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Review Article

SECONDARY METABOLITES AND METABOLIC ENGINEERING

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Abstract: Unraveling plant secondary metabolism is the way to successful applications in molecular farming, health food, functional food, and plant resistance. Various pathways have been altered using genes encoding biosynthetic enzymes or regulatory proteins and show enormous potential for the genetic engineering of plant secondary metabolism. Recent achievements have been made in the metabolic engineering of plant secondary metabolism. Various pathways have been altered using genes encoding biosynthetic enzymes or genes encoding regulatory proteins.

Key words: Secondary metabolism, Metabolic engineering

INTRODUCTION

Plant secondary metabolism has multiple functions throughout the plant's life cycle. These functions can be classified as mediators in the interaction of the plant with its environment, such as plant–insect, plant–microorganism and plant–plant interactions[1,2]. The production of secondary metabolites forms part of the plant's defence system, for example, the constitutive production of antifeedants and phytoanticipins, and the inducible production of phytoalexins [2]. Secondary metabolism also plays a role in plant reproduction, for example, in attracting pollinators and in male fertility. Secondary metabolites determine important aspects of human food quality (taste, colour and smell), and plant pigments are important for the diversity of ornamental plants and flowers. Moreover, several plant secondary metabolites are used for the production of medicines, dyes, insecticides, flavours and fragrances.

SECONDARY PRODUCTS

The sum of all of the chemical reactions that take place in an organism constitutes metabolism. Metabolism is a dynamic process where molecules are constantly turning over the composition of a cell at any given time represent the balance between synthesis and degradation. Much of that carbon and energy ends up in protein, nucleic acids, lipids and other molecules that are common to all organisms, but in plants a significant proportion of assimilated carbon and energy is diverted to the synthesis of molecules that may have no obvious role in growth and development. The molecules are known as secondary product. The latter generally occurs in low quality and their production may be widespread or restricted to particular families, genera, or even species. The secondary products with an emphasis on the biosynthesis physiology and ecological roles are of four major classes viz., (i) terpenes, phenolic compounds, (ii) saponins, (iii) cardiacglycosis, cyanogenic glycosides, glucosinolates and (iv) alkaloids.

(i) Terpenoids: The terpenoids are a functionally and chemically diverse group of molecules. They are generally lipophilic substances derived from a single five carbon unit. The terpenoids family includes hormones (gibberellins and abscisic acid); the carotenoid pigments sterols e.g. ergosterol, sitosteral, cholesterol and sterol derivatives (Fig. 1) and many of the essential oils that give plants their distinctive odours and flavours. All terpenes and terpene derivative share a common biosynthetic pathways called mevalonic acid pathway after a key intermediate.

Terpenoids biosynthesis begins with acetyl coenzyme-A, an intermediate in the respiratory breakdown of carbohydrate and fatty acid metabolism. Three molecules of acetyl-CoA condense in a two step reaction to form hydroxymethylglutaryl CoA, which is then reduced to mevalonic and MVA then undergoes a two step phosphorylation, at the expense of two molecules of ATP to form C6 mevalonic acid pyrophosphate. Two more condensation with IPP give rise first to the C15 intermediate farnesyl pyrophosphate and then to the Cro geranyl geranyl pyrophosphate. FPP is a branch point that can give rise to C15 sesquiterpenes possibly including abscisic acid. The vast majority of terpenoids are secondary metabolite many of which appear to act as toxins or feeding deterrents to herbivorous insects.

Steroids and Sterols: Steroids are known as triterpenoids. They are synthesized from the acylic triterpenoid and qualana. Steroids with an alcohol group are known as sterols. The most abundant sterols in higher plants are stigmasterol and β-sitosterol which together often constitute more than 70% of the total sterols. Sterols are planner molecule and their packing tend to increase the viscosity and stability of membranes. Some sterols may have a protective function, such as the phytoecolysones, which have structure similar to the insect molting hormones when ingested by insect herbivores, phyto-ecdysores disrupt the insects molting cycle (Fig.2).

Saponins: Saponins are terpene glycosides. They may be steroid glycosides, steroid alkaloids sides triterpene glycosides. They may occur as aglycones which are known as sapogenins. The combination of hydrophobic triterpene with a hydrophilic sugar gives saponines the properties of a surfactant or detergent. The name saponine is derived from the soapwort or bouncing bet which at one time was employed as a soap substitute. Commercially, Saponins from the bark of Quillaja saponaria have been used as surfactants in photographic film in shampoo, liquid detergents and beverages (Fig. 3).

Cardiac glycosides: Cardiac glycosides or cardinolides have a wide distribution. *Digitalis purpurea, D. lanata*. The two principal cardinolides in digitalis are digitoxin and its close analog digoxin. Digitalis is also the source of a saponin, digitonin (Fig.4). Digitalis has been used for its therapeutic value in treating heart condition such as arteriosclerosis. Another common source of cardionlides are the milkweeds *Asclepias* and *Calotropis*.

