Heteroscyphus planus*) (Nabeta *et al* 1995) and hornwort (*Anthoceros punctatus*) (Itoh *et al* 2000). Analysis of the labelling patterns using quantitative [^{13}C]-NMR spectroscopy of the sesquiterpenes bisabololoxide-A and chamazulene, isolated from the hydrodistillate of the labelled chamomile (*M. recutita*) flowers showed that the two of the isoprene building blocks were predominantly formed via the non-MVA pathway, whereas the third unit was of mixed origin (Adam and Zapp 1998; Adam *et al* 1999). Thus, the sub-cellular compartmentation of two independent IPP pathways could play a crucial role in regulating the biosynthesis of

Table 1. Terpenoids synthesized via non-MVA pathway in various plant species.

Terpenoids	Plant species	Reference
Hemiterpenes		
Isoprene	*Chlidonium majus*, *Populus nigra*, *Salix viminalis*	Zeidler *et al* (1997)
Monoterpenes		
Borneol	*Conocephalum conicum*	Theil and Adam (2002)
Cineole	*Eucalyptus globules*	Rieder *et al* (2000)
Geraniol	*Pelargonium graveolens*	Eisenreich *et al* (1997)
	Vitis vinifera	Lunn and Wüst (2002)
Linalyl acetate	*Mentha citrata*	Fowler *et al* (1999)
Menthone, menthol, menthofuran	*Mentha piperita*	Eisenreich *et al* (1997)
Pulegone	*Mentha pulegium*	Eisenreich *et al* (1997)
Thymol	*Thymus vulgaris*	Eisenreich *et al* (1997)
Sesquiterpenes		
Bisaboloxide A and B, Chamazulene	*Matricaria recutita*	Adam and Zapp (1998)
Sesquiterpenoids derivatives	*Hordeum vulgare*	Maier *et al* (1998)
Diterpenes		
Ginkgolides	*Gingko biloba*	Schwarz (1994)
Marrubiin	*Marrubium vulgare*	Knoss *et al* (1997)
Taxol	*Taxus chinensis*	Eisenreich *et al* (1996)
Neo-epi-verrucosane	*Fossombronia alaskana*	Eisenreich *et al* (1999)
Carotenoids		
b-Carotene	*Capsicum annuum*	Fellermeier *et al* (1999)
	Catharanthus roseus	Arigoni *et al* (1997)
	Lemna gibba	Lichtenthaler *et al* (1997)
	Liriodendron tulipifera	Sagner *et al* (1998)
	Narcissus pseudonarcissus	Fellermeier *et al* (1999)
Lutein	*Catharanthus roseus*	Arigoni *et al* (1999)
Lycopene	*Lycopersicon esculentum*	Zeidler *et al* (1998)
Components with isoprenoid origin		
Abscisic acid	*Persea americana*	Hirai *et al* (2000)
	Persea americana	Milborrow and Lee (1998)
ent-Kaurene (gibberellins precursor)	*Arabidopsis thaliana*	Okada *et al* (2002)
Loganin (terpenoid indole alkaloid)	*Rauwolfia serpentina*	Eichinger *et al* (1999)
Phytol (prenyl chain of chlorophyll)	*Catharanthus roseus*	Arigoni *et al* (1997)
	Dacus carota	Lichtenthaler *et al* (1997)
	Hordeum vulgare	Zeidler *et al* (1998)
	Lemna gibba	Lichtenthaler *et al* (1997)
Plastoquinone	*Lemna gibba*	Lichtenthaler *et al* (1997)
Secologanin (terpenoid indole alkaloid)	*Catharanthus roseus*	Contin *et al* (1998)

various classes of plant-derived terpenoids (Lichtenthaler 1999; Rohmer 1999; Eisenreich *et al* 2001).

5. Discovery of genes, enzymes and intermediates involved in non-MVA pathway in plants

After the discovery of non-MVA pathway, extensive studies have been done to elucidate the complete enzymatic steps involved in non-MVA pathway in plants; however, few steps still remain to be elucidated. Various genes, enzymes and intermediates involved were first discovered in *Escherichia coli* and extensively studied (figure 2B; Eisenreich *et al* 2001; Rohdich *et al* 2001), but some orthologous genes encoding various enzymes of non-MVA pathway have also been demonstrated in plant species (table 2).

