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Review

Current status of metabolic phytochemistry

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I would like to dedicate this review article to Prof. Dr. A. Wilhelm Alfermann on the occasion of his 65th birthday with many thanks for his continuous support.

Abstract

This review will give selected examples of topics of current research into plant secondary metabolism. Besides detection, isolation and characterisation of enzymes and genes involved in the formation of natural products, the structures of enzymes after crystallisation are now being investigated and this information gives us hints on the catalytic mechanisms as well as probable evolutionary origins of these enzymes. Manipulation of natural product formation is achieved by overexpression or down-regulation of genes encoding biosynthetic enzymes or regulators (transcription factors) as well as by transfer of those genes into foreign organisms (bacteria, yeast, plants). Techniques, strategies and methods are used to investigate plant secondary metabolism ensuring that this field remains challenging.

Keywords: Natural product biosynthesis; Biosynthetic enzymes; Metabolic engineering; Overexpression; RNAi; Transcription factors; Enzyme structure elucidation

Contents

1. Introduction

In this article the title "Current status of metabolic phytochemistry" was interpreted in such a way that recent discoveries in the wide field of plant metabolism should be reviewed. This, of course, is not possible in a few pages since our understanding of plant metabolism has increased dramatically in the last decades. The topics given here are restricted to plant secondary metabolism although it has become clear that a strict line between primary and secondary metabolism cannot be drawn. Even with this restriction a comprehensive review is not possible. The paper will pick out some topics that appeared interesting to the author (therefore the choice will be rather subjective) and will present only few examples. Where appropriate, recent reviews

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will be cited to enable further reading. Key examples will be noted to illustrate the following approaches:

- The discovery of new enzymes and genes of plant natural product biosyntheses.
- The identification and application of transcription factors involved in the regulation of secondary metabolism.
- The down-regulation or overexpression of plant genes and their impact on natural product accumulation.
- The structural elucidation of proteins involved in plant secondary metabolism.
- The transfer of plant or microbial genes of secondary metabolism into *Escherichia coli* or *Saccharomyces cerevisiae* for the heterologous production of plant natural compounds (''combinatorial biosynthesis'').

It should be stressed at this point that the described examples do not depict the pioneering work that has been done in the last decades on these fields but advanced approaches that are based on these early findings.

2. Discovery of new enzymes and genes of plant natural product biosyntheses

The attempts and the success to identify enzymes and genes of plant secondary metabolism in the past have led to our current knowledge of metabolic pathways leading to natural products. The biosynthetic steps to major compounds such as flavonoids, phenolic derivatives, terpenes and many alkaloids have been clarified and numerous enzymes characterised; more and more genes are known as well. This research of course has not come to an end, simply for the reason that we by far do not yet know all secondary compounds present in plants and this knowledge increases from day to day. In this section only a few new achievements in plant metabolism restricted to recent advances in unravelling metabolic pathways leading to different phenolic compounds will be exemplified.

The introduction of the 3,4-dihydroxy substitution of hydroxycinnamic acids and derivatives has been a long posed question, and a ''4-coumarate 3-hydroxylase'' has long been searched for. Different enzymes have been reported to be involved in the ortho-hydroxylation of 4-coumaric acid or 4-coumaroyl-CoA, e.g. phenolase-type enzymes (see e.g. Stafford, 1974; Mayer, 1987), an enzyme with the same substrate requirements as phenolases but insensitive to tentoxin (Kojima and Takeuchi, 1989), a soluble 4-coumaroyl-CoA 3-hydroxylase dependent on FAD, NADPH and O_2 (Kamsteeg et al., 1981), a Zn^{2+}-dependent enzyme acting on 4-coumaroyl-CoA (Kneusel et al., 1989) or a FAD/FMN- and NAD(P)H-dependent enzyme in particulate fractions hydroxylating 4-coumaric acid (Boniwell and Butt, 1986). The 3-hydroxylation of 4-coumaroylquinate and 4-coumaroylshikimate by cytochrome P450-dependent monooxygenases was reported by Heller and Kühnl (1985) and Kühnl et al. (1987). Similarly, the introduction of the 3- and 3′-hydroxyl groups into the aromatic rings at the ester stage by cytochrome P450 monooxygenases was also found in the biosynthesis of rosmarinic acid (Petersen et al., 1993; Petersen, 1997). The clue for the introduction of the 3-hydroxyl group into hydroxycinnamoyl units was identified recently: the 3-hydroxylation takes place at the aromatic ring of esterified 4-coumaric acid (Schoch et al., 2001; Franke et al., 2002). 4-Coumaric acid is first coupled with quinic or shikimic acid (Hoffmann et al., 2003), then the 3-hydroxylation is catalysed by a cytochrome P450 belonging to the CYP98 family and then the caffeoyl moiety can be retransferred to coenzyme A. Caffeoyl-CoA then enters into further biosynthetic pathways (Fig. 1). This makes chlorogenic acid and/or 4-caffeoylshikimic acid central intermediates in the biosynthesis of phenylpropanoid derivatives including lignin. A cytochrome P450 (CYP98A6) catalysing the introduction of the caffeic acid substitution pattern of rosmarinic acid at the ester stage was cloned from *Lithospermum erythrorhizon* (Matsuno et al., 2002); a similar hydroxylase (CYP98A14) is known from *Coleus blumei*. cDNAs for (putative) hydroxycinnamoyltransferases involved in the formation of the 4-coumaroylshikimic/quinic esters have been cloned from, e.g. tobacco (Hoffmann et al., 2003; Niggeweg et al., 2004) and tomato (Niggeweg et al., 2004) as well as an increasing number of other plant species. These enzymes belong to the superfamily of BAHD acyltransferases that are involved in plant and fungal secondary metabolism in using CoA-activated acids as substrates for acyl transfer (St. Pierre and De Luca, 2000; D'Auria, 2006). Chlorogenic acid (=caffeoylquinic acid) serves two purposes, firstly as a (putative) defense compound and secondly as an intermediate in phenylpropanoid biosyntheses. Different hydroxycinnamoyltransferases (HCTs) seem to be involved in the formation of chlorogenic acid for these two functions since overexpression and/or silencing of different HCT genes leads to either alterations in chlorogenic acid levels or lignin amounts and composition (see below). The enzyme involved in rosmarinic acid biosynthesis, transferring a hydroxycinnamoyl moiety from hydroxycinnamoyl-CoA to a hydroxyphenyllactate, belongs to the same BAHD acyltransferase superfamily and the full-length cDNA has been cloned recently (Berger et al., 2006).

The formation of phenolic volatile oil components like eugenol and isoeugenol, present for example in the scent of *Petunia* flowers or in the peltate glandular trichomes of basil, has been shown to take place via an ester stage. A hydroxycinnamyl alcohol (e.g. coniferyl alcohol) is coupled with an acetate moiety (acetyl-CoA:coniferyl alcohol acetyltransferase; Dexter et al., 2007) and the acetate ester is cleaved concomitantly with a reduction step by a NADPH-dependent reductase (eugenol synthase, isoeugenol synthase; Koeduka et al., 2006). The acetyltransferase is another member of the BAHD acyltransferase superfamily, whereas the reductase belongs to the PIP-family of reductases. Other scent compounds of *Petunia* are, e.g.

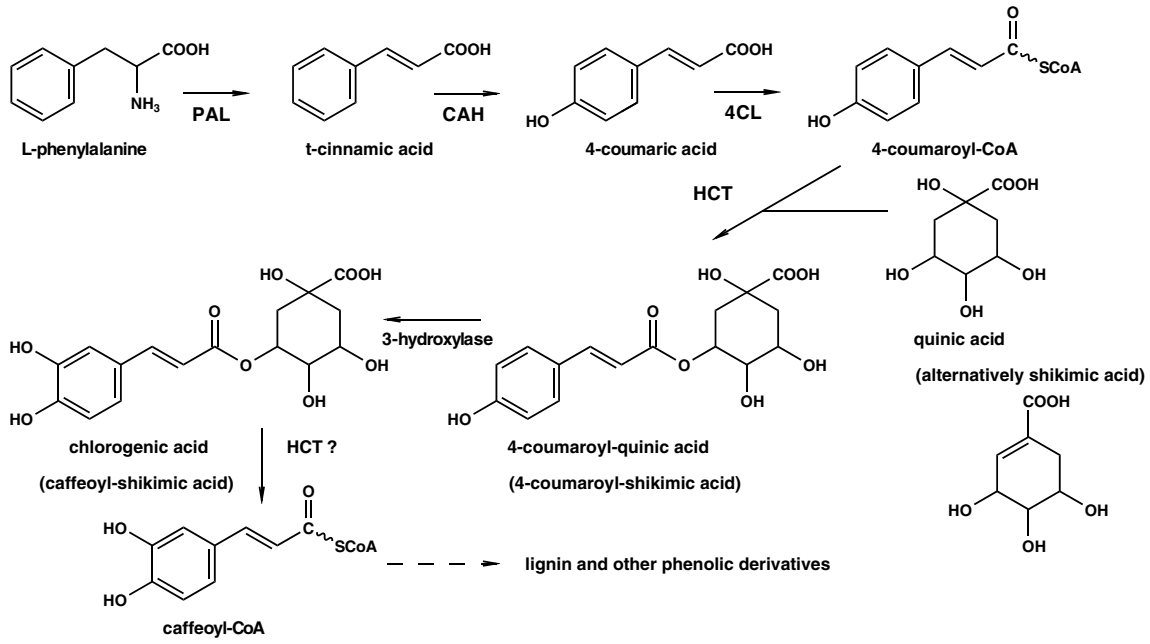

Fig. 1. Current view of phenylpropanoid metabolism. Enzymes involved are phenylalanine ammonia-lyase (PAL), cinnamic acid 4-hydroxylase (CAH), 4-coumarate coenzyme A-ligase (4CL), hydroxycinnamoyl-CoA:quinate/shikimate hydroxycinnamoyltransferase (HCT), 4-coumaroylquinate/shikimate 3-hydroxylase (3-hydroxylase).

benzylbenzoate and phenylethylbenzoate, the formation of which is catalysed by a benzoyltransferase, again a member of the BAHD acyltransferases (Boatright et al., 2004); the genes of other enzymes involved in *Petunia* scent production have been identified as well (Dexter et al., 2007). Other scent and aroma compounds (e.g. benzylbenzoate, geranylacetate, cinnamylacetate) are synthesised by related enzymes, e.g. in *Clarkia breweri*, roses, strawberries or bananas (D'Auria et al., 2002; Shalit et al., 2003; Beekwilder et al., 2004). The scent of *Petunia* flowers has been genetically altered by transferring the cDNA encoding a rose alcohol acetyltransferase driven by the CaMV 35S-promoter into the plant. The *Petunia* flowers newly produced phenylethylacetate and benzylacetate which were not present in control flowers (Guterman et al., 2006).

