Mol Biotechnol (2010) 46:94–104
DOI 10.1007/s12033-010-9257-6

REVIEW

Production of Plant Bioactive Triterpenoid Saponins: Elicitation Strategies and Target Genes to Improve Yields

Anna C. A. Yendo · Fernanda de Costa · Grace Gosmann · Arthur G. Fett-Neto

Published online: 4 March 2010

Abstract Triterpenoid saponins are a class of plant secondary metabolites with structure derived from the precursor oxidosqualene in which one or more sugar residues are added. They have a wide range of pharmacological applications, such as antiplatelet, hypocholesterolemic, antitumoral, anti-HIV, immunoadjuvant, anti-inflammatory, antibacterial, insecticide, fungicide and anti-leishmanial agents. Their accumulation in plant cells is stimulated in response to changes mediated by biotic and abiotic elicitors. The enhancement of saponin yields by methyl jasmonate in plants and cell cultures in several species indicates the involvement of these metabolites in plant defence mechanisms. The elucidation of their biosynthesis at the molecular level has advanced recently. Most studies to date have focused on the participation of early enzymes in the pathway, including oxidosqualene cyclase, squalene synthase and dammarenediol synthase, as well as in isolating and characterizing genes that encode β-amyrin synthase. Yields of bioactive saponins in various plant species and experimental systems have been successfully increased by treating cells and tissues with jasmonate or by exposing these to oxidative stress. These elicitation and molecular studies are consolidating a robust knowledge platform from which to launch the development of improved sources for commercial supply of bioactive saponins.

Keywords Biosynthetic pathways · β-Amyrin synthase · Methyl jasmonate · Ginsenosides · 2,3-Oxidosqualene

A. C. A. Yendo · F. de Costa
Department of Botany, Graduate Program in Botany, Federal University of Rio Grande do Sul (UFRGS), Av. Bento Gonçalves 9500, Porto Alegre, RS 91501-970, Brazil

G. Gosmann
Graduate Program in Pharmaceutical Sciences, Faculty of Pharmacy, UFRGS, Av. Ipiranga 2752, Porto Alegre, RS 90610-000, Brazil

A. G. Fett-Neto (✉)
Plant Physiology Laboratory, Graduate Program in Cell and Molecular Biology, Center for Biotechnology, Federal University of Rio Grande do Sul (UFRGS), P.O. Box 15005, Porto Alegre, RS 91501-970, Brazil
e-mail: fettneto@cbiot.ufrgs.br

Saponin Structure and Occurrence

Saponins are a class of high molecular weight secondary metabolites widely distributed in plants. Their relevance is a consequence of their industrial use and potential pharmacological activity as antiplatelet, hypocholesterolemic, antitumoral [1–3], anti-HIV [3], immunoadjuvant [4, 5], anti-inflammatory [6, 7], antibacterial, insecticide, fungicide [8] and anti-leishmanial [9, 10] agents.

These metabolites are characterized by the presence of a skeleton derived from a 30-carbon 2,3-oxidosqualene precursor. Besides showing a complex and diverse molecular structure, they can be classified in two subgroups, according to their polar nucleus, known as aglycone or sapogenin; triterpenoid (C30) or steroidal (C18–C29). This polar nucleus, linked to one or more sugar residues, is responsible for the main characteristics of these compounds, i.e. the amphiphilic nature and the marked ability to form foam in water [11].

Secondary metabolites, which often occur in low concentration, do not participate directly in plant growth and development. Their production and accumulation are frequently stimulated in response to environmental changes [12]. One of the most important roles of secondary metabolites in plants is to protect them from herbivore and

pathogen attacks and to improve their survival under abiotic stress. Therefore, some strategies and molecular targets for the production of metabolites in culture based on this principle have been developed in order to increase yields of secondary metabolites of interest. This approach represents an important step in understanding secondary metabolite biosynthesis and achieving sustained production of these molecules.

The various biological properties, allied to the diversity of the reported compounds, make saponins promising candidates for the development of pharmaceutical and/or nutraceuticals in natural or synthetic forms. There is significant demand for saponins, particularly due to their presence in phytomedicines and as modern immunoadjuvants in commercial vaccines. The annual global market for herbal medicines was over US$ 63 billion in 2003 [13], and the ever growing vaccine market is approaching US$ 15 billion [14]. Since the knowledge of agents and conditions that stimulate saponin accumulation is of great interest, several studies have been carried out with that aim. The elucidation of the steps of their biosynthesis at the molecular level is well underway. So far, however, cell and tissue culture systems have failed to afford commercially attractive yields of these metabolites. This review presents and discusses the literature reported in recent years aiming at the identification of key factors and molecular targets, which control the biosynthesis and accumulation of bioactive triterpenoid saponins in plants. This information will hopefully aid in the design of protocols to improve sustainable production of these molecules of interest.

Biosynthesis of Triterpenoid Saponins

Earlier Steps: IPP, FPP, Squalene, 2,3-Oxidosqualene, Triterpenoid Skeleton

Triterpenoid saponins are synthesized starting from the isoprenoid pathway through farnesyl diphosphate (FPP) by the cyclization of 2,3-oxidosqualene, leading to the formation of triterpenoid skeletons such as oleanane (β-amyrin), ursane (α-amyrin), lupeol or dammarane. The subsequent structural modifications, like oxidation, substitution and glycosylation, mediated by various enzymes, result in different skeletons [15].

Saponin biosynthesis derives from the isoprenoid pathway, in which two molecules of isopentenyl diphosphate are condensed with one molecule of dimethylallyl diphosphate by the head to tail link, creating the molecule FPP (15C). Two FPP molecules are joined by the link tail to tail, originating the 30 carbon product squalene, a reaction catalysed by squalene synthase (SS). Squalene is oxidized by squalene epoxidase (SE) to 2,3-oxidosqualene, the first step leading to the cyclizations, which will originate the triterpenoid saponins [11]. Their principal route of formation is from β-amyrin, one of the most common triterpenes in plants. It is made of a pentacyclic carbon skeleton, derived from the precursor 2,3-squalene after a series of processes mediated by 2,3-oxidosqualene cyclase (OSC) and β-amyrin synthase (B-AS) (Fig. 1).

Ginsenosides are likely the best studied bioactive saponins at the molecular level to date. Their aglycones can be classified as oleananes and dammaranes. The first step for the ginsenoside pathway of the dammarane-type skeleton is the cycling of 2,3-oxidosqualene to dammarenediol-II, a reaction catalysed by an enzyme from the squalene group: dammarenediol synthase (DDS) (Fig. 2). Han et al. [16] investigated the expression of DDS and other genes involved in ginsenoside biosynthesis. The silencing of the gene encoding DDS led to an 84.5% decrease in ginsenoside production.

Later Steps: Oxygenation and Glycosylation of the Triterpene Skeleton

In an effort to characterize the genes involved in ginsenoside biosynthesis, about 12,000 expressed sequence tags (ESTs) of five *Panax ginseng* libraries were sequenced, yielding candidate genes for 4 oxydosqualene cyclases, 9 cytochrome P450 oxygenases and 12 glycosyltransferases (GTs) [17]. It is believed that a hydroxylation by a cytochrome P450 dependent enzyme [18] and glycosylation via GT, as well as subsequent formation of ginsenosides [19], take place after cyclization.

Glycosyltransferases transfer a glycosyl group from a donator to a hydroxyl group of a receptor and, in general, the ones that employ secondary metabolites as substrate constitute a small fraction of the total protein in plant cells and have an important role in the final steps of biosynthesis. Yue and Zhong [20] isolated a GT of *Panax notoginseng*. Named UGRdGT, this enzyme is involved in the synthesis of Rb1 ginsenoside from Rd.

An overview scheme of some triterpenoid saponins derived from β-amyrin in plants is shown in Fig. 3. In spite of the large structural diversity of the various metabolites, the sequential reactions of oxygenation and glycosylation in the production of saponins are a common feature among pathways.