5.1 *Synthesis of DOXP*

The first dedicated step of the pathway is synthesis of DOXP, which is catalyzed by thiamine diphosphate (TPP) dependent DOXP synthase (DXS) via a transketolase-like decarboxylation from pyruvate and GAP. Genomic database searches revealed putative DXS gene (*dxs*) orthologues in certain plant species. Several groups have independently isolated and cloned the genes encoding DXS from various plant species such as in *M. piperita* (Lange *et al* 1998), *Capsicum annuum* (Bouvier *et al* 1998) and periwinkle (*Catharenthus roseus*) suspension cultures (Chahed *et al* 2000; Veau *et al* 2000). In *Arabidopsis thaliana*, *CLA1* gene was found showing high similarity to DXS from other plants studied (Araki *et al* 2000; Estévez *et al* 2000, 2001), and its putative plastidic localization has also been shown (Araki *et al* 2000). Two distinct classes of DXS-like cDNAs (MtDXS1 and MtDXS2) were identified, cloned and expressed in *E. coli* from the model legume *Medicago truncatula*, and the deduced mature proteins encoded by these two genes showed the plastid-targeting sequences, and 70% identity in their amino acid sequences (Walter *et al* 2002). Detailed studies have also shown that DXS requires TPP and divalent cations, such as Mg^{2+} or Mn^{2+}, for its activity. The gene encoding this protein from various plant species are in the nuclear genome and are preceded by putative N-terminal plastid targeting sequences (Bouvier *et al* 1998; Lange *et al* 1998; Estévez *et al* 2000; Lois *et al* 2000).

Table 2. Enzymes of non-MVA pathway characterized/demonstrated in various plants.

Enzyme	Plant species	Reference
DOXP synthase	*Arabidopsis thaliana*	Araki *et al* (2000); Estévez *et al* (2000)
	Capsicum annuum	Bouvier *et al* (1998)
	Catharanthus roseus	Chahed *et al* (2000)
	Lycopersicon esculentum	Lois *et al* (2000)
	Mentha piperita	Lange *et al* (1998)
	Medicago truncatula	Walter *et al* (2002)
DOXP reductoisomerase	*Arabidopsis thaliana*	Carretero-Paulet *et al* (2002); Schwender *et al* (1999)
	Catharanthus roseus	Veau *et al* (2000)
	Mentha piperita	Lange and Croteau (1999a); Mahmoud and Croteau (2001)
CDP-ME synthase	*Arabidopsis thaliana*	Rohdich *et al* (2000a)
	Capsicum annuum	Luttgen *et al* (2000)*
CDP-ME kinase	*Lycopersicon esculentum*	Rohdich *et al* (2000b)
	Mentha piperita	Lange and Croteau (1999b)
MECP synthase	*Capsicum annuum*	Fellermeier *et al* (2001)*; Herz *et al* (2000)*
	Catharanthus roseus	Veau *et al* (2000)
	Narcissus pseudonarcissus	Fellermeier *et al* (1999)*
Terminal enzymes		
HMBPP synthase	*Arabidopsis thaliana*	Hecht *et al* (2001)*
HMBPP reductase	*Capsicum annuum*	Adam *et al* (2002)*

*These studies only demonstrated the presence of such enzymes in various plant system either by sequence homology to characterized *E. coli* gene or demonstrating their possible role by incorporation of labelled substrates into specific terpenoids using isolated chromoplasts. CDP, cytidine diphosphate; DOXP, 1-deoxy-D-xylulose-5-phosphate; ME, 2-C-methyl-D-erythritol; MECP, 2-C-methyl-D-erythritol 2,4-cyclodiphosphate; HMBPP, 1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate.