The strategy to identify new enzymes and genes of plant secondary metabolism has often changed. In the past the tedious purification of an enzyme to near homogeneity, followed by peptide microsequencing, synthesis of degenerate oligonucleotides and screening of cDNA libraries was the method of choice to get hold of a specific gene. Nowadays, establishment of EST (expressed sequence tag) databases or even whole genome sequencing (e.g. the genomes of *Arabidopsis thaliana* or *Medicago truncatula*) together with polymerase chain reaction (PCR) using conserved sequence motifs in different enzyme classes facilitates the identification of genes putatively involved in the reaction of interest (D'Auria and Gershenzon, 2005; Fridman and Pichersky, 2005). On the other hand, cDNAs isolated in full-length by a more random approach and heterologously expressed in *E. coli* or yeast often do not easily reveal their substrate(s) accepted and reactions performed. A favourable situation exists for enzymes which transfer a group that can be radioactively labeled (e.g. methyl from *S*-adenosyl-methionine (SAM) or glucose from uridine diphosphate (UDP)-glucose) rendering a radioactive reaction product. Much more tedious is the search for the substrates of cytochrome P450s (CYPs). Genome projects have shown that species can contain several hundred cytochrome P450 sequences (e.g. 272 in *A. thaliana*, Schuler and Werck-Reichhart, 2003). Cytochrome P450 cDNAs can be rather easily isolated with help of conserved sequence motifs. However, the identification of a suitable substrate after expression in microbial or insect cell cultures often is tedious, if not impossible. For this reason many CYP sequences are deposited in databases without known function and the same is true for other enzyme classes. Function assignment is often done only due to sequence homologies and in many cases these assigned functions lateron prove to be false.

3. Identification and application of transcription factors involved in plant secondary metabolism

A promising way of inducing whole biosynthetic pathways would be the overexpression of regulatory transcription factors that are involved in switching on genes of secondary metabolic pathways. Many of these can be induced by internal or external signals, for example elicitors or methyl jasmonate mimicking an infection. Other signals are hormones, UV or light that switch on the formation of certain compounds. External signals are often mediated by internal signal compounds, e.g. jasmonates.

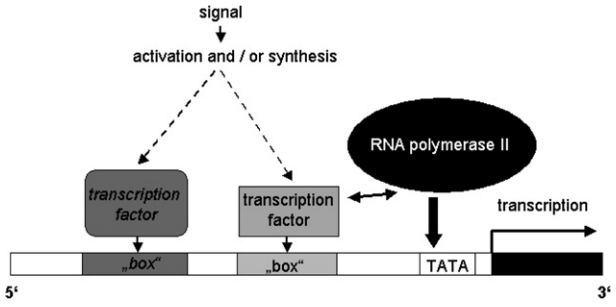

Fig. 2. Simplified scheme of the action of transcription factors: External or internal signals mediate the activation and/or synthesis of transcription factors. These proteins can bind to specific binding boxes in the promoter region of certain genes and thus influence the transcription rate of the respective genes by RNA polymerase II. Different transcription factors can interact with each other and/or with RNA polymerase II.

Some transcription factors for secondary pathways are already known: ORCA transcription factors are involved in the regulation of terpenoid indole alkaloid biosynthesis in *Catharanthus roseus* and a set of MYB-like and basic helix-loop-helix (bHLH) transcription factors regulate flavonoid biosyntheses in different plants. The expression of transcription factors of course must be regulated by higher regulators, but this is not yet fully understood (Vom-Endt et al., 2002). Transcription factors are sequence-specific DNA-binding proteins that interact with the promoter regions of target genes (Fig. 2), and modulate the rate of initiation of transcription by RNA polymerase II (Vom-Endt et al., 2002). Genes that are regulated coordinately often have similar binding sites in their promoters.

The tissue-specific regulation of flavonoid/anthocyanin biosynthesis is mediated by the concerted action of two families of transcription factors, the MYB-proteins and the basic helix-loop-helix proteins (bHLH); proteins of both families can in some cases interact physically. Well investigated plants are *A. thaliana*, *Antirrhinum majus*, *Zea mays* and *Petunia* where differing nomenclatures for the transcription factors are used. In maize the biosynthesis of anthocyanins as well as their transport into the vacuole is regulated by *C1* (MYB) and *R* (bHLH). Involvement of the MYB-like factor *P* induces the formation of dihydroflavonol reductase and leads to flavan-4-ol and phlobaphene formation acting as insecticidal compounds in the plant cell walls (Mol et al., 1998; Grotewold et al., 1998; Gantet and Memelink, 2002). In *Arabidopsis* the *C1*-ortholog *PAP1* is responsible for pigmentation, the interacting bHLH transcription factor seems to be constitutively expressed (Vom-Endt et al., 2002). Seed coat pigmentation by condensed tannins is additionally affected by *TT2* (MYB) and *TT8* (bHLH).

A MYB-like transcription factor *AtMYB4* regulates the expression of the UV-protecting sinapic acid esters in *Arabidopsis* in a different way. The transcription factor represses the formation of cinnamic acid 4-hydroxylase and some other biosynthetic enzymes. Sinapate ester formation is upregulated by UV-induced downregulation of *AtMYB4* expression (Jin et al., 2000).

C. roseus (Apocynaceae) is known for its production of terpenoid indole alkaloids (TIAs). The dimeric alkaloids vinblastine and vincristine are only produced in minute amounts in the plant (0.001–0.005% of the dry weight). These alkaloids and their semisynthetic derivatives are inhibitors of microtubule polymerisation and are used to treat certain cancers (e.g. leukemia, melanoma or Morbus Hodgkin). The main monomeric alkaloids present in *C. roseus* are ajmalicine, catharanthine, serpentine and vindoline. The precursors are tryptamine, formed from tryptophan by tryptophan decarboxylase (TDC), and secologanin, synthesised via the methylerythritolphosphate pathway. Tryptamine and secologanin are coupled by strictosidine synthase to strictosidine (Fig. 3) which is then transformed to the monomeric and dimeric TIAs. A number of *C. roseus* genes involved in the biosynthesis of TIAs and the formation of the primary precursors for these alkaloids have been cloned (Memelink et al., 2001). All tested biosynthetic genes were found to be inducible by the treatment with jasmonate. The promoter of strictosidine synthase has been studied for elements responsible for this jasmonate responsiveness and two regions named BA and JERE (*j*asmonate and *e*thylene *r*esponsive *e*lement) near the TATA box have been identified. Additionally a G-box was found near the JERE region which binds the bHLH factor CrMYC as well as the repressing factor CrGBF (*C. roseus* G-box binding factor; Gantet and Memelink, 2002). Mutational analysis showed that the JERE region is absolutely essential for jasmonate induction whereas mutation of the BA region only leads to reduced induction levels. An elicitor-inducible but jasmonate-independent MYB-like factor CrBPF1 binds to this BA region (Van der Fits et al., 2000). Jasmonate-dependent ORCA (*o*ctadecanoid acid *r*esponsive *Catharanthus A*P2-domain proteins) transcription factors (ORCA2, ORCA3) having an AP2/ERF DNA binding domain bind to the JERE region of the strictosidine synthase promoter (Menke et al., 1999; Van der Fits and Memelink, 2001). Thus the signaling pathways leading to the formation of these two transcription factors must be different. Protein phosphorylation and increase in cytosolic Ca^{2+}-levels are involved in

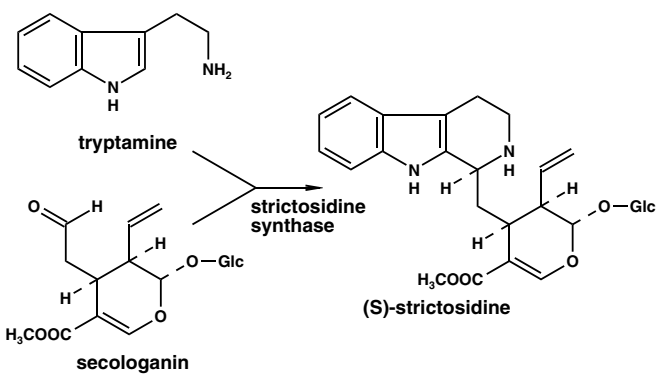

Fig. 3. Reaction of strictosidine synthase from *Catharanthus roseus* involved in the biosynthesis of terpenoid indole alkaloids.

the elicitor-dependent signalling pathway leading to the activation of BA (Van der Fits et al., 2000). Jasmonates on the other hand induce *orca* gene expression as well as the activation of existing ORCA proteins. Not all the known genes of TIA biosynthesis are induced by ORCAs. Genes encoding, e.g. geraniol 10-hydroxylase, strictosidine β-glucosidase and acetyl-CoA:4-*O*-deacetylvindoline 4-*O*-acetyltransferase do not react on *orca* overexpression (Van der Fits and Memelink, 2000; Memelink et al., 2001). Consequently, overexpression of ORCA proteins did not lead to an increased accumulation of TIAs although the level of the precursor tryptamine was increased. Obviously the second precursor secologanin was rate-limiting. Feeding of loganin (a precursor for secologanin) to *C. roseus* cell cultures therefore resulted in an increased alkaloid level (Van der Fits and Memelink, 2000). These findings show that overexpression of one or more transcription factors might be useful to enhance the accumulation of secondary metabolites by switching on whole biosynthetic pathways and probably also precursor formation. For this reason, the research to identify more transcription factors involved in secondary metabolite biosyntheses should be intensified.