Genes and Metabolic Engineering Strategies of Triterpenoid Saponin Biosynthesis

The application of technologies to obtain desired metabolic profiles has been based on the balance between result output and application flexibility. Nevertheless, many

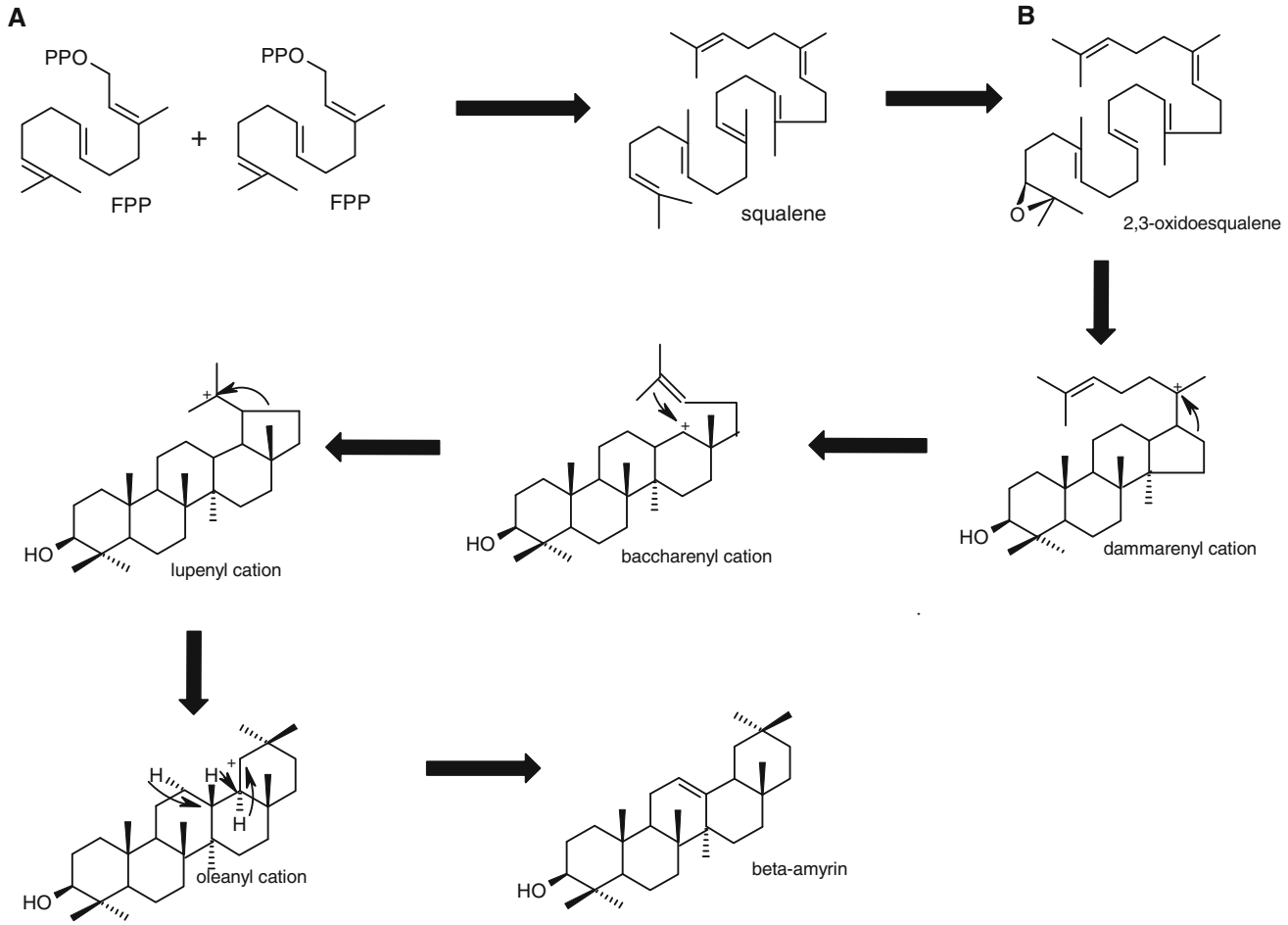

Fig. 1 **a** Synthesis of squalene from condensation of farnesildiphosphate (FPP). **b** Cyclization of 2,3-oxidosqualene to triterpene β-amyrin mediated by β-amyrin synthase

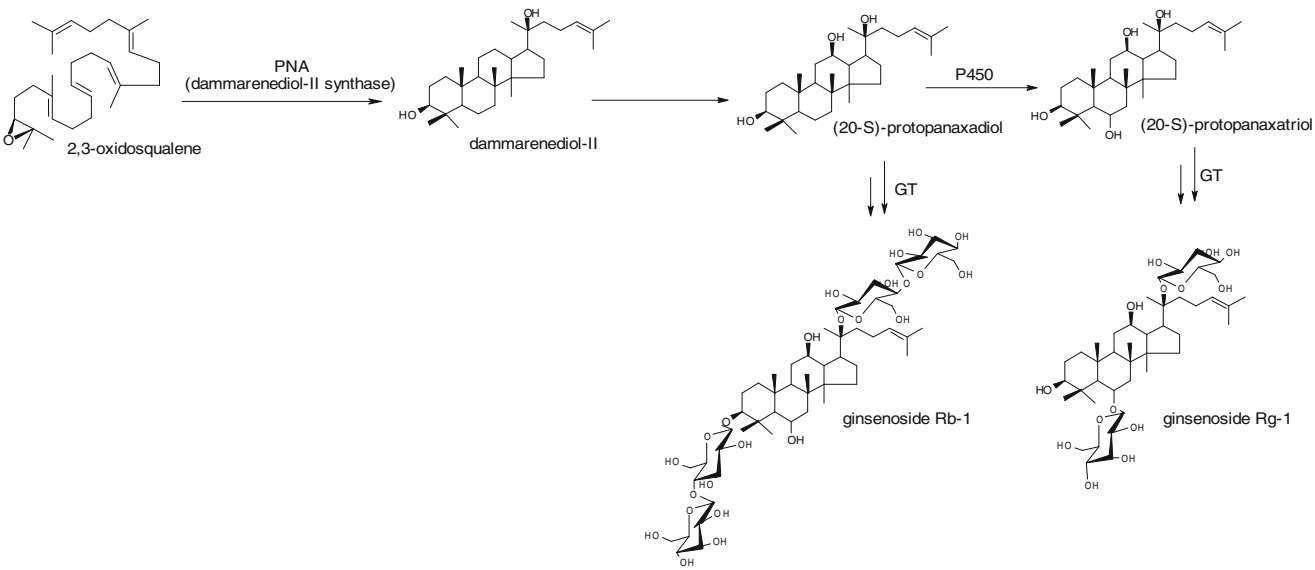

Fig. 2 Biosynthesis of dammarene type ginsenosides in *Panax ginseng* from 2,3-oxidosqualene. *PNA* gene encodes dammarenediol-II synthase, P450 is a cytochrome P450 oxygenase, GT is a glycosyltransferase

