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REVIEW

Diversity, regulation, and genetic manipulation of plant mono- and sesquiterpenoid biosynthesis

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Abstract Among plant secondary metabolites, terpenoids are the most abundant and structurally diverse group. In addition to their important roles in pollinator attraction and direct and indirect plant defense, terpenoids are also commercially valuable due to their broad applications in the cosmetic, food, and pharmaceutical industries. Because of their functional versatility and wide distribution, great efforts have been made to decipher terpenoid biosynthetic pathways, to investigate the molecular mechanism determining their structural diversity, and to understand their biosynthetic regulation. Recent progress on the manipulation of terpenoid production in transgenic plants not only holds considerable promise for improving various plant traits and crop protection but also increases our understanding of the significance of terpenoid metabolites in mediating plant-environment interactions.

Keywords Plant terpenoid biosynthesis · Structural diversity · Regulation · Genetic manipulation · Transgenic plants

Introduction

Terpenoids are the most abundant and structurally diverse group, comprising over 40,000 individual compounds [1]. A large number of these compounds are of plant origin and play numerous biological roles in higher plants. Some terpenoids are present in most of the plant species, and they are essential for plant growth and development [2]. For example, sterols (C_{30}) are essential components of membranes, carotenoids (C_{40}) function as photosynthetic pigments, gibberellins (C_{20}) and abscisic acid (C_{15}) are phytohormones, and ubiquinones are involved in mitochondrial electron transport. On the other hand, many terpenoids including monoterpenoids (C_{10}), sesquiterpenoids (C_{15}) and diterpenoids (C_{20}) are only found in certain species or taxonomically related groups. They are considered secondary metabolites and play important roles in plant-plant and plant-environment interactions [3, 4].

Terpenoids are extremely variable in chemical structure, exhibiting hundreds of different carbon skeletons. However, they share a common feature of biosynthesis, and the classification of terpenoids is based on the number of common five-carbon isoprene units in the skeletal structure. The specific focus of this review is on the origins of the mono- and sesquiterpenoids, the C_{10} and C_{15} members of the terpenoid family. Mono- and sesquiterpenoids are two of the most heavily studied classes, largely due to their wide distribution throughout the plant kingdom and their essential roles in both plants and human society. They are the common components of floral scents and essential oils that can function as pollinator attractants [5–7] or as repellents or phytoalexins against herbivores or pathogens [8, 9]. Importantly, some volatile mono- and sesquiterpenoids can be emitted from leaves or flowers in response to herbivore attack to attract natural enemies of herbivores, either predators or parasitoids [10–12]. In addition, herbivore-induced volatile monoterpenoids can also act as airborne signals to prime neighboring plants against future insect attack. For example, three terpenoids including the monoterpene (E)-β-ocimene were emitted from lima bean leaves in response to damage by spider mites (*Tetranychus urticae*). These volatile terpenoids elicited defense-related

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gene expression in neighboring uninfested lima bean leaves [13]. While terpenoids are usually studied in above-ground tissues, the novel functions of sesquiterpenoids as signal molecules in the below-ground environment have also been identified recently. For example, the sesquiterpene *β*-caryophyllene was found to be released from maize roots in response to attack by the beetle *Diabrotica virgifera virgifera* as a volatile signal to attract predatory nematodes, which indirectly protect the plant from further damage [14].

The functional versatility of mono- and sesquiterpenoids is reflected in their great structural diversity. For example, maize contains as many as 100 different terpenoids, most of which are sesquiterpene hydrocarbons. Interestingly, terpenoid composition can not only be markedly different from one species to another, but within a single species it often exhibits pronounced qualitative or quantitative variations among different varieties or ecotypes [15, 16]. Although recent progress in terpenoid biosynthesis has provided new insights into the mechanisms determining terpenoid diversity and distribution, we do not yet have a comprehensive understanding of this immense diversity.

Humans have also benefited from many terpenoids. For example, the monoterpene limonene is widely used as a fragrance in cosmetics products. Nootkatone, an important flavor constituent of grapefruit, is commonly used in the beverage industry as a flavor additive [17]. Artemesinin, a sesquiterpenoid in wormwood, has proved to be an effective antimalarial compound [18]. The anti-inflammatory and potential anticancer compound zerumbone from shampoo ginger has recently become the attractive subject of pharmacological investigations [19–22].

The ecological and commercial importance of terpenoids has prompted the rapid development of engineering terpenoid production in transgenic plants. Recent progress in deciphering terpenoid biosynthetic pathways and the identification of genes and enzymes involved in these pathways has made genetic manipulation in plants highly feasible. This manipulation can improve a large number of traits in crops, including pest resistance, weed control, increased aroma of fruits and vegetables, and the production of medicinal compounds. In addition, transgenic plants with modified terpenoid profile can provide a valuable tool for studying the biosynthesis and regulation of these compounds and their ecological functions in plant-environment interactions.

In this article, we describe the recent advances in understanding the molecular mechanisms of plant terpenoid biosynthesis and regulation and the genetic manipulation of terpenoid production in transgenic plants. Here, we limit our review to the mono- and sesquiterpenoids as defined above.

Terpenoid biosynthesis and its subcellular compartmentation in plants

All terpenoids are derived from the five-carbon building blocks isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP). The sequential head-to-tail condensation of IPP and DMAPP generates *trans*- (or *E*-) isoprenyl diphosphates, *2E*-geranyl diphosphate (GPP, C_{10}), *2E, 6E*-farnesyl diphosphate (*E, E*-FPP, C_{15}), and *2E, 6E, 10E*-geranylgeranyl diphosphate (*E, E, E*-GGPP, C_{20}). These reactions are catalyzed by short-chain *trans*- (or *E*-) prenyltransferases, GPP synthases (GPS), FPP synthases (FPS), and GGPP synthases (GGPS), respectively (Fig. 1). Subsequent cyclizations of prenyl phosphates by the action of terpene synthases (TPSs) generate the olefinic parent skeletons, monoterpenes (C_{10}), sesquiterpenes (C_{15}), and diterpenes (C_{20}), respectively. Pairwise condensation of FPP and GGPP generates the classes of triterpenes (C_{30}) and tetraterpenes (C_{40}), respectively. Further modifications of the parent skeleton types by a series of different enzymes (P450 hydroxylases, dehydrogenases, reductases, and methyl transferases) give rise to the large variety of different terpenoid metabolites [23]. Recently, a new pathway for mono- and sesquiterpene biosynthesis from *cis*- (or *Z*-) isoprenyl diphosphates has been reported. In wild tomato trichomes, a *cis*- (or *Z*-) FPP synthase (*z*FPS) catalyzes the formation of *Z, Z*-FPP from IPP and DMAPP, and a sesquiterpene synthase (Santalene and Bergamotene Synthase [SBS]) uses *Z, Z*-FPP as a substrate to produce the tomato class II sesquiterpenes. More surprisingly, SBS can also use neryldiphosphate (NPP), the *cis*-isomer of GPP, and convert it into a monoterpene. These novel findings provide new insights into terpene biosynthesis in higher plants [24].

