Acta Pharmaceutica Sinica B 2021;11(7):1813–1834

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Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

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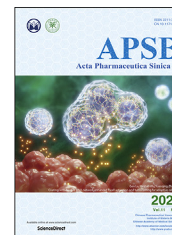

REVIEW

Ginsenosides in *Panax* genus and their biosynthesis

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Received 8 September 2020; received in revised form 3 December 2020; accepted 11 December 2020

KEY WORDS

Ginsenoside;
Panax species;
Biosynthetic pathway;
Transcription factors;
Non-coding RNAs;
Biotechnological approach

Abstract Ginsenosides are a series of glycosylated triterpenoids which belong to protopanaxadiol (PPD)-, protopanaxatriol (PPT)-, ocotillol (OCT)- and oleanane (OA)-type saponins known as active compounds of *Panax* genus. They are accumulated in plant roots, stems, leaves, and flowers. The content and composition of ginsenosides are varied in different ginseng species, and in different parts of a certain plant. In this review, we summarized the representative saponins structures, their distributions and the contents in nearly 20 *Panax* species, and updated the biosynthetic pathways of ginsenosides focusing on enzymes responsible for structural diversified ginsenoside biosynthesis. We also emphasized the transcription factors in ginsenoside biosynthesis and non-coding RNAs in the growth of *Panax* genus plants, and highlighted the current three major biotechnological applications for ginsenosides production. This review covered advances in the past four decades, providing more clues for chemical discrimination and assessment on certain ginseng plants, new perspectives for rational

Abbreviations: α-AS, α-amyrin synthase; ABA, abscisic acid; ADP, adenosine diphosphate; AtCPR (ATR), *Arabidopsis thaliana* cytochrome P450 reductase; BARS, baruol synthase; β-AS, β-amyrin synthase; CAS, cycloartenol synthase; CDP, cytidine diphosphate; CPQ, cucurbitadienol synthase; CYP, cytochrome P450; DDS, dammarenediol synthase; DM, dammarenediol-II; DMAPP, dimethylallyl diphosphate; FPP, farnesyl pyrophosphate; FPPS (FPS), farnesyl diphosphate synthase; GDP, guanosine diphosphate; HEJA, 2-hydroxyethyl jasmonate; HMGR, HMG-CoA reductase; IPP, isopentenyl diphosphate; ITS, internal transcribed spacer; JA, jasmonic acid; JA-Ile, (+)-7-*iso*-jasmonoyl-L-isoleucine; JAR, JA-amino acid synthetase; JAZ, jasmonate ZIM-domain; KcMS, *Kandelia candel* multifunctional triterpene synthases; LAS, lanosterol synthase; LUP, lupeol synthase; MEP, methylerythritol phosphate; MeJA, methyl jasmonate; MVA, mevalonate; MVD, mevalonate diphosphate decarboxylase; NDP, nucleotide diphosphate; OA, oleanane or oleanic acid; OAS, oleanolic acid synthase; OCT, ocotillol; OSC, oxidosqualene cyclase; PPD, protopanaxadiol; PPDS, PPD synthase; PPT, protopanaxatriol; PPTS, PPT synthase; RNAi, RNA interference; SA, salicylic acid; SE (SQE), squalene epoxidase; SS (SQS), squalene synthase; SPL, squamosa promoter-binding protein-like; SUS, sucrose synthase; TDP, thymine diphosphate; UDP, uridine diphosphate; UGPase, UDP-glucose pyrophosphosphprylase; UGT, UDP-dependent glycosyltransferase; WGD, whole genome duplication.

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Peer review under responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2020.12.017

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evaluation and utilization of ginseng resource, and potential strategies for production of specific ginsenosides.

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1. Introduction

Panax genus consists of about 20 species or variants[1–3] (Table 1[4–19]) among which *Panax ginseng* C.A. Meyer (Asian or Korean ginseng), *Panax quinquefolius* L (America ginseng), and *Panax notoginseng* (Burkill) F.H. Chen (Sanchi ginseng) are especially famous for their wide consumption as food, dietary supplements, functional food and medicinal materials for thousands of years, thus becoming the three best-selling ginseng products[20]. In addition to the above mentioned valuable species, *Panax japonicus* C.A. Meyer and its variants[21,22], *Panax vietnamensis* and its variants[15,23], and *Panax zingiberensis*[24] are also used as medicinal plants. Ginsenosides (here mean saponins from *Panax* species) are main bioactive constituents of *Panax* species, which can be divided into dammarane- and oleanane (OA)-type saponins according to their skeletons. Dammarane-type ginsenosides can be sub-grouped into protopanaxadiol (PPD)-, protopanaxatriol (PPT)- and ocotillol (OCT)-type[25] (Fig. 1). Pharmacological researches demonstrated that ginsenosides have multiple bioactivities including anti-inflammation[26–28], anti-cancer[29–31], anti-diabetic[32,33], cardiovascular-protective[34,35], and others[36–40].

Till 2013, the global market of ginseng products is estimated to be more than USD 2000 million with *P. ginseng* taking the largest proportion, followed by *Panax quinuefolius*, *P. notoginseng*, and *P. japonicus*[20]. In 2016, fresh ginseng output in China was 28,900 tons, leading to about USD 7500 million value in Jilin ginseng industry[41]. As the demand for ginseng products increases, ginseng cultivation and alternative approaches to produce ginseng products have become hot research topics[42]. Hence, a thorough understanding of the structures, distributions, and biosynthetic pathways of ginsenosides is important for quality evaluation of herbal medicines, rational utilization of natural resource and production of ginsenosides by biotechnology.

There are excellent reviews available including those concerning ginsenosides focusing on structures or their bioactivities[43,44], on isolation and analysis[45–47], on special ginsenoside such as OCT-type saponins and saponin stereoisomers[48,49], on metabolic regulation[50–53], and on elicitation strategy and signal transductions[54]. In contrast, reviews on ginsenoside biosynthesis are limited and mainly focusing on sapogenin biosynthetic genes and their applications[55].

In this review, we summarized the representative saponins of every known *Panax* species, reviewed the biosynthetic pathways from the formation of both the sapogenin and the sugar donor aspects, highlighted the enzymatic processes leading to ginsenoside structure diversities, and provided new insights for understanding the biological origin of diversified ginsenosides. We discussed the topic in four main sections: (1) the distribution of representative ginsenosides from nearly 20 *Panax* species including species-specific ginsenosides, malonylated ginsenosides, and minor ginsenosides, (2) the pathways of saponin biosynthesis including enzymes in saponin skeleton biosynthesis, in UDP-sugar formation, and in skeleton decoration, emphasizing OSCs, CYPs, and UGTs that responsible for saponin structure diversities, (3) transcription factors (TFs) in ginsenoside biosynthesis and non-coding RNAs (ncRNAs) regulating ginseng plant growth, and (4) the advanced efforts on biotechnological approaches for the production of ginsenosides in recent years.

2. Diverse ginsenosides in *Panax* species as natural resources

About 300 ginsenosides have been isolated and identified from different *Panax* species[25]. Some of them are important as chemical markers for their distinctive distributions, parts of them are attracting as natural resources for their high content in plants, and a few of them are becoming hot molecules for their particular bioactivities. It is of great significance to summarize representative saponins of each *Panax* species, which will benefit for the clarification of their divergent biosynthesis pathways to provide biotechnological approaches and produce the hot compounds.

About 20 *Panax* species, distinctly growing in the eastern North America and East Asia, have been reported[1–3]. Phylogenic analysis with internal transcribed spacer (ITS)[56], chloroplast DNA (cpDNA) restriction site and ITS[57], *trn*C-*trn*D[1], or *trn*K and 18S rRNA sequence[58] all showed the diversities and complexes of the East Asia species. Zuo et al.[59] later classified them as seven well-divergent species (*Panax trifolius*, *Panax pseudoginseng*, *Panax stipuleanatus*, *P. notoginseng*, *P. ginseng*, *P. quinquefolius*, and *P. japonicus* C.A. Mey.) and a not well-defined group named *Panax bipinnatifidus* species complex using DNA barcode. The *P. bipinnatifidus* species complex that was mainly located in Sino-Himalayan region and was previously treated as part of *P. pseudoginseng*[56], was separated into 10 groups using amplified fragment length polymorphism (AFLP) makers[60], and *P. zingiberensis*, *P. vietnamensis*, *Panax wangianus* and *P. bipinnatifidus* Clades split from this species complex as analyzed by restriction-site associated DNA sequencing (RAD-seq) method[61]. East Asia was the diversification centers of *Panax* species, and southeastern Yunnan of China might have acted as the corridor for *Panax* species genetic exchanges and dispersals[62]. During the dispersal and evolution process, *P. ginseng*, *P. quinquefolius*, and *P. japonicus* (from Japan) become tetraploid (*P. zingiberensis* may also be a tetraploid) by whole genome duplication (WGD) events to adapt the cooler environments, while most of the other species are diploid[61–63]. A phylogenetic tree of *Panax* plants based on widely used ITS gene[3,56,57] were constructed here to better understand their evolution relationships (Fig. 2). As displayed, the 29 ginseng samples were clustered into 8 Clades (or Subclades).

Saponins in plants of Clades 1–3 were OA- or dammarane-type, while saponins in species of Clades 5 and 6 were mainly dammarane-type. Their content was varying from 0.06 mg/g in *P. trifolius* of Clade 8, 5.8–15.6 mg/g in *P. ginseng* of Clade 1, 55.0–70.4 mg/g in *P. notoginseng* of Clade 6, 68.1–167.1 mg/g in *P. vietnamensis* of Clade 5 to 192.8–296.2 mg/g in *P. japonicus* of Clade 1 (Table 1). Base on

Table 1 Representative saponins in main root (or rhizome) of *Panax* species.