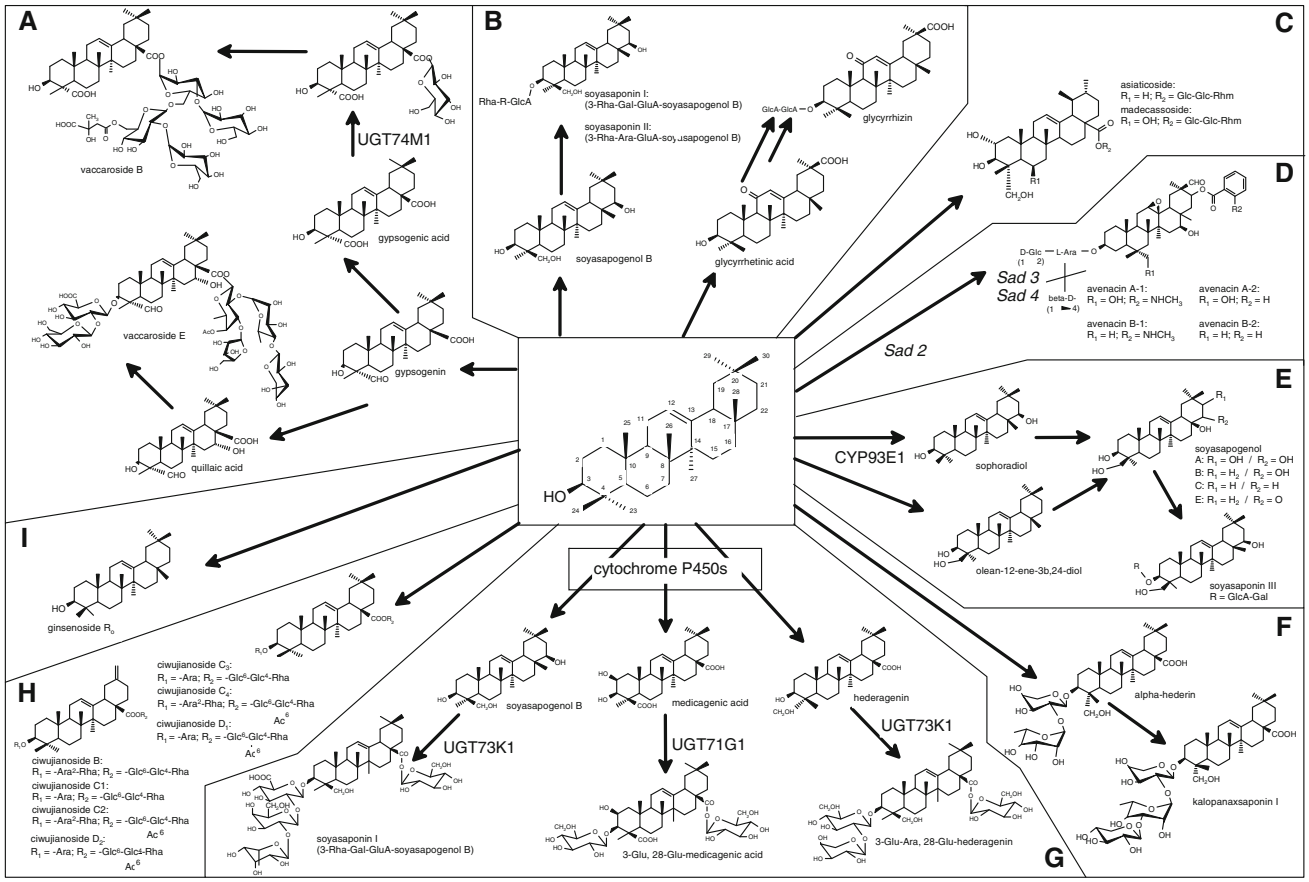

Fig. 3 Schematic summary of biosynthetic pathways of bioactive triterpenoid saponins in plants derived from β-amyrin, indicating identified enzymes. *Sad1*, *Sad2*, *Sad3* encode β-amyrin synthase, cytochrome P450 monooxygenase, and glycosyltransferase, respectively. *Capital letters* indicate pathways occurring in different species: A—*Saponaria vaccaria*, B—*Glycyrrhiza glabra*, C—*Centella asiatica*, D—*Avena strigosa*, E—*Glycine max*, F—*Nigella sativa*, G—*Medicago truncatula*, H—*Eleutherococcus senticosus*, I—*Panax ginseng*

obstacles remain, mainly in the studies on triterpenoid saponins. Resources such as ESTs, for example, are useful in the identification of putative biosynthetic genes, mostly when incorporated with the knowledge of the place and/or specific conditions in which the metabolites are synthesized [17, 21]. However, for many plant species that accumulate triterpenoid saponins this knowledge is not available.

The isolation of two cDNAs was obtained from molecular studies in *Centella asiatica.* The first, named *CaFPS*, encodes a FPP synthase. The sequence contains 1,029 nucleotides and encodes 343 amino acids, displaying 84, 79 and 72% of identity with the FPP synthase of *Artemisia annua*, *Arabidopsis thaliana* and *Oryza sativa*, respectively. Southern blot analysis indicated that there is a single copy of this gene in the genome of *C. asiatica* [22].

The overexpression of the SS-encoding gene (*PgSSI*) in *P. ginseng* positively regulated genes encoding SS, SE, B-AS, as well as cycloartenol synthase (CAS), the latter being specific for sterols and steroidal saponins; the positive regulation by the overexpressed gene increased yields of phytosterols and triterpenoid saponins [23]. That was the first report showing a gene for SS regulating triterpene biosynthesis. The expression of this gene in leaves of *Eleutherococcus senticosus* corroborates this fact. The expression of *PgSSI* gene increased not only the production of phytosterols, but also the synthesis of ciwujianosides (triterpenoid saponins which aglycones are noroleandienolic acid and oleanolic acid) [24]. The antisense suppression of *CAS* caused an increase of 50–100% in ginsenoside content of hairy roots of *P. ginseng* [25]. This effect was associated with lower CAS and higher DDS activity, presumably due to increased availability of 2,3-oxidosqualene, the common precursor to phytosterols and ginsenosides. The corresponding reduction in phytosterol contents lead to a slower initial growth rate in the antisense CAS hairy roots [25].

Successful cloning of the cDNA of dammarenediol-II synthase (DDS, also known as PNA) in roots of *P. ginseng* was reported by Tansakul and collaborators [26]. The dammarane-type aglycones, protopanaxadiol and protopanaxatriol are hardly detected in roots and adventitious

roots of *Panax*, suggesting higher expression levels of hydroxylases and GTs compared to that of DDS. The formation of dammarenediol-II can be a limiting step in ginsenoside biosynthesis, which was supported by a larger production of ginsenosides via overexpression of a DDS encoding gene.

A cDNA isolated from *C. asiatica*, named *CabAS*, has similarities with sequences reported for B-AS, and, based on the predicted sequence of amino acids, it was initially believed that it encoded an enzyme, which synthesized β-amyrin [27]. The analysis by Southern blot indicated a single copy of this gene, whereas northern blot revealed that the gene was only expressed in leaves, the organs where asiaticosides are accumulated. The expression of *CabAS* in leaves is positively regulated by MeJA. The expression of this cDNA, together with that of the cDNA that encodes for SS (*CaSQS*), was compared with the production of centellosides (asiaticoside, madecassoside and their respective aglycones) in plants and callus cultures [28]. It was shown that a lower expression of *CaSQS* and *CabAS* in roots was associated with lower concentration of centellosides.

Further characterization of *CabAS* was carried out by functional expression of the gene in a lanosterol synthase-deficient yeast mutant. Liquid chromatography and mass spectrometry of the recombinant enzyme product showed the absence of β-amyrin and the presence of dammarenediol [29]. Therefore, *CabAS* was renamed to *C. asiatica* dammarenediol synthase (*CaDDS*). Although tetracyclic triterpene saponins such as ginsenoside have not been reported for *C. asiatica*, it is possible that new saponins of this class are produced by the species. It has been been suggested that transformation of *P. ginseng* with *CaDDS* could improve dammarane-type ginsenoside production by upregulating triterpene aglycone formation [29].

Besides *Centella* and ginseng, studies with other species have provided new data regarding enzymes involved in the biosynthesis of triterpenoid saponins. The cDNA for the triterpene synthase of *Euphorbia tirucalli* (first report for the family Euphorbiaceae) showed 82% of identity in amino acid sequence with that of B-AS of *P. ginseng* [30].