It is well known that in higher plants two independent pathways, localized in different intracellular compartments, are involved in the biosynthesis of IPP and DMAPP. In the cytosol, IPP is derived from the classic mevalonic acid (MVA) pathway that begins with the condensation of acetyl-CoA [25], whereas in plastids, IPP is generated from pyruvate and glyceraldehyde 3-phosphate (GA-3P) through the methylerythritol phosphate (MEP) pathway [26, 27]. It was previously assumed that the cytosolic pool of IPP provides the precursor for the production of sesquiterpenes and triterpenes, while the plastidial pool of IPP serves as the precursor for the production of monoterpenes, diterpenes, and tetraterpenes. However, there is increasing evidence suggesting that cross-talk occurs between the two different biosynthetic pathways [28–34]. In glandular trichomes of peppermint, the cytoplasmic MVA pathway is blocked at 3-hydroxy-3-methylglutaryl-CoA reductase, a rate-controlling enzyme of the MVA pathway, and both plastidial

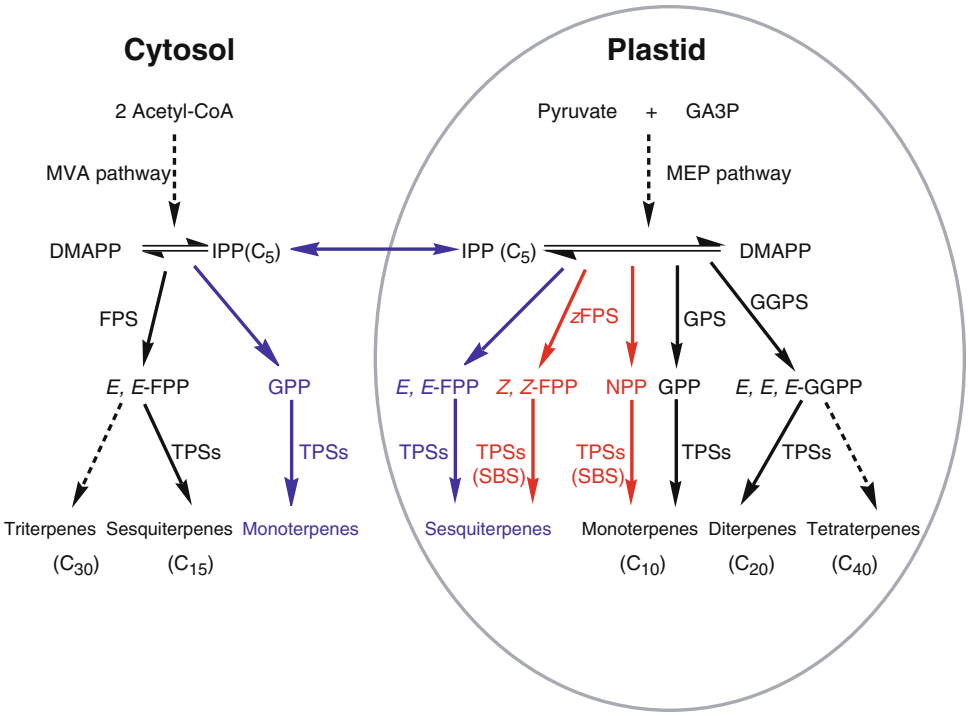

Fig. 1 The two terpenoid biosynthetic pathways producing different terpene classes in plants and their intracellular compartmentation. Both the cytosolic mevalonate (MVA) pathway and the plastidial methylerythritol phosphate (MEP) pathway lead to the formation of the C_5 units IPP and DMAPP. Generally, the MVA pathway is responsible for the production of sesquiterpenes (C_{15}) and triterpenes (C_{30}), whereas the MEP pathway is responsible for monoterpenes (C_{10}), diterpenes (C_{20}), and tetraterpenes (C_{40}). However, cross-talk between the two pathways, including the exchange of IPP between two compartments, the cytosolic monoterpene biosynthesis and plastidial sesquiterpene biosynthesis, has been demonstrated (indicated in *blue*). In addition, the biosynthesis of terpenes from *cis*-isoprenyl diphosphates in chloroplasts of tomato trichomes has been recently discovered (indicated in *red*). *Solid and dashed arrows* represent single and multiple enzymatic steps, respectively

monoterpene and cytosolic sesquiterpene biosynthesis rely exclusively on plastidic-derived IPP [28]. Recent studies with snapdragon flowers have also indicated that the MEP pathway provides IPP precursors for both monoterpene and sesquiterpene biosynthesis, and the trafficking of IPP occurs unidirectionally from the plastids to cytosol [31]. In addition, in some cases, both pathways can even cooperate to supply IPP precursors for biosynthesis of certain terpenoids. For example, the chamomile sesquiterpenes consist of two C_5 isoprene units derived from the MEP pathway, with a third unit being formed via both pathways [32, 33]. Towler and Weathers [34] also provided the indirect evidence that artemisinin is probably biosynthesized from IPP pools derived from both the plastid and the cytosol. Despite these observations, the relative contribution of each pathway to the biosynthesis of the various classes of terpenoids remains largely unknown.

Besides the transport of the IPP precursors between different compartments, the intracellular localization of the different terpenoid biosynthesis is also not as strictly defined as previously believed. It has been well established that monoterpene biosynthesis localizes in plastids, the formation site of the precursor GPP, whereas sesquiterpenes are synthesized in the cytosol/endoplasmic reticulum compartment, the site of FPP formation. However, two different strawberry monoterpene synthase proteins, which respectively generate linalool and several olefinic monoterpenes, lack the plastid-targeting signals and have been shown to be targeted to the cytosolic compartment [35]. Similarly, transgenic tomato plants carrying a lemon basil α-zingiberene synthase can also produce monoterpenes in the cytosol [36]. In contrast, two sesquiterpene synthases, STC1 and SBS from maize and tomato, respectively, contain putative N-terminal plastid-targeting peptides [24, 37]. Green fluorescent protein (GFP) experiments with SBS demonstrated that SBS is localized in the chloroplast, thus providing the evidence that sesquiterpene biosynthesis can take place in the plastids [24]. These findings raise new questions about the fundamental processes in plants that regulate how and where terpenoids are produced, transported and accumulated in the plant cell. An outline of the two terpenoid biosynthetic pathways in plants and their intracellular compartmentation is shown in Fig. 1.