Phenolics: The shikimic acid pathway gives rise to the aromatic amino acids which in turn, may be directed towards either primary or secondary products. The family of secondary metabolites derived from the aromatic amino acids are known generally as phenolics, polyphenols or phenylpropane derivates.

Phenolics or polyphenols are a large and chemically diverse family of compounds ranging from simple phenolic acids to very large and complex polymers such as tannins and lignin. The biosynthesis of most phenolics being with the tryptophan. The aromatic amino acids are in turn, synthesized from phosphoenolpyruvate and erythrose-4-phosphate by a sequence of reactions, known as the shikimic acid pathway. The latter is common to bacteria, fungi and plant but is not found in animals. Phenylalanine and tryptophan are consequently among the ten amino acids. Considered essential to animals and represent the principal source of all aromatic molecules in animals. Synthesis of the aromatic amino acids begin with the condensation of one molecule of erythrose-4-P from the pentose phosphate respiratory pathway with one molecule of PEP from glycolysis. Resulting 7-carbon sugar is then cyclized and then reduced to form shikimate. Phenolic acids are plant metabolites widely spread throughout the plant kingdom. Recent interest in phenolic acids stems from their potential protective role, through ingestion of fruits and vegetables, against oxidative damage diseases (coronary heart disease, stroke, and cancers). Phenolic compounds are essential for the growth and production of plants, and are produced as a response for defending injured plants against pathogens. Phenolic acid compounds seem to be universally distributed in plants. They have been the subject of a great number of chemical, biological, agricultural, and medical studies. Phenolic acids form a diverse group that includes the widely distributed hydroxybenzoic and hydroxycinnamicacids (Fig. 5).

Plant phenolic compounds are diverse in structure but are characterized by hydroxylated aromatic rings (e.g. flavan-3-ols) phenol, the parent compound, used as an disinfectant and for chemical synthesis. Examples of phenolic compounds are capsaicin, the pungent compound of chilli peppers, tyrosine, an amino acid, the neurotransmitters serotonin, dopamine, adrenaline, and noradrenaline. L-DOPA, a drug to treat Parkinson's disease. Eugenol, the main constituent of the essential oil of clove, chavibetol from betel, estradiol and other estrogens and alkaloid of psilocybe mushrooms.