Several studies support the role of DXS in controlling the biosynthesis of various classes of terpenoids in plants. A positive correlation was suggested between the levels of DXS transcript and protein and the accumulation of various plastidic isoprenoid products in transgenic *A. thaliana* engineered to under-expressing or over-expressing DXS gene (Estévez *et al* 2001). Similar positive correlation between DXS transcript and specific terpenoids production has also been observed in various plants, such as in lycopene production in tomato (*Lycopersicum esculentum*) during fruit ripening (Lois *et al* 2000), apocarotenoids in roots from monocots after colonization by mycorrhizal fungi (Walter *et al* 2000, 2002), terpenoid indole alkaloids in periwinkle (*C. roseus*) cell suspension culture upon hormonal induction (Chahed *et al* 2000), and carotenoids in pepper (*C. annuum*) fruit during chloroplast to chromoplast transition (Bouvier *et al* 1998).

5.2 *Synthesis of MEP*

In the second step DOXP is transformed into 2-C-methyl-D-erythritol-4-phosphate (MEP). The formation of MEP from DOXP, involves an intramolecular rearrangement and reduction step catalyzed by enzyme DOXP reductoisomerase (DXR) in the presence of NADPH. In plants, two different groups at the same time have cloned the genes encoding DXR from *A. thaliana* (Schwender *et al* 1999) and *M. piperita* (Lange and Croteau 1999a), and expressed these in *E. coli*. Recently, Carretero-Paulet *et al* (2002) have cloned a single copy gene (cDNA) from *Arabidopsis* encoding the DXR, the protein sequence analysis predicted the presence of an N-terminal transit peptide located to plastids, with a conserved cleavage site, and a conserved proline-rich region at the N-terminus of the mature protein (Carretero-Paulet *et al* 2002). In transgenic peppermint (*M. piperita*), over-expressing DXR led to an increase of essential oil monoterpenes in its leaf tissues compared to the wild type, and conversely, partial DXR gene silencing in some of the engineered peppermint plants led to a reduction of essential oil accumulation (Mahmoud and Croteau 2001). A positive correlation was found between the accumulation of apocarotenoids in mycorrhizal roots from monocots with increase in DXR transcript (Walter *et al* 2000), and similar observation was also reported with indole alkaloids accumulation in periwinkle cell suspension culture (Veau *et al* 2000). However, in tomato (*L. esculentum*) fruit during ripening, neither DXR transcripts nor protein level increased, despite the massive carotenoids accumulation, suggesting a non-limiting role for DXR in this system (Rodriguez-Concepcion *et al* 2001). The kinetic properties of the DXR enzyme from *M. piperita* (Lange and Croteau 1999a) have shown that the enzyme can be inhibited by fosmidomycin, as reported in some other plant species (Zeidler *et al* 1998; Fellermeier *et al* 1999; Lichtenthaler 2000).

5.3 *Synthesis of CDP-ME*

The next step of the pathway is the conversion of MEP to 4-(diphosphocytidyl)-2-C-methyl-D-erythritol (CDP-ME) in a cytidine triphosphate (CTP)-dependent reaction. In plants, the gene encoding CDP-ME synthase was first cloned from *A. thaliana*, and a fragment encoding the protein minus a potential plastid-targetting sequence was expressed in a recombinant *E. coli* (Rohdich *et al* 2000a). In a separate study it has been shown that radiolabelled CDP-ME is incorporated efficiently into carotenoids of red pepper (*C. annuum*) demonstrating the essential role for this enzyme (Herz *et al* 2000), similar to the recombinant CDP-ME synthase from *Arabidopsis* (Rohdich *et al* 2000a). The *Arabidopsis* enzyme requires a divalent cation, preferably Mg^{2+}, and the recombinant protein shows a tendency for self-aggregation depending on the buffer system, also the sequence analysis of the *Arabidopsis* protein sequence revealed a putative plastid import sequence (Rohdich *et al* 2000a).