4. Inhibition and overexpression of biosynthetic enzymes

Knowing the genes of biosynthetic pathways enables researchers to increase the enzyme activities by overexpression of the respective genes under control of a constitutive or inducible strong promoter thus surpassing slow biosynthetic steps. On the other hand, antisense techniques and more recently RNA interference (RNAi) can lead to lower enzyme activities which can help to suppress the formation of unwanted natural compounds or to channel metabolites into pathways leading to desired products. Our current lack of understanding of metabolic networks and transport and accumulation processes in the cells might, however, lead to rather unexpected results when interfering with metabolic pathways (Winkel, 2004; Dixon, 2005).

RNA interference (RNAi) techniques are based on the fact that cells are able to recognise and destroy double-stranded RNA (dsRNA). In normal metabolism dsRNA does not occur in plant cells in high amounts except, e.g. in short stretches in tRNAs. DsRNA often is the genetic material of viruses and viroids and thus the cells are supposed to fight against infections by destroying this dsRNA. This mechanism has been found in all eukaryotic organisms (Tang and Galili, 2004; Qi and Hannon, 2005; Mansoor et al., 2006). Three different mechanisms are known: silencing by microRNAs (miRNAs), transcriptional gene silencing (TGS) and posttranscriptional gene silencing (PTGS). Only the latter will be addressed here since it is the approach most often used to manipulate plant secondary metabolic pathways. In short, sense and antisense DNA for the gene of interest are introduced into a plasmid (e.g. pHANNIBAL; Wesley et al., 2001) separated by an intron and under the control of a constitutive promoter (“hairpin cassette”). This hairpin cassette is transferred into a transformation plasmid which is used to transform plant cells; this can be done, e.g. by *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes*. After transcription the RNA will form a dsRNA hairpin (hpRNA) with a loop formed by the intron. This dsRNA is recognised by an enzyme called “dicer” which cleaves the RNA into small fragments with a length of 21–25 base pairs (siRNAs). The small RNA fragments are bound by a *R*NA-*i*nduced *s*ilencing *c*omplex (RISC) which is able to recognise mRNAs with sequence similarities to the siRNAs and degrades these (and/or similar) mRNAs. Translation of these mRNAs thus is not possible (Fig. 4).

The caffeine content in coffee (*Coffea arabica*, *Coffea canephora*) was reduced by RNAi-techniques by Ogita et al. (2003, 2004). The biosynthesis of caffeine from xanthosine includes three *N*-methylation steps: xanthosine is methylated by xanthosine methyltransferase (XMT), the ribosyl moiety is then removed from 7-methylxanthosine and 7-methylxanthine is further methylated by 7-*N*-methylxanthine 3-*N*-methyltransferase (theobromine synthase, MXMT) resulting in theobromine which is methylated in position 1 to caffeine by 3,7-dimethylxanthine 1-*N*-methyltransferase (DXMT) (Fig. 5; Ogita et al., 2004). Theobromine synthase from *C. arabica* (*CaMXMT1*) was silenced by RNAi techniques resulting in significantly lower transcript levels of *CaMXMT1*. In some transformants the transcript levels of the other two methyltransferases were reduced as well. In leaves of regenerated plantlets the theobromine and caffeine contents were reduced down to 28% and 46% of the controls, respectively.

Morphine accumulation in *Papaver somniferum* (Fig. 6) was reduced by silencing of codeinone reductase (COR; Allen et al., 2004). COR is encoded by a gene family and catalyses the last but one step of morphine biosynthesis, the NADPH-dependent reduction of codeinone to codeine

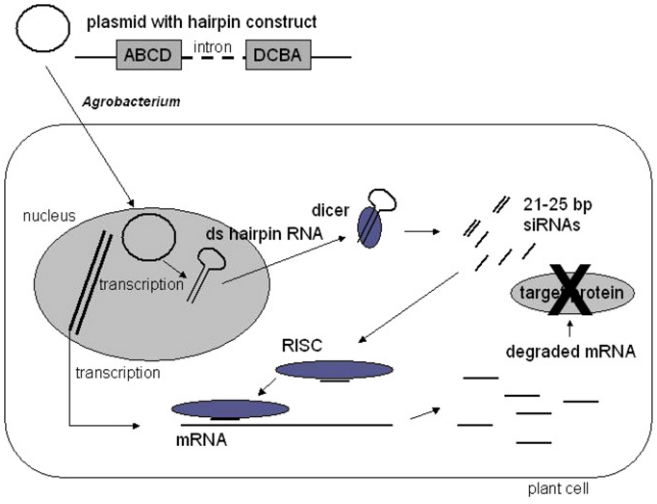

Fig. 4. Simplified scheme of down-regulation of metabolic activities by RNAi-techniques.

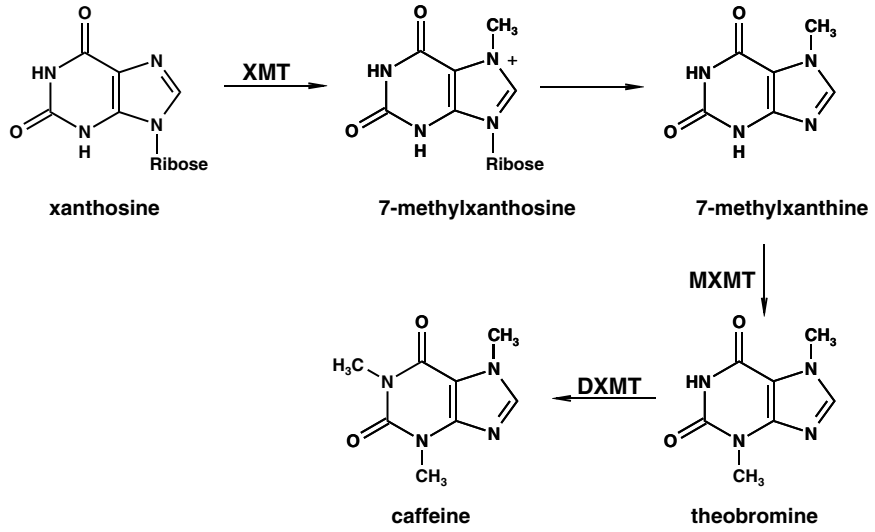

Fig. 5. Biosynthesis of caffeine in *Coffea* spec. (Ogita et al., 2004). XMT = xanthosine 7-*N*-methyltransferase, MXMT, 7-*N*-methylxanthine 3-*N*-methyltransferase (theobromine synthase); DXMT, 3,7-dimethylxanthine 1-*N*-methyltransferase.

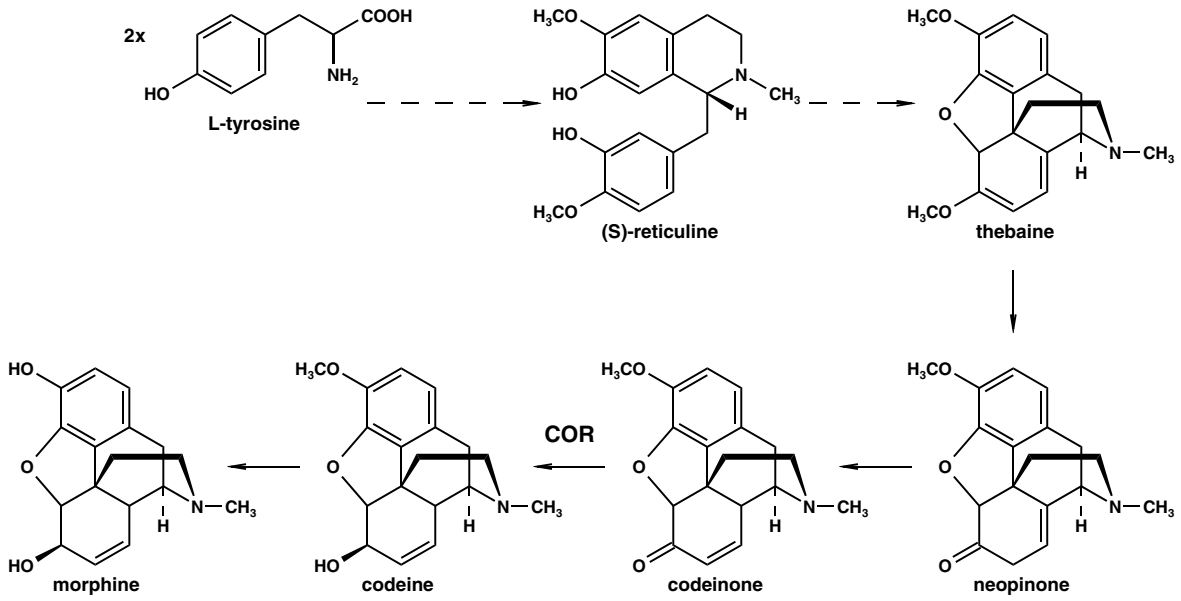

Fig. 6. Biosynthesis of morphine in *Papaver somniferum* (according to Allen et al., 2004). COR = codeinone reductase. Broken arrows indicate that several biosynthetic steps are involved.