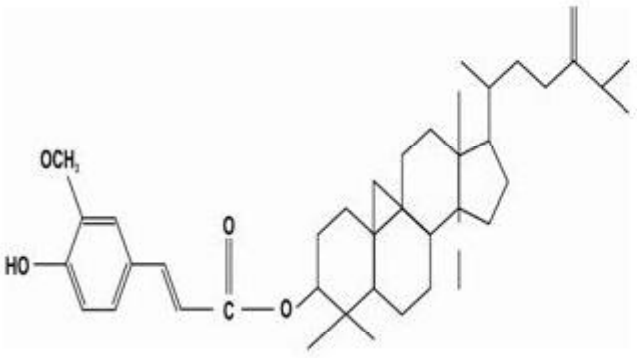

Fig .1 Structure of Terpenoids ORYZANOL

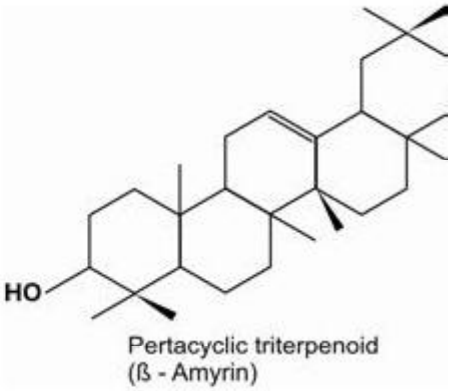

Fig.2

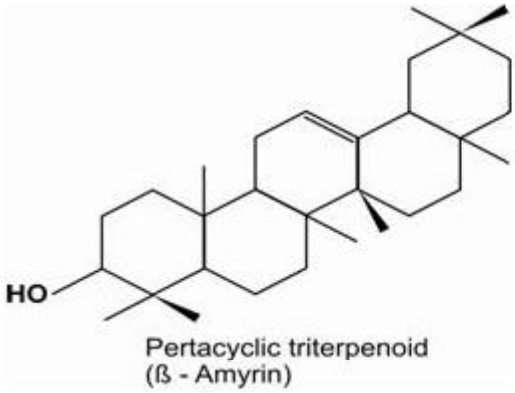

Fig. 3

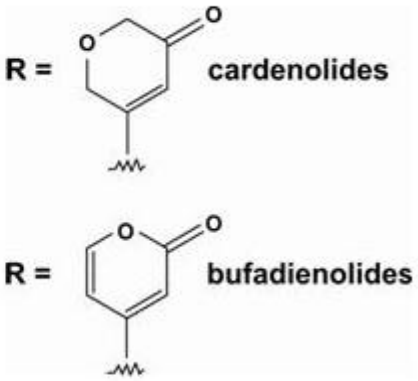

Fig.4

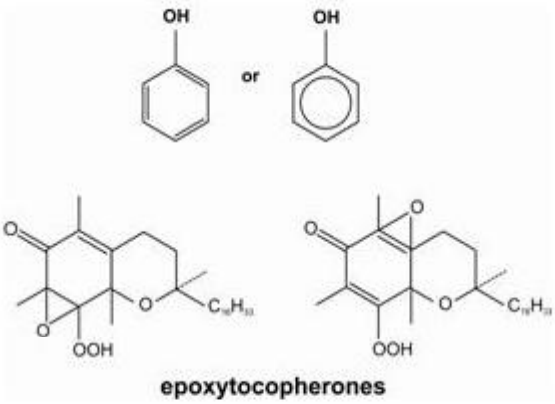

Fig.5

Coumarins: The Coumarins are a widespread group of lactone formed by ring closure of hydroxycinnamic acid. Coumarin it self is not toxic, it can be converted by fungi to a toxic product dicoumarol, that is typically found in moldy hay. Dicoumarel causes fatal hemorrhaging in cattle by inhibiting vitamin K, an essential cofactor in blood clotting (Figs. 6 A,B).

Lignin: Lignin is a highly branched polymer of three simple phenolic alcohols. Lymnospermum lignin is comprised mainly of coniferyl alcohol sub units while an comprised mainly of subunits while angiosperm lignin is a mixture of coniferyl and sinapyl alcohol subunits. The alcohols are oxidised to free radical by plant enzyme peroxidase. Lignin is found in cell walls, especially the secondary walls of tracheary element. The elements in the xylem, where it contributes mechanical strength and rigidity to woody stems. Lignins and other phenolic derivatives accumulate at the site of fungal penetration presumably slowing the rate of cell degradation.

Tannins: The name tannin is derived from use of plant extract to tan animal hides that is to convert hides to leather. Condensed tannins are polymers of flavonoid units linked by strong carbon bonds. These bonds are not subject to hydrolysis but can be oxidized by strong acid to release anthocyanidins.

Alkaloids: Alkaloids, the term is linguistically derived from the Arabic word a/-qali, the plant from which soda was first obtained are nitrogenous compounds that constitute the pharmacologically active "basic principles" of predominantly, although not exclusively, flowering plants. Since the identification of the first alkaloid, morphine, from the opium poppy, *Papaver somniferum,* by Sertürner in 1806, 10,000 alkaloids have been isolated and their structures elucidated [3]. Historically, the use of alkaloid-containing plant extracts as potions, medicines, and poisons can be traced back almost to the start of civilization. Famed examples include Socrates' death in 399 B.C. by consumption of coniine-containing hemlock (*Conium maculatum*) and Cleopatra's use during the last century B.C. of atropine-containing extracts of Egyptian henbane (*Hyoscyamus muficus*) to dilate her pupils and thereby appear more alluring. Medieval European women utilized extracts of deadly nightshade, *Atropa belladonna*, for the same purpose, hence the name belladonna. Although coniine is too toxic to find therapeutic use today outside of homeopathy, tropicamide, an anticholinergic that is a synthetic derivative of atropine, is routinely used in eye examinations to dilate the pupil. Tropicamide has also recently shown promise as an early diagnostic tool in the detection of Alzheimer's disease [4]. A tonic prepared from the bark of *Cinchona officinalis* that contains the antimalarial drug quinine greatly facilitated European exploration and inhabitation of the tropics during the past two centuries.

The alkaloids are a very large and heterogeneous family of secondary metabolites that are of interest primarily because of their pharmacological properties and medical applications. Alkaloids generate vary degrees of physiological and psychological response in human largely by interfering with neurotransmitters. Alkaloids or alkaloid rich extract have been used for variety of pharmacological purpose such as muscle relaxant, tranquilizer pain killer and mind altering drug. Most alkaloids are synthesized from a few common amino acids (tyrosin, tryptophan and lysine).