In *Aster sedifolius*, a new gene for B-AS was isolated from leaves and roots (*AsOXA1*) with function in the production of astersedifoliosides (A, B and C). This gene shares an identity of 79% with *PNY2* of *P. ginseng*, and the analysis by Southern blot demonstrates that it belongs to a multi-gene family with similar sequences [15]. A B-AS gene was isolated from *Gentiana straminea*, displaying 76% similarity at deduced peptide level with the corresponding enzyme of *P. ginseng* [31]. The 756 residue enzyme has key features of triterpene synthases, such as four QW and one DCTAE motif, and its heterologous expression in microbial systems was shown to yield a gene product with appropriate size and antibody reactivity. The gene was mostly expressed in leaves [31].

Transformation of *Medicago truncatula* with the gene of B-AS from *A. sedifolius* (*AsOXA1*) driven by the strong promoter 35S resulted in enhanced accumulation of triterpenes, such as bayogenin, medicagenic acid and zanhic acid in leaves and bayogenin, hederagenin, soyasapogenol E and 2 β-hydroxyoleanolic acid in roots [32]. A good correlation was found between *B-AS* expression levels and triterpene accumulation. In addition, plants expressing *AsOXA1* showed consistently better nodulation rates (higher number and size of nodules per root dry weight) when compared to control plants. This enhancement was concomitant with a significant increase in soyasapogenol B in the transformed plants [32].

A cDNA corresponding to a gene encoding a β-AS (*SvBS*) was identified from *Saponaria vaccaria*. The expression of *SvBS* is larger in leaves than in roots and germinated seeds, and its sequence of amino acids has identity of 81% with *Glycyrrhiza glabra* and of 80% with *M. truncatula* [33].

Most saponins from the Caryophyllaceae family are based on β-amyrin. The more common aglycones are quillaic acid, gypsogenic acid and gypsogenin. The ones found in *S. vaccaria* are monodesmosidics (vaccaroside B) and bidesmosidics (vaccaroside E) and, based on the structures, the synthesis steps seem to include β-amyrin oxidation in positions 16, 23 and/or 28, as well as glycosylation in position 28. For the biosynthesis of bidesmosidic saponins, the glycosylation must also occur in position 3, as well as sugar acylation, with acetyl and 2-hydroxy-2-methylglutaryl [33].

Monodesmosidic saponins are formed from the transference of one activated monosaccharide (like UDP-Glc), which first step would probably be an ester link of glucose with the carboxyl group in C28. Afterwards, additional sugars can be transferred and also acylated. In the study of the steps involved in the assembly of monodesmosides, a cDNA encoding a GT (UGT74M1) was also identified. UGT74M1 appears to take part in monodesmoside biosynthesis by the formation of a hexose ester in C28 of specific sapogenins [33].

It was also identified a cDNA that encodes for a triterpene hydroxylase in *Glycine max* [18], which hydroxylates the C-24 position of β-amyrin and sophoradiol. The expression of the gene *CYP93E1* (that encodes for a cytochrome P-450 oxygenase) together with *B-AS* led to the product olean-12-ene-3β, 24-diol (24-hydroxy-β-amyrin), an intermediate in the soyasaponin biosynthesis of legumes.

Medicago truncatula contains more than 30 saponins, characterized by the soyasapogenol B and E aglycones, medicagenic acid, hederagenin and bayogenin. It seems that the biosynthesis of these saponins includes a series of hydroxylations/oxidations, which are dependent on

Table 1 Examples of target genes to modify the production of triterpenoid saponins

Enzyme	Gene	Enzyme products/target metabolites	Species	Tissue	References
B-AS	*AsOXA1*	Bayogenin, medicagenic and zanhic acid, hederagenin, soyasapogenol E and 2β-hydroxyoleanolic acid	*Medicago truncatula*	Leaves and roots	Confalonieri et al. [32]
	SvBS	Monodesmosidic saponines	*Saponaria vaccaria*	Leaves, roots, and germinated seeds	Meesapyodsuk et al. [33]
	AsbAS1	Avenacins	*Avena strigosa*	Roots	Qi et al. [36]
	βAS	Oleanolic acid	*Gentiana straminea*	Leaves, roots and stems	Liu et al. [31]
	AsOXA1	Astersedifoliosides A,B and C	*Aster sedifolius*	Leaves and roots	Cammareri et al. [15]
Carboxylic acid GT	*pSv33B05*	Monodesmosidic saponines	*Saponaria vaccaria*	Leaves and roots	Meesapyodsuk et al. [33]
CAS	*CAS*	Ginsenosides	*Panax ginseng*	Hairy roots	Liang et al. [25]
CYP450 oxygenase	*CYP93E1*	Olean-12ene-3b,24-diol and soyasapogenol B	*Glycine max*	Leaves	Shibuya et al. [18]
Dammarenediol synthase	*DDS*	Ginsenosides	*Panax ginseng*	Roots	Han et al. [16]
	CaDDS	Unknown tetracyclic triterpene saponin	*Centella asiatica*	Leaves	Kim et al. [29]
Dammarenediol-II synthase	*PNA*	Ginsenosides	*Panax ginseng*	Hairy roots	Tansakul et al. [26]
FPS	*CaFPS*	Farnesyldiphosphate	*Centella asiatica*	Leaves	Kim et al. [22]
GT	*Sad 3 / Sad 4*	Avenacin A1	*Avena strigosa*	Roots	Mylona et al. [38]
Monoxygenase	*AsCyp51H10*	Avenacins			Qi et al. [37]
SS	*PgSS1*	Phytosterols and ciwujianosides	*Eleutherococcus senticosus*	Whole plants	Seo et al. [24]
SS and B-AS	*CaSQS + CabAS*	Centellosides	*Centella asiatica*	Callus, leaves and roots	Mangas et al. [28]
SS, SE, B-AS, CAS	*PgSS1*	Phytosterols and ginsenosides	*Panax ginseng*	Adventitious roots	Lee et al. [23]

cytochrome P450 and reactions of transference of sugar catalysed by GTs. Two uridine diphosphate GTs have already been functionally characterized: UGT73K1, specific for hederagenin and soyasapogenol B and E; and UGT71G1, specific for medicagenic acid, apparently acting in position C23 and C28, since that GT does not glycosylate in the hydroxyl in C3. Besides these GTs, *β-AS* are mostly expressed in flowers, roots, stems and petioles [34].

The relevance of GTs in the control of triterpenoid saponin biosynthesis has also been observed for *P. ginseng*. Three ESTs related to GTs were found to be up regulated by MeJA treatment of hairy roots of *P. ginseng*, and this response was correlated with higher triterpenoid saponin accumulation [35].

Most of the studies with triterpenoid saponins focus on dicotyledonous species. A notable exception, however, are avenacins, triterpenoid saponins produced in the oat *Avena strigosa*. The gene *AsbAS1* (*Sad1*), which encodes B-AS, is grouped (in non- conserved sections in other cereals) with other genes necessary for at least four distinctive processes in avenacin biosynthesis: the cyclization of 2,3-oxidosqualene, β-amyrin oxidation, glycosylation and acylation [36]. Whereas *Sad 1* encodes B-AS, *Sad 2* (or AsCyp51H10) encodes a cytochrome P450 monoxygenase [37]. *Sad 3* and/or *Sad 4* may encode GTs, which are required for the addition of a D-glucose molecule in β-1, 4 link to the triterpenoid skeleton of avenacin A-1 or be involved in the regulation of avenacin A-1 glucosylation or its transport/sequestering [38].

A summary of representative identified genes for which changes in expression have been associated with modified production/accumulation of triterpenoid saponins is shown in Table 1.

Environmental Control of Triterpenoid Saponin Production

Elicitors can be defined as molecules originated from various sources, which may trigger both physiologic and

morphologic responses, as well as phytoalexin accumulation. Abiotic elicitors include metal ions and inorganic compounds, whereas biotic elicitors derive from fungus, bacteria, virus, plant cell walls or even molecules accumulated by the plant upon pathogen and/or herbivore attack [39].