Diversity and plasticity of terpenoid formation

Terpenoids exhibit a tremendous range of diversity in carbon skeletons and functional groups. Recent progress in terpenoid biosynthesis has provided valuable insights into the mechanisms determining terpenoid diversity. The most significant in this regard are studies of terpene synthases (TPSs), a large group of enzymes that are responsible for most of the structural varieties of terpenoids. As described above, TPSs are the branch point enzymes that convert diphosphates to the parent carbon skeletons of terpenoids. A striking feature of TPSs is their astonishing capability to produce multiple products from a single substrate with high regio- and stereospecificity. This ability results from their unique catalytic mechanism that involves manipulation of carbocations, intramolecular additions and rearrangements [38]. Generally, individual intermediates have a variety of metabolic fates in the reaction, resulting in the formation of multiple products. For example, in grand fir, δ-selinene synthase and γ-humulene synthase generate 34 and 52 different sesquiterpenes, respectively [39].

Despite the relatively high degree of structural relatedness among TPSs, comparative sequence analyses have indicated that there is no clear relationship between phylogenic organization and catalytic specificities [40]. Therefore, it is extremely difficult to predict the product profiles of TPSs based solely on their primary sequence. Thanks to the availability of the three-dimensional structures of three plant TPSs [41–43], it has become possible to identify the active sites and to determine the regions or amino acid residues that control the product specificity and selectivity of TPSs. Domain-swapping experiments based on amino acid comparisons of two phylogenetically related sesquiterpene synthases, 5-epi-aristolochene (TEAS) and vetispiradiene synthases (HVS), demonstrated that the first 261 amino acids and the last 106 residues could be interchanged between the two proteins without affecting the product profile, and the middle regions between amino acids 261 and 342 of TEAS and amino acids 386 and 449 of HVS are responsible for the product specificity. Chimeric enzymes containing both of these functional domains unexpectedly generated products of both parent enzymes at various ratios [44]. Because TEAS and HVS share the first two catalytic reaction steps and differ in the terminal step, Greenhagen et al. [45] developed a contact mapping strategy to identify amino acids that contribute to discrete reaction steps. By employing this strategy as well as mutational replacement of variable residues within and surrounding the active sites of the two enzymes, the activities of TEAS and HVS could be interconverted. These results facilitated the use of homology modeling and site-directed mutagenesis to pinpoint which amino acid residues determine the product outcome.

TPS4 and TPS5 are two closely related maize sesquiterpene synthases that produce the same complex sesquiterpene mixture in different proportions. Comparative mutational analyses have indicated that the product selectivity of the two enzymes is controlled by only four amino acids at the catalytic site [16]. Additional studies of TPS4 by modeling and mutagenesis showed that two different functional pockets in the active site contribute to the formation of multiple products by TPS4. One pocket appears to be the site of the initial steps of the reaction including substrate binding, isomerization, and the first cyclization. A conformational change of the bisabolyl cation intermediate results in a shift from one pocket to the other, where it is then converted to a different group of products by further cyclization [46].

Studies of the maize TPS4 and TPS5 enzymes also found that, although both B73 and Delprim varieties contain TPS4 and TPS5 genes, each variety has only one functional allele, suggesting the contributions of gene duplication and allelic variation of TPSs to the terpenoid diversity and distribution [16]. Differences in the terpenoid profile between different varieties or cultivars have also been detected in other plant species. For example, in the headspace of cultivated strawberry, only the monoterpene linalool and the sesquiterpene nerolidol were observed. However, wild strawberry emitted several monoterpenes that are not detected in the cultivated varieties. This marked difference is due to two different TPSs, FaNES1 and FvPINS, that are expressed in the cultivated and wild varieties, respectively [35]. In another example, the glandular trichomes of two tomato species, *Solanum lycopersicum* and its wild relative *Solanum habrochaites* LA1777, also have different terpenoid profiles. *S. lycopersicum* mainly produces monoterpenes and traces of class I sesquiterpenes, whereas LA1777 produces mainly class II sesquiterpene carboxylic acids (SCAs), small amounts of class I sesquiterpenes, and traces of monoterpenes [47]. Class I sesquiterpenes comprise germacrenes as well as α-humulene and β-caryophyllene, whereas class II sesquiterpenes consist of α-santalene, α-bergamotene, and β-bergamotene, which are the likely precursors of SCAs. The biosynthesis of the two distinct classes of sesquiterpenes is controlled by two independent loci. A locus on chromosome 6 consists of two gene clusters of sesquiterpene synthases, of which only one is genetically associated with the biosynthesis of class I sesquiterpenes. At a second locus of LA1777 on chromosome 8, SBS gene is responsible for the biosynthesis of class II sesquiterpenes [24, 48]. These studies shed light on the molecular mechanism resulting in terpenoid diversity under selective pressure during evolution and domestication.

TPSs not only can produce multiple products from a single substrate but also exhibit tremendously functional flexibility. For example, Yoshikuni et al. [49] identified the candidate 'plasticity' residues that are not essential for catalytic functionality but may determine product selectivity in grand fir γ-humulene synthase, and then they developed an approach for systematic recombination of these residues and successfully constructed seven novel sesquiterpene synthases with improved specificities by screening fewer than 2,500 mutants. This functional plasticity of TPSs would seem to be very important in natural evolution, allowing plants to adapt to various environmental changes.

Another intriguing feature of TPSs is that some of them, such as FaNES1 and SBS, can accept both GPP and FPP as their substrates to produce a mixture of mono- and sesquiterpenes [24, 35]. GFP localization experiment showed that FaNES1 localizes to the cytosol and a change in subcellular localization may lead to the enzyme encountering both GPP and FPP substrates. In another example, overexpression of the lemon basil α-zingiberene synthase (ZIS) gene in tomato fruits led to the unexpected enhanced accumulation of both mono- and sesquiterpenes. In vitro experiment also showed that the recombinant ZIS can accept GPP as a substrate to produce monoterpenes, suggesting that when sufficient GPP is available, ZIS could have monoterpene synthase activity in addition to sesquiterpene synthase activity [36]. These observations indicate that the subcellular sites of TPS activities and substrate availability also regulate the terpenoid diversity.

In addition to the contribution of TPSs, the diversity of terpenoids can be further amplified by the modification of the parent hydrocarbon skeletons, such as the addition of hydroxyl groups, further oxidation to ketones, demethylation, and methyl transference, which affect various chemical properties of the molecules that influence their biological activities. A good example of the action of terpene-modifying enzymes is the biosynthesis of a monoterpenoid (−)-menthol in peppermint. Briefly, menthol biosynthesis begins with the formation of (−)-limonene, a reaction catalyzed by a limonene synthase. Subsequent modifications include hydroxylation of (−)-limonene to (−)-*trans*-isopiperitenol by P450 limonene-3-hydroxylase, dehydrogenation of (−)-*trans*-isopiperitenol to (−)-isopiperitenone by isopiperitenol dehydrogenase, generation of (+)-*cis*-isopulegone by (−)-isopiperitenone reductase, isomerization of (+)-*cis*-isopulegone to (+)-pulegone by isopulegone isomerase, reduction of (+)-pulegone to (−)-menthone by (+)-pulegone reductase, and finally formation of (−)-menthol by (−)-menthone reductase [50].