(Unterlinner et al., 1999). Transgenic plants with a hairpin construct with sense and antisense sequences of COR showed strongly reduced transcript levels, reduced COR activity and an altered alkaloid spectrum. (*S*)-Reticuline and its methylated derivatives (codamine, laudanine, laudanosine) were the main alkaloids found in the latex of transformed plants. Interestingly, not codeinone (the substrate of COR) was accumulated but (*S*)-reticuline which is seven enzymatic steps upstream of COR. The contents of morphine, codeine, thebaine and oripavine were strongly reduced. The transcript levels for morphine biosynthetic enzymes before and after the intermediate reticuline were not affected. The basis for this unexpected effect is not yet known.

The principle of overexpression is easily explained: the gene of interest is placed under the control of a constitutive or strong inducible eukaryotic promoter and transferred to plant cells. The enzyme of interest is produced in high amounts and may eventually lead to a higher production of a secondary metabolite. However, the outcome of these experiments can be unpredictable: overexpression of an enzyme may disturbe the cells' metabolism and thus affect the viability of the cells. Moreover co-suppression sometimes leads to lower than normal levels of the target enzyme.

The tropane alkaloids hyoscyamine and scopolamine accumulated by several species of the Solanaceae are used as anticholinergic agents. Although the market volume of these tropane alkaloids is not very high, there are no other classes of compounds that might be able to substitute for these alkaloids and the demand of scopolamine is about ten times that of hyoscyamine/atropine (Oksman-Caldentey and Arroo, 2000). The biosynthesis starts from ornithine finally leading to the tropinone moiety, and phenylalanine which gives the tropic acid moiety. Hyoscyamine is converted in two steps to scopolamine by hyoscyamine 6β-hydroxylase (H6H), a 2-oxoglutarate-dependent dioxygenase (Hashimoto and Yamada, 1986). In order to increase the levels of scopolamine, *Hyoscyamus niger* was transformed with cDNAs encoding H6H and putrescine *N*-methyltransferase (PMT), the enzyme channeling putrescine into tropane alkaloid biosynthesis, either singly or simultaneously (Zhang et al., 2004). Hairy root cultures harbouring these cDNAs were analysed for their alkaloid production. Although the alkaloid levels and composition were quite variable, the best transgenic line expressing both *pmt* and *h6h* displayed a scopolamine accumulation of 411 mg/l which is 941% of the wild-type culture. Expressing only *pmt* did not significantly increase the accumulation of tropane alkaloids. Hairy roots only overexpressing *h6h* showed also an elevated scopolamine accumulation of 184 mg/l in the best line. These experiments prove that direction of putrescine into tropane alkaloid biosynthesis by overexpressed PMT and the increased pulling force of the final enzyme of the biosynthetic pathway H6H both add to the increased accumulation of scopolamine.

Chlorogenic acid, an ester of caffeic acid and quinic acid (Fig. 1), is an antioxidative phenolic compound occurring widely in the plant kingdom and therefore also in food (Clifford, 1999). It is considered to be a health-promoting compound and therefore a higher concentration of chlorogenic acid might be beneficial in food plants. Furthermore a high chlorogenic acid content might increase the pathogen resistance of plants. Hydroxycinnamoyl-CoA:quinate hydroxycinnamoyltransferase (HQT) catalyses the transfer of the hydroxycinnamoyl moiety from the respective CoA-ester to quinic acid (Fig. 1). The *hqt* gene was either silenced or overexpressed in tomato (Niggeweg et al., 2004). Silencing was achieved by RNA interference. A plasmid leading to the formation of a hairpin RNA was transiently expressed in tomato cells. The chlorogenic acid levels were reduced by 98%, the levels of other phenolics were not affected and also lignin levels were normal. Overexpression of *hqt* under the control of the double CaMV35S promoter resulted in a threefold increase in HQT activity and a 85% increase of the chlorogenic acid contents. Again lignin formation was not affected. Concomitantly the antioxidative capacity of the tomato plants and the pathogen resistance against *Pseudomonas syringae* were increased. The fact that lignin formation was not affected by the activity of HQT although the formation of chlorogenic acid is considered to be an essential step in the formation of lignin precursors (Schoch et al., 2001) probably is due to a second, slightly different hydroxycinnamoyltransferase (HCT; Hoffmann et al., 2003). Silencing of this HCT in *A. thaliana* and *Nicotiana benthamiana* resulted in strongly reduced HCT activity (about 1% of control levels) and changes in lignin amounts (reduction by approximately 15%) and composition (Hoffmann et al., 2004, 2005). The percentage of syringyl residues were strongly reduced in lignin of the transgenic plants.

Overexpression of transcription factors can switch on whole biosynthetic pathways. This was shown for the MYB transcription factor *ant1* from tomato (Mathews et al., 2003). Overexpression of the gene for this transcription factor resulted in upregulation of early (e.g. chalcone synthase) and late (e.g. dihydroflavonol synthase, glucosyltransferases) enzymes of anthocyanin biosynthesis as well as proteins involved in anthocyanin transport into the vacuole. Leaves, flowers and shoots of the tomato plants showed purple coloration and the anthocyanin contents were increased up to 470-fold from 7.6 μg/g fresh weight in wildtype seedlings to 3574 μg/g fresh weight in the best anthocyanin-producing line (Mathews et al., 2003).

5. Structure elucidation of enzymes involved in plant secondary metabolism

The possibility to heterologously express cDNAs encoding enzymes of plant secondary metabolism in order to produce high amounts of the proteins as well as facilitated purification by attaching "tags" (e.g. His-tag, GST-tag) to the protein has greatly improved the possibilities to get hold of sufficient amounts of highly pure proteins that are needed for protein crystallisation and structure elucidation. This has been hampered in earlier times by the usually very low protein quantities of secondary metabolic enzymes present in plant cells and the tedious purification procedures with heavy losses of the wanted enzyme. The knowledge of the structures of enzymes involved in secondary metabolism can not only provide us with insights into the architecture of active sites, but also give us clues to understand reaction mechanisms as well as the evolutionary origin of these enzymes in proteins involved in primary tasks (Noel et al., 2005). Even when sequence homologies are quite low, structural features might have been conserved during evolution. As an example, the structure elucidation of the entry-point enzyme of phenolic metabolism, phenylalanine ammonia-lyase (PAL), from *Petroselinum crispum* has shown that the enzyme probably has evolved from histidine ammonia-lyase (HAL) which is active in the degradation of histidine to glutamate in primary metabolism (Schwede et al., 1999; Ritter and Schulz, 2004). PAL was crystallised from parsley and HAL from *Pseudomonas putida* (the protein structures can be seen in the pdb-database under 1w27 and 1b8f). Both enzymes are active as homotetramers. Their structures were found to be quite similar and are characterised by a predominance of α-helices.

In contrast to HAL, however, PAL has an additional "shielding" domain which is thought to strengthen the interaction with the core domain and which limits the accessibility of the active center (Ritter and Schulz, 2004). Both, PAL and HAL act without cofactor although a strong electrophilic group is needed for the abstraction of ammonia. The reaction mechanism for the elimination of ammonia has been clarified. The electrophilic prosthetic 4-methylidene-imidazole-5-one (MIO) group is formed autocatalytically from the tripeptide Ala-Ser-Gly in positions 202–204 of PAL. This MIO group is attacked by and bound to the phenyl ring of phenylalanine resulting in stabilisation of the positive charge of the substrate phenyl group. The hydrogen of the β-C-atom of the side chain is abstracted by Tyr351 from another subunit. The amino group is eliminated and the MIO group rearranged (Ritter and Schulz, 2004).

Recently, the first structure of a member of the superfamily of BAHD acyltransferases, vinorine synthase from *Rauvolfia serpentina* has been published (pdb: 2bgh; Ma et al., 2005). The structure showed, that although the conserved DFGWG-motif at the C-terminus of the protein was shown to be important for catalysis it is not involved in forming the active center but is supposed to play a structural role.

The structures of different plant polyketide synthases type III have been solved during the last few years, e.g. chalcone synthase from alfalfa (pdb: 1bi5; Ferrer et al., 1999), pyrone synthase from *Gerbera hybrida* (pdb: 1qlv; Jez et al., 2000), stilbene synthases from *Pinus sylvestris* and *Arachis hypogaea* (pdb: 1u0u; Austin et al., 2004; pdb: 1xes, Ng et al., in press; pdb: 1z1e; Shomura et al., 2005) or a pentaketide chromone synthase from *Aloe arborescens* (pdb: 2d3m; Morita et al., 2006). These structures are helpful in determining the differences in the active centers that finally lead to the acceptance of different starter molecules, different numbers of reaction cycles and different reaction products.

6. Combinatorial biosynthesis of plant natural compounds

After the successful identification of enzymes and genes involved in plant secondary metabolite biosyntheses a recent field of research is to engineer whole biosynthetic pathways into microorganisms. Ligation of cDNAs into plasmids, transfer into foreign organisms and heterologous expression in *E. coli*, yeast or insect cell cultures is routine for DNAs encoding single biosynthetic enzymes in every molecular genetic laboratory nowadays. Advances have, however, been made to concomitantly transfer and express a number of cDNAs of a biosynthetic pathway in the same heterologous organism and thus enabling this organism to catalyse several successive biosynthetic steps leading to plant metabolites such as taxoids, artemisinin precursors or flavonoids. This will be exemplified below.