Jasmonic acid (JA) and its methyl jasmonate ester (MeJA) derive from the catabolism of linolenic acid and act as secondary messengers that modulate several physiological processes in plants, including root development, senescence and defense response against pathogen and herbivore attacks. JA triggers or increases the biosynthesis of various secondary metabolites, which are important in plant adaptation mainly upon biotic environmental challenges. Recently, it has been established that the active form of JA is actually JA-Ile, a complex with isoleucine that regulates selective proteolysis of sequestering proteins (JAZ) capable of blocking the action of MYC2 transcription factors, which transactivate JA responsive genes [40]. JA and its derivatives are the most frequently employed elicitors in studies aiming at the induction of triterpenoid compounds.

The increase of madecassoside, asiaticoside and their respective sapogenins in cell cultures, roots and whole plants of *C. asiatica* was reported after the application of 100 μM of MeJA in culture medium [41, 42]. The stimulation of soyasaponin biosynthesis and the participation of JA in the positive regulation of B-AS, SS and glycuronic acid-UDP: soyasapogenol B glucuronosyltransferase (enzyme catalysing the last step of soysaponin biosynthesis) were also observed in cell cultures of *Glycyrrhiza glabra* [43].

The treatment with jasmonate yielded greater accumulation of *β*-amyrin [44], as well as B-AS activity, SS and SE in *M. truncatula* [45]. A strong co-ordinated induction of transcripts encoding B-AS, SS and one form of SE correlated with triterpene accumulation upon MeJA treatment in cell suspensions of *M. truncatula* [46]. B-AS activity and oleanolic acid accumulation were promoted by treatment with 0.1 mM MeJA in *Gentiana straminea* [31]. JA or yeast extract were effective in promoting oleanolic acid production in cell cultures of *Calendula officinalis* [47]. MeJA also stimulated the expression of an SE gene (*NSSQE1*) in *Nigella sativa* plants [48]. The gene was found to be expressed in all organs, including roots, which do not produce triterpenoid saponins, leading the authors to suggest that it is probably a housekeeping gene in triterpene metabolism in this species. MeJA stimulated the production of α-hederin and of kalopanaxsaponin I in *Nigella sativa* [49], of ginsenoside in roots and callus of *P. ginseng* [50, 51] and of ginsenosides Rg1, Re, Rb1 and Rd in cell cultures of *P. notoginseng* [52].

Exposure to MeJA at 0.1 mM in hairy roots of *P. ginseng* induced the expression of several genes involved in triterpene biosynthesis, such as SS, SE and DDS, with a slight decrease being observed for CAS [35]. The content of protopanaxadiol-type saponins (Rb group) increased between 5.5 and 9.7 times after MeJA treatment, whereas that of protopanaxatriol-type saponins (Rg group) increased between 1.9 and 3.8 times. Interestingly, Rg1 ginsenoside was negatively affected by the same treatment, unlike what was observed for cell cultures of *P. notoginseng* [52].

Some studies employed new JA derivatives, as well as JA mixed with other substances. Two-hydroxyethyl jasmonate (HEJ) proved to be a powerful stimulator of ginsenoside production in cell cultures of *P. notoginseng* [53]. A combination of ethephon (2-chlorethyl phosphonic acid), a synthetic precursor of the phytohormone ethylene, with MeJA increased in synergic fashion the production of ginsenosides in *P. ginseng* [54]. In contrast, addition of gibberellin A3 to cell cultures of *Glycyrrhiza glabra* caused down regulation of steady-state mRNA encoding B-AS [43]. Another phytohormonal product, the synthetic cytokinin thidiazuron (TDZ, 1-phenyl-3(1,2,3-thidiasol-5-yl) urea), resulted in a larger production of asiaticosides in whole plants of *C. asiatica*, whereas the synthetic auxin 2,4-D (2,4-dichlorophenoxyacetic acid) caused reduced accumulation of the same metabolites [55]. In the case of TDZ, it was suggested that the observed effect might have been a consequence of biomass gain, although the proportion of increment of accumulation of asiaticosides was quite superior to the increment in biomass. On the other hand, the accumulation of an immunoadjuvant fraction of *Quillaja brasiliensis* saponins was not significantly affected by different auxins applied in the rooting of microcuttings cultivated in vitro [56]. JA spraying on leaves or UV light exposure stimulated saponin accumulation in leaves of *Q. brasiliensis* (J.D. Fleck, G. Gosmann and A. Fett-Neto, unpublished results). Similarities between JA and UV-induced metabolic profiles have been previously reported for secondary metabolites, such as monoterpene indole alkaloids [57].

A permeabilization and feeding strategy was described for the production of centellosides in *C. asiatica*. Cell cultures at the mid to late log phase of growth were treated with dimethyl sulfoxide (DMSO) or DMSO plus α-amyrin dissolved in acetone [58]. DMSO alone was capable of effectively permeabilizing cells, increasing eight-fold the total centelloside content in medium. The same treatment also had a positive effect on centelloside production, increasing it by 213% relative to control concentrations. The combined application of DMSO and α-amyrin (1% v/v and 0.01% mg/ml) improved the total centelloside content by approximately four-fold, presumably due to improved uptake of α-amyrin [58]. Besides showing that α-amyrin is an adequate substrate for conversion into centellosides, these experiments suggested that enzymes involved in later

steps of centelloside biosynthesis are not the bottleneck for low product formation in control cell cultures in presence of adequate substrate amounts.

Light and temperature conditions favourable for ginsenoside production are within conventional ranges used for in vitro culture. It has been suggested that the accumulation of ginsenosides can be promoted with the exposure of hairy roots to white fluorescent light, right after a period of culture in the dark [59]. The use of 200 μM of salicylic acid, a pathogen attack signalling molecule in plants, also induced the accumulation of ginsenosides [50]. Two oligosaccharides from *Paris polyphylla* var. *yunnanensis* stimulated saponin production in *P. ginseng* hairy roots [60]. In cell cultures, the induction of the accumulation of ginsenosides was obtained with the supply of additional oxygen (40%) [61], or by means of exposure to osmotic stress achieved by supplementation with sorbitol and with sorbitol combined with sucrose [12]. In root cultures of *P. ginseng*, exposure to 25 μM of copper increased hydrogen peroxide and superoxide generation, and improved ginsenoside production by approximately 35% after 20 days [62]. Since the production of reactive oxygen species is a common theme in the signal transduction of all of these environmental stress responses, ginsenoside metabolism seems to be modulated by oxidative stress.

The overt evidence for the participation of MeJA as a positive regulator of production and accumulation of ginsenosides led to more detailed studies, providing

Table 2 Examples of treatments effective for increasing production and/or accumulation of triterpenoid saponins

Species	Treatment	Induced genes, enzymes or phytochemicals	Tissue	References
Calendula officinalis	MeJA or yeast extract	Oleanolic acid	Cell culture	Wiktorowska et al. [47]
Centella asiatica	TDZ	Asiaticoside	Whole plant	Kim et al. [55]
	Auxin			
	MeJA	Asiaticoside/madecassoside/ madecasid/asiatic acid		Mangas et al. [41]
		Asiaticoside		Kim et al. [42]
	DMSO + α-amyrin	Asiaticoside/madecassoside/ madecasid/asiatic acid	Cell culture	Hernandez-Vazquez et al. [58]
Gentiana straminea	MeJA	B-AS activity and oleanolic acid accumulation	Leaves, roots and stems	Liu et al. [31]
Glycyrrhiza glabra		Soyasaponin	Cell culture	Hayashi et al. [43]
Medicago truncatula		*β*-Amyrin and soyasapogenol B and E glycosides		Broeckling et al. [44]
		B-AS, SS, SE		Suzuki et al. [45]
Nigella sativa		α-Hederin/kalopanaxsaponin I	Shoots	Scholz et al. [49]
		SE gene (*NSSQE1*)	Seedlings/plants	Lipinski et al. [48]
Panax ginseng		Ginsenosides	Adventitious roots	Ali et al. [50]
				Lee et al. [23]
			Cell culture	Thanh et al. [51]
		SS, SE, DDS	Hairy roots	Kim et al. [35]
	Linolenic acid	Ginsenosides	Adventitious roots	Dewir et al. [65]
	Sorbitol		Cell culture	Wu et al. [12]
	Sorbitol + sucrose			
	Salicylic acid		Adventitious roots	Ali et al. [50]
	Oxygen (40%)		Cell culture	Thanh et al. [61]
	Ethephon + MeJA		Adventitious roots	Bae et al. [54]
	Light		Hairy roots	Yu et al. [59]
	Oligosaccharides			Zhou et al. [60]
	Copper		Adventitious roots	Ali et al. [62]
Panax notoginseng	MeJA	Ginsenosides	Cell culture	Hu and Zhong [63]
		Ginsenosides Rg_1, Re, Rb_1, Rd		Wang et al. [52]
	HEJ	Ginsenosides		Wang et al. [53]
				Hu and Zhong [63]