Spatial and temporal regulation of plant terpenoid biosynthesis

Terpenoid biosynthesis in plants can be spatially and temporally regulated during development and in response to biotic and abiotic factors, such as insect or pathogen damage, light intensity, temperature, humidity and nutrient availability [51–57].

Mono- and sesquiterpenoids are often emitted from specific floral tissues at particular times or developmental stages to attract pollinators. In snapdragon, the emission of two main floral scent compounds, the monoterpene myrcene and (E)-β-ocimene, occurs in the upper and lower petal lobes and is regulated developmentally, following diurnal rhythms controlled by a circadian clock. The developmental and rhythmic emission of the two monoterpenes correlates with the weak diurnal oscillation of the corresponding monoterpene synthase genes [58]. In *Nicotiana suaveolens*, the nocturnal emission of several monoterpenes from petals and stigmas is a consequence of the transcriptional regulation of the 1,8-cineole synthase by the circadian clock [59]. These specific changes in terpenoid emission that follow diurnal, nocturnal, or circadian rhythms over the flower lifespan may be associated with the appearance of insects that pollinate the flowers. For example, flowers that are pollinated by night-active insects such as moths may have their scent output at maximum levels in the early evening, coincident with the period of maximum feeding activity of the nocturnal insects [60, 61].

Besides serving as attractants for species-specific pollinators, terpenoid volatiles are also known to play a significant role in plant defense. Tholl et al. [15] revealed that in *Arabidopsis* flowers, terpene volatiles consisting predominantly of sesquiterpenes are not synthesized in petals but in stigma, nectaries, sepals, and anthers. Given the fact that *Arabidopsis* is largely self-pollinating, these terpenoid volatiles may serve to inhibit microbial infection or herbivorous damage at the vulnerable sites. In addition, some terpenoids are also frequent constituents of essential oils and resins and are constitutively accumulated in highly specialized secretory structures, such as the glandular trichomes of mints [62, 63] and resin ducts of conifers [64–66]. Some of these constitutive terpenoids can be released from storage structures against herbivory [67, 68]. Furthermore, their biosynthesis can be restricted to a brief period during leaf development or fruit ripening [69, 70]. More commonly, de novo terpenoid biosynthesis, including the formation of new storage structures such as resin ducts or oil glands, can be induced locally and systematically in response to fungal elicitation or herbivoral attack [14, 71–75]. For instance, in conifers, herbivore attack and jasmonate treatment triggered the production of oleoresin

containing mono-, sesqui-, and diterpenoid components, which is often accompanied by extensive cellular differentiation, leading to the de novo formation of traumatic resin ducts [71, 73]. More recently, Opitz et al. [76] also demonstrated that elevated levels of terpenoids in cotton leaves after real and simulated herbivory are due to the increased filling of existing glands as well as the production of additional storage glands.

Research over the past few decades has begun to unveil some of the regulatory mechanisms by which terpenoid biosynthesis is induced following damage. It has been discovered that induced terpenoid biosynthesis appears to be regulated by the activities of relevant biosynthetic enzymes. One of the best-studied examples is the induction of sesquiterpenoid accumulation in elicitor- or pathogen-challenged tobacco cell suspension cultures. The rapid increase of antibiotic sesquiterpenoids in challenged cells results from the coordinated induction of sesquiterpene synthase activities and suppression of squalene synthase, the first committed enzyme in sterol biosynthesis [77, 78]. These observations are consistent with a number of similar studies with other plant species [79, 80], suggesting that the induction of terpenoid biosynthesis may be associated with other branch pathways sharing the same precursor pools. Despite these findings, the signal transduction cascades resulting in the activation of the terpenoid biosynthetic pathways and the regulation elements that control the induced cellular differentiation leading to the new formation of secretory structures are far from being understood.

Manipulation of terpenoid production in transgenic plants

Genetic manipulation of terpenoid biosynthesis in plants is currently a very active research area. Over the past few years, many attempts have been made to manipulate terpenoid production in transgenic plants by genetic engineering to alter fragrance and flavor profiles of flowers and fruits and to improve direct and indirect defenses (reviewed in [81–85]). Although the feasibility of enhancing terpenoid yield by overexpressing the enzymes that produce terpene precursors or modify parent terpene structures has been demonstrated [86–89], most of the studies on manipulation of terpenoid production focused on the overexpression of TPSs in transgenic plants under constitutive promoters.

The introduction of three lemon monoterpene synthases into tobacco is one of the successful examples of altering scent profiles by genetic engineering. Transgenic tobacco plants emitted olfactorily detectable amounts of monoterpenoid volatiles from flowers and leaves [90]. Importantly, the relatively high levels of the introduced monoterpenoids in flowers did not affect the endogenous linalool biosynthesis, suggesting that a precursor pool is available to introduced monoterpene synthases. The first attempt to improve the flavor of the fruits was performed by overexpression of a *Clarkia breweri* S-linalool synthase (LIS) gene in tomato under the control of the fruit-specific E8 promoter. This resulted in the accumulation of low levels of linalool and 8-hydroxylinalool in ripening fruits [91]. In a more successful experiment, the ectopic expression of the lemon basil geraniol synthase (GES) gene under the control of the fruit ripening-specific polygalacturonase (PG) promoter led to the accumulation of high levels of geraniol and its derivatives in tomato fruit, which profoundly affected tomato flavor [92].

In addition to the impact on the quality of flavor and scent, manipulation of terpenoid production in plants has also been proven to be an effective strategy for improving direct and indirect defenses. Overexpression of FaNES1, a strawberry linalool/nerolidol synthase gene in *Arabidopsis* produced high levels of linalool that significantly repelled aphids [93]. Moreover, transgenic potato plants that express the same gene were more attractive to beneficial predatory mites than the wild-type plants [83], thus indirectly protecting plants from insect damage. In another study, the introduction of a maize sesquiterpene synthase (TPS10) to *Arabidopsis* led to the strong emission of several herbivore-induced sesquiterpenoids, which are exploited by the females of the parasitoid *Cotesia marginiventris* to locate their lepidopteran hosts [94]. These studies indicated that metabolic engineering of terpenoids in plants also provides a valuable tool to evaluate the role of some volatile terpenoids in mediating tritrophic interactions.