The biosynthesis of the diterpenoid alkaloid paclitaxel (taxol®, Fig. 7), a natural product of *Taxus* species (Taxaceae; Croom, 1995), comprises 19 enzymatic steps from geranylgeranyl diphosphate. The biosynthetic steps include cyclisation, several hydroxylations, oxidations and acylations and a final side chain assembly (DeJong et al., 2006). Paclitaxel has become a very important anti-cancer drug (Goldspiel, 1997) and the demand for this compound from natural sources can hardly be satisfied. Besides the use of *Taxus* cell cultures (www.phytonbiotech.com) elucidation of the biosynthetic pathway of paclitaxel (Jennewein and Croteau, 2001; Jennewein et al., 2004) and afterwards an establishment of a part of the biosynthetic pathway in yeast by transferring plasmids with the respective open reading frames under the control of inducible or constitutive promoters was tried. *S. cerevisiae* was transformed with three plasmids carrying the cDNAs for five consecutive biosynthetic enzymes: geranylgeranyl diphosphate synthase, taxadiene synthase, taxadiene 5α-hydroxylase, taxadienol 5-*O*-acetyltransferase and taxoid 10β-hydroxylase. Two of these enzymes are cytochrome P450-dependent monooxygenases. The yeast internal NADPH: cytochrome P450 reductase was used for supplementing electrons from NADPH to these cytochromes. The endogenous supply of isoprenoid precursors was used. All enzymes could be detected immunologically as well as by showing their activities in enzyme/microsomal preparations from the transformed yeast cells. Taxadiene as the first taxoid product was detected after feeding of radiolabeled precursors. However, the expected final product taxadien-5α-acetoxy-10β-ol was not accumulated to measurable amounts in the yeast cells; only very low amounts of taxadien-5α-ol were detected. Obviously the two cytochrome P450-dependent hydroxylation steps were rate-limiting (DeJong et al., 2006).

A similar approach was used to express enzymes involved in the formation of the putative artemisinin precursor artemisinic acid (Fig. 8). Artemisinin is isolated from *Artemisia annua* (Asteraceae) as a compound active against multi-drug-resistant *Plasmodium* species causing malaria (Balint, 2001; Sriram et al., 2004). Artemisinin is a sesquiterpene lactone with an endoperoxide bridge. Farnesyl diphosphate originating from the mevalonate pathway is cyclised by amorphadiene synthase to amorphadiene (Mercke et al., 2000; Wallaart et al., 2001) which is then oxidised in three consecutive steps to artemisinic acid by a single cytochrome P450 (CYP71AV1; Teoh et al., 2006). Yeast was transformed with the coding sequences for amorphadiene synthase, for CYP71AV1 and for NADPH:cytochrome P450 reductase from *A. annua* (Ro et al., 2006). The availability of farnesyl diphosphate was increased by upregulating the mevalonate pathway and downregulating sterol biosynthesis. This yeast strain produced artemisinic acid, a precursor for artemisinin, and excreted it from the cytoplasm. It could be removed from the cell wall space of sedimented yeast cells with alkaline buffer. A yield of 115 mg artemisinic acid per

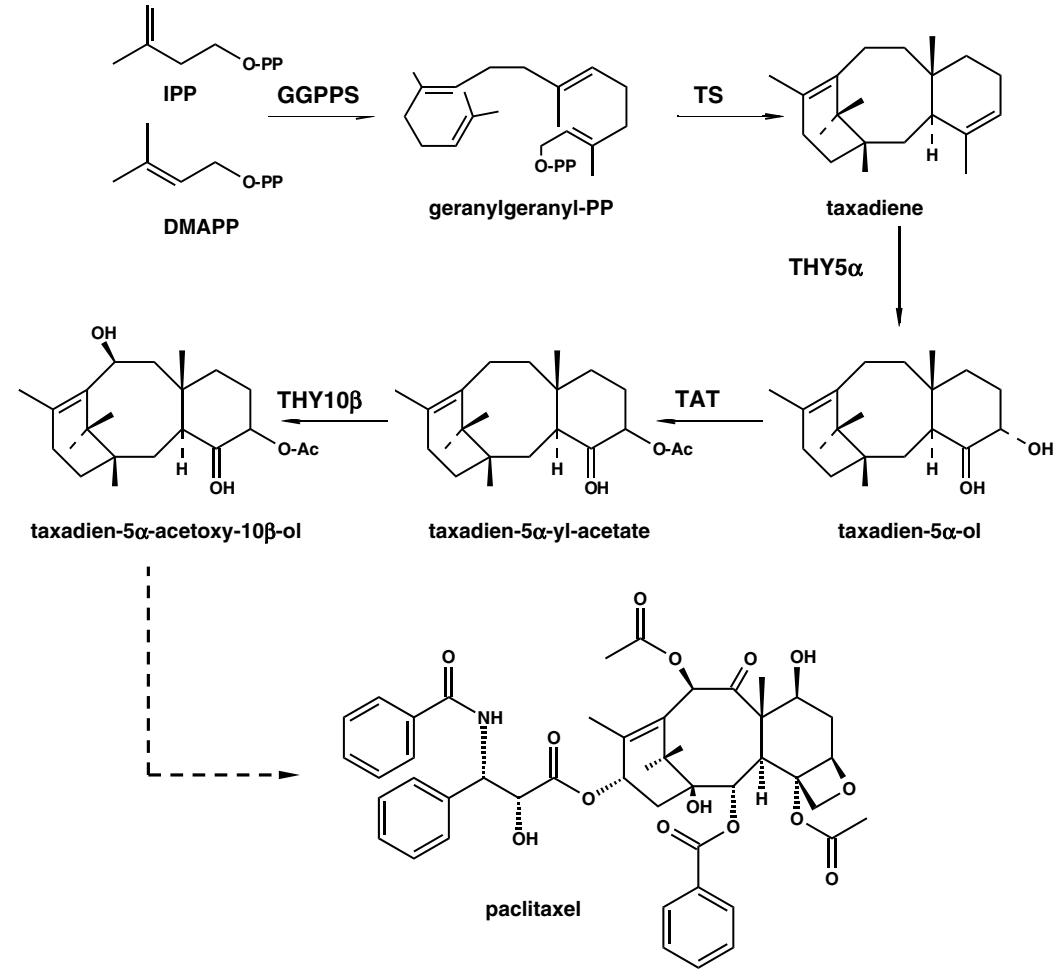

Fig. 7. Paclitaxel biosynthesis from isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). GGPPS, geranylgeranyl diphosphate synthase; TS, taxadiene synthase; THY5α, taxadiene 5α-hydroxylase; TAT, taxadienol 5-*O*-acetyltransferase; THY10β, taxoid 10β-hydroxylase (according to DeJong et al., 2006). The broken arrow indicates multiple biosynthetic steps.

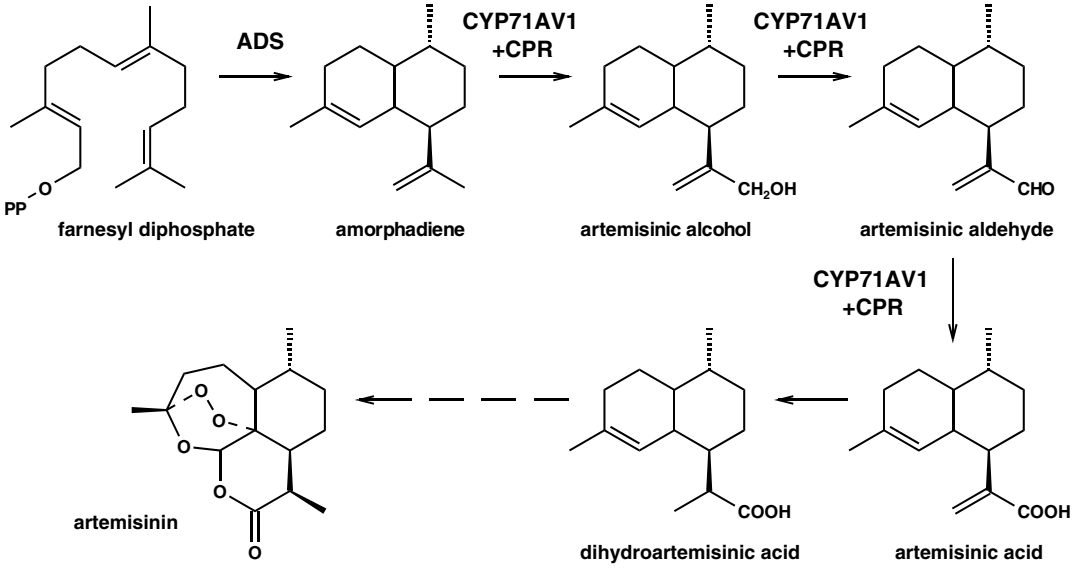

Fig. 8. Biosynthesis of artemisinin (according to Ro et al., 2006). ADS, amorphadiene synthase; CYP71AV1, oxidising cytochrome P450 in artemisinin biosynthesis; CPR, NADPH:cytochrome P450 reductase. The broken arrow indicates more than one biosynthetic step.

litre of culture could be produced. The authors claim that the specific productivity for artemisinic acid in engineered yeast is nearly two orders of magnitude higher than in *A. annua* (Ro et al., 2006). Artemisinic acid can serve as a precursor for the semi-synthesis of therapeutic artemisinin or its derivatives.