information on gene expression and the participation of certain enzymes. From cell cultures of *P. notoginseng*, Hu and Zhong [63] cloned three cDNA fragments of genes encoding SS, SE and CAS; the accumulation of these transcripts was investigated in cell cultures elicited with HEJ and MeJA. A positive regulation of genes encoding SS and SE with treatment with MeJA and, mainly, with HEJ, was observed possibly as a result of enhanced contents of endogenous JA, subsequently leading to ginsenoside accumulation. Positive regulation was also observed for a late enzyme in the pathway, UDGP-ginsenoside Rd GT, leading to the formation of Rb1ginsenoside [53]. The data point to a co-ordinated regulation of ginsenoside biosynthesis by JA and its derivatives. Auxin and MeJA simultaneous treatment has also yielded synergistic increases in ginsenoside production by adventitious roots of *P. ginseng* [64]. In the same type of root culture, the application of the jasmonate precursor linolenic acid at 1 μmol/l on the 40th day of culture resulted in a three-fold increase in total ginsenoside yield after 7 days, particularly those of the Rb group, without causing growth reduction [65]. The strategy of using octadecanoid derivatives to enhance saponin production has also important implications for large-scale culture systems. Factors affecting biomass accumulation and ginsenoside production in large-scale adventitious roots of ginseng have recently been reviewed [66].

Further studies on *P. ginseng* reported the MeJA-mediated induction of transcription of *SE*, *B-AS*, *SS* (PgSSI) [23] and *OSC* [19]. Data to date clearly indicates that the exposure of cells, organs or plants to jasmonate and its derivatives has been one of the main strategies to stimulate the production of bioactive saponins. A list of examples of elicitation strategies to improve saponin yields is shown in Table 2.

The increased yield of triterpenoid saponins by JA or its methyl ester treatment in most studies, not only in cell and root cultures but also in plants, may simply reflect the involvement of saponins in mechanisms of plant defense. Octadecanoid signaling molecules such as JA perform a significant role in the transduction process of the signals that regulate plant defense genes. Furthermore, the enzyme induction of the early steps of the biosynthetic pathway leads to the formation of both triterpenoid compounds and steroids, denoting a broader role to those elicitors. The mechanisms of action of these and several other factors are not clearly elucidated yet, and future studies with that aim should be pursued.

Concluding Remarks

The generation of ESTs from cDNAs of a specific plant tissue is an efficient approach to identify genes that are involved in the biosynthesis of secondary metabolites. The identification of consistent and robust elicitation signals for triterpene biosynthesis suggests that subtractive hybridization methods for comparative gene expression in elicited and control conditions may also be a useful strategy to identify genes involved in the production of these metabolites. Through genomic sequence, heterologous gene expression, enzymology and analytical chemistry, an increasing number of triterpenes has been associated to the enzymes that produce them and the genes encoding these enzymes. Although a few 2,3-oxidosqualene cyclases have been characterized, the development in the area is fast-growing. Most of these OSCs originate from dicotyledonous species, but detailed studies with oat, for instance, provide important evolutionary and comparative insights about the biosynthesis of triterpenes in monocotyledonous plants.

A great deal of the present studies concentrates on the first biosynthetic phase of triterpenoid saponins, mainly isolating and characterizing genes that encode B-AS. This enzyme, as exclusive member of the triterpenoid route, is considered a limiting step in the production and accumulation of saponins (Fig. 3). The knowledge of the genes that encode B-AS, as well as of the ones that encode the subsequent enzymes of the pathway, in addition of the regulatory factors modulating enzyme activity and biosynthetic gene expression, are key elements for a rational and sustainable production of bioactive triterpene saponins at industrial scale.

References

1. Yan, M. C., Liu, Y., Chen, H., Ke, Y., Xu, Q. C., & Cheng, M. S. (2006). Synthesis and antitumor activity of two natural N-acetylglucosamine-bearing triterpenoid saponins: Lotoidoside D and E. *Bioorganic & Medicinal Chemistry Letters, 16*, 4200–4204.
2. Ma, Y. X., Fu, H. Z., Li, M., Sun, W., Xu, B., & Cui, J. R. (2007). An anticancer effect of a new saponin component from *Gymnocladus chinensis* Baillon through inactivation of nuclear factor-kappa B. *Anti-Cancer Drugs, 18*(1), 41–46.
3. Kuo, R. Y., Qian, K., Morris-Natschke, S. L., & Lee, K. H. (2009). Plant-derived triterpenoids and analogues as antitumor and anti-HIV agents. *Natural Products Reports, 26*, 1321–1344.
4. Fleck, J. D., Kauffmann, C., Spilki, F., Lencina, C. L., Roehe, P. M., & Gosmann, G. (2006). Adjuvant activity of *Quillaja brasiliensis* saponins on the immune responses to bovine herpesvirus type 1 in mice. *Vaccine, 24*, 7129–7134.
5. Sun, H. X., Ye, Y. P., Pan, H. J., & Pan, Y. J. (2004). Adjuvant effect of *Panax notoginseng* saponins on the immune responses to ovalbumin in mice. *Vaccine, 22*, 3882–3889.
6. Kupeli, E., Tatli, I. I., Akdemir, Z. S., & Yesilada, E. (2007). Bioassay-guided isolation of anti-inflammatory and antinociceptive glycoterpenoids from the flowers of *Verbascum lasianthum* Boiss. ex Bentham. *Journal of Ethnopharmacology, 110*, 444–450.
7. Ponou, B. K., Barboni, L., Teponno, R. B., Mbiantcha, M., Nigueleface, T. B., Park, H. J., et al. (2008). Polyhydroxyoleanane-type triterpenoids from *Combretum molle* and their anti-inflammatory activity. *Phytochemistry Letters, 1*, 183–187.

