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REVIEW ARTICLE

Genes and Regulatory Mechanisms for Ginsenoside Biosynthesis

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Abstract

Panax ginseng is a medicinal plant belonging to the Araliaceae family. Ginseng is known as the king of oriental medicine, which has been practiced since ancient times in East Asian countries and globally in the modern era. Ginseng is used as an adaptogen, and research shows that it has several pharmacological benefits for various ailments such as cancer, inflammation, diabetes, and neurological symptoms. The pharmacological benefits of ginseng are attributed to the triterpenoid saponin ginsenosides found throughout the *Panax ginseng* species, which are abundant in its root and are found exclusively in *P. ginseng* and *Panax quinquefolius*. Recently, with the completion of the entire ginseng genome sequencing and the construction of the ginseng genome database, it has become possible to access information about many genes newly predicted to be involved in ginsenoside biosynthesis. This review briefly summarizes the current progress in ginseng genome analysis and genes involved in ginsenoside biosynthesis, proposing directions for functional studies of the predicted genes related to ginsenoside production and its regulation.

Keywords Ginseng genome · Tetraploid · Ginsenosides · Shade growth · Metabolic regulation

Introduction

Panax ginseng Meyer is popularly known as the king of oriental medicine in Asia and has been