In total, about 13,000 plant species are known to have been used as drugs throughout the world [5]. Approximately 25% of contemporary materia medica is derived from plants and used either as pure compounds (such as the narcotic analgesic morphine, the analgesic and antitussive codeine, and the chem-otherapeutic agents vincristine and vinblastine) oras teas and extracts (Fig.7). Plant constituents have also served as models for modern synthetic drugs, such as atropine for tropicamide, quinine for chloroquine, and cocaine for procaine and tetracaine. In fact, active plant extract screening programs continue to result in new drug discoveries. The most recent examples of anticancer alkaloids are taxo1 from the western yew, *Taxus brevifolia*, and camptothecin (and derivatives currently in clinical trials) from the Chinese "happy tree," xi shu (*Camptotheca acuminata*), both of which were originally isolated and assayed for biological activity. In other areas, there are intense searches for nove 1 antivirals and antimalarials. Stimulants like caffeine in coffee and tea and nicotine in cigarettes are the alkaloids.

Alkaloids belong to the broad category of secondary metabolites. This class of molecule has historically been defined as naturally occurring substances that are not vital to the organism that produces them. Alkaloids have traditionally been of interest only due to their pronounced and various physiological activities in animals and humans. A picture has now begun to emerge that alkaloids do have important ecochemical functions in the defense of the plant against path-ogenic organisms and herbivores or, as in the case of