E. coli and *S. cerevisiae* have successfully been used for the production of flavonoids and isoflavonoids. These compounds are interesting from a pharmaceutical point of view because of their antioxidative and anti-cancer as well as general health-promoting activities (Bravo, 1998; Middleton et al., 2000; Nijveldt et al., 2001; Le Marchand, 2002). The enzymes and genes of these biosynthetic pathways have been identified and isolated from several plant species, and some of the early biosynthetic steps are catalysed by microbial enzymes as well. A plasmid was constructed with an artificial gene cluster consisting of phenylalanine ammonia-lyase (PAL) from *Rhodotorula rubra*, 4-coumarate CoA-ligase from *Streptomyces coelicolor* A3(2) and chalcone synthase from *Glycyrrhiza echinata* and introduced into *E. coli*. The bacteria were able to produce pinocembrin from phenylalanine and naringenin from tyrosine, because PAL also displayed the activity of tyrosine ammonia-lyase (Hwang et al., 2003). A similar approach was followed by Watts et al. (2004) using genes from *A. thaliana* or alternatively a tyrosine ammonia-lyase from *Rhodobacter sphaeroides* resulting in naringenin production (20.8 mg/l) in engineered *E. coli* cultures. Addition of a chalcone isomerase from *Pueraria lobata* and bacterial genes for acetyl-CoA carboxylase (thus filling up the malonyl-CoA pool) increased the yields of the flavanones to about 60 mg/l (Miyahisa et al., 2005) and led to the accumulation of the natural stereospecific flavanones (2*S*)-naringenin and (2*S*)-pinocembrin (Fig. 9). The same authors furthermore added the cDNA for flavone synthase I from *P. crispum*, a soluble 2-oxoglutarate-dependent dioxygenase, to the same *E. coli* strain and found apigenin (13.0 mg/l) production after feeding of tyrosine and chrysin (9.4 mg/l) after supplementation with phenylalanine. The flavonols kaempferol (15.1 mg/l) and galangin (1.1 mg/l) were produced when cDNAs for flavanone 3β-hydroxylase from *Citrus sinensis* and flavonol synthase from *Citrus unshiu*, both soluble 2-oxoglutarate-dependent dioxygenases, were additionally transferred to the bacteria (Miyahisa et al., 2006). In all these complex systems for flavonoid production the authors only used the genes of soluble enzymes that can be readily expressed in bacteria. The diversity of the products was achieved by feeding different amino acids (phenylalanine, tyrosine) to the bacteria thus avoiding the step from cinnamic acid to 4-coumaric acid catalysed by cinnamic acid 4-hydroxylase, a membrane-bound cytochrome P450 enzyme system. For the production of isoflavonoids, e.g. genistein, the expression of a cytochrome P450 cannot be circumvented since the ring migration establishing the isoflavonoid skeleton is such a step. This was overcome by using yeast cells as producers for this enzyme system. Katsuyama et al. (2007) combined soluble enzymes

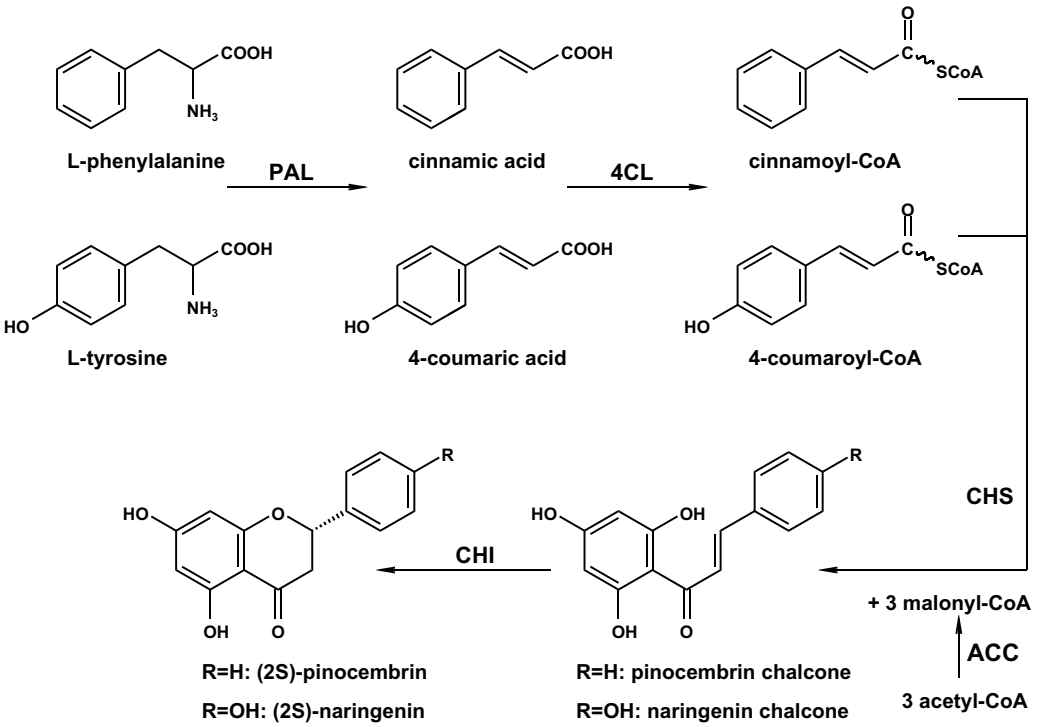

Fig. 9. Production of the flavanones (2*S*)-pinocembrin and (2*S*)-naringenin by *Escherichia coli* transformed with phenylalanine ammonia-lyase (PAL) from *Rhodotorula rubra*, 4-coumarate CoA-ligase (4CL) from *Streptomyces coelicolor*, chalcone synthase (CHS) from *Glycyrrhiza echinata*, chalcone isomerase from *Pueraria lobata* and acetyl-CoA carboxylase (ACC) from *Corynebacterium glutamicum* (according to Miyahisa et al., 2005).

engineered into *E. coli* and a membrane-bound enzyme system in *S. cerevisiae* for the production of genistein. Genistein has become an important medicinal compound due to its phytoestrogenic activity (Dixon and Ferreira, 2002). The bacteria harboured the cDNAs for PAL, 4-coumarate CoA-ligase, chalcone synthase and chalcone isomerase and additionally an acetyl-CoA carboxylase for a better supply of malonyl-CoA and produced naringenin which was excreted to the medium. Yeast cells carried the cDNAs for isoflavone synthase and an internal cytochrome P450 reductase which is known to be able to transfer electrons to plant cytochrome P450s. Coincubation of *E. coli* and yeast cells resulted in the production of genistein which was formed from 2,5,7,4′-tetrahydroxyisoflavone in an acid environment. The genistein yield was reported to be 5.8 mg/l.

These three examples show that plant natural products can be produced with the help of microorganisms, provided that the plant genes are expressed under the control of a suitable promoter and in a suitable environment. Bacteria are useful for soluble enzymes whereas the eukaryotic *S. cerevisiae* provides the necessary membranes of the endoplasmic reticulum for active cytochrome P450 enzyme systems.

Transfer of whole biosynthetic pathways into plants usually not expressing this biosynthetic pathway requires the transfer of several genes in a functional way into the same plant. Different techniques can be used for this purpose. The most prominent example where enzymes from different origin have been engineered into one species is the so-called "Golden Rice" expressing the biosynthetic pathway to β-carotene (provitamin A) in the rice endosperm (Ye et al., 2000; Al-Babili and Beyer, 2005). A phytoene synthase and a lycopene β-cyclase from *Narcissus pseudonarcissus* and a bacterial phytoene desaturase from *Erwinia uredovora* were transferred to rice under the control of constitutive or endosperm-specific promoters. The usually white rice grains of transgenic plants were yellow and accumulated around 2 μg β-carotene per gram rice grain (Ye et al., 2000). The recently announced "Golden Rice 2" has increased levels of β-carotene reaching up to 37 μg/g (Paine et al., 2005).

7. Conclusion

The topics treated in this review have shown the breadth of research methodologies and the exciting depth of recent research in plant secondary metabolism in the last decades of the last and the first years of this 21st century. We are learning about catalytic mechanisms and primary evolutionary origins of enzymes. The roles and functions of the so-called "secondary" products in the whole plant are getting clearer and we learn that many compounds exert various roles and have functions in primary processes as well. Secondary metabolism is integrated in networks with primary metabolism, and interfering with one reaction in this network may have unforeseen effects in the whole plant system. Still the biosynthetic pathways for most of the structurally known natural compounds (and of course for all those that still have to be structurally elucidated!) are unknown – fields of research that can be and should be "occupied" by young scientists in future. Young researchers are *strongly* encouraged to get involved in one or the other field of plant secondary metabolism – it is always interesting and worthwhile, although often hard!

References

Al-Babili, S., Beyer, P., 2005. Golden Rice – five years on the road – five years to go? Trends Plant Sci. 10, 565–573.

Allen, R.S., Millgate, A.G., Chitty, J.A., Thisleton, J., Miller, J.A.C., Fist, A.J., Gerlach, W.L., Larkin, P.J., 2004. RNAi-mediated replacement of morphine with the nonnarcotic alkaloid reticuline in opium poppy. Nat. Biotechnol. 22, 1559–1566.

Austin, M.B., Bowman, M.E., Ferrer, J.L., Schröder, J., Noel, J.P., 2004. An aldol switch discovered in stilbene synthases mediates cyclization specificity of type III polyketide synthases. Chem. Biol. 11, 1179–1194.

Balint, G.A., 2001. Artemisinin and its derivatives: an important new class of antimalarial agents. Pharmacol. Ther. 90, 261–265.

Beekwilder, J., Alvarez-Huerta, M., Neef, E., Verstappen, F.W.A., Bouwmeester, H.J., Aharoni, A., 2004. Functional characterization of enzymes forming volatile esters from strawberry and banana. Plant Physiol. 135, 1865–1878.

Berger, A., Meinhard, J., Petersen, M., 2006. Rosmarinic acid synthase is a new member of the superfamily of BAHD acyltransferases. Planta 224, 1503–1510.

Boatright, J., Negre, F., Chen, X., Kish, C.M., Wood, B., Peel, G., Orlova, I., Gang, D., Rhodes, D., Dudareva, N., 2004. Understanding in vivo benzenoid metabolism in petunia petal tissue. Plant Physiol. 135, 1993–2011.

Boniwell, J.M., Butt, V.S., 1986. Flavin nucleotide-dependent 3-hydroxylation of 4-hydroxyphenylpropanoid carboxylic acids by particulate preparations from potato tubers. Z. Naturforsch. 41c, 56–60.

Bravo, L., 1998. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr. Rev. 56, 317–333.

Clifford, M.N., 1999. Chlorogenic acids and other cinnamates – nature, occurrence and dietary burden. J. Sci. Food Agric. 79, 362–372.

Croom Jr., E.M., 1995. *Taxus* for taxol and taxoids. In: Suffness, M. (Ed.), Taxol: Science and Applications. CRC, Boca Raton, pp. 37–70.