No.	Species	Origin	Representative saponins (content, mg/g)	Group	Ref.
1	*P. assamicus* Ban.	Sikkim, India	–	–	4
2	*P. bipinnatifidus* Seem.	Sikkim, India; Nepal	Rb1 (5.2), Rd (1.8), Re (1.5), Rg2 (7.1), Rg1 (2.9), P-RT1 methyl ester, S-R2 methyl ester	III	5,6
3	*P. ginseng* C.A. Mey. (Asian or Korean ginseng)	Northeast China; Korea	Rb1 (15), mRb1 (10), Rb2 (2), mRb2 (2), Rc (3), mRc (2), Rd (1), Re (6), Rg1 (15), Rf (4)	I	7
4	*P. japonicus* C.A. Mey. (Chikusetsu-ninjin, Japanese ginseng)	Japan	Ro (107.9–163.0), C-IV (51.8–82.4), C-III (30.4–44.7), C-IVa (4.3–6.7), Re (0–0.7), N-R2 (0.6–1.1)	III	8
5	*P. japonicus* C.A. Mey. (Satsuma-ninjin, Japanese ginseng)	Southern Kyusyu, Japan	Ro (37.9), C-IV (38.2), C-IVa (2.3), C-III (2.4), Rb1 (3.4), Rc (1.0), Re (2.4), Rg1 (5.0), N-R2 (2.0)	III	8
6	*P. japonicus* C.A. Mey. (Zhujie Shen, Bai Sanchi)	Yunnan, China	Ro (55.9–79.9), C-IVa (10.9–20.0), C-IV (16.5–33.2), Rg1 (3.6–4.1), Re (2.2–5.3), Rb1 (1.2–3.3), Rd (0.25–0.73), N-R2 (0–0.43), M-R2 (0–6.7)	III	8
7	*P. japonicus* C.A. Mey. var. *major* (Burk.) Y. Wu et K.M. Feng (Zhuzi Shen, Daye Sanqi)	Southwest China	Ro (74.1–165.7), C-IVa (22.2–84.2), C-IV (0–1.4), Rd (0.3–11.6), Rb1 (2.1–4.7), Rg1 (0–4.5), Re (0–4.9), N-R2 (0.26–1.0)	III	8
8	*P. japonicus* C.A. Mey. var. *bipinnatifidus* (Seem.) C.Y. Wu et K.M. Feng (feather-leaf bamboo ginseng)	Southwest China	Ro (92), Rb1 (2.9), Re (2.5), Rd (0.75), N-R2 (0.32), C-IVa (9.7), C-IV (0.83)	III	8
9	*P. japonicus* C.A. Mey. var. *angustifolius* (Burkill) C.Y. Cheng et C.Y. Chu (narrow-leaved Japanese ginseng)	Sichuan, China	C-IV (40.2–45.7), C-IVa (29.4–47.0), Ro (55–81.8), Rb1 (1.4–2.4), Rd (0.4–0.75)	III	8
10	*P. notoginseng* (Burk.) F.H. Chen (Sanchi ginseng)	Yunnan, China	N-R1 (5.3–7.2), Rg1 (29.6–39.1), Rb1 (26.7–30.6), Rd (5.7–8.4), Re (3.8–5.1), Rc (1.1–1.5)	I	9,10
11	*P. pseudoginseng* Wall. var. *elegantior* (Burk.) Hoo et Tseung (pearl pseudoginseng)	China; Nepal; India; Bhutan	Rb1 (7), Re (3), Rg1 (3), Rg2 (0.3), Rd (1), Ro (5), N-R1 (2), N-R2 (0.9), M-R2 (1), P-RT1 (4), P-F11 (0.1), P(*S*)-F11 (0.6), P-RT2 (0.2), C-IVa (0.4), G-XVII (0.8) (isolated yield from rhizomes)	III	19
12	*P. pseudoginseng* Wall. subsp. *himalaicus* (Himalayan ginseng)	Bhutan	Ro (4/72.5), P-RT1 (15/–), C-IVa (17/6), C-IV (–/3), Rb1 (3/10.5), Rd (2/–), Rg1 (4/-), RT3 (1/–)	III	11,12
13	*P. quinquefolius* L. (American ginseng)	North America	Rb1 (20), mRb1 (18), Re (12), Rg1 (10), Rc (3), mRc (1.5), Rd (2), mRd (2), P-F11 (1.5)	I	7
14	*P. sikkimensis* Ban.	Sikkim, India	Rb1, Re, Rg1, Rg2, Rb2 (in cell culture)	–	13
15	*P. sokpayensis* Shiva K. Sharma et Pandit	Sikkim, India	Rb1 (5.2), Rd (7.9), Re (4.4), Rf (0.6), Rg1 (2.4), Rg2 (8.0)	I	5
16	*P. stipuleanatus* H.T. Tsai et K.M. Feng (Pingbian Sanchi)	Southwest China; North Vietnam	S-R2 (6.3–59.5), S-R1 (1.1–4.6), C-IV (0–4.0)	II	8,18
17	*P. trifolius* L. (Dwarf ginseng)	North America	Total ginsenosides (Re, Rf, Rg2 and Ro, etc.) content is about 0.06 mg/g	I	14
18	*P. vietnamensis* Ha et Grushv. (Vietnamese ginseng)	Southwest China; North Vietnam	M-R1 (7.2), M-R2 (93.5), P-RT4 (2.4), V-R11 (8.7), V-R1+V-R2 (33.7), Rb1 (8.1), Rb2 (3.6), Rd (2.3), Rg1 (10.9), Re (1.1), N-R1 (3.5), N-R2 (3.0)	I	15
19	*P. vietnamensis* var. *fuscidiscus*	Southwest China; North Vietnam	M-R2 (57.8–84.8), Rg1 (23.1–56.7), N-R1 (7.6), N-R2 (0.7–1.7), Re (1.0–3.7), Rb1 (6.2–15.6), Rb2 (3.9), Rc (0.8–2.3), Rd (0.2–6.0)	I	8
20	*P. vietnamensis* var. *langbianensis*	Vietnam	–	–	3
21	*P. wangianus* S.C. Sun	Meghalaya, India	–	–	16

(*continued on next page*)

Table 1 (*continued*)

No.	Species	Origin	Representative saponins (content, mg/g)	Group	Ref.
22	*P. zingiberensis* C.Y. Wu et K.M. Feng (ginger ginseng)	Southwest China; North Vietnam	Ro (68.3−93.9), C-IV (27.3−38.5), C-IVa (7.8−8.0), Rg1 (20.5−23.8), Rb1 (1.4−3.4), Rc (0.3−0.9), N-R2 (0−0.2), Z-R1 (0.8)	III	8,17

Group I plants mainly contain dammarane-type ginsenosides; Group II plants mainly contain OA-type saponins; Group III plants contain plenty of both dammarane-type and OA-type saponins. Saponin content is obtained from one of the cited literatures as a reference, and it varies due to different growing areas and ages of plants as well as sample preparation and analytical method. The saponin contents of *P. pseudoginseng* Wall. subsp. *himalaicus* and *P. japonicus* C.A. Mey. growing in different places are quite distinct from each other as described in literatures. C-: chikusetsusaponin, G-: gypenoside, M-: majonoside, N-: notoginsenoside, P-: 24(*R*)-pseudoginsenoside, P(*S*)-: 24(*S*)-pseudoginsenoside, S-: stipuleanoside, V-: vinaginsenoside R1, Z-: zingibroside.

chemical profiles, *Panax* genus could be divided into three groups, although they were classified into two groups previously[8]. *P. ginseng*[7], *P. notoginseng*[10], *P. quinquefolius*[7], and *P. vietnamensis* and its varieties[15,22] were classified in Group I characterized by high amounts of dammarane-type ginsenosides. *P. stipuleanatus* was a Group II species for having high content of OA-type ginsenosides only[8]. Group III species included *P. zingiberensis*[8], *P. bipinnatifidus* Seem.[5,6,64], *P. japonicus* and its varieties[8] for containing both large amount of OA-type ginsenosides and dammarane-type ginsenosides. The grouping of other ginseng species and their reported representative saponins were listed in Table 1.

2.1. *Representative ginsenosides*

The composition of ginsenosides varies within the same species grown in different area. For example, *P. japonicus* collected from China (named Zhujieshen) and Japan (named Chikusetsu-ninjin) are distinct in ginsenosides composition. Although both of them had plenty of OA-type Ro (55.9−163.0 mg/g), C-IV (16.5−82.4 mg/g) and C-IVa (4.3−20.0 mg/g), the latter was rich in PPD-type chikusetsusaponin III (C-III) (30.4−44.7 mg/g) but the former did not have this compound[8]. The former also had a considerable amount of Rg1, Re, Rb1 and Rd, while the latter only had minor of Re, N-R2, Rg1 and Rb1[8] (Table 1). Satsuma-ninjin, a special population of *P. japonicus* in southern Kyusyu Japan, which had plenty of Ro (37.9 mg/g), C-IV (38.2 mg/g), and considerable amount of C-IVa, C-III, Rb1, Rc, Re, Rg1 and N-R2[8] (Table 1), is also distinct to *P. japonicus* in China. C-III is a marker constituent in *P. japonicus* from Japan, present in both Chikusetsu-ninjin and Satsuma-ninjin[8].

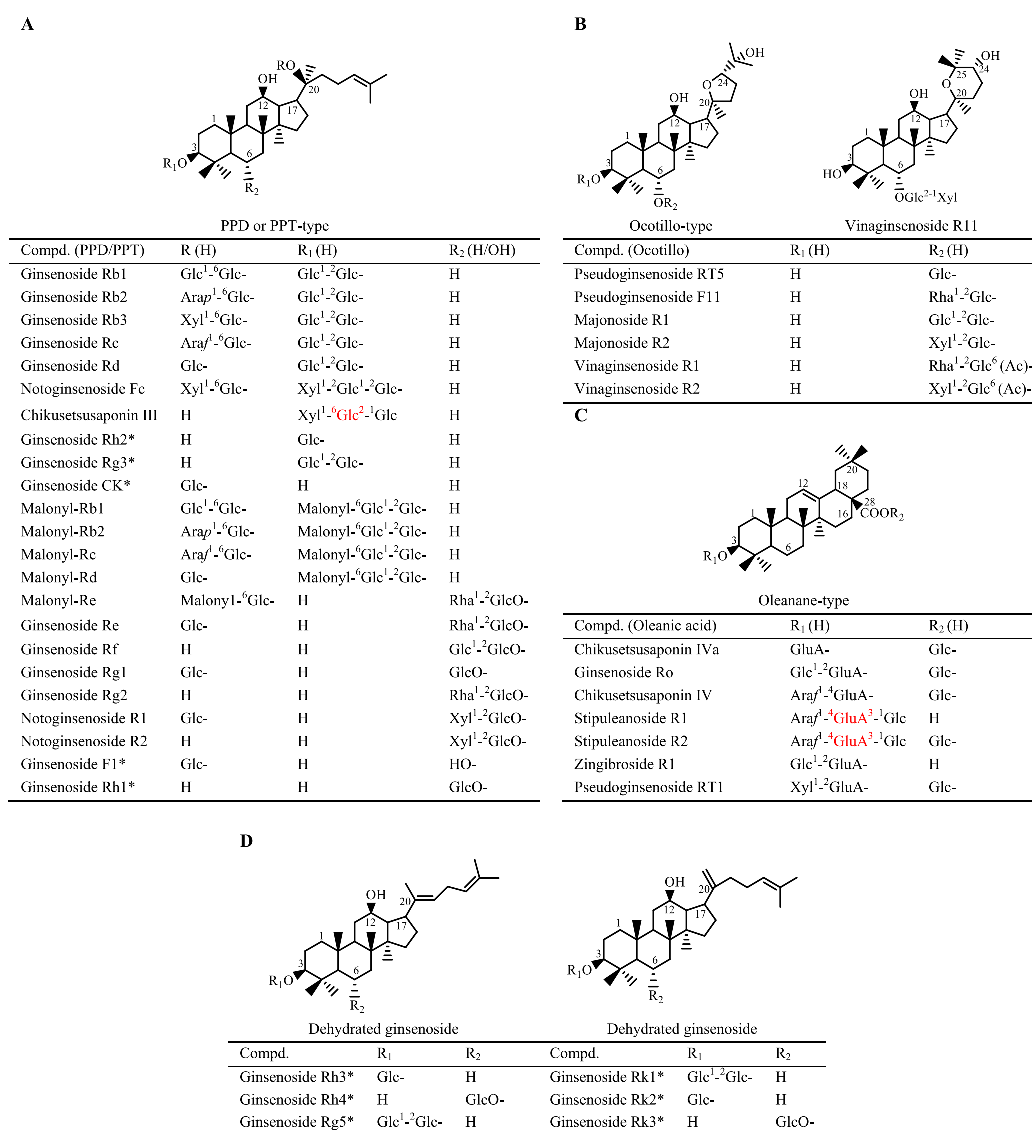
In addition, the content and the ratios of individual ginsenosides are varied in different parts within a species. For instance, the total saponin content in the rhizomes, main roots, fine roots, leaves, and stems of *P. ginseng* was about 142.4, 63, 142.5, 92 and 8.6 mg/g, respectively[7]. The most abundant ginsenosides were Rb1, Rg1, malonylginsenoside Rb1 (mRb1), Re, Rf, Rb2, mRb2, Rc and mRc in the main roots (their contents ranging from 2 to 15 mg/g, Table 1), Re (22 mg/g), Rg1 (10 mg/g), Rd (15 mg/g), mRd (12 mg/g), Rb2 (6 mg/g), mRb2 (4.5 mg/g), Rc (6 mg/g) and mRc (3 mg/g) in leaves, and Re (3 mg/g), Rg1 (2 mg/g) and mRd (1 mg/g) in stems, respectively[7]. Ginsenoside Rb1 and mRb1, the two most plentiful saponins in underground parts of *P. ginseng*, could not be quantified in some aerial parts due to the low concentrations[7]. As for *P. quinquefolius*, the total saponin content in fine roots, main roots, rhizomes, stems, and leaves was about 116, 76, 107, 13 and 37 mg/g, respectively. The major ginsenosides in leaves of *P. quinquefolius* were ginsenosides Re (10 mg/g), P-F11 (6 mg/g), mRb2 (4 mg/g), Rg1 (3 mg/g), Rb3 (2−3 mg/g) and mRd (2 mg/g), while those in its main roots were Rb1, mRb1, Re, Rg1, Rc, mRc, Rd, mRd, and P-F11 (1.5−20 mg/g, Table 1)[7]. Regarding to *P. notoginseng*, the content of total ginsenosides in the main roots, rhizomes, stems, and leaves was about 75.7−89.8, 137.5, 10.8 and 109.2 mg/g, respectively[9]. The leaves had high content of PPD-type saponins including ginsenosides Rb3 (25.4−32.6 mg/g), Rc (14.0−16.3 mg/g), Rb2 (4.8−6.7 mg/g), notoginsenoside Fc (N-Fc, 8.2−13.4 mg/g), and traceable N-Fe (0−0.58 mg/g) and N-Fd (0−0.98 mg/g), but no PPT-type ginsenosides[65], whereas the main roots were rich in both PPT-type ginsenosides Rg1, Re and N-R1, and PPD-type ginsenosides Rb1, Rd and Rc[9] (1.1−39.1 mg/g, Table 1). Ginsenoside Rf, P-F11 and N-R1 were regarded as distinctive compounds in

P. ginseng, *P. quinquefolius* and *P. notoginseng*, respectively[66]. Malonylginsenosides, such as mRc and mRb2, were also considered as differential compounds between *P. ginseng* and *Panax notginseng*[66]. In *P. vietnamensis*, total saponin content was about 195.2, 155.9 and 139.3 mg/g in rhizomes, radices, and fine roots, respectively, with majonoside R2 (M-R2), Rg1, Rb1, vinaginsenoside R11 (V-R11), V-R1, V-R2, and M-R1 being the richest[15]. M-R2, the characteristic and the most abundant saponin in roots of *P. vietnamensis*, was not found in its leaves[15].