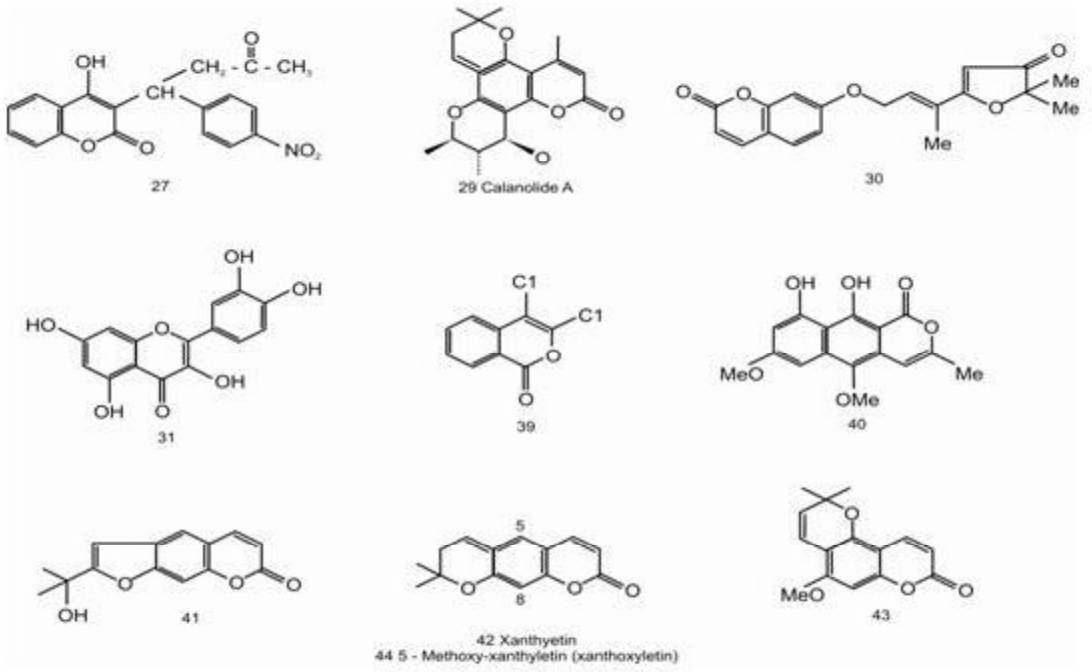

Fig.6 A Natural Isocoumarins

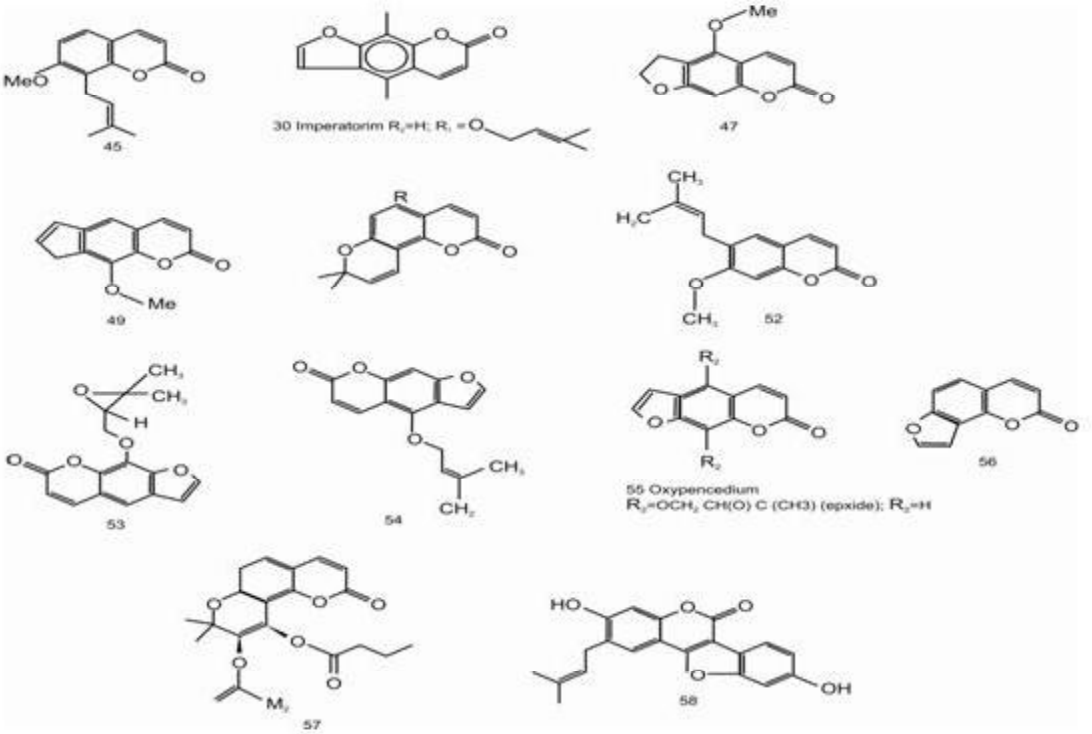

Fig.6 B Natural Furanocoumarins

pyrrolizidine alkaloids, as pro-toxins for insects, which further modifiy the alkaloids and then incorporate them into their own defense secretions [6].

Metabolic engineering: Metabolic engineering is a promising approach for the value addition of the medicinal and economically important crops. In the past 10 years quite some research has been done in this field [7], a major constraint has been the poor characterization of plant secondary metabolic pathways at the level of biosynthetic inter-mediates and enzymes. During 1970s the use of plant cell cultures as experimental systems was introduced and since then, 80 new enzymes that catalyze steps in the biosynthesis of the indole, isoquinoline, tropane, pyrrolizidine, and purine classes of alkaloids have been discovered and partially characterized. The best-studied pathway at the genetic level is the one leading to the formation of flavonoids and anthocyanins [8,9]. Most of the genes in the anthocyanin pathway have been cloned. This achievement was facilitated by the fact that flower colour is an easily screenable phenotype. Other secondary metabolite pathways that have extensively been studied at the level of intermediates and enzymes mainly lead to pharmaceutically important products such as indole and isoquinoline alkaloids [10,11]. From these pathways, however, only a relatively small number of genes have been identified so far.

Genetic engineering of a secondary metabolic pathway aims to either increase or decrease the quantity of a certain compound or group of compounds [4,7]. To decrease the production of a certain unwanted group of compound(s) several approaches are possible. An enzymatic step in the pathway can be knocked out, for example, by reducing the level of the corresponding mRNA via antisense, cosuppression or RNA interference technologies, or by overexpressing an antibody against the enzyme. The antisense gene approach has been successfully used for changing flower colours [12].

Flavonoids and anthocyanins: Flavonoid and anthocyanin biosynthesis was the first target for genetic engineering [9]. Numerous experiments have been performed involving the overexpression of various pathway genes, aiming, for example, to produce new flower colours by introducing new compounds in the plant. Higher levels of anthocyanins and flavonoids in food are an interesting objective because of their antioxidant activity. Many studies are underway in tomato. Chalcone isomerase (CHI), an early enzyme of the flavonoid pathway, was found to be the key to increase flavonol production [13]. Overexpression of the Petunia CHI gene led to a 78-fold increase of flavonoid levels in the tomato peel. Upon processing such tomatoes, a 21-fold increase of flavonols in tomato paste was achieved, if compared with non-transgenic controls. Except for the flavonoid content, neither transgenic plants nor the paste of the transgenic tomatoes could be distinguished from their respective controls. Thus showing that it is feasible to increase the level of compounds that are beneficial for health in tomato-based products.

Alkaloids (indole alkaloids biosynthesis genes): Fifteen terpenoid indole alkaloids are industrially important, including the antitumour alkaloids vinblastine, vincristine and camptothecin owing to this fact, the terpenoid indole alkaloid pathway has been the target of numerous genetic engineering attempts. These alkaloids share the pathway leading to the intermediate strictosidine, and from this point the pathways in various alkaloid-producing plant species diverge. The extensive efforts have been concentrated on mapping the early part of the pathway and on over-expression of early genes, aiming to increase the metabolic flux into the alkaloid pathway. In particular, the genes encoding tryptophan decarboxylase (TDC) and strictosidine synthase (STR) have been studied extensively in *Catharanthus roseus* cell cultures. Overexpression of TDC resulted in higher levels of the immediate product tryptamine, but not in increased levels of alkaloids; in the case of STR higher levels of alkaloids was recorded [3]. Feeding such cell lines with tryptophan and terpenoid intermediates showed that they have the capacity for high alkaloid production (up to 1100 μmol/L) [14], indicating that the terpenoid branch of the pathway is limiting. Such studies indicate that there may be multiple rate-limiting steps.