5.4 *Synthesis of CDP-ME2P*

The further step in the pathway is the phosphorylation of the 2-hydroxy group of CDP-ME converting into 4-(diphosphocitidyl)-2-C-methyl-D-erythritol-2-phosphate (CDP-ME2P) by an enzyme termed as CDP-ME kinase. Though there are few radiotracer studies in plants, which demonstrated the possible role of CDP-ME kinase in the formation of CDP-ME2P, the complete enzymology and its molecular analysis from plant system is not available. [^{14}C]-labelled CDP-ME (the substrate for CDP-ME kinase) was efficiently converted into carotenoids by isolated chromoplast of *C. annuum* (Luttgen *et al* 2000), suggesting the possible role of this enzyme. The putative catalytic domain of a homologous gene in tomato (*L. esculentum*) with predicted similarity to CDP-ME kinase from *E. coli* has also been deduced and the enzyme was expressed in *E. coli* (Rohdich *et al* 2000b). A partially purified enzyme (showing similarity to CDP-ME kinase of *E. coli*) from *M. piperita* showed the phosphorylation of isopentenyl monophosphate (IMP) to IPP, and it was therefore concluded as the final step in IPP biosynthesis (Lange and Croteau 1999b). However, the same CDP-ME kinases from both tomato and *E. coli*, have not shown this catalytic step (Rohdich *et al* 2000b), since in the later studies the phosphorylation of IMP to IPP was found too slow to be relevant. Orthologuous genes from tomato and peppermint carry putative plastid target sequences and

putative ATP binding sites (Lange and Croteau 1999b; Rohdich *et al* 2000b).

5.5 *Synthesis of MECP and IPP*

The next step in this pathway is the transformation of CDP-ME2P into a cyclic intermediate 2-C-methyl-D-erythritol 2,4 cyclodiphosphate (MECP), catalyzed by MECP synthase. The gene encoding such enzyme has been demonstrated in *Arabidopsis* (Cunningham *et al* 2000), but the enzyme was not fully characterized. Lange and Croteau (1999b) suggested the kinase from peppermint (*M. piperita*), that catalyzed phosphorylation of IMP to IPP as the terminal enzyme completing the IPP pathway. The gene from *M. piperita* also showed a high homology (when its plastid targeted peptide sequence was excluded) to the gene in *E. coli* encoding enzyme known to complete the IPP pathway (Cunningham *et al* 2000; Herz *et al* 2000; Luttgen *et al* 2000). A *lytB* cDNA (similar gene proposed to be involved in the terminal steps of non-MVA pathway for IPP in *E. coli*) from the *Adonis aestivalis* was found to enhance accumulation of carotenoids in engineered *E. coli*, but the enzyme in the plant system has been studied only to a limited extent (Cunningham *et al* 2000).

The conversion of MECP into IPP is the terminal step, and the genes, enzymes and catalytic reactions involved are not fully characterized in plants. However, from a structural point of view, a ring-opening reaction, two dehydration steps and two reduction steps could be required in the formation of IPP and DMAPP (Dewick 2002). Few studies in *E. coli* suggested the conversion of MECP to IPP and DMAPP via an intermediate 1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate (HMBPP), involving two subsequent steps catalyzed by two enzymes HMBPP synthase and HMBPP reductase (figure 2B) (cited in Carretero-Paulet *et al* 2002). Amino acid sequence analysis revealed an orthologous gene encoding HMBPP synthase in *A. thaliana* that was highly homologous to *ispG* gene of *E. coli* which was shown to catalyze the reaction MECP to HMBPP by HMBPP synthase (Hecht *et al* 2001). Adam *et al* (2002) have shown that the low catalytic activity from isolated chromoplasts of red pepper (*C. annuum*) was enhanced by the addition of purified HMBPP reductase protein from *E. coli*. Moreover, there are now growing evidences (Dewick 2002) that IPP and DMAPP are synthesized via independent mechanisms in the late steps of this non-MVA pathway, as suggested by a few radiotracer studies in plants (Arigoni *et al* 1999; McCaskill and Croteau 1999; Rieder *et al* 2000). A detailed knowledge about the genes, enzymes and reaction intermediates of non-MVA pathway could lead to the development of novel plant-derived drugs of terpenoid origin and production of various commercially important terpenes in plants through modern biotechnological approaches.

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