Traditionally, rhizomes and roots of ginseng species are used for medicinal therapy and healthcare. Leaves of *P. ginseng*, *P. notoginseng*, and *P. quinquefolius* are all good source of specific ginsenosides that attract researchers to explore as alternative medicinal material resources. As for most of the other *Panax* species, ginsenosides compositions in their aerial parts still require to be explored.

2.2. Malonylginsenosides

Malonylginsenosides, a kind of acidic ginsenosides, comprise a large proportion of saponins in *Panax* genus[67–69]. The ratio of the total content of neutral ginsenosides (unmalonylated ginsenosides herein, Rb1, Rb2, Rb3, Rc, Rd, Rg1 and Re) to the corresponding malonylginsenosides in flowers of *P. notoginseng*, *P. ginseng* and *P. quinquefolius* were 5.52 ± 1.33%, 3.2 ± 0.64% and 2.39 ± 0.57%, respectively[69]. The content of mRb1 in the roots of *P. ginseng* and *P. quinquefolius* was up to 10 and 18 mg/g, respectively[7]. Recently, more than 100 malonylginsenosides have been tentatively characterized from *P. ginseng*, *P. quinquefolius* and *P. notoginseng*[68]. About 20 malonylginsenosides have been separated and identified from roots and flower buds of *P. ginseng*[67,70]. Malonyl groups were found linking to different positions of the first or the second glucose residues in C-3/C-20 of



Compd. (PPD/PPT)	R (H)	R$_1$ (H)	R$_2$ (H/OH)
Ginsenoside Rb1	Glc^{1}-^{6}Glc-	Glc^{1}-^{2}Glc-	H
Ginsenoside Rb2	Ara*p*^{1}-^{6}Glc-	Glc^{1}-^{2}Glc-	H
Ginsenoside Rb3	Xyl^{1}-^{6}Glc-	Glc^{1}-^{2}Glc-	H
Ginsenoside Rc	Ara*f*^{1}-^{6}Glc-	Glc^{1}-^{2}Glc-	H
Ginsenoside Rd	Glc-	Glc^{1}-^{2}Glc-	H
Notoginsenoside Fc	Xyl^{1}-^{6}Glc-	Xyl^{1}-^{2}Glc^{1}-^{2}Glc-	H
Chikusetsusaponin III	H	Xyl^{1}-^{6}Glc^{2}-^{1}Glc	H
Ginsenoside Rh2*	H	Glc-	H
Ginsenoside Rg3*	H	Glc^{1}-^{2}Glc-	H
Ginsenoside CK*	Glc-	H	H
Malonyl-Rb1	Glc^{1}-^{6}Glc-	Malonyl-^{6}Glc^{1}-^{2}Glc-	H
Malonyl-Rb2	Ara*p*^{1}-^{6}Glc-	Malonyl-^{6}Glc^{1}-^{2}Glc-	H
Malonyl-Rc	Ara*f*^{1}-^{6}Glc-	Malonyl-^{6}Glc^{1}-^{2}Glc-	H
Malonyl-Rd	Glc-	Malonyl-^{6}Glc^{1}-^{2}Glc-	H
Malonyl-Re	Malony1-^{6}Glc-	H	Rha^{1}-^{2}GlcO-
Ginsenoside Re	Glc-	H	Rha^{1}-^{2}GlcO-
Ginsenoside Rf	H	H	Glc^{1}-^{2}GlcO-
Ginsenoside Rg1	Glc-	H	GlcO-
Ginsenoside Rg2	H	H	Rha^{1}-^{2}GlcO-
Notoginsenoside R1	Glc-	H	Xyl^{1}-^{2}GlcO-
Notoginsenoside R2	H	H	Xyl^{1}-^{2}GlcO-
Ginsenoside F1*	Glc-	H	HO-
Ginsenoside Rh1*	H	H	GlcO-

Compd. (Ocotillo)	R$_1$ (H)	R$_2$ (H)
Pseudoginsenoside RT5	H	Glc-
Pseudoginsenoside F11	H	Rha^{1}-^{2}Glc-
Majonoside R1	H	Glc^{1}-^{2}Glc-
Majonoside R2	H	Xyl^{1}-^{2}Glc-
Vinaginsenoside R1	H	Rha^{1}-^{2}Glc^{6} (Ac)-
Vinaginsenoside R2	H	Xyl^{1}-^{2}Glc^{6} (Ac)-

Compd. (Oleanic acid)	R$_1$ (H)	R$_2$ (H)
Chikusetsusaponin IVa	GluA-	Glc-
Ginsenoside Ro	Glc^{1}-^{2}GluA-	Glc-
Chikusetsusaponin IV	Ara*f*^{1}-^{4}GluA-	Glc-
Stipuleanoside R1	Ara*f*^{1}-^{4}GluA^{3}-^{1}Glc	H
Stipuleanoside R2	Ara*f*^{1}-^{4}GluA^{3}-^{1}Glc	Glc-
Zingibroside R1	Glc^{1}-^{2}GluA-	H
Pseudoginsenoside RT1	Xyl^{1}-^{2}GluA-	Glc-

Compd.	R$_1$	R$_2$	Compd.	R$_1$	R$_2$
Ginsenoside Rh3*	Glc-	H	Ginsenoside Rk1*	Glc^{1}-^{2}Glc-	H
Ginsenoside Rh4*	H	GlcO-	Ginsenoside Rk2*	Glc-	H
Ginsenoside Rg5*	Glc^{1}-^{2}Glc-	H	Ginsenoside Rk3*	H	GlcO-

Figure 1 Representative and hot ginsenosides in *Panax* species. (A) PPD or PPT-type ginsenosides. (B) Ocotillo-type ginsenosides. (C) Oleanane-type ginsenosides. (D) Dehydrated ginsenosides. Ginsenoside metabolites (hot compounds) are marked by asterisk. Chikusetsusaponin III, stipuleanoside R1 and R2 are branched glycosides, sapogenins link to C-1 of Glc or GluA (marked by red).

PPD-type and C-6/C-20 of PPT-type malonylginsenosides, some of which had potential antidiabetic activities[67].

2.3. Minor ginsenosides

Minor ginsenosides usually refer to saponins that naturally occur at low concentrations (less than 0.1%) in *Panax* species with the most important being ginsenoside artifacts. The artifacts are mainly deglycosylated or dehydrated products or 20(*R*)-epimers. Some of them are hot molecules due to the high value as medicinal and healthcare materials. Major ginsenosides can be converted to minor ones through the drying and steaming process, and thus red ginseng is enriched in minor ginsenosides as it is made through these processes[71]. Microbiota-mediated metabolites of ginsenosides is another source of minor ones[72]. Ginsenosides Rg3 and Rh2 are two minor ginsenosides known for their anticancer activities[30,31]. Ginsenoside compound K (CK) is another minor saponin which has been paid much attention for its multiple bioactivities[73,74]. Ginsenosides Rg5 and Rk1, both of which showed potential anticancer activities[75,76], are two dehydration products of ginsenoside Rg3, and are enriched in steamed ginseng products[77]. The 20(*R*)-epimers of both Rg3 and Rh2 have also gained wide attention for their bioactivities[78,79]. Our group have revealed that notoginsenoside Ft1, a rare 20(*R*)-epimer of PPD-type saponin, has potential hemostatic, pro-apoptotic, angiogenesis and other activities[80–82]. Minor PPT-type ginsenoside Rh1, which content is increased in red ginseng[83], has antioxidant, anti-inflammatory, and immunomodulatory effects[84].

3. Biosynthetic pathways of ginsenosides

As mentioned above, ginsenosides are distinguished from other saponins by dammarane- or OA-type triterpene scaffolds decorated with one or more sugar chains. Correspondingly, the biosynthesis of ginsenosides contains three main processes, the formation of ginsenoside skeletons, the synthesis of sugar donors, and the skeletons modification processes.

3.1. Ginsenoside skeleton formation

(3*S*)-2,3-Oxidosqualene, the mono-oxidative product of squalene by SE[85], is the key precursor for the biosynthesis of both dammarane-type ginsenosides *via* DM cyclized by dammarenediol-II synthase (DDS) and OA-type ginsenosides *via* β-amyrin catalyzed by β-amyrin synthase (β-AS)[86,87] (Fig. 3). Squalene comes from the condensation reaction of isopentenyl diphosphate (IPP or IDP) and dimethylallyl diphosphate (DMAPP or DMADP) which are originated from either the cytosol MVA pathway or the plastid MEP pathway[88,89]. The modified MVA pathway discovered in archaea may also contribute to the formation of IPP through phosphomevalonate decarboxylase (MVAPD) and isopentenyl phosphate kinase (IPK) catalyzing reactions instead of phosphomevalonate kinase (PMK) and mevalonate diphosphate decarboxylase (MVD)[90,91].

Both MVA pathway and MEP pathway are involved in ginsenoside biosynthesis, and MVA pathway plays a more important role[89]. HMGR was considered as a rate limiting enzyme and was

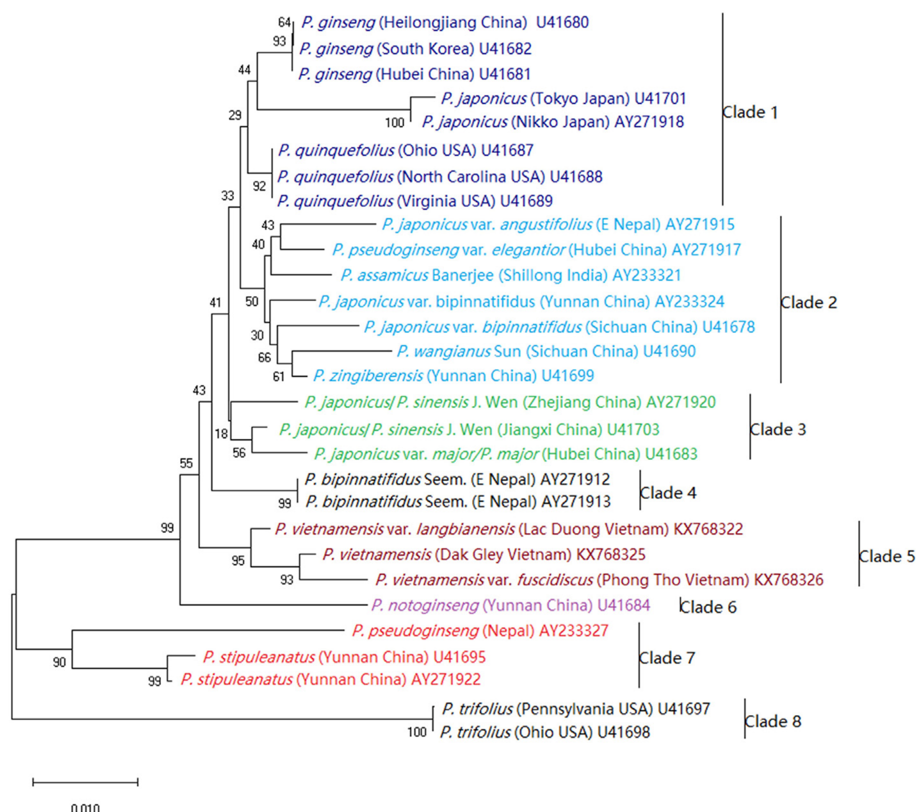


Figure 2 Phylogenetic tree of *Panax* plants based on ITS gene by Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The ID of ITS in GenBank are shown in the tree. *P. japonicus* from China was also treated as *P. sinensis* by Jun Wen et al.[56].

always selected as regulatory target in MVA pathway[92]. Overexpression of *HMGR* enhanced the production of ginsenoside in *P. ginseng* and *P. notoginseng*[92,93]. While MVD, another enzyme in MVA pathway played a key role in phytosterol rather than ginsenoside biosynthesis in *P. ginseng*[94].