Tropane alkaloids and pyrrolidine alkaloids: Extensive research has already been done on the genetic engineering of the pharmaceutically important tropane alkaloids [15]. In particular, the conversion of hyoscyamine to the much more valuable scopolamine is the major goal of these studies. The enzyme hyoscyamine-6β-hydroxylase (H6H) catalyzes this conversion. By the overexpression of the gene encoding H6H in *Hyoscyamus muticus* hairy root cultures, a 100-fold increase of scopolamine levels could be reached compared with controls that produced hyoscyamine as the major alkaloid [16].

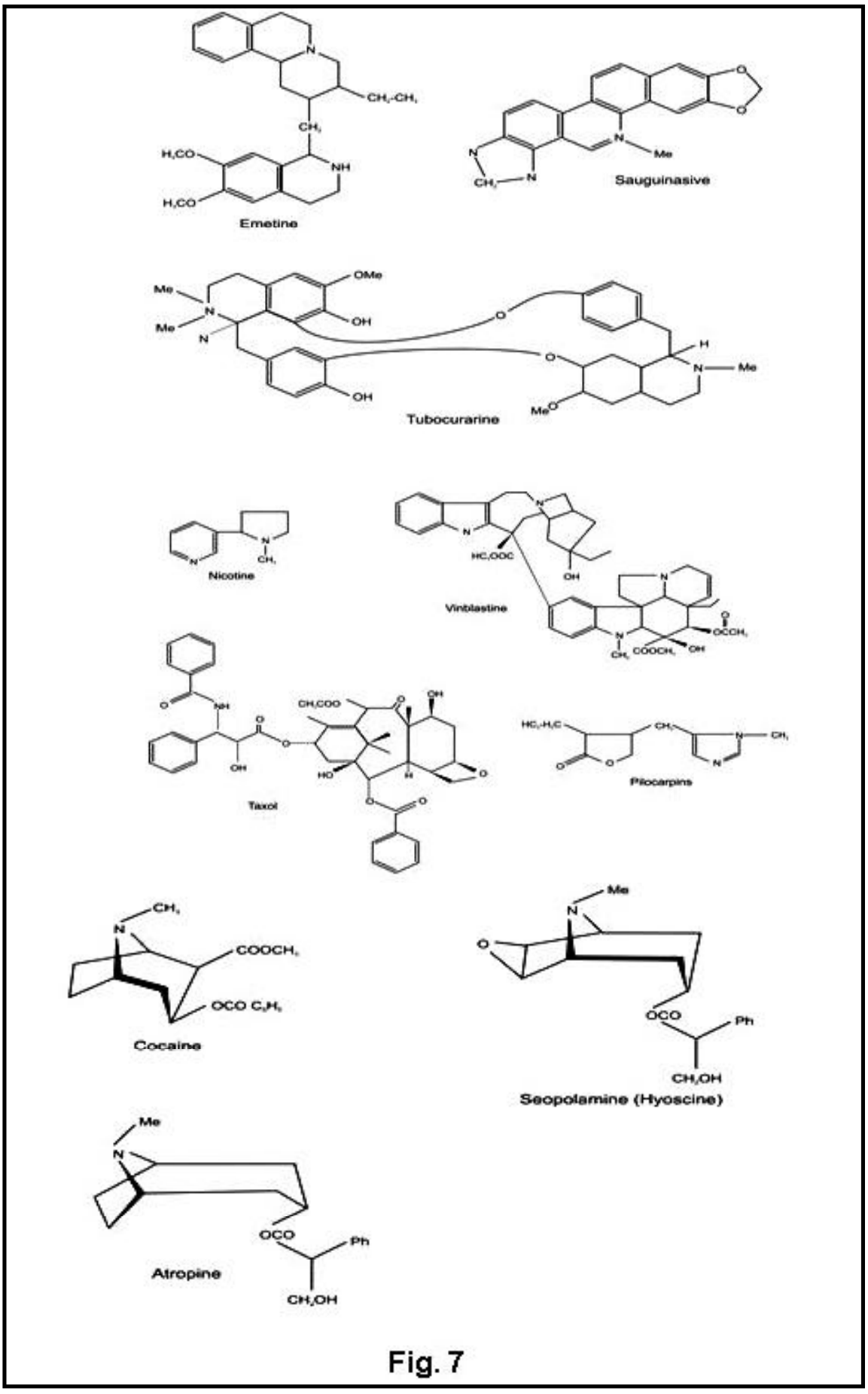
Emetine
Sauguinasive
Tubocurarine
Nicotine
Vinblastine
Taxol
Pilocarpins
Cocaine
Seopolamine (Hyoscine)
Atropine

Fig. 7

The hyoscyamine level (about 10-fold higher than for scopolamine in the transgenic roots) was similar in transgenic and control cell lines. Our group has also worked in this area and we have overexpressed adc/odc genes in *Datura innoxia* and has increased the level of hyosyamine by 89% [17].