Engineering of sesquiterpenoid production in plants seems more difficult and less successful than the generation of monoterpenoids due to the lack of sufficient precursor for the introduced sesquiterpene synthases. In a recent study, transgenic tomato fruits overexpressing ZIS gene under the control of the tomato PG promoter produced high levels of α-zingiberene and various quantities of 14 other sesquiterpenes without affecting plant phenotype except for a slight decrease in carotenoid content, implying that there was sufficient cytosolic supply of FPP in ripening tomato fruits [36]. In another study, enhancement of sesquiterpenoid production was achieved by switching the subcellular localization of FENS1 to the mitochondria. This manipulation resulted in substantial emission of (3S)-(E)-nerolidol and a degradation product of nerolidol, C11 homoterpene 4,8-dimethyl-1,3(E),7-nonatriene [(E)-DMNT] from *Arabidopsis*, and both of these terpenoids could attract carnivorous predatory mites, the natural enemies of spider mites [95]. More significantly, Wu et al. [96] developed a new engineering strategy for high-level sesquiterpenoid

biosynthesis in plants by coexpression of a FPP synthase and a sesquiterpene synthase, while diverting the subcellular compartment of the two enzymes from cytosol to plastids. This strategy boosted the production of two sesquiterpenes, patchoulol and amorpha-4,11-diene more than 1,000-fold, highlighting the importance of compartmentalization of both precursor pools and introduced enzymes in the regulation of terpenoid biosynthesis. This success also indicated the potential of using metabolic engineering to explore plant-based production platforms for commercially interesting terpenoids.

Although significant progress has been made in manipulation of terpenoid production in plants in the past few years, many studies have also revealed the limitations and problems that have to be surmounted. It has been shown that the introduction of a single gene has only limited value for substantial production of the desired compound [91, 97, 98]. In addition, in many cases, the intended terpenoids produced by transgenic plants were further modified by the endogenous enzymes, resulting in different terpenoid derivatives based on the entire biochemical repertoire of the plant used. For example, while engineering petunia and carnation plants with LIS gene, the synthesized linalool was partially converted into linalyl β-D-glucoside and linalyl oxides, respectively [97, 98]. Therefore, it is difficult to predict the metabolic fate of the introduced terpenoids. More importantly, high-level expression of an introduced gene in plants can lead to deleterious effects on plant growth and development [92, 93, 96]. In fact, transgenic tobacco plants producing exceptionally high levels of patchoulol exhibited leaf chlorosis, vein clearing, and a reduction in stature [96]. In another experiment, high geraniol accumulation in transgenic tomato fruits was at the expense of a 50% decrease in lycopene content [92]. These phenotypic changes result either from the toxicity of the newly synthesized terpenoids or from diversion of the carbon flux of the primary metabolism pathway, which causes a deficiency of some essential metabolites such as sterols and carotenoids.

Conclusions and prospects

Recent progress in the biochemical and molecular genetic analysis of terpenoid biosynthesis has provided new insight into the mechanism directing terpenoid diversity, distribution, and regulation. The two elaborate biosynthetic pathways localized in separate subcellular compartments and the cross-talk between the two pathways reflect the complexity of terpenoid biosynthesis in plants. The subcellular compartmentation of precursors and enzymes, the multifunctional feature of TPSs, and the evolutionary process of TPS genes, including gene duplication, subsequent neo-functionalization, and the allelic variations all contribute to the remarkable diversity and plasticity of terpenoids, which might allow plants to adapt easily to continuous environmental changes.

The spatial and temporal regulation of terpenoid biosynthesis during development and in response to environmental stresses is closely related to their ecological or physiological functions, such as attracting pollinators, repelling pathogens and herbivores, and attracting the natural enemies of herbivores. The combination of genomic, proteomic and metabolomic tools, as well as the establishment of in vitro cell culture systems should help elucidate the regulatory mechanism of terpenoid biosynthesis.

Recent efforts to manipulate terpenoid production in plants have demonstrated the feasibility of enhancing plant defenses and improving the scent and aroma quality of flowers and fruits by metabolic engineering. However, many attempts have resulted in disappointing enhancement of terpenoids or in unpredicted metabolic consequences due to our insufficient understanding of plant metabolic networks and their regulation. To explore plant systems as an efficient terpenoid production platform, more sophisticated strategies for engineering multistep pathways should be developed either by simultaneously overexpressing multiple genes in a pathway and/or blocking those in another competing pathway, or through the use of transcriptional regulators to control several endogenous genes.

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References

1. Buckingham J (2004) Dictionary of natural products, web version 2004. Chapman & Hall, London
2. Chappell J (1995) The biochemistry and molecular biology of isoprenoid metabolism. Plant Physiol 107:1–6
3. Dudareva N, Pichersky E, Gershenzon J (2004) Biochemistry of plant volatiles. Plant Physiol 135:1893–1902
4. Facchini PJ, Chappell J (1992) Gene family for an elicitor-induced sesquiterpene cyclase in tobacco. Proc Natl Acad Sci USA 89:11088–11092
5. Dobson HEM (1994) Floral volatiles in insect biology. In: Bernays EA (ed) Insect-plant interactions, vol 5. CRC Press, Boca Raton, pp 47–81
6. Dudareva N, Pichersky E (2000) Biochemical and molecular genetic aspects of floral scents. Plant Physiol 122:627–633
7. Pichersky E, Gershenzon J (2002) The formation and function of plant volatiles: perfumes for pollinator attraction and defense. Curr Opin Plant Biol 5:237–243