3.1.1. Squalene biosynthesis enzymes

Squalene is synthesized from two FPP molecules by SS, while FPP is condensed by FPPS that catalyzes the sequential addition of two molecules of IPP to DMAPP to form first geranyl diphosphate (GPP) and then FPP. *PnFPPS* was highly expressed in flowers, leaves and stems of four years old *P. notoginseng*, but less in roots[95]. Expression patterns showed that the total saponins accumulation might result firstly from the contribution of *FPS*, then from *SS* and *DS*[96]. Overexpression of *PgFPPS* in *P. ginseng* hairy roots led to total ginsenosides increasing to approximately 2.4-fold of the control[94].

Three SS namely, PgSS1, PgSS2, and PgSS3, were functionally characterized from *P. ginseng*. *PgSS1* (mRNA) was highly expressed in all organs, whereas *PgSS2* and *PgSS3* were only found in specific tissues[97]. Co-overexpression of *PnSS* and *PnHMGR* in *P. notoginseng* cells, resulted in total ginsenosides increasing to 3- and 1.5-fold of the control and the cell line overexpressed *PnHMGR* alone, respectively[92].

3.1.2. Squalene epoxidases (SEs)

SE catalyzes the oxygenation of squalene to (3*S*)-2,3-oxidosqualene, and is one of the rate-limiting enzymes in this pathway (Fig. 3). Four to twelve SE isoforms were predicted in *P. ginseng*[98,99] and five isoforms were in *P. notoginseng*[100]. PnSE1 and PnSE2, belonging to two separate large groups, were similar to PgSE1 and PgSE2, respectively[95]. Phylogenetic analysis showed that the SEs from *P. ginseng*, *P. vietnamensis*, and *P. notoginseng* formed a clade, while the SE of *Panax quinquefolium* clustered outside[101], possibly due to the incompletely identification of multiple divergent SE homologs in *P. quinquefolium*. *In situ* hybridization experiments indicated that both *PgSE1* and *PgSE2* accumulated preferentially in vascular bundle tissue and resin ducts of petioles. RNAi of *PgSE1* completely suppressed *PgSE1* transcription but strongly upregulated *PgSE2* and *PNX* (cycloartenol synthase gene) mRNA, leading to the reduction of ginsenosides but the enhancement of phytosterols[85].

3.1.3. Oxidosqualene cyclases (OSCs)

The OSCs-catalyzed cyclization of (3*S*)-2,3-oxidosqualene is the first committed step in the biosynthesis of diverse triterpenoid saponins and phytosterols[102]. To date, more than 80 functionally different OSCs have been characterized in plants, contributing to the formation of diverse triterpenoid and steroid skeletons[103,104].

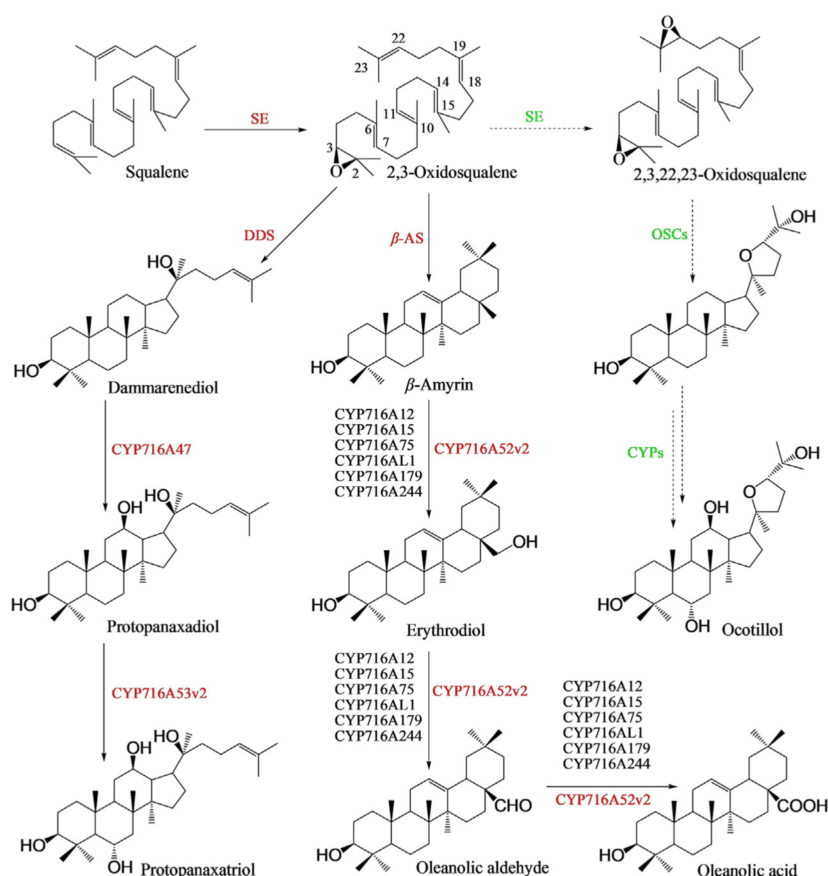


Figure 3 CYPs in the biosynthesis of different ginsenoside skeletons. CYPs marked in red and dark are identified from *Panax* species and other species, respectively. Solid arrows are proven pathways, dashed arrows are unproven pathways, and double arrows are multiple steps (similarly hereinafter). The abbreviations are indicated as followed, *β*-AS: *β*-amyrin synthase, CYP (s): cytochrome P450 (s), DDS: dammarenediol synthase, OSC (s): oxidosqualene cyclase (s), SE: squalene epoxidase.

There are about 13−19 genetically different *OSCs* in *P. ginseng* genome, encoding lanosterol synthase (LAS), cycloartenol synthase (CAS), DDS and β-AS[98,99]. DDS cyclizes (3*S*)-2,3-oxidosqualene to DM, which can be further oxidized to PPD and PPT. Several isoforms of DDS have been characterized including PgDDS from *P. ginseng*[105], PqDDS from *P. quinquefolius*[106], PnDDS from *P. notoginseng*[95,101] and CaDDS from *Centella asiatica*[107]. DDS within *Panax* species shows about 97% identity with each other[101], while CaDDS from *C. asiatica* shows about 78% identity with DDS from *Panax* species. β-AS from *P. ginseng* (PNY1 and PNY2), which catalyzes the formation of β-amyrin, has 56% identity with PgDDS[108]. The OSCs catalyzing the formation of the skeleton of M-R2, a major ginsenoside in *P. vietnamensis*[15], has not been reported yet. It was deduced that such OSCs might use (3*S*,22*S*)-2,3,22,23-dioxidosqualene as substrate to generate 3-epicabraleadiol (Fig. 4). This speculation was supported by the result that incubating (3*S*,22*S*)-2,3,22,23-dioxidosqualene with *Arabidopsis* lupeol synthase 1 (AtLUP1) expressed by yeast could produce epoxydammaranes and olefinic analogues, one of which was 3-epicabraleadiol, the precursor of ocotillol[109]. V-R11 with the content being about 8.7 mg/g in *P. vietnamensis* rhizome[15], has a similar skeleton with M-R2. It might also generate from (3*S*,22*S*)-2,3,22,23-dioxidosqualene but *via* (20*S*,24*S*)-20,25-epoxy-24-hydroxy-dammarenediol by the same or similar OSC with that of M-R2.

OSCs can be divided into two groups based on the different reaction mechanisms, the protosteryl cation mechanism and the dammarenyl cation mechanism[103,104]. The protosteryl cation serves as an intermediate leading to tetracyclic cycloartenol, lanosterol and cucurbitadienol *via* chair-boat-chair (CBC) conformation. The dammarenyl cation makes tetracyclic dammarenediol-II and baruol, or pentacyclic lupeol, β-amyrin, α-amyrin, germanicol and a series of other uncommon triterpenes *via* chair-chair-chair (CCC) conformation[110,111]. The cyclization mechanisms from oxidosqualene to triterpenoids and phytosterols are summarized in several articles[104,112]. Briefly, the cyclization involves four steps: 1) substrate binding and folding, 2) reaction initiation by protonation of the 2,3-epoxide, 3) cyclization, sometimes ring expansion and (or) skeleton rearrangement by 1,2-methyl and (or) -hydride shifts, and 4) termination by deprotonation or hydrated reactions. Here, we compared the different cyclization mechanisms of DDS, β-AS and other OSCs to fully understand the relationships between them (Fig. 4).

DDSs are uncommon in nature, which result in the unique ginsenoside scaffolds predominant in *Panax* species, and β-ASs are widespread in plant kingdom such as in Araliaceae[113],

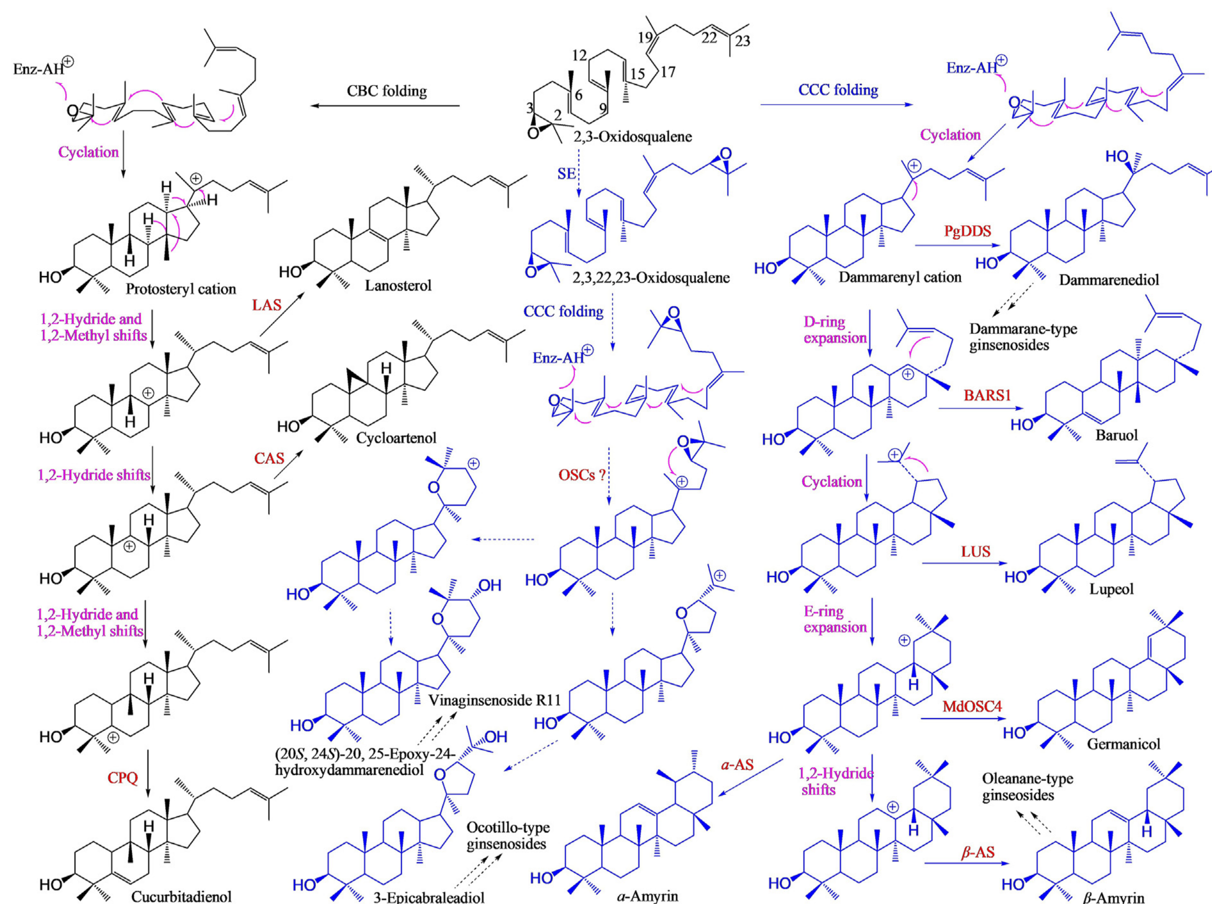


Figure 4 Comparison of different cyclization mechanisms among DDS, β-AS and other OSCs. Products labeled in black are generated *via* protosteryl cation, while products labeled in green are synthesized *via* dammarenyl cation. The abbreviations are indicated as follows, α-AS: α-amyrin synthase, β-AS: β-amyrin synthase, BARS1: baruol synthase, CAS: cycloartenol synthase, CCC: chair-chair-chair, CBC: chair-boat-chair, CPQ: cucurbitadienol synthase, LAS: lanosterol synthase, LUP: lupeol synthase, MdOSC4: oxidosqualene cyclase 4 of *Malus domestica*, PgDDS: dammarenediol synthase of *P. ginseng*.