8. Sparg, S. G., Light, M. E., & Staden, J. (2004). Biological activities and distribution of plant saponins. *Journal of Ethnopharmacology, 94*, 219–243.
9. Rajput, V. K., & Mukhopadhyay, B. (2008). Concise synthesis of a pentasaccharide related to the anti-leishmanial triterpenoid saponin isolated from *Maesa balansae*. *Journal of Organic Chemistry, 73*, 6924–6927.
10. Vermeersch, M., Foubert, K., da Luz, R. I., Puyvelde, V., Pieters, L., Cos, P., et al. (2009). Selective antileishmania activity of 13, 28-epoxy-oleanane and related triterpene saponins from the plant families Myrsinaceae, Primulaceae, Aceraceae and Icacinaceae. *Phytotherapy Research, 23*, 1404–1410.
11. Vincken, J. P., Heng, L., Groot, A., & Gruppen, H. (2007). Saponins, classification and occurrence in the plant kingdom. *Phytochemistry, 68*, 275–297.
12. Wu, J. Y., Wong, K., Ho, K. P., & Zhou, L. G. (2005). Enhancement of saponin production in *Panax ginseng* cell culture by osmotic stress and nutrient feeding. *Enzyme and Microbial Technology, 36*, 133–138.
13. Higson, A. P., & Hamer, A. (2009). Specialty non-food crops. In A. E. Osbourn & V. Lanzotti (Eds.), *Plant-derived natural products* (pp. 569–584). New York, NY: Springer.
14. Taylor, K., Nguyen, A., & Stéphenne, J. (2009). The need for new vaccines. *Vaccine, 27* S, G3–G8.
15. Cammareri, M., Consiglio, M. F., Pecchia, P., Corea, G., Lanzotti, V., Iebas, J. I., et al. (2008). Molecular characterization of β-amyrin synthase from *Aster sedifolius* L. and triterpenoid saponin analysis. *Plant Science, 175*, 255–261.
16. Han, J. Y., Kwon, Y. S., Yang, D. C., Jung, Y. R., & Choi, Y. E. (2006). Expression and RNA interference-induced silencing of the dammarenediol synthase gene in *Panax ginseng*. *Plant Cell Physiology, 47*, 1653–1662.
17. Jung, J. D., Park, H. W., Hahn, Y., Hur, C. G., In, D. S., Chung, H. J., et al. (2003). Discovery of genes for ginsenoside biosynthesis by analysis of ginseng expressed sequence tags. *Plant Cell Reports, 22*, 224–230.
18. Shibuya, M., Hoshino, M., Katsube, Y., Hayashi, H., Kushiro, T., & Ebizuka, Y. (2006). Identification of β-amyrin and sophoradiol 24-hydroxylase by expressed sequence tag mining and functional expression assay. *The FEBS Journal, 273*, 948–959.
19. Choi, D. W., Jung, J. D., Ha, Y. I., Park, H. W., In, D. S., Chung, H. J., et al. (2005). Analysis of transcripts in methyl jasmonate-treated ginseng hairy roots to identify genes involved in the biosynthesis of ginsenosides and other secondary metabolites. *Plant Cell Reports, 23*, 557–566.
20. Yue, C. J., & Zhong, J. J. (2005). Purification and characterization of UDPG: ginsenoside Rd glucosyltransferase from suspended cells of *Panax notoginseng*. *Process Biochemistry, 40*, 3742–3748.
21. Wu, Q., Song, J., Sun, Y., Suo, F., Li, C., Luo, H., et al. (2010). Transcript profiles of *Panax quinquefolius* from flower, leaf and root bring new insights into genes related to ginsenosides biosynthesis and transcription regulation. *Physiologia Plantarum, 138*, 134–149.
22. Kim, O. K., Ahn, J. C., Hwang, S. J., & Hwang, B. (2005). Cloning and expression of a farnesyl diphosphate synthase in *Centella asiatica* (L.) Urban. *Molecules and Cells, 19*, 294–299.
23. Lee, M. H., Jeong, J. H., Seo, J. W., Shin, C. G., Kim, Y. S., In, J. G., et al. (2004). Enhanced triterpene and phytosterol biosynthesis in *Panax ginseng* overexpressing squalene synthase gene. *Plant Cell Physiology, 45*, 976–984.
24. Seo, J. W., Jeong, J. H., Shin, C. G., Lo, S. C., Han, S. S., Yu, K. W., et al. (2005). Overexpression of squalene synthase in *Eleutherococcus senticosus* increases phytosterol and triterpene accumulation. *Phytochemistry, 66*, 869–877.
25. Liang, Y. L., Zhao, S. J., & Zhang, X. (2009). Antisense suppression of cycloartenol synthase results in elevated ginsenoside levels in *Panax ginseng* hairy roots. *Plant Molecular Biology Reporter, 27*, 298–304.
26. Tansakul, P., Shibuya, M., Kushiro, T., & Ebizuka, Y. (2006). Dammarenediol-II synthase, the first dedicated enzyme for ginsenoside biosynthesis, in *Panax ginseng*. *FEBS Letters, 580*, 5143–5149.
27. Kim, O. K., Kim, M. Y., Huh, S. M., Bai, D. G., Ahn, J. C., & Hwang, B. (2005). Cloning of a cDNA probably encoding oxidosqualene cyclase associated with asiaticoside biosynthesis from *Centella asiatica* (L.) Urban. *Plant Cell Reports, 24*, 304–311.
28. Mangas, S., Moyano, E., Osuna, L., Cusido, R. M., Bonfill, M., & Palazón, J. (2008). Triterpenoid saponin content and the expression level of some related genes in calli of *Centella asiatica*. *Biotechnology Letters, 30*, 1853–1859.
29. Kim, O. T., Lee, J. W., Bang, K. H., Kim, Y. C., Hyun, D. Y., Cha, S. W., et al. (2009). Characterization of dammarenediol synthase in *Centella asiatica* (L.) Urban. *Plant Physiology and Biochemistry, 47*, 998–1002.
30. Kajikawa, M., Yamato, K. T., Fuzukawa, H., Sakai, Y., Uchida, H., & Ohyama, K. (2005). Cloning and characterization of a cDNA encoding β-amyrin synthase from petroleum plant *Euphorbia tirucalli* L. *Phytochemistry, 66*, 1759–1766.
31. Liu, Y. L., Cai, Y. F., Zhao, Z. J., Wang, J. F., Li, J., Xin, W., et al. (2009). Cloning and functional analysis of a β-amyrin synthase gene associated with oleanolic acid biosynthesis in G*entiana straminea* maxim. *Biological and Pharmaceutical Bulletin, 32*, 818–824.
32. Confalonieri, M., Cammareri, M., Biazzi, E., Pecchia, P., Fevereiro, M. P. S., Balestrazzi, A., et al. (2009). Enhanced triterpene saponin biosynthesis and root nodulation in transgenic barrel medic (*Medicago truncatula* Gaertn.) expressing a novel β-amyrin synthase (AsOXA1) gene. *Plant Biotechnology Journal, 7*(2), 172–182.
33. Meesapyodsuk, D., Balsevich, J., Reed, D. W., & Covello, P. S. (2007). Saponin biosynthesis in *Saponaria vaccaria.* cDNAs encoding β-amyrin synthase and a triterpene carboxylic acid glucosyltransferase. *Plant Physiology, 143*, 959–969.
34. Achnine, L., Huhman, D. V., Farag, M. A., Sumner, L. W., Blount, J. W., & Dixon, R. A. (2005). Genomics-based selection and functional characterization of triterpene glycosyltransferases from the model legume *Medicago truncatula*. *Plant Journal, 41*, 875–887.
35. Kim, O. T., Bang, K. H., Kim, Y. C., Hyun, D. Y., Kim, M. Y., & Cha, S. W. (2009). Upregulation of ginsenoside and gene expression related to triterpene biosynthesis in ginseng hairy root cultures elicited by methyl jasmonate. *Plant Cell, Tissue and Organ Culture, 98*, 25–33.
36. Qi, X., Bakht, S., Leggett, M., Maxwell, C., Melton, R., & Osbourn, A. (2004). A gene cluster for secondary metabolism in oat: implications for the evolution of metabolic diversity in plants. *Proceedings of the National Academy of Sciences of the United States of America, 101*, 8233–8238.
37. Qi, X., Bakht, S., Qin, B., Leggett, M., Hemmings, A., Mellon, F., et al. (2006). A different function for a member of an ancient and highly conserved cytochrome P450 family: from essential sterols to plant defense. *Proceedings of the National Academy of Sciences of the United States of America, 103*, 18848–18853.
38. Mylona, P., Owatworakit, A., Papadopoulou, K., Jenner, H., Qin, B., Findlay, K., et al. (2008). Sad3 and Sad4 are required for saponin biosynthesis and root development in oat. *Plant Cell, 20*, 201–212.