Isoquinoline alkaloids: Pharmaceutically important group of plant secondary metabolites comprises the isoquinoline alkaloids, which include, among others, the important medicines morphine and codeine. The pathways to several of these alkaloids have been elucidated in the past, opening the way for metabolic engineering. Yamada and co-workers [18] hypothesized that overexpression of an enzyme at a branch-point in a pathway should lead to an increased flux through the affected branch. In the biosynthesis of berberine, the enzyme (S)-scoulerine 9-O-methyltransferase (SMT) is such an enzyme that might control the ratio of coptisine and berberine plus columbamine in *Coptis japonica* cells . Over-expression of this gene resulted in a 20% increase in enzyme activity, with an increase of berberine and columbamine from 79% of the total alkaloid content in wild-type cells to 91% in transgenic cells. This observation proves that fluxes at a branch point can be changed by metabolic engineering. Overexpression of *C. japonica* SMT gene in a plant cell culture of *Eschscholzia californica*, a plant lacking this enzyme, resulted in the production of columbamine.

Monoterpenes and sesquiterpenes: Terpenoids are by far the largest group of plant secondary metabolites. Following the recent discovery of the role of the 2-C-methyl-D-erythritol-4-phospate (MEP) pathway in the biosynthesis of plastidial terpenoids, such as the carotenoids and monoterpenes and diterpenes, several genes of this pathway have been cloned [10,19,20]. Modifying the MEP pathway is potentially useful for a wide range of applications. A co-suppression and an antisense strategy used to knock out a cytochrome P450 enzyme in tobacco trichome glands, for example, conferred an increased resistance to aphids [19]. There was a clear shift in the cembranoid spectrum, with a 19-fold increase of the diterpenoid cembratriene-ol and a decrease in its oxidation product cembratriene-diol. In another example, overexpression of a chimeric farnesyl diphosphate synthase gene in Artemisia annua was reported to increase the flux in the sesquiterpenoid biosynthetic pathway leading to a twofold to threefold increase in the antimalaria drug [7]. In tomatoes, overexpression of an S-linalool synthase transgene increased many fold the production of the monoterpenoid flavor compound S-linalool, compared with control plants, although no changes in the levels of other terpenoids were observed [21].

Carotenoids: Carotenoid biosynthetic pathway is an interesting target for genetic engineering as carotenoids are important colour compounds in flowers, food and fruits, they are also antioxidants, and vitamin A is formed from β-carotene [22]. Vitamin A deficiency is widespread. The introduction of β-carotene biosynthesis into the major staple food rice, by over-expression of phytoene synthase, phytoene desaturase and lycopene β-cyclase, is thus an important achievement [8]. β-Carotene (provitamin A) levels of 2 mg/kg dry rice endosperm were achieved. In contrast to rice, where over-expression of a plant phytoene synthase (originating from narcissus) only results in the production of phytoene, in canola seed specific overexpression of the bacterial gene results in a 50-fold increase of β-carotene levels in seeds [23]. In tomato, β-carotene content was increased up to threefold by overexpression of a bacterial phytoene desaturase in tomato plastids. However, the total carotenoid content, including the direct product of the enzyme lycopene, was decreased [13]. Several of the carotenoid enzymes were upregulated. The decrease of total carotenoid content is probably due to feedback inhibition some where in the pathway. The expression of a bacterial phytoene synthase in tomato fruits resulted in a two fold to four fold increase of total carotenoids. Levels of other plastidial isoprenoids were not affected, neither were the activities of various enzymes in the pathway [24]. Overexpression of the lycopene β-cyclase gene (βLcy) using a specific promoter increased levels of the direct product of the enzyme, β-carotene, in tomatoes sev-enfold [25]. By introduction of an algal gene encoding β-carotene ketolase into tobacco, astaxanthin production was achieved in chromoplasts, mainly in the nectaries. The total carotenoid level was increased in the flowers of the transgenic plants [26].