D'Auria, J.C., 2006. Acyltransferases in plants: a good time to be BAHD. Curr. Opt. Plant Biol. 9, 331–340.

D'Auria, J.C., Gershenzon, J., 2005. The secondary metabolism of *Arabidopsis thaliana*: growing like a weed. Curr. Opin. Plant Biol. 8, 308–316.

D'Auria, J.C., Chen, F., Pichersky, E., 2002. Characterization of an acyltransferase capable of synthesizing benzylbenzoate and other volatile esters in flowers and damaged leaves of *Clarkia breweri*. Plant Physiol. 130, 466–476.

DeJong, J.H.M., Liu, Y., Bollon, A.P., Long, R.M., Jennewein, S., Williams, D., Croteau, R.B., 2006. Genetic engineering of taxol biosynthetic genes in *Saccharomyces cerevisiae*. Biotechnol. Bioeng. 93, 212–224.

Dexter, R., Qualley, A., Kish, C.M., Ma, C.J., Koeduka, T., Nagegowda, D., Dudareva, N., Pichersky, E., Clark, D., 2007. Characterization of a petunia acetyltransferase involved in the biosynthesis of the floral volatile isoeugenol. Plant J. 49, 265–275.

Dixon, R.A., 2005. Engineering of plant natural product pathways. Curr. Opin. Plant Biol. 8, 329–336.

Dixon, R.A., Ferreira, D., 2002. Molecules of interest: Genistein. Phytochemistry 60, 205–211.

Ferrer, J.L., Jez, J.M., Bowman, M.E., Dixon, R.A., Noel, J.P., 1999. Structure of chalcone synthase and the molecular basis of plant polyketide biosynthesis. Nat. Struct. Biol. 6, 775–784.

Franke, R., Humphreys, J.M., Hemm, M.R., Denault, J.W., Ruegger, M.O., Cusumano, J.C., Chapple, C., 2002. The Arabidopsis *REF8* gene encodes the 3-hydroxylase of phenylpropanoid metabolism. Plant J. 30, 33–45.

Fridman, E., Pichersky, E., 2005. Metabolomics, genomics, proteomics, and the identification of enzymes and their substrates and products. Curr. Opin. Plant Biol. 8, 242–248.

Gantet, P., Memelink, J., 2002. Transcription factors: tools to engineer the production of pharmacologically active plant metabolites. Trends Pharmacol. Sci. 23, 563–569.

Goldspiel, B.R., 1997. Clinical overview of the taxanes. Pharmacotherapy 17, 1105–1255.

Grotewold, E., Chamberlin, M., Snook, M., Siame, B., Butler, L., Swenson, J., Maddock, S., St Clair, G., Bowen, B., 1998. Engineering secondary metabolism in maize cells by ectopic expression of transcription factors. Plant Cell 10, 721–740.

Guterman, I., Masci, T., Chen, X., Negre, F., Pichersky, E., Dudareva, N., Weiss, D., Vainstein, A., 2006. Generation of phenylpropanoid pathway-derived volatiles in transgenic plants: rose alcohol acetyltransferase produces phenylethyl acetate and benzyl acetate in petunia flowers. Plant Mol. Biol. 60, 555–563.

Hashimoto, T., Yamada, Y., 1986. Hyoscyamine 6β-hydroxylase, a 2-oxoglutarate-dependent dioxygenase, in alkaloid-producing root cultures. Plant Physiol. 81, 619–625.

Heller, W., Kühnl, T., 1985. Elicitor induction of a microsomal 5-*O*-(4-coumaroyl)shikimate 3′-hydroxylase in parsley cell suspension cultures. Arch. Biochem. Biophys. 241, 453–460.

Hoffmann, L., Maury, S., Martz, F., Geoffroy, P., Legrand, M., 2003. Purification, cloning, and properties of an acyltransferase controlling shikimate and quinate ester intermediates in phenylpropanoid metabolism. J. Biol. Chem. 278, 95–103.

Hoffmann, L., Besseau, S., Geoffroy, P., Ritzenthaler, C., Meyer, D., Lapierre, C., Pollet, B., Legrand, M., 2004. Silencing of hydroxycinnamoyl-coenzyme A shikimate/quinate hydroxycinnamoyltransferase affects phenylpropanoid biosynthesis. Plant Cell 16, 1446–1465.

Hoffmann, L., Besseau, S., Geoffroy, P., Ritzenthaler, C., Meyer, D., Lapierre, C., Pollet, B., Legrand, M., 2005. Acyltransferase-catalysed *p*-coumarate ester formation is a committed step of lignin biosynthesis. Plant Biosyst. 139, 50–53.

Hwang, E.I., Kaneko, M., Ohnishi, Y., Horinouchi, S., 2003. Production of plant-specific flavanones by *Escherichia coli* containing an artificial gene cluster. Appl. Environ. Microbiol. 69, 2699–2706.

Jennewein, S., Croteau, R., 2001. Taxol: Biosynthesis, molecular genetics and biotechnological applications. Appl. Microbiol. Biotechnol. 57, 13–19.

Jennewein, S., Wildung, M.R., Chau, M., Walker, K., Croteau, R., 2004. Random sequencing of an induced *Taxus* cell cDNA library for identification of clones involved in Taxol biosynthesis. Proc. Natl. Acad. Sci. USA 101, 9149–9154.

Jez, J.M., Austin, M.B., Ferrer, J.L., Bowman, M.E., Schröder, J., Noel, J.P., 2000. Structural control of polyketide formation in plant-specific polyketide synthases. Chem. Biol. 7, 919–930.

Jin, H., Cominelli, E., Bailey, P., Parr, A., Mehrtens, F., Jones, J., Tonelli, C., Weisshaar, B., Martin, C., 2000. Transcriptional repression by AtMYB4 controls production of UV-protecting sunscreens in *Arabidopsis*. EMBO J. 19, 6150–6161.

Kamsteeg, J., van Brederode, J., Verschuren, P.M., van Nigtevecht, G., 1981. Identification, properties and genetic control of *p*-coumaroyl-coenzyme A 3-hydroxylase isolated from petals of *Silene dioica*. Z. Pflanzenphysiol. 102, 435–442.

Katsuyama, Y., Miyahisa, I., Funa, N., Horinouchi, S., 2007. One-pot synthesis of genistein from tyrosine by coincubation of genetically engineered *Escherichia coli* and *Saccharomyces cerevisiae* cells. Appl. Microbiol. Biotechnol. 73, 1143–1149.

Kneusel, R.E., Matern, U., Nicolay, K., 1989. Formation of *trans*-caffeoyl-CoA from *trans*-4-coumaroyl-CoA by Zn^{2+}-dependent enzymes in cultured plant cells and its activation by an elicitor-induced pH shift. Arch. Biochem. Biophys. 269, 455–462.

Koeduka, T., Fridman, E., Gang, D.R., Vassao, D.G., Jackson, B.L., Kish, C.M., Orlova, I., Spassova, S.M., Lewis, N.G., Noel, J.P., Baiga, T.J., Dudareva, N., Pichersky, E., 2006. Eugenol and isoeugenol, characteristic aromatic constituents of spices, are biosynthesized via reduction of a coniferyl alcohol ester. Proc. Natl. Acad. Sci. USA 103, 10128–10133.

Kojima, M., Takeuchi, W., 1989. Detection and characterization of *p*-coumaric acid hydroxylase in mung bean, *Vigna mungo*, seedlings. J. Biochem. 105, 265–270.

Kühnl, T., Koch, U., Heller, W., Wellmann, E., 1987. Chlorogenic acid biosynthesis: characterization of a light-induced microsomal 5-*O*-(4-coumaroyl)-D-quinate/shikimate 3′-hydroxylase from carrot (*Daucus carota* L.) cell suspension cultures. Arch. Biochem. Biophys. 258, 226–232.

Le Marchand, L., 2002. Cancer preventive effects of flavonoids – a review. Biomed. Pharmacother. 56, 296–301.

Ma, X., Koepke, J., Panjikar, S., Fritzsch, G., Stöckigt, J., 2005. Crystal structure of vinorine synthase, the first representative of the BAHD superfamily. J. Biol. Chem. 280, 13576–13583.

Mansoor, S., Amin, I., Hussain, M., Zafar, Y., Briddon, R.W., 2006. Engineering novel traits in plants through RNA interference. Trends Plant Sci. 11, 559–565.

Mathews, H., Clendennen, S.K., Caldwell, C.G., Liu, X.L., Connors, K., Matheis, N., Schuster, D.K., Menasco, D.J., Wagoner, W., Lightner, J., Wagner, D.R., 2003. Activation tagging in tomato identifies a transcriptional regulator of anthocyanin biosynthesis, modification, and transport. Plant Cell 15, 1689–1703.

Matsuno, M., Nagatsu, A., Ogihara, Y., Ellis, B.E., Mizukami, H., 2002. CYP98A6 from *Lithospermum erythrorhizon* encodes 4-coumaroyl-4′-hydroxyphenyllactic acid 3-hydroxylase involved in rosmarinic acid biosynthesis. FEBS Lett. 514, 219–224.

Mayer, A.M., 1987. Polyphenol oxidases in plants – recent progress. Phytochemistry 26, 11–20.

Memelink, J., Verpoorte, R., Kijne, J.W., 2001. ORCAnization of jasmonate responsive gene expression in alkaloid metabolism. Trends Plant Sci. 6, 212–219.

Menke, F.L.H., Champion, A., Kijne, J.W., Memelink, J., 1999. A novel jasmonate- and elicitor-responsive element in the periwinkle secondary metabolite biosynthetic gene Str interacts with a jasmonate- and elicitor-inducible AP2-domain transcription factor, ORCA2. EMBO J. 18, 4455–4463.