Umbelliferae[114], Leguminosae[115] and other families[116,117]. DDS and β-AS from *Panax* genus are single product producing enzymes, while some other OSCs are multifunctional enzymes. For example, CrAS from *Catharanthus roseus* generated α- and β-amyrin in a 5:1 ratio[116], and MdOSC4 from *Malus domestica* catalyzed the formation of germanicol, β-amyrin, and lupeol in the proportion of 82:14:4[110]. In addition, a single mutation enabled lupeol synthase (LUP) had the function of β-AS[118]. All these results indicated that β-AS, α-AS and LUP were genetically and functionally closely related with each other.

The function of DDS and β-AS has also been investigated *in vivo*. Silencing of *DDS* expression in transgenic *P. ginseng* resulted in a reduction of ginsenoside production to 84.5%, indicating that DDS played a vital role in ginsenoside biosynthesis[108]. Transgenic *P. notoginseng* cells with *β-AS* from *P. japonicus* (*Pjβ-AS*) produced C-IV and C-IVa, two OA-type saponins[119], and transgenic *Pjβ-AS* rice produced OA-type sapogenin, demonstrating that β-AS was engaged in the biosynthesis of the precursor of OA-type ginsenosides[87]. Suppressing *β-AS*, *PNY1* and *PNY2* in *P. ginseng* hairy root resulted in the obvious decrease of OA-type ginsenoside (Ro) but the increase of dammarane-type ginsenosides (Rb1 and Rg1) and total ginsenosides[120].

3.2. Cytochrome P450s (CYPs)

Cytochrome P450s (CYPs) are heme-containing oxygenases with primary monooxygenase activity that introduce an oxygen atom from oxygen molecules into hydrophobic substrates generating more hydro-soluble products[121]. The oxygen atoms introduced by CYPs provide anchoring points for further decorations, significantly expanding the skeleton diversity[122], which is another essential process for the structural diversities of ginsenosides.

In plants, CYPs constitute one of the largest family of enzymatic proteins[123], but the reported CYPs related to the formation of ginsenosides are limited. Three CYPs, CYP716A47 (PPDS), CYP716A53v2 (PPTS) and CYP716A52v2 (OAS), have been identified from *P. ginseng* through homology-based cloning and *in vivo* or *in vitro* activity screening (Fig. 3)[124–126]. CYP716A47 could oxidize C-12 of DM to produce PPD which was further hydroxylated at C-6 by CYP716A53v2 to generate PPT. Different from the monooxygenase activity of CYP716A47 and CYP716A53v2, CYP716A52v2 was a multifunctional oxygenase participating in the oleanolic acid biosynthesis. It oxidized β-amyrin at the C-28 position to form oleanolic acid *via* erythrodiol intermediate through three-step reactions (Fig. 3)[127–130]. Six other CYPs, with 70% similarity to CYP716A52v2 including MtCYP716A12 from *Medicago truncatula*[128], VvCYP716A15 from *Vitis vinifera*[127], GuCYP716A179 from *Glycyrrhiza uralensis*[130], CrCYP716AL1 from *C. roseus*[131], EsCYP716A244 from *Eleutherococcus senticosus*[113], and MlCYP716A75 from *Maesa lanceolata*[129], had the same activities with CYP716A52v2, but the first four enzymes could also catalyze the oxidation of C-28 of α-amyrin and lupeol[127,130,131]. All the three characterized CYPs in *P. ginseng* belong to CYP716A subfamily, yet their functions are quite different from each other and they diversified ginsenoside structures further.

3.3. Biosynthesis of UDP-sugars

Sugar moieties are highly related with the biological activities of ginsenosides. Nucleoside diphosphate (NDP)-sugars were used as sugar-donors for glycosides biosynthesis, and UDP-sugars are the preferred ones for the glycosylation of secondary metabolites in plants[132,133]. Various sugar donors contribute to ginsenoside structure diversities leading to function and bioactivity diversified ginsenosides. UDP-α-D-glucose (UDP-Glc) was mostly used in ginsenoside biosynthesis, and β-D-glucosidic linkages were found almost in every ginsenosides[134,135] (Fig. 1). Apart from UDP-Glc, other sugar donors are also participating in the ginsenoside skeleton modifications such as UDP-GluA, UDP-Xyl, UDP-Gal, UDP-Ara*p*, UDP-Ara*f* and UDP-Rha.

Biosynthetic pathways for these UDP-sugars are diverse with the “salvage” and the interconversion pathways being the two main routes in plants[133]. Sucrose synthase (SUS) and UDP-glucose pyrophosphosphprylase (UGPase) are the two key enzymes respectively utilizing sucrose and α-D-glucose-1-phosphate (G1P) as the direct precursors in UDP-Glc biosynthesis to produce UDP-Glc[133]. The biosynthesis of UDP-sugars has not been explored in *Panax* species, let alone which route has the highest metabolic flux efficiency to produce specific ginsenosides. The conservative pathways of UDP-sugars in other plants will provide a reference for the study in ginseng species.

3.4. UDP-dependent glycosyltransferases (UGTs)

The glycosylation of most ginsenoside triterpene scaffolds is catalyzed by UGTs using UDP-sugars mentioned above as sugar donors to form diverse ginsenosides. Ginsenoside sapogenins are further decorated at C-3 or/and C-20 hydroxyl groups of PPD-type, C-6 or/and C-20 hydroxyl groups of PPT-type and C-3 hydroxyl or/and C-28 carboxyl groups of OA-type saponins. This is the third step for structural diversities of ginsenosides.

The nomenclature of UGTs is based on the homology of their amino acid sequences. A sequence homology >40% defines the family, while a homology >60% defines the subfamily[136]. Plant UGTs belonging to superfamily 1 GTs are assigned to the UGT family 71–100. The superfamily 1 GTs exhibit inverting catalytic mechanism and depict GT-B fold which contains two Rossmann folds in the N- and C-terminal residues, respectively[134,135,137]. The ginseng genome encodes a large diverse set of UGTs. About 225 or 226 *UGTs* have been predicted, accounting for one of the largest gene families in ginseng[98,99]. The encoding enzymes were assigned to 24 subfamilies, with UGT73 being the most abundant family, followed by UGT74 and UGT94[98]. A total of 158 *UGTs* encoding predominate UGT71, UGT73, UGT74, UGT85, UGT91 and UGT94 subfamilies were predicted in genome of *P. notoginseng*, and five of them, PnUGT1–5, were functionally characterized[100]. To date, dozens of UGTs that participate in the biosynthesis of dammarane-type saponins have been identified from *Panax* species (Fig. 5). These UGTs belonged to UGT71, UGT74 or UGT94 families. UGTPg1 (PgUGT71A53) catalyzed the glycosylation of the hydroxyl group at the C20 position in PPD and PPT to produce compound K and ginsenoside F1, respectively[138–140]. UGTPg100 (PgUGT71A54) specifically glycosylated the C6-OH of PPT to produce ginsenoside Rh1[139,140]. While UGTPg101 (PgUGT71A55) could glycosylate both C20-OH of PPT and C6-OH of F1 to generate ginsenoside F1 and Rg1, respectively[139,140]. PgUGT74AE2 and two of its homologs, UGTPg45 (PgUGT74AE4) and Pq3-*O*-UGT1, catalyzed the glycosylation of the C3-OH of PPD to produce ginsenoside Rh2. UGTPg29 (PgUGT94Q2) and its homologs Pq3-*O*-UGT2 could transfer glucose onto the C-2′ hydroxyl group of the first glucose residue at C-3 of ginsenoside Rh2 to produce ginsenoside Rg3[140–144], which were proved by over expression and RNAi

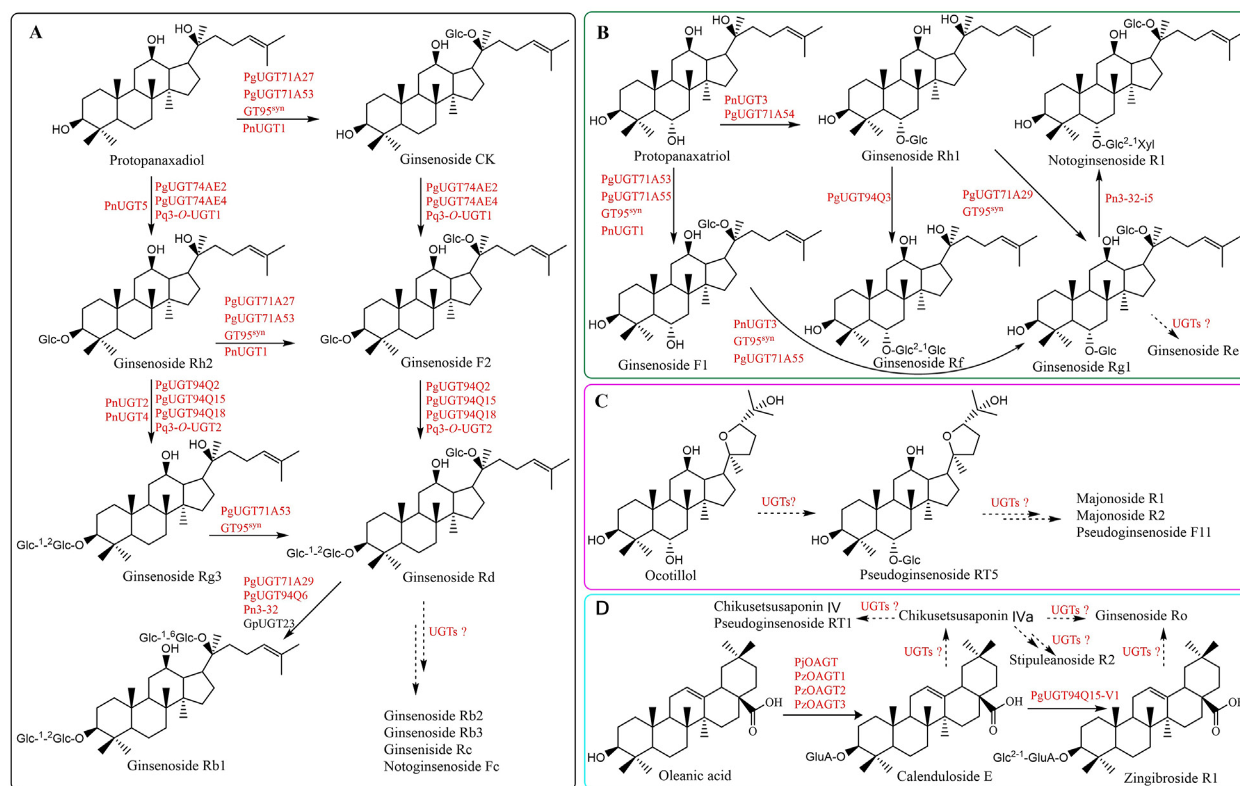


Figure 5 UGTs catalyzing ginsenoside biosynthesis identified from *Panax* species (A)−(D) show UGTs involved in PPD-, PPT-, OCT- and OA-type ginsenoside biosynthesis, respectively. The abbreviations are indicated as follows, Pg: *P. ginseng*, Pj: *P. japonicus* var. *major*, Pn: *P. notginseng*, Pq: *P. quinquefolius*, Pz: *P. zingiberensis*, Gp: *G. pentaphyllum*.