Benzoic acid derivatives: Salicylic acid (SA) is an important signalling molecule in plants involved in systemic acquired resistance after challenge with plant pathogens. Although the evidence is incomplete, it has long been thought that this compound was formed via phenylalanine. More recently, it was discovered that SA produced in response to pathogen infection is formed from chorismate via conversion to isochorismate by isochorismate synthase (ICS)

[27]. Microorganisms produce SA from chorismate via conversion to isochorismate by ICS followed by the splitting off of the pyruvate group by isochorismate pyruvate lyase (IPL). Plants overexpressing the bacterial enzymes ICS and IPL in the plastids showed a normal phenotype, but an increased resistance against viral and fungal infections [11]. The SA level was increased 1000-fold compared with wild-type plants, but growth was not affected. It seems that the second enzyme, IPL, is the rate-limiting step for SA formation. A fused protein of two bacterial enzymes has been constructed and was introduced into *Arabidopsis* [28].The plants with the fused enzyme in the cytosol showed a two- to three-fold increase in SA levels, whereas after expression in plastids a 20-fold increase was observed. The plants clearly showed reduced growth, which might have been due to depletion of isochorismate for phylloquinone production. In the transgenic tobacco IPL activity was limiting for SA production [29], therefore, the overexpressed ICS produced sufficient isochorismate for both SA and phylloquinone biosynthesis. This example shows that engineering of two or more steps in a pathway requires a correct balance between the activity of the overexpressed enzymes, to avoid affecting negatively the precursor availability for other pathways.

Cyanogenic glucosides: An example of the expression of a complete secondary metabolite biosynthesis pathway in a heterologous plant species is provided by the expression of cyanogenic glucoside biosynthesis genes from Sorghum bicolor in Arabidopsis [34]. Sorghum contains the cyanogenic glucoside dhurrin, which is hydrolysed by a β-glucosidase upon tissue damage. The resulting cyanide release is an effective pest deterrent and insecticide. Dhurrin is synthesised from tyrosine via the action of two multifunctional cytochrome P450 enzymes (CYPs) and a specific UDPG-glucosyltransferase. Overexpression of the first enzyme (CYP79A1) of the pathway in Arabidopsis led to the formation of p-hydroxybenzylglucosinolates, which are not native to this plant species [35,36]. Arabidopsis plants overexpressing both Sorghum CYP genes produced various glucosides of p-hydroxybenzoic acid, formed after decomposition of the nitrile, but no dhurrin. Apparently, none of the many glucosyltransferases occurring in Arabidopsis was able to glucosylate p-hydroxymandelonitrile to form dhurrin. The overexpression of the specific Sorghum glucosyltransferase in combination with the two CYP genes resulted in dhurrin production in Arabidopsis. The dhurrin-producing transgenic Arabidopsis released high levels of cyanide upon tissue damage, indicating that dhurrin is hydrolysed by an endogenous β-glucosidase. Leaf tissue from the transgenic Arabidopsis plants was rejected by larvae of the flea beetle *Phyllotreta nemorum*, and larvae feeding on transgenic leaves died. High levels of a foreign metabolite were thus produced in a plant species without negative effects on growth and with positive effects on resistance against pests. The results show that an entire biosynthetic pathway can be transferred to a heterologous plant species, and suggest that it may be possible to transfer other pathways in the future [37].

More often, the goal is to increase the production of certain compounds in the normal producing plant species or to transfer (part of) a pathway to other plant species or other microorganisms. Also, there is interest in the production of novel compounds not yet produced in nature by plants. To increase the production of a (group of) compound(s), two general approaches have been followed. Firstly, methods have been employed to change the expression of one or a few genes, thereby overcoming specific rate-limiting steps in the pathway, to shut down competitive pathways, and to decrease catabolism of the product of interest. Secondly, attempts have been made to change the expression of regulatory genes that control multiple biosynthesis genes. Recent examples of the different approaches are reviewed here, covering several major secondary metabolite groups.

Challenges ahead: In the past few years, several secondary metabolism genes have been overexpressed in the original plant or in other plant species. In some cases, overexpression resulted in an improved production of the desired products, whereas in other cases only an increase in the level of the direct product of the overexpressed enzyme was achieved. However, from these results no general conclusions can be drawn about the chance that a certain approach will be successful. This is partly because the differences in transgene expression levels in individual transformants are not well understood, and partly because of differences observed between different plant species. In several cases, overexpression results in the production of unexpected products, demonstrating the complexity of the metabolic networks and our lack of knowledge of these networks and their regulation.

The picture that evolves from the studies on biosynthetic pathways and metabolic engineering is that once the plant cell factory has been assembled, based on the genetic information present in the cell and on its environment, the cells can produce all kind of compounds, without the need for further genetic regulation. The fluxes through the pathways are controlled to a great extent at the level of enzymes and intracellular and intercellular transport. Fluxes through metabolic pathways are thus not only determined by gene expression levels, but also by post-translational regulation of enzyme activity and enzyme and metabolite compartmentation and transport. The major challenge for the coming years is to obtain more information about regulation at all these levels — genes, enzymes, compartmentation, transport and accumulation. This will open the way for successful strategies for altering the accumulation of certain compounds.

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