39. Zhao, J., Davis, L. C., & Verpoorte, R. (2005). Elicitor signal transduction leading to production of plant secondary metabolites. *Biotechnology Advances, 23*, 283–333.
40. Staswick, P. (2008). JAZing up jasmonte signaling. *Trends in Plant Science, 13*, 66–71.
41. Mangas, S., Bonfill, L., Osuna, L., Moyano, E., Tortoriello, J., Cusido, R. M., et al. (2006). The effect of methyl jasmonate on triterpene and sterol metabolisms of *Centella asiatica*, *Ruscus aculeatus* and *Galphimia glauca* cultured plants. *Phytochemistry, 67*, 2041–2049.
42. Kim, O. K., Kim, M. Y., Hong, M. H., Ahn, J. C., & Hwang, B. (2004). Stimulation of asiaticoside accumulation in the whole plant cultures of *Centella asiatica* (L.) Urban by elicitors. *Plant Cell Reports, 23*, 339–344.
43. Hayashi, H., Huang, P., Takada, S., Obinata, M., Inoue, K., Shibuya, M., et al. (2004). Differential expression of three oxidosqualene cyclase mRNAs in *Glycyrrhiza glabra*. *Biological and Pharmaceutical Bulletin, 27*, 1086–1092.
44. Broeckling, C. D., Huhman, D. V., Farag, M. A., Smith, G. D. M., Mendes, P., Dixon, R. A., et al. (2005). Metabolic profiling of *Medicago truncatula* cell cultures reveals the effects of biotic and abiotic elicitors on metabolism. *Journal of Experimental Botany, 56*, 323–336.
45. Suzuki, H., Reddy, M. S. S., Naoumkina, M., Aziz, N., May, G. D., Huhman, D. V., et al. (2005). Methyl jasmonate and yeast elicitor induce differential transcriptional and metabolic re-programming in cell suspension cultures of the model legume *Medicago truncatula*. *Planta, 220*, 696–707.
46. Suzuki, H., Achnine, L., Xu, R., Matsuda, S. P. T., & Dixon, R. A. (2002). A genomics approach to the early stages of triterpene saponin biosynthesis in *Medicago truncatula*. *Plant Journal, 32*, 1033–1048.
47. Wiktorowska, E., Dlugosz, M., & Janiszowska, W. (2010). Significant enhancement of oleanolic acid accumulation by biotic elicitors in cell suspension cultures of *Calendula officinalis* L. *Enzyme and Microbial Technology, 46*, 14–20.
48. Lipinski, M., Scholz, M., Pieper, K., Fischer, R., Pruefer, D., & Muller, K. J. (2009). A squalene epoxidase from *Nigella sativa* participates in saponin biosynthesis and mediates terbinafine resistance in yeast. *Central European Journal of Biology, 4*, 163–169.
49. Scholz, M., Lipinski, M., Leupold, M., Luftmann, H., Harig, L., Ofirc, R., et al. (2009). Methyl jasmonate induced accumulation of kalopanaxsaponin I in *Nigella sativa*. *Phytochemistry, 70*, 517–522.
50. Ali, M. B., Yu, K. W., Hahn, E. J., & Paek, K. Y. (2006). Methyl jasmonate and salicylic acid elicitation induces ginsenosides accumulation, enzymatic and non-enzymatic antioxidant in suspension culture *Panax ginseng* roots in bioreactors. *Plant Cell Reports, 25*, 613–620.
51. Thanh, N. T., Murthy, H. N., Yu, K. W., Hahn, E. J., & Paek, K. Y. (2005). Methyl jasmonate elicitation enhanced synthesis of ginsenoside by cell suspension cultures of *Panax ginseng* in 5-l balloon type bubble bioreactors. *Applied Microbiology and Biotechnology, 67*, 197–201.
52. Wang, W., Zhang, Z. Y., & Zhong, J. J. (2005). Enhancement of ginsenoside biosynthesis in high-density cultivation of *Panax notoginseng* cells by various strategies of methyl jasmonate elicitation. *Applied Microbiology and Biotechnology, 67*, 752–758.
53. Wang, W., Zhao, Z. J., Xu, Y., Qian, X., & Zhong, J. J. (2006). Efficient induction of ginsenoside biosynthesis and alteration of ginsenoside heterogeneity in cell cultures of *Panax notoginseng* by using chemically synthesized 2-hydroxyethyl jasmonate. *Applied Microbiology and Biotechnology, 70*, 298–307.
54. Bae, K. H., Choi, Y. E., Shin, C. G., Kim, Y. Y., & Kim, Y. S. (2006). Enhanced ginsenoside productivity by combination of ethephon and methyl jasmonate in ginseng (*Panax ginseng* C.A. Meyer) adventitious root cultures. *Biotechnology Letters, 28*, 1163–1166.
55. Kim, O. K., Kim, M. Y., Huh, S. M., Ahn, J. C., Seong, N. S., & Hwang, B. (2004). Effect of growth regulators on asiaticoside production in whole plant cultures of *Centella asiatica* (L.) Urban. *Journal of Plant Biology, 47*, 361–365.
56. Fleck, J. D., Schwambach, J., Almeida, M. E., Yendo, A. C. A., Costa, F., Gosmann, G., et al. (2009). Immunoadjuvant saponin production in seedlings and micropropagated plants of *Quillaja brasiliensis*. *In Vitro Cell and Developmental Biology—Plant, 45*, 715–720.
57. Pasquali, G., Porto, D. D., & Fett-Neto, A. G. (2006). Metabolic engineering of cell cultures versus whole plant complexity in production of bioactive monoterpene indole alkaloids: recent progress related to old dilemma. *Journal of Bioscience and Bioengineering, 101*, 287–296.
58. Hernandez-Vazquez, L., Bonfill, M., Moyano, E., Cusido, R. M., Navarro-Ocaña, A., & Palazon, J. (2010). Conversion of α-amyrin into centellosides by plant cell cultures of *Centella asiatica*. *Biotechnology Letters, 32*, 315–319.
59. Yu, K. W., Murthy, H. N., Hahn, E. J., & Paek, K. Y. (2005). Ginsenoside production by hairy root cultures of *Panax ginseng*: influence of temperature and light quality. *Biochemical Engineering Journal, 23*, 53–56.
60. Zhou, L., Cao, X., Zhang, R., Peng, Y., Zhao, S., & Wu, J. (2007). Stimulation of saponin production in *Panax ginseng* hairy roots by two oligosaccharides from *Paris polyphylla* var yunnanensis. *Biotechnology Letters, 29*, 631–634.
61. Thanh, N. T., Murthy, H. N., Yu, K. W., Jeong, C. S., Hahn, E. J., & Paek, K. Y. (2006). Effect of oxygen supply on cell growth and saponin production in bioreactor cultures of *Panax ginseng*. *Journal of Plant Physiology, 163*, 1337–1341.
62. Ali, M. B., Hahn, E. J., & Paek, K. Y. (2006). Copper-induced changes in the growth, oxidative metabolism, and saponin production in suspension culture roots of *Panax ginseng* in bioreactors. *Plant Cell Reports, 25*, 1122–1132.
63. Hu, F. X., & Zhong, J. J. (2008). Jasmonic acid mediates gene transcription of ginsenoside biosynthesis in cell cultures of *Panax notoginseng* treated with chemically synthesized 2-hydroxyethyl jasmonate. *Process Biochemistry, 43*, 113–118.
64. Kim, Y. S., Yeung, E. C., Hahn, E. J., & Paek, K. Y. (2007). Combined effects of phytohormone, indole butyric acid. and methyl jasmonate on root growth and ginsenoside production in adventitious root cultures of *Panax ginseng* C.A. Meyer. *Biotechnology Letters, 29*, 1789–1792.
65. Dewir, Y. H., Chakrabarty, D., Wu, C. H., Hahn, E. J., Jeon, W. K., Paek, K. Y. 2009. Influences of polyunsaturated fatty acids (PUFAs) on growth and secondary metabolite accumulation in *Panax ginseng* C.A. Meyer adventitious roots cultured in air-lift bioreactors. *South African Journal of Botany*. doi:10.016/j.sajb.2009.10.008 .
66. Paek, K. Y., Murthy, H. N., Hahn, E. J., & Zhong, J. J. (2009). Large scale culture of ginseng adventitious roots for production of ginsenosides. *Advances in Biochemical Engineering and Biotechnology, 113*, 151–176.