8. Langenheim JH (1994) Higher plant terpenoids: a phytocentric overview of their ecological roles. J Chem Ecol 20:1223–1280
9. Gershenzon J, Dudareva N (2007) The function of terpene natural products in the natural world. Nat Chem Biol 3:408–414
10. Pare PW, Tumlinson JH (1999) Plant volatiles as a defense against insect herbivores. Plant Physiol 121:325–331
11. Dicke M, van Loon JJA (2000) Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. Entomol Exp Appl 97:237–249
12. Kessler A, Baldwin IT (2001) Defensive function of herbivore-induced plant volatile emissions in nature. Science 291:2141–2144
13. Arimura G, Ozawa R, Shimoda T, Nishioka T, Boland W, Takabayashi J (2000) Herbivory-induced volatiles elicit defence genes in lima bean. Nature 406:512–515
14. Rasmann S, Köllner TG, Degenhardt J, Hiltpold I, Toepfer S, Kuhlmann U, Gershenzon J, Turlings TCJ (2005) Recruitment of entomopathogenic nematodes by insect-damaged maize roots. Nature 434:732–737
15. Tholl D, Chen F, Petri J, Gershenzon J, Pichersky E (2005) Two sesquiterpene synthases are responsible for the complex mixture of sesquiterpenes emitted from *Arabidopsis* flowers. Plant J 42:757–771
16. Köllner TG, Schnee C, Gershenzon J, Degenhardt J (2004) The variability of sesquiterpenes emitted from two Zea mays cultivars is controlled by allelic variation of two terpene synthase genes encoding stereoselective multiple product enzymes. Plant Cell 16:1115–1131
17. Berry R, Wagner CJ, Moshonas MG (1967) Flavor studies of nootkatone in grapefruit juice. J Food Sci 32:75–78
18. Klayman DL (1985) Qinghaosu (artemisinin): an antimalarial drug from China. Science 228:1049–1055
19. Ozaki Y, Kawahara N, Harada M (1991) Anti-inflammatory effect of Zingiber cassumunar Roxb and its active principles. Chem Pharm Bull 39:2353–2356
20. Murakami A, Tanaka T, Lee JY, Surh YJ, Kim HW, Kawabata K, Nakamura Y, Jiwajinda S, Ohigashi H (2004) Zerumbone suppresses skin tumor initiation and promotion stages in mice: involvement of induction of anti-oxidative and phase II drug metabolizing enzymes and inhibition of NF-kB-mediated cyclooxygenase-2 expression. Int J Cancer 110:481–490
21. Xian MJ, Ito K, Nakazato T, Shimizu T, Chen CK, Yamato K, Murakami A, Ohigashi H, Ikeda Y, Kizaki M (2007) Zerumbone, a bioactive sesquiterpene, induces G2/M cell cycle arrest and apoptosis in leukemia cells via a Fas- and mitochondria-mediated pathway. Cancer Sci 98:118–126
22. Yu FN, Okamoto S, Nakasone K, Adachi K, Matsuda S, Harada H, Misawa N, Utsumi R (2008) Molecular cloning and functional characterization of α-humulene synthase, a possible key enzyme of zermbone biosynthesis in shampoo ginger (Zingiber zerumbet Smith). Planta 227:1291–1299
23. McGarvey D, Croteau R (1995) Terpenoid metabolism. Plant Cell 7:1015–1026
24. Sallaud C, Rontein D, Onillon S, Jabes F, Duffe P, Giacalone C, Thoraval S, Escoffier C, Herbette G, Leonhardt N, Causse M, Tissier A (2009) A novel pathway for sesquiterpene biosynthesis from Z,Z-farnesyl pyrophosphate in the wild tomato Solanum habrochaites. Plant Cell 21:301–317
25. Newman JD, Chappell J (1999) Isoprenoid biosynthesis in plants: carbon partitioning within the cytoplasmic pathway. Crit Rev Biochem Mol Biol 34:95–106
26. Eisenreich W, Schwarz M, Cartayrade A, Arigoni D, Zenk MH, Bacher A (1998) The deoxyxylulose phosphate pathway of terpenoid biosynthesis in plants and microorganisms. Chem Biol 5:R221–R233
27. Lichtenthaler HK (1999) The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. Annu Rev Plant Physiol Plant Mol Biol 50:47–65
28. McCaskill D, Croteau R (1995) Monoterpene and sesquiterpene biosynthesis in glandular trichomes of peppermint (Mentha × piperita) rely exclusively on plastid-derived isopentenyl diphosphate. Planta 197:49–56
29. Hemmerlin A, Hoeffler JF, Meyer O, Tritsch D, Kagan IA, Grosdemange-Billiard C, Rohmer M, Bach TJ (2003) Cross-talk between the cytosolic mevalonate and the plastidial methylerythritol phosphate pathways in tobacco bright yellow-2 cells. J Biol Chem 278:26666–26676
30. Laule O, Furholz A, Chang HS, Zhu T, Wang X, Heifetz PB, Gruissem W, Lange BM (2003) Crosstalk between cytosolic and plastidial pathways of isoprenoid biosynthesis in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 100:6866–6871
31. Dudareva N, Andersson S, Orlova I, Gatto N, Reichelt M, Rhodes D, Boland W, Gershenzon J (2005) The nonmevalonate pathway supports both monoterpene and sesquiterpene formation in snapdragon flowers. Proc Natl Acad Sci USA 102:933–938
32. Adam KP, Zapp J (1999) Biosynthesis of the isoprene units of chamomile sesquiterpenes. Phytochemistry 48:953–959
33. Adam KP, Thiel R, Zapp J (1999) Incorporation of 1-[1–13C]deoxy-D-xylulose in chamomile sesquiterpenes. Arch Biochem Biophys 369:127–132
34. Towler MJ, Weathers PJ (2007) Evidence of artemisinin production from IPP stemming from both the mevalonate and the nonmevalonate pathways. Plant Cell Rep 26:2129–2136
35. Aharoni A, Giri AP, Verstappen FWA, Bertea CM, Sevenier R, Sun ZK, Jongsma MA, Schwab W, Bouwmeester HJ (2004) Gain and loss of fruit flavor compounds produced by wild and cultivated strawberry species. Plant Cell 16:3110–3131
36. Davidovich-Rikanati R, Lewinsohn E, Bar E, Iijima Y, Pichersky E, Sitrit Y (2008) Overexpression of the lemon basil α-zingiberene synthase gene increases both mono- and sesquiterpene contents in tomato fruit. Plant J 56:228–238
37. Shen B, Zheng Z, Dooner HK (2000) A maize sesquiterpene cyclase gene induced by insect herbivory and volicitin: characterization of wild-type and mutant alleles. Proc Natl Acad Sci USA 97:14807–14812
38. Bohlmann J, Meyer-Gauen G, Croteau R (1998) Plant terpenoid synthases: molecular biology and phylogenetic analysis. Proc Natl Acad Sci USA 95:4126–4133
39. Steele CL, Crock J, Bohlmann J, Croteau R (1998) Sesquiterpene synthases from grand fir (Abies grandis): comparison of constitutive and wound-induced activities, and cDNA isolation, characterization and bacterial expression of delta-selinene synthase and gamma-humulene synthase. J Biol Chem 273:2078–2089
40. Trapp S, Croteau R (2001) Genomic organization of plant terpene synthases and molecular evolutionary implications. Genetics 158:811–832
41. Starks CM, Back KW, Chappell J, Noel JP (1997) Structural basis for cyclic terpene biosynthesis by tobacco 5-epi-aristolochene synthase. Science 277:1815–1820
42. Whittington DA, Wise ML, Urbansky M, Coates RM, Croteau R, Christianson DW (2002) Bornyl diphosphate synthase: structure and strategy for carbocation manipulation by a terpenoid cyclase. Proc Natl Acad Sci USA 99:15375–15380
43. Hyatt DC, Youn B, Zhao Y, Santhamma B, Coates RM, Croteau RB, Kang C (2007) Structure of limonene synthase, a simple model for terpenoid cyclase catalysis. Proc Natl Acad Sci USA 104:5360–5365
44. Back KW, Chappell J (1996) Identifying functional domains within terpene cyclases using a domain-swapping strategy. Proc Natl Acad Sci USA 93:6841–6845