experiments concerning *Pq3-O-UGT1*, *Pq3-O-UGT2* and *PgUGT94Q2* in transgenic *P. quinquefolius* and *P. ginseng* hairy roots[143,144]. UGT71A29 has high similarity with UGTPg1 at amino acid level (96.47%). It could glycosylate Rh1 and Rd to produce ginsenosides Rg1 and Rb1, respectively[145]. Both Pn3-32 from *P. notoginseng* and GpUGT23 from *Gynostemma pentaphyllum* belong to UGT94 family. Both of them have low identity with UGT71A29 (less than 30%) but could also convert ginsenoside Rd to Rb1, and Pn3-32 could also catalyze F1 to form Rg1[135,146]. Interestingly, Pn3-32-i5, another UGT94 family member from *P. notoginseng*, could convert ginsenoside Rg1 to notoginsenoside R1 by adding a xylose moiety thus was identified as UDP-Xylose transferase, although it has >90% identity with Pn3-32 and PgUGT94Q2[146]. A serial of UGTs in UGT94 family with high amino acid identity, ranging from 85.87% to 99.78%, from *P. ginseng* and *P. notoginseng* have been cloned and characterized, recently[140]. Most of them had the activity of catalyzing Rh2 to produce Rg3 (PgUGT94Q18 showed the highest activities), some of them could extend the sugar chain at C3-*O*-Glc and C20-*O*-Glc of PPD-type saponins, or C6-*O*-Glc and C20-*O*-Glc of PPT-type saponins. Particularly, PgUGT94Q15-V1 could lengthen the sugar chain at C3-*O*-Glc of calunduloside E to produce zingibroside R1, an OA-type saponin discovered in *P. zingiberensis*[140]. Our team found that GT95^{syn} (belong to UGT71 family) originated from *P. notoginseng*, could transform 20(*R*)-PPD and 20(*R*)-PPT to produce 20(*R*)-CK and 20(*R*)-F1, respectively[147]. GTK1 and GTC1 from *Bacillus subtilis* were able to glycosylate the C3-OH of 20(*R*)-PPD to form 20(*R*)-Rh2[147]. The functional elucidated UGTs provided useful tools for production of diversified ginsenosides including those (*R*)- and (*S*)-isomers in the future.

By transcriptomic analysis and *in vitro* activity screening, other UGTs, namely OAGT, OAGT1, OAGT2, and OAGT3, particularly transferring glucuronic acid at the C-3 position of OA yielding calenduloside E, have also been characterized[24]. Three of them (OAGT1-3) were from *P. zingiberensis* and one (OAGT) was from *P. japonicus* var. *major*[24] (Fig. 5D). Although UGTs glycosylating C28-carboxyl of OA have not been reported, UGT74M1 from *Saponaria vaccaria* could transfer UDP-Glc to C28-carboxyl of gypsogenic acid, a substrate with similar structure to OA[148].

Whole genome sequencing of *P. ginseng*[98,99] and *P. notoginseng*[100,149,150] provided a genome-scale metabolic network and a holistic view of ginsenoside biosynthesis. The chromosome-level genome assembly and detailed transcriptional analysis of *P. notoginseng* combined with multi-omics analysis showed the biosynthesis and regulation of saponins at temporal and spatial levels, and would facilitate the identification of new functional genes including UGTs[100]. Transcriptomics of other *Panax* species[24,151−155] have also been reported, which will prompt identification of new UGTs in those *Panax* species.

3.5. Acyltransferases

Ginsenoside are always malonylated at the hydroxyl groups of sugar chains to generate malonylginsenosides, represented by mRb1, mRb2, mRc, mRd, and mRe. This is the last process to diversify the structure of ginsenosides. None of the enzymes responsible for linking those malonyl groups to ginsenosides has

been reported till now, although BAHD acyltransferases have been extensively reported in other plants[156]. For example, malonyltransferases, the BAHD family members using malonyl-CoA thioesters as the acyldonors, are versatile plant acyltransferases. Diverse malonyltransferases responsible for malonylating anthocyanin, flavonoid, isoflavonoid, and isoflavone glucosides have been functionally characterized in plants[157−160]. Such work would benefit for the researches of acyltransferases in ginsenoside biosynthesis.

4. Regulators

As well known, the biosynthesis of plant secondary metabolites is controlled by sophisticated regulatory networks in which regulators such as transcription factors (TFs) and non-coding RNAs (ncRNAs) play important roles. Thus, exploring regulators in ginsenoside biosynthesis is very helpful to decipher the accumulation patterns of these compounds in ginseng species. It has been reported that some TFs and ncRNAs could be stimulated by biotic and abiotic signals and participated in the regulation of ginsenoside biosynthesis.

4.1. Transcription factors (TFs)

A total of 2150 transcription factors belonging to 57 different families were identified from *P. notoginseng*[100], and 851 transcriptional regulators were predicted in *P. ginseng*[99]. TFs of WRKY, bHLH, MYB and AP2/ERF TF families play key roles in ginsenoside biosynthesis (Fig. 6). JA responsive genes involved in ginsenoside biosynthesis are regulated by different TFs[161−163]. MeJA and its analogues, the best effective elicitor on ginsenoside accumulation, were often used to stimulate ginsenoside biosynthesis in plant tissue cultures[52]. (+)-7-*iso*-Jasmonoyl-L-isoleucine (JA-Ile) was considered as the endogenous bioactive jasmonate[164], and MeJA was cleaved by esterases and subsequently converted to JA-Ile by JA-amino acid synthetase (JAR1) before working as an elicitor[165]. Jasmonate ZIM-domain (JAZ) proteins are among the most important components of the jasmonate pathway, acting as both repressors of downstream TFs and co-receptors of the hormone, together with the COI1 receptor[166]. Crosstalk between JA and other hormones is beyond this topic and can be find elsewhere[167,168].

Six *PgbHLH* genes were discovered to be potentially involved in the regulation of ginsenoside biosynthesis through tissue-specific expression and chemical constituents analysis[162]. In transgenic *PnbHLH P. notoginseng* cells, the expression levels of the four key genes involved in the biosynthesis of triterpenoid saponins namely, *PnDS*, *PnSS*, *PnSE* and *PnFPS*, were upregulated and the total saponin contents reached to about 2-fold in the transgenic *PnbHLH1* cell lines compared with the wild cell lines[169].

PqWRKY1, one of the WRKY family genes which could be induced by MeJA, was a positive regulator related to osmotic stress and ginsenoside biosynthesis in *P. quinquefolius*[161]. The expression level of *HMGR*, *FPS2*, *SQS1*, and *SQE2* in transgenic *PqWRKY1 Arabidopsis thaliana* were upregulated to 1−5-fold of the control, and the salt and drought tolerance of the transgenic plant was increased correspondingly[161]. Nine *PgWRKYs* (*PgWRKY*1–9) in *P. ginseng* have been identified recently, each of which includes one WRKYGQK sequence motif and one C2H2-type zinc-finger motif. Some of them could respond to cold, salt (NaCl) and various hormone external signals[170,171]. Notably, hypothermia stimulus experiments showed that the expression level of five ginsenoside biosynthetic genes viz., *GPS*, *SS*, *CYP716A53v2*, *UGT74AE2* and *UGT94Q2*, and three *PgWRKYs* viz., *PgWRKY1*, *PgWRKY3* and *PgWRKY8* had a strong positive correlation with the production of total ginsenosides, suggesting these *PgWRKY*s might participate in the biosynthesis of ginsenosides through regulating the pathway genes[171].

PgMYB1, a R2R3-type gene encoding a 238 amino acid protein, was expressed at higher level in roots, leaves, and lateral roots than in stems and seeds in *P. ginseng*. The transcription of *PgMYB1* could be upregulated by ABA, SA, NaCl, and cold (chilling), and downregulated by MeJA[172], while *PgMYB2*, another R2R3-type *MYB* gene, was significantly induced by MeJA and highly expressed in ginseng roots. PgMYB2 could bind to the promoter of *DDS,* and the transient expression of *PgMYB2* in ginseng leaves promoted the expression of *PgDDS*[163], indicating that PgMYB2 was possibly responsible for the biosynthesis of ginsenosides.

PnERF1, an AP2/ERF-type TF from *P. notoginseng*, was isolated by full-length cDNA cloning using Rapid Amplification of cDNA Ends (RACE) method[173]. In *PnERF1*-overexpressing *P. notoginseng* cell lines, the transcription levels of *DS* and *SS* were upregulated to 1.6- and 1.9-times of that in the control, and consequently six major monomer ginsenosides (Rg3, Rh1, Rd, Rg1, F1 and Re), especially Re and Rg1 were increased[173], suggesting that PnERF1 was related to the biosynthesis of ginsenosides.

4.2. ncRNAs

As reported, a total of 3688 mRNA-like non-coding RNAs (mlncRNAs), a class of lncRNAs, were identified in *P. ginseng*. Approximately 40% of the identified mlncRNAs were processed into small RNAs, implying their regulatory roles *via* small RNA-mediated mechanisms[174]. 73 conserved microRNAs (miRNAs) belonging to 33 families, and 28 non-conserved ones belonging to 9 families were identified from *P. ginseng*. Five of them were dehydration-responsive and ten of them were heat responsive miRNAs, and there was a crosstalk among some of the stress-responsive miRNAs[175]. In *P. notoginseng* roots, miR156 and miR166 were considered as the largest miRNA families, while miR156i and miR156g were the highest abundant ones[176]. Another report showed that miR156 family and one of its Squamosa Promoter-Binding Protein-Like (SPL) target genes had inverse expression levels, which was tightly correlated with greater root biomass contents[177].

5. Biotechnological approaches for ginsenoside production

Recent advances in understanding the ginsenoside biosynthesis are now opening the way to access these biologically important compounds applying time-saving and low-cost biotechnologies. Plant cell and tissue culture, synthetic biology-based microbial cell factory, and *in vitro* cascade reactions have been developed in ginsenoside production in recent years (Fig. 7).

5.1. Plant cell and tissue culture

Plant cell and tissue culture of ginseng is an effective approach for production of saponins[51,55]. Hairy roots (HR), adventitious roots

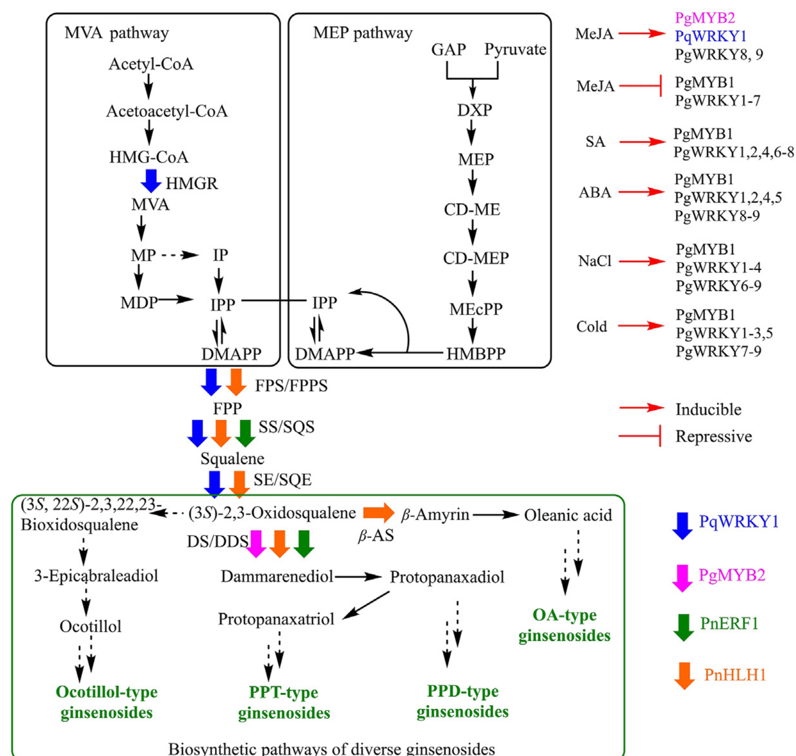


Figure 6 Transcription factors identified involved in ginsenoside biosynthesis. HMGR, FPPS, SS, SE, β-AS and DDS are regulated by at least one TF from *Panax* species, TFs regulating other steps of ginsenoside biosynthesis is unresolved. The color of the hollow arrow corresponds to the color of the transcription factors, which regulate the enzyme encoding gene. The abbreviations are indicated as follows, β-AS: β-amyrin synthase, CD-ME: 4-diphosphocytidyl-2-*C*-methylerythritol, CD-MEP: 4-diphosphocytidyl-2-*C*-methyl-D-erythritol 2-phosphate, DS/DDS: dammarenediol-II synthase, DMAPP: dimethylallyl diphosphate, DXP: 1-deoxyxylulose 5-phosphate, FPP: farnesyl diphosphate, FPS/FPPS: farnesyl diphosphate synthase, GAP: glyceraldehyde-3-phosphate, HMBPP: 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate, HMG-CoA (*S*)-3-hydroxy-3-methylglutaryl-coenzyme A, HMGR: HMG-CoA reductase, IP: isopentenyl phosphate, IPP: isopentenyl diphosphate, MDP: mevalonate-5-diphosphate, MEcPP: 2-*C*-methyl-D-erythritol-2,4-cyclodiphosphate, MEP: methylerythritol phosphate, MP: mevalonate-5-phosphate, MVA (3*R*)-3,5-dihydroxy-3-methylpentanoic acid, SS/SQS: squalene synthase, SE/SQE: squalene epoxidases.