Mercke, P., Bengtsson, M., Bouwmeester, H.J., Posthumus, M.A., Brodelius, P.E., 2000. Molecular cloning, expression, and characterization of amorpha-4,11-diene synthase, a key enzyme of artemisinin biosynthesis in *Artemisia annua* L. Arch. Biochem. Biophys. 381, 173–180.

Middleton Jr., E., Kandaswami, C., Theoharides, T.C., 2000. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol. Rev. 52, 673–751.

Miyahisa, I., Kaneko, M., Funa, N., Kawasaki, H., Kojima, H., Ohnishi, Y., Horinouchi, S., 2005. Efficient production of (2*S*)-flavanones by *Escherichia coli* containing an artificial biosynthetic gene cluster. Appl. Microbiol. Biotechnol. 68, 498–504.

Miyahisa, I., Funa, N., Ohnishi, Y., Martens, S., Moriguchi, T., Horinouchi, S., 2006. Combinatorial biosynthesis of flavones and flavonols in *Escherichia coli*. Appl. Biotechnol. Microbiol. 71, 53–58.

Mol, J., Grotewold, E., Koes, R., 1998. How genes paint flowers and seeds. Trends Plant Sci. 3, 212–217.

Morita, H., Kondo, S., Abe, T., Noguchi, H., Sugio, S., Abe, I., Kohno, T., 2006. Crystallization and preliminary crystallographic analysis of a novel plant type III polyketide synthase that produces pentaketide chromone. Acta Cryst. F62, 899–901.

Ng, S.H., Chirgadze, D., Spiteller, D., Li, T.L., Spencer, J.B., Blundell, T.L., in press. Crystal structure of stilbene synthase from *Pinus sylvestris*.

Niggeweg, R., Michael, A., Martin, C., 2004. Engineering plants with increased levels of the antioxidant chlorogenic acid. Nat. Biotechnol. 22, 746–754.

Nijveldt, R.J., van Nood, E., van Hoorn, D.E.C., Boelens, P.G., van Norren, K., van Leeuwen, P.A.M., 2001. Flavonoids: a review of probable mechanisms of action and potential applications. Am. J. Clin. Nutr. 74, 418–425.

Noel, J.P., Austin, M.B., Bomati, E.K., 2005. Structure–function relationships in plant phenylpropanoid biosynthesis. Curr. Opin. Plant Biol. 8, 249–253.

Ogita, S., Uefuji, H., Yamaguchi, Y., Koizumi, N., Sano, H., 2003. Producing decaffeinated coffee plants. Nature 423, 823.

Ogita, S., Uefuji, H., Morimoto, M., Sano, H., 2004. Application of RNAi to confirm theobromine as the major intermediate for efficient biosynthesis in coffee plants with potential for construction of decaffeinated varieties. Plant Mol. Biol. 54, 931–941.

Oksman-Caldentey, K.M., Arroo, R., 2000. Regulation of tropane alkaloid metabolism in plants and plant cell cultures. In: Verpoorte, R., Alfermann, A.W. (Eds.), Metabolic Engineering of Plant Secondary Metabolism. Kluwer, Dordrecht, pp. 253–281.

Paine, J.A., Shipton, C.A., Chaggar, S., Howells, R.M., Kennedy, M.J., Vernon, G., Wright, S.Y., Hinchliffe, E., Adams, J.L., Silverstone, A.L., Drake, R., 2005. Improving the nutritional value of Golden Rice through increased pro-vitamin A content. Nat. Biotechnol. 23, 429–430.

Petersen, M., 1997. Cytochrome P-450-dependent hydroxylation in the biosynthesis of rosmarinic acid in *Coleus*. Phytochemistry 45, 1165–1172.

Petersen, M., Häusler, E., Karwatzki, B., Meinhard, J., 1993. Proposed biosynthetic pathway for rosmarinic acid in cell cultures of *Coleus blumei* Benth. Planta 189, 10–14.

Qi, Y., Hannon, G.J., 2005. Uncovering RNAi mechanisms in plants: Biochemistry enters the foray. FEBS Lett. 579, 5899–5903.

Ritter, H., Schulz, G.E., 2004. Structural basis for the entrance into the phenylpropanoid metabolism catalysed by phenylalanine ammonia-lyase. Plant Cell 16, 3426–3436.

Ro, D.K., Paradise, E.M., Ouellet, M., Fisher, K.J., Newman, K.L., Ndungu, J.M., Ho, K.A., Eachus, R.A., Ham, T.S., Kirby, J., Chang, M.C.Y., Withers, S.T., Shiba, Y., Sarpong, R., Keasling, J.D., 2006. Production of the antimalarial drug precursor artemisinic acid in engineered yeast. Nature 440, 940–943.

Schoch, G., Goepfert, S., Morant, M., Hehn, A., Meyer, D., Ullmann, P., Werck-Reichhart, D., 2001. CYP98A3 from *Arabidopsis thaliana* is a 3′-hydroxylase of phenolic esters, a missing link in the phenylpropanoid pathway. J. Biol. Chem. 276, 36566–36574.

Schuler, M.A., Werck-Reichhart, D., 2003. Functional genomics of P450s. Annu. Rev. Plant Biol. 54, 629–667.

Schwede, T.F., Rétey, J., Schulz, G.E., 1999. Crystal structure of histidine ammonia-lyase revealing a novel polypeptide modification as the catalytic electrophile. Biochemistry 38, 5355–5361.

Shalit, M., Guterman, I., Volpin, H., Bar, E., Tamari, T., Menda, N., Adam, Z., Zamir, D., Vainstein, A., Weiss, D., Pichersky, E., Lewinsohn, E., 2003. Volatile ester formation in roses. Identification of an acetyl-coenzyme A:geraniol/citronellol acetyltransferase in developing rose petals. Plant Physiol. 131, 1868–1876.

Shomura, Y., Torayama, I., Suh, D.Y., Xiang, T., Kita, A., Sankawa, U., Miki, K., 2005. Crystal structure of stilbene synthase from *Arachis hypogaea*. Proteins 60, 803–806.

Sriram, D., Rao, V.S., Chandrasekhara, K.V., Yogeeswari, P., 2004. Progress in the research of artemisinin and its analogues as antimalarials: an update. Nat. Prod. Rep., 503–527.

Stafford, H.A., 1974. The metabolism of aromatic compounds. Annu. Rev. Plant Physiol. 25, 459–486.

St. Pierre, B., De Luca, V., 2000. Evolution of acyltransferase genes: origin and diversification of the BAHD superfamily of acyltransferases involved in secondary metabolism. Rec. Adv. Phytochem. 34, 285–316.

Tang, G., Galili, G., 2004. Using RNAi to improve plant nutritional value: from mechanism to application. Trends Biotechnol. 22, 463–469.

Teoh, K.H., Polichuk, D.R., Reed, D.W., Nowak, G., Covello, P.S., 2006. *Artemisia annua* L. (Asteraceae) trichome-specific cDNAs reveal CYP71AV1, a cytochrome P450 with a key role in the biosynthesis of the antimalarial sesquiterpene lactone artemisinin. FEBS Lett. 580, 1411–1416.

Unterlinner, B., Lenz, R., Kutchan, T.M., 1999. Molecular cloning and functional expression of codeinone reductase: the penultimate enzyme in morphine biosynthesis in the opium poppy *Papaver somniferum*. Plant J. 18, 465–475.

Van der Fits, L., Memelink, J., 2000. ORCA3, a jasmonate-responsive transcriptional regulator of plant primary and secondary metabolism. Science 289, 295–297.

Van der Fits, L., Memelink, J., 2001. The jasmonate-inducible AP2/ERF-domain transcription factor ORCA3 activates gene expression via interaction with a jasmonate responsive promoter element. Plant J. 25, 43–53.

Van der Fits, L., Zhang, H., Menke, F.L.H., Deneka, M., Memelink, J., 2000. A *Catharanthus roseus* BPF-1 homologue interacts with an elicitor-responsive region of the secondary metabolite biosynthetic gene *Str* and is induced by elicitor via a jasmonate-independent signal transduction pathway. Plant Mol. Biol. 44, 675–685.

Vom-Endt, D., Kijne, J.W., Memelink, J., 2002. Transcription factors controlling plant secondary metabolism: what regulates the regulators? Phytochemistry 61, 107–114.

Wallaart, T.E., Bouwmeester, H.J., Hille, J., Poppinga, L., Maijers, N.C., 2001. Amorpha-4,11-diene synthase: cloning and functional expression of a key enzyme in the biosynthetic pathway of the novel antimalarial drug artemisinin. Planta 212, 460–465.

Watts, K.T., Lee, P.C., Schmidt-Dannert, C., 2004. Exploring recombinant flavonoid biosynthesis in metabolically engineered *Escherichia coli*. ChemBioChem 5, 500–507.

Wesley, S.V., Helliwell, C.A., Smith, N.A., MingBo, W., Rouse, D.T., Liu, Q., Gooding, P.S., Singh, S.P., Abbott, D., Stoutjesdijk, P.A., Robinson, S.P., Gleave, A.P., Green, A.G., Waterhouse, P.M., 2001. Construct design for efficient, effective and high throughput gene silencing in plants. Plant J. 27, 581–590.

Winkel, B.S.J., 2004. Metabolic channeling in plants. Annu. Rev. Plant Biol. 55, 85–107.

Ye, X., Al-Babili, S., Kloti, A., Zhang, J., Lucca, P., Beyer, P., Potrykus, I., 2000. Engineering provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science 287, 303–305.

Zhang, L., Ding, R., Chai, Y., Bonfill, M., Moyano, E., Oksman-Caldentey, K.M., Xu, T., Pi, Y., Wang, Z., Zhang, H., Kai, G., Liao, Z., Sun, X., Tang, K., 2004. Engineering tropane biosynthetic pathway in *Hyoscyamus niger* hairy root cultures. Proc. Natl. Acad. Sci. USA 101, 6786–6791.

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