45. Greenhagen BT, O'Maille PE, Noel JP, Chappell J (2006) Identifying and manipulating structural determinates linking catalytic specificities in terpene synthases. Proc Natl Acad Sci USA 103:9826–9831
46. Köllner T, O'Maille PE, Gatto N, Boland W, Gershenzon J, Degenhardt J (2006) Two pockets in the active site of maize sesquiterpene synthase TPS4 carry out sequential parts of the reaction scheme resulting in multiple products. Arch Biochem Biophys 448:83–92
47. Besser K, Harper A, Welsby N, Schauvinhold I, Slocombe S, Li Y, Dixon RA, Broun P (2009) Divergent regulation of terpenoid metabolism in the trichomes of wild and cultivated tomato species. Plant Physiol 149:499–514
48. van der Hoeven RS, Monforte AJ, Breeden D, Tanksley SD, Steffens JC (2000) Genetic control and evolution of sesquiterpene biosynthesis in Lycopersicon esculentum and L. hirsutum. Plant Cell 12:2283–2294
49. Yoshikuni Y, Ferrin TE, Keasling JD (2006) Designed divergent evolution of enzyme function. Nature 440:1078–1082
50. Croteau RB, Davis EM, Ringer KL, Wildung MR (2005) (-)-Menthol biosynthesis and molecular genetics. Naturwissenschaften 92:562–577
51. Van Poecke RMP, Posthumus MA, Dicke M (2001) Herbivore-induced volatile production by *Arabidopsis thaliana* leads to attraction of the parasitoid Cotesia rubecula: chemical, behavioral, and gene-expression analysis. J Chem Ecol 27:1911–1928
52. Rodriguez-Saona C, Crafts-Brandner SJ, Cañas LA (2003) Volatile emissions triggered by multiple herbivore damage: beet armyworm and whitefly feeding on cotton plants. J Chem Ecol 29:2539–2550
53. Phillips MA, Croteau R (1999) Resin based defenses in conifers. Trends Plant Sci 4:184–190
54. Staudt M, Bertin N (1998) Light and temperature dependence of the emission of cyclic and acyclic monoterpenes from holm oak (Quercus ilex L.) leaves. Plant Cell Environ 21:385–395
55. Llusià J, Peñuelas J (2000) Seasonal patterns of terpene content and emission from seven Mediterranean woody species in field conditions. Am J Bot 87:133–140
56. Gouinguene SP, Turlings TC (2002) The effects of abiotic factors on induced volatile emissions in corn plants. Plant Physiol 129:1296–1307
57. Hakola H, Tarvainen V, Bäck J, Ranta H, Bonn B, Rinne J, Kulmala M (2006) Seasonal variation of mono- and sesquiterpene emission rates of Scots pine. Biogeosciences 2:1697–1717
58. Dudareva N, Martin D, Kish CM, Kolosova N, Gorenstein N, Fäldt J, Miller B, Bohlmann J (2003) (E)-beta-ocimene and myrcene synthase genes of floral scent biosynthesis in snapdragon: function and expression of three terpene synthase genes of a new terpene synthase subfamily. Plant Cell 15:1227–1241
59. Roeder S, Hartmann AM, Effmert U, Piechulla B (2007) Regulation of simultaneous synthesis of floral scent terpenoids by the 1,8-cineole synthase of Nicotiana suaveolens. Plant Mol Biol 65:107–124
60. Loughrin JH, Hamilton-Kemp TR, Andersen RA, Hildebrand DF (1990) Volatiles from flowers of Nicotiana sylvestris, N. otophora and Malus × domestica: headspace components and day/night changes in their relative concentrations. Phytochemistry 29:2473–2477
61. Heath RR, Landolt PJ, Dueben B, Lenczewski B (1992) Identification of floral compounds of night-blooming jessamine attractive to cabbage looper moths. Environ Entomol 21:854–859
62. Gershenzon J, Maffei M, Croteau R (1989) Biochemical and histochemical localization of monoterpene biosynthesis in the glandular trichomes of spearmint (Mentha spicata). Plant Physiol 89:1351–1357
63. McCaskill D, Gershenzon J, Croteau R (1992) Morphology and monoterpene biosynthetic capabilities of secretory cell clusters isolated from glandular trichomes of peppermint (Mentha piperita L.). Planta 187:445–454
64. Banan MW (1936) Vertical resin ducts in the secondary wood of the abietineae. New Phytol 35:11–46
65. Nagy NE, Franceschi VR, Solheim H, Krekling T, Christiansen E (2000) Wound-induced traumatic resin duct development in stems of Norway spruce (Pinaceae): anatomy and cytochemical traits. Am J Bot 87:302–313
66. McKay SA, Hunter WL, Godard KA, Wang SX, Martin DM, Bohlmann J, Plant AL (2003) Insect attack and wounding induce traumatic resin duct development and gene expression of (-)-pinene synthase in Sitka spruce. Plant Physiol 133:368–378
67. Degenhardt DC, Lincoln DE (2006) Volatile emissions from an odorous plant in response to herbivory and methyl jasmonate exposure. J Chem Ecol 32:1573–1561
68. Loughrin JH, Manukian A, Heath RR, Turlings TC, Tumlinson JH (1994) Diurnal cycle of emission of induced volatile terpenoids by herbivore-injured cotton plant. Proc Natl Acad Sci USA 91:11836–11840
69. Gershenzon J, McConkey ME, Croteau R (2000) Regulation of monoterpene accumulation in leaves of peppermint. Plant Physiol 122:205–214
70. Sharon-Asa L, Shalit M, Frydman A, Bar E, Holland D, Or E, Lavi U, Lewinsohn E, Eyal Y (2003) Citrus fruit flavor and aroma biosynthesis: isolation, functional characterization, and developmental regulation of Cstps1, a key gene in the production of the sesquiterpene aroma compound valencene. Plant J 36:664–674
71. Martin D, Tholl D, Gershenzon J, Bohlmann J (2002) Methyl jasmonate induces traumatic resin ducts, terpenoid resin biosynthesis, and terpenoid accumulation in developing xylem of Norway spruce stems. Plant Physiol 129:1003–1018
72. Schnee C, Köllner TG, Gershenzon J, Degenhardt J (2002) The maize gene terpene synthase 1 encodes a sesquiterpene synthase catalyzing the formation of (E)-β-farnesene, (E)-nerolidol, and (E, E)-farnesol after herbivore damage. Plant Physiol 130:2049–2060
73. Arimura G, Huber DPW, Bohlmann J (2004) Forest tent caterpillars (Malacosoma disstria) induce local and systemic diurnal emissions of terpenoid volatiles in hybrid poplar (Populus trichocarpa × deltoides): cDNA cloning, functional characterization, and patterns of gene expression of (−)-germacrene D synthase, PtdTPS1. Plant J 37:603–616
74. Miller B, Madilao LL, Ralph S, Bohlmann J (2005) Insect-induced conifer defense: white pine weevil and methyl jasmonate induce traumatic resinosis, de novo formed volatile emissions, and accumulation of terpenoid synthase and putative octadecanoid pathway transcripts in Sitka spruce. Plant Physiol 137:369–382
75. Cheng AX, Xiang CY, Li JX, Yang CQ, Hu WL, Wang LJ, Lou YG, Chen XY (2007) The rice (E)-b-caryophyllene synthase (OsTPS3) accounts for the major inducible volatile sesquiterpenes. Phytochemistry 68:1632–1641
76. Opitz S, Kunert G, Gershenzon J (2008) Increased terpenoid accumulation in cotton (Gossypium hirsutum) foliage is a general wound response. J Chem Ecol 34:508–522
77. Threlfall DR, Whitehead IM (1988) Coordinated inhibition of squalene synthetase and induction of enzymes of sesquiterpenoid phytoalexin biosynthesis in cultures of Nicotiana tabacum. Phytochemistry 27:2567–2580
78. Vögeli U, Chappell J (1988) Induction of sesquiterpene cyclase and suppression of squalene synthetase activities in plant cell cultures treated with fungal elicitor. Plant Physiol 88:1291–1296