(AR), and suspension cells (SC) were three commonly used materials. Generally, hairy roots were generated directly from explants infected by *Agrobacterium rhizogenes*[178]. Adventitious roots and suspension cells were induced from the explants *via* calli usually on Murashige and Skoog (MS) medium supplemented with plant growth regulators (PGRs), mostly 2,4-dichlorophenoxy acetic acid (2,4-D) or sometimes α-naphthalene acetic acid (NAA) and kinetin instead of 2,4-D for health and safety considerations[50,51]. Subculture of calli on liquid MS medium without any PGRs forms suspension cells[179]. Furthermore, adventitious roots were developed from calli cultured on MS medium supplemented with indole-3-butyric acid (IBA)[180]. Hairy root and adventitious root culture showed great advantages in both stable biomass growth and high ginsenoside production. However, hairy roots require *A. rhizogenes* induction and sustained antibiotics, which might have harmful effect to health. Currently, ginseng adventitious root culture has been industrialized with high ginsenoside yield and good safety[51].

MS or modified MS media were mostly used in plant cell and tissue cultures of *Panax* species, while B5 and SH (Schenk and Hildebrandt) media were occasionally used[53]. Sucrose in the concentration of 3%−5% was preferred carbon source[181]. Macroelement concentrations and nitrogen source showed important effects on ginseng adventitious roots growth and ginsenoside accumulation in modified SH medium cultures[182]. Appropriate temperature and light intensity also have profound effects on biomass growth and ginsenoside accumulation. In a hairy root culture experiment, the optimum temperature for total ginsenosides production (10.5 mg/g DW, 133.4 mg/L) was at 25 °C and the optimum temperature for biomass growth was under 20 and 13 °C cycle of day (12 h) and night (8 h)[183]. Fluorescent light (FL) irradiation was good for ginsenoside production (30.2 ± 0.9 mg/L), while red and dark light were favorable for hairy root growth, so two stage bioreactor culture methodology was proposed[183].

Application of elicitors are effective to improve ginsenoside production. Among these elicitors, MeJA and its analogues were most effective[52]. In a MeJA elicited experiment, all the MVA pathway genes except *IDI* were up-regulated. What's more, *HMGR* which encoded the enzyme that catalyzed the rate-limiting step in this pathway, was induced up to 178-fold in leaves and 6-fold in roots[141]. In hormone elicited experiments, two stage culture strategies were adopted to reduce the impact on biomass growth and get high content of ginsenoside. A two stage culture of *P. notoginseng* adventitious roots experiment showed that the highest total content of saponins (71.94 mg/g) was achieved after being treated with 5 mg/L JA, which was 2.09-fold higher than native roots and 8.45-fold higher than that in the control[184]. The ginsenoside content in adventitious roots of *P. ginseng* elicited with 150 μmol/L of MeJA showed up to 60 mg/g DW, which was about 20-fold enhancement compared to roots without MeJA

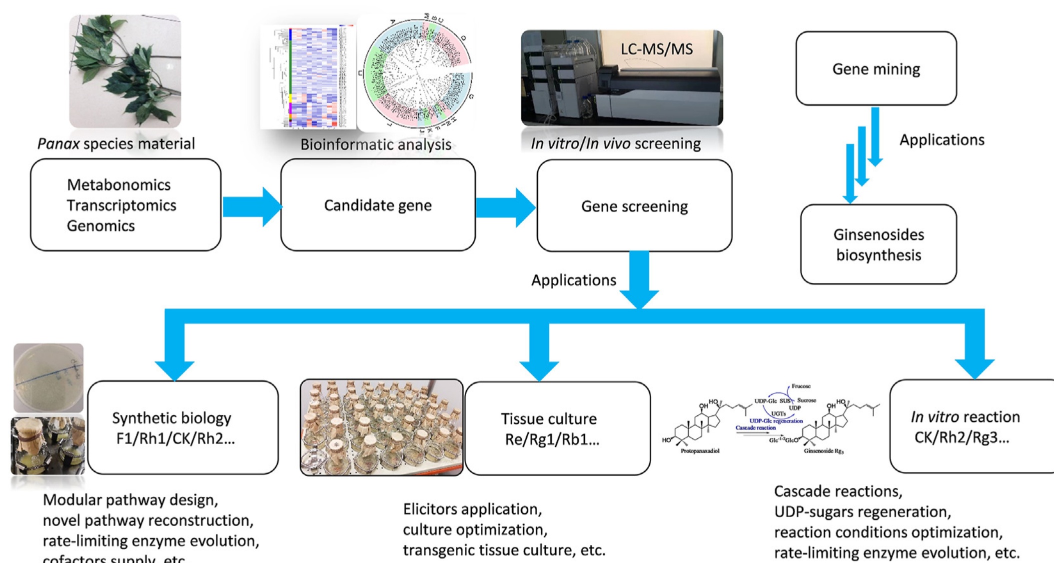


Figure 7 Omics-based gene mining technology and their applications in ginsenoside biosynthesis.

induction[185]. MeJA or JA treatment can obviously increase ginsenoside content and change the ratio of PPD- and PPT-type ginsenosides[179,185], probably by upregulating the expression level of *CYP716A47* and *CYP716A53v2* in a different degree[125]. Interestingly, both JA (5 mg/L) and methyl dihydrojasmonate (MDJ, 5 mg/L) treated *P. notoginseng* adventitious roots obviously increased both Rd and Rg group ginsenoside biosynthesis[184]. Another synthesized elicitor, 2-hydroxyethyl jasmonate (HEJ) could increase both protopanaxdiol 6-hydroxylase (P6H) and UDPG-ginsenoside Rd glucosyltransferase (UGRdGT) activities and simultaneously increased Rb1 and Rg group ginsenosides content in *P. notoginseng* cell cultures[186]. Abiotic elicitors such as, polyunsaturated fatty acids (PUFAs, JA precursor), ABA and SA[187−189], and biotic elicitors such as, yeast extracts and microbes were also used to increase saponins content[190−192]. JA, reactive oxygen species (ROS), Ca^{+} and ethylene signaling are involved in ginsenoside biosynthesis as reviewed by Rahimi et al[54].

Transgenic method is another effective way to improve ginsenoside production in tissue and cell culture. *HMGR*, *SS,* and *FPS* were three mostly used functional genes in transgenic ginseng cells and tissues. Overexpression of *PnHMGR* in *P. notoginseng* cells increased total ginsenoside content from about 20 to 40 mg/g, and co-overexpressions of *PnHMGR* and *PnSS* increased total ginsenoside titer to about 60 mg/g, which was about 2- and 3-times higher than that of the control, respectively[92]. In *P. ginseng* hairy roots, transgenic lines transformed with *PgFPS* showed the highest total ginsenoside content (36.42 mg/g), which was 2.4-fold higher compared with wild-type control[94]. Besides, transcription factors such as, *PnbHLH1* and *PnERF1* were also selected to improve ginsenoside biosynthesis. Transgenic cell lines of *P. notoginseng* with *PnbHLH1* increased total ginsenoside content by 1.7−2.2-fold compared with that in control cell lines[169]. In another experiment, the content of total saponins in *PnERF1* transgenic *P. notoginseng* increased about 2-fold from 40 mg/g in control cell lines to 80 mg/g in the top transgenic cell lines[173]. Transgenic ginseng plants with stable heredity and high saponin content productivity need to be further investigated. Main achievements of ginseng tissue and cell cultures in recent years are summarized in Table 2[92,94,169,173,178,184,186−188,191-215].

5.2. *Synthetic biology-based microbial cell factories*

Synthetic biology-based microbe cell factories especially heterogeneous expression systems such as engineered yeast and *Escherichia coli* provide efficient tools for various natural products synthesis[216−218]. The identification of PgDDS and CYP716A47 was conducive to producing useful dammarane-type ginsenosides by genetic engineering approaches[86,124], and with the identification of UGTPg1, the first glycosyltransferase in ginsenoside biosynthesis, ginsenoside compound K was produced with 1.4 mg/L in engineered yeast[138]. By co-expression of truncated *HMGR* (*tHMGR*), *DDS* and *β-AS*, *OAS*, *PPDS*, *PPTS* and *A. thaliana* cytochrome P450 reductase gene (At*CPR)* in *Saccharomyces cerevisiae*, 17.2 mg/L PPD, 15.9 mg/L PPT and 21.4 mg/L oleanolic acid were produced, simultaneously[219]. Overexpression of *tHMGR* was an effect method to increased PPD production, and simultaneously overexpressing *tHMGR*, *FPS*, *SS*, *SE* and *PPDS* led to 262-fold increase of PPD production. Finally, 1.19 g/L PPD and 1.55 g/L DM were produced in engineered *S. cerevisiae via* two-phase extractive fermentation[220]. Increasing coupling efficiency between PPDS and ATR1 by protein fusion was another strategy to improve PPD production and reduce the ratio of DM to PPD[221]. PPD content reached up to 4.25 g/L in 5 L fed-batch fermentation by PPDS−ATR1 protein fusion and *S. cerevisiae* ROS tolerance enhancing[222]. Protein and metabolic engineering were two other good choices to improve ginsenoside titers. By introducing semi-rationally designed mutant glycosyltransferase gene into yeast and further metabolic engineering, including preventing Rh2 hydrolysis and increasing UDP-Glc precursor supply, ginsenoside Rh2 were produced at about 300 mg/L in a 5 L bioreactor by fed-batch fermentation[223]. In a recent study, firstly *via* modular engineering of the MVA pathway and optimization of CYPs expression levels, and then by increasing the copy numbers of UGTs and engineering its promoter to increase expression levels, at last through direct evolution of UGT bioparts, the highest titer of PPD and Rh2 was reached up to 11.02 and 2.25 g/L in 10 L fed-batch fermentation, respectively[224]. Moreover, biosynthesis of bioactive unnatural ginsenosides also made great success, the titers of 3*β*-*O*-Glc-DM and 20*S*-*O*-Glc-DM, two

Table 2 Recent achievements in the production of ginsenosides by plant cell and tissue cultures (from 2006 to 2020).

No.	Plant	Cultured material	Basal medium	Elicitor/Transgenic gene	Maximum titers	Ref.
1	*P. ginseng*	AR	MS	MeJA, SA	40.4 mg/g DW with MeJA, 30.7 mg/g DW with SA	193
2	*P. ginseng*	AR	MS	SA	About 1 mg/g DW	194
3	*P. ginseng*	AR	MS	Nitrogen-fixing bacteria	105.6 mg/g DW	192
4	*P. ginseng*	AR	MS	Ethephon, MeJA	About 30 mg/g DW	195
5	*P. ginseng*	AR	MS	Gamma irradiation	63.2 mg/L	196
6	*P. ginseng*	AR	MS	–	15.9 mg/g DW	197
7	*P. ginseng*	AR	MS	MeJA	50 mg/g DW	198
8	*P. ginseng*	AR	MS	–	63 mg/L	199
9	*P. ginseng*	AR	MS	–	15.1 mg/g DW	200
10	*P. ginseng*	HR	MS, SH	*PgFPS*	36.4 mg/g DW	94
11	*P. ginseng*	HR	MS	Tween 80, Tween 20, MeJA	60–80 mg/g DW	201
12	*P. notoginseng*	SC	MS	MeJA, 2-hydroxyethyl jasmonate	28.9 mg/g DW (Re, Rg1, Rb1 and Rd)	186
13	*P. ginseng*	SC	MS	–	13.6 mg/g DW	200
14	*P. ginseng*	SC	67-V	*N*,*N*′-dicyclohexylcarbodiimide (DCCD), sodium nitroprusside	3.4 mg/g DW	202
15	*P. ginseng*	SC	67-V	NH_4VO_3, $NaVO_3$, $VOSO_4$, $NiSO_4$, $CuSO_4$, $MnSO_4$	5.6 mg/g DW	203
16	*P. ginseng*	SC	MS	Casein hydrolysate	4.9%	214
17	*P. japonicus*	SC	MS	Casein hydrolysate	3.2%	214
18	*P. japonicus*	SC	MS	–	2%–3%	204
19	*P. japonicus*	SC	MS	Casein hydrolysate	49.4 mg/g DW	205
20	*P. notoginseng*	AR	MS	JA, methyl dihydrojasmonate (MDJ)	71.94 mg/g DW	184
21	*P. notoginseng*	Calli	MS	*PnSS* and *PnHMGR*	About 60 mg/g	92
22	*P. notoginseng*	SC	MS	2-Hydroxyethyl jasmonate, MeJA	>40 mg/g DW	206
23	*P. notoginseng*	SC	MS	Phenobarbital	56.4 mg/g calculated by Re and Rg1	207
24	*P. notoginseng*	SC	MS	MeJA, HEJA	Rg1 (47.4 mg/L), Re (52.3 mg/L), Rb1 (190 mg/L), Rd (12.1 mg/L)	208
25	*P. notoginseng*	SC	MS	*PnbHLH1*	About 90 mg/g	169
26	*P. notoginseng*	SC	MS	*PnERF1*	About 80 mg/g	173
27	*P. quinquefolius*	AR	MS	4 mg/L *Alternaria panax* extracts	276 mg/L	191
28	*P. quinquefolius*	HR	B5	*trans*-Anethole	27.79 mg/g DW	209
29	*P. quinquefolius*	HR	MS, B5	–	Crude ginsenoside 0.2 g/g DW	178
30	*P. quinquefolius*	HR	B5	ABA	14.4 mg/g DW	187
31	*P. quinquefolius*	SC	MS	SA	About 70.5 mg/L	188
32	*P. quinquefolius*	SC	MS	Inorganic salt or culture filtrates of microorganisms	Total ginsenoside content increased 2- (54.3 mg/L) to 3.2-fold compared with control	210
33	*P. quinquefolius*	SC	MS	Lactoalbumin hydrolysate, MeJA	45.93 mg/L	211
34	*P. quinquefolius*	SC	MS	–	30 mg/g DW	212
35	*P. vietnamensis*	AR	MS	JA, ABA, SA, YE, CH	M-R2 (2.83%), Rg1 (0.32%), Rb1 (0.85%)	215
36	*P. vietnamensis*	SC	MS	Yeast extract (YE), chitosan (CH)	–	213
37	*P. sikkimensis*	SC	MS	SA	102.2 mg/L	188

The abbreviations are indicated as follows, HR: hairy roots; AR: adventitious roots; SC: suspension cells.

Table 3 Recent achievements in ginsenoside biosynthesis in yeast cell factories.

No.	Compd.	Titer (g/L)	Strategy	Ref.
1	CK	1.4×10^{-3}	Coexpression or overexpression of *DDS*, *CYP716A47*, *ATR2-1*, *UGTPg1*, *tHMGR* and *UPC2.1*	138
2	CK	1.17	Overexpression of *FPPS*, SS, SE and MVA pathway genes, codon optimization of *Pn3-29*, integration three copies of *PgDDS*, six copies of *PgPPDS*, two copies of *VvCPR*, insertion of *Pn3-29* and UDP-glucose synthetic pathway genes into the chromosomal LPP1 site, inactivation of LPP1 genes, cloning *Pn3-29* into the high-copy 2 micron plasmid	146
3	DM	1.55	Codon optimization of PPDS, coexpression or overexpression of *tHMGR*, *FPPS*, *SS, SE*, *DDS*, *PPDS* and *AtCPR1*	220
	PPD	1.19		
4	DM	7.10−8.09	Modular engineering of MVA pathway, optimization of CYPs expression level, integrating multicopy of *UGT*, and engineering UGT promoter	224
	PPD	9.05−11.02		
	Rh2	2.25		
5	3*β*-*O*-Glc-DM	2.4	Chassis cell optimization, integrating multicopy of *DDS* and *UGT*, block competitive pathway, and overexpression rate-limiting enzymes and transcriptional activator	225
	20*S*-*O*-Glc-DM	5.6		
6	3*β*,12*β*-Di-*O*-Glc-PPD	9.05×10^{-3}	Coexpression of *DDS*, *PPDS*, *ATR1*, *UGT109A1* and overexpression of *tHMGR*	226
7	PPD	17.2×10^{-3}	Coexpression of truncated *HMGR* (*tHMGR*), *DDS* and *β-AS*, *OAS*, *PPDS*, *PPTS* and *AtCPR*	219
	PPT	15.9×10^{-3}		
	OA	21.4×10^{-3}		
8	PPD	1.44	Construction of PPDS−ATR1 fusion protein, and overexpression of *tHMGR*, *FPPS*, *SS* and *SE*	221
9	PPD	4.25	Overexpression of *tHMGR*, *FPPS*, *SS*, *SE*, *SSD1* and *YBP1,* and construction of PPDS−ATR1 fusion protein	222
10	Rg3	1.3×10^{-3}	Coexpression of *DDS*, *PPDS*, *ATR2*, *UGT74AE2* and *UGT 94Q2* and replace *ERG7* promoter with methionine-repressible *MET3*	141
11	Rh2	17.0×10^{-3} (1.5 μmol/g)	Coexpression of *tHMGR*, *FPPS*, *SS*, *SE*, *DDS*, *PPDS*, *ATR2.1*, *UGTPg45* and *UGTPg29*	142
	Rg3	49.8×10^{-3} (3.5 μmol/g)		
12	Rh2	0.3	Directed evolution of UGT51, block Rh2 hydrolysis, and increasing UDP-Glc supply	223

saponins with anti-colon cancer activities, were reached to 2.4 and 5.6 g/L through fed-batch fermentation, respectively[225]. Main achievements and strategies in ginsenoside biosynthesis are summarized in Table 3[138,141,142,146,219–226].

5.3. *In vitro* cascade reactions

In vitro enzymatic methods especially cascade reactions and in-site UDP-sugars regeneration systems provided a green chemistry approach for efficient glycosylation in natural products synthesis[227,228]. Ginsenoside Rh2 was synthesized in 0.20 g/L by coupling promiscuous glycosyltransferase and sucrose synthase in one-pot reactions, representing the first attempt of ginsenoside biosynthesis *in vitro*[229]. The buffers, co-solvents, different substrate and enzyme ratios were often considered in cascade reactions.

Biotechnology-based approaches are independent of climate, season, cropland, and don't need pesticides, herbicides, and chemical fertilizers, thus providing environmentally friendly alternative ways for producing secondary metabolites[230]. The three main methods described above have their own advantages and challenges. The products of plant cell and tissue culture are not only ginsenosides but also ginseng peptides and polysaccharides, which are similar to that of the natural herbs and have great advantages in producing functional foods, cosmetics and healthcare products. Besides, plant cell and tissue cultures do not need to completely elucidate the biosynthetic pathways of ginsenosides. The challenge of this approach is that safety concerns must be paid on the culture extracts when using as cosmetics and foods products. Under these circumstances, the toxicological evaluation of plant cell and tissue cultures is required[51], and using food-conform culture medium and replacing synthetic phytohormones like 2,4-D with natural ones such as indole-3-acetic acid, zeatin is necessary[230]. Another challenge is that high quality facilities and instruments are required for large-scale cultures of plant cell and tissues. Synthetic biology-based approaches are dependent on the elucidation of the biosynthetic pathway of target ginsenosides and require elaborate design, construction and verification before application, but it is conductive to obtaining single compounds such as certain sapogenins and their monosaccharide products, which are the start materials for new drug development or additives of healthcare products[142,224,225]. Besides, it usually utilizes microbial cell factories which have sophisticated fermentation processes and facilities for large-scale production. Similarly, the merits of *in vitro* cascade reactions are to obtain single products for pharmaceutical industries, and are convenient to obtain a series of structural analogues to provide leading compounds for new drug research and development. This method brings forward higher requests to the stabilities and activities of enzymes and is dependent on natural resources or other strategies for starting materials.

6. Prospectives

Panax genus plants together with their secondary metabolites, mostly ginsenosides, are valuable resource for medicinal therapy and healthcare. Distinct multiple biological activities of structural diversity ginsenosides are resulted from both the specific triterpene skeletons and various sugar moieties. New functional enzymes, tissue specific regulators and various catalytic complexes (metabolons) are three possible factors that result in ginsenoside structure diversities.

β-AS which produce β-amyrin, the precursor of OA-type ginsenosides, is not uncommon in plant kingdoms. However, DDS that has high sequence similarity with β-AS is a new functional OSC in *Panax* genus, producing DM, the precursor of PPD- and PPT-type ginsenosides. Moreover, OCT-type ginsenosides, another kind of dammarane-type ginsenosides with a new skeleton that was different from PPD- and PPT-type ones, were discovered in *P. vietnamensis* and *P. quinquefolius*. These findings predict the possible existence of other specialized OSCs in *Panax* species. The diverse sugar chains of ginsenosides indicate different new functional UGTs may play key roles in ginsenoside glycosylation. UGTs responsible for the formation of ginsenoside Re, ginsenoside Ro, chikusetsusaponin IVa and majonoside R2 have not been fully characterized.

PPT-type ginsenosides, a kind of predominant bioactive compounds in *P. notoginseng* roots, are not present in leaves of *P. notoginseng*. The tissue distribution difference of PPT-type ginsenosides reminds us that PPTS may be regulated by tissue specific regulators in *P. notoginseng*. Another possibility is that two different metabolons may play roles in PPD and PPT biosynthesis, respectively.

Catalytic complexes which are not deeply researched are widespread in life cycles. The fact that all OCT-type ginsenosides are C-6 hydroxylated saponins tell us a catalytic complex may exist (maybe SE, OSC, CYPs and other partners form a catalytic complex) and play an important role in OCT-type ginsenoside biosynthesis. Identification of these metabolons will help us to manipulate ginsenoside biosynthesis.

Moreover, how do the enzymes of multigene family members coordinate to produce a particular saponin? How do the lipophilic aglycones and hydrophilic sugar donors transport between different organelles, tissues or organs and which organelle is the main accumulating and assembling location for UGTs catalyzed glycosylation? To comprehensively decipher the biosynthesis and regulatory mechanism of ginsenosides will benefit further biotechnological production of such valuable natural products.

Acknowledgments

We thank Dr. Xin Ju for his valuable comments on the manuscript. This work was supported by National Natural Science Foundation of China (No. 81673540, No. 81530096), Natural Science Foundation of Shanghai (No. 16ZR1434100, China), and Shanghai local Science and Technology Development Fund Program guided by the Central Government (YDZX20203100002948, China).

Author contributions

Zhengtao Wang and Shujuan Zhao gave the outline of the review and conducted the modifying work. Maoqi Hou retrieved the literatures and drafted the manuscript. Rufeng Wang participated in revising the manuscript.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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