79. Brindle PA, Kuhn P, Tbxelfall DR (1988) Biosynthesis and metabolism of sesquiterpenoid phytoalexins and triterpenoids in potato cell suspension cultures. Phytochemistry 27:133–150
80. Tjamos EC, Kuc JA (1982) Inhibition of steroid glyoalkaloid accumulation by eicosapentaenoic and arachidonic acid in potato. Science 217:542–544
81. Mahmoud SS, Croteau R (2002) Strategies for transgenic manipulation of monoterpene biosynthesis in plants. Trends Plant Sci 7:366–373
82. Aharoni A, Jongsma MA, Bouwmeester HJ (2005) Volatile science? Metabolic engineering of terpenoids in plants. Trends Plant Sci 10:594–602
83. Aharoni A, Jongsma MA, Kim TY, Ri MB, Giri AP, Verstappen FWA, Schwab W, Bouwmeester HJ (2006) Metabolic engineering of terpenoid biosynthesis in plants. Phytochem Rev 5:49–58
84. Pichersky E, Dudareva N (2007) Scent engineering: toward the goal of controlling how flowers smell. Trends Biotechnol 25:105–110
85. Dudareva N, Pichersky E (2008) Metabolic engineering of plant volatiles. Curr Opin Biotechnol 19:181–189
86. Mahmoud SS, Croteau R (2001) Metabolic engineering of essential oil yield and composition in mint by altering expression of deoxyxylulose phosphate reductoisomerase and menthofuran synthase. Proc Natl Acad Sci USA 98:8915–8920
87. Chen D, Ye H, Li G (2000) Expression of a chimeric farnesyl diphosphate synthase gene in Artemisia annua L. transgenic plants via Agrobacterium tumefaciens-mediated transformation. Plant Sci 155:179–185
88. Mahmoud SS, Williams M, Croteau R (2004) Cosuppression of limonene-3-hydroxylase in peppermint promotes accumulation of limonene in the essential oil. Phytochemistry 65:547–554
89. Lücker J, Schwab W, Franssen MC, Van Der Plas LH, Bouwmeester HJ, Verhoeven HA (2004) Metabolic engineering of monoterpene biosynthesis: two-step production of (+)-trans-isopiperitenol by tobacco. Plant J 39:135–145
90. Lücker J, Schwab W, Hautum BV, van der Plas LHW, Bouwmeester HJ, Verhoeven HA (2004) Increased and altered fragrance of tobacco plants after metabolic engineering using three monoterpene synthases from lemon. Plant Physiol 134:510–519
91. Lewinsohn E, Schalechet F, Wilkinson J, Matsui K, Tadmor K, Nam KH, Amar O, Lastochkin E, Larkov O, Ravid U, Hiatt W, Gepstein S, Pichersky E (2001) Enhanced levels of the aroma and flavor compound S-linalool by metabolic engineering of the terpenoid pathway in tomato fruits. Plant Physiol 127:1256–1265
92. Davidovich-Rikanati R, Sitrit Y, Tadmor Y, Iijima Y, Bilenko N, Bar E, Carmona B, Fallik E, Dudai N, Simon JE, Pichersky E, Lewinsohn E (2007) Enrichment of tomato flavor by diversion of the early plastidial terpenoid pathway. Nat Biotechnol 25:899–901
93. Aharoni A, Giri AP, Deuerlein S, Griepink F, de Kogel WJ, Verstappen FWA, Verhoeven HA, Jongsma MA, Schwab W, Bouwmeester HJ (2003) Terpenoid metabolism in wild-type and transgenic *Arabidopsis* plants. Plant Cell 15:2866–2884
94. Schnee C, Kollner TG, Held M, Turlings TCJ, Gershenzon J, Degenhardt J (2006) The products of a single maize sesquiterpene synthase form a volatile defense signal that attracts natural enemies of maize herbivores. Proc Natl Acad Sci USA 103:1129–1134
95. Kappers IF, Aharoni A, Van Herpen TWJM, Luckerhoff LLP, Dicke M, Bouwmeester HJ (2005) Genetic engineering of terpenoid metabolism attracts bodyguards to *Arabidopsis*. Science 309:2070–2072
96. Wu SQ, Schalk M, Clark A, Miles RB, Coates R, Chappell J (2006) Redirection of cytosolic or plastidic isoprenoid precursors elevates terpene production in plants. Nat Biotechnol 24:1441–1447
97. Lücker J, Bouwmeester HJ, Schwab W, Blaas J, van der Plas LH, Verhoeven HA (2001) Expression of Clarkia S-linalool synthase in transgenic petunia plants results in the accumulation of S-linalyl-β-D-glucopyranoside. Plant J 27:315–324
98. Lavy M, Zuker A, Lewinsohn E, Larkov O, Ravid U, Vainstein A, Weiss D (2002) Linalool and linalool oxide production in transgenic carnation flowers expressing the Clarkia breweri linalool synthase gene. Mol Breed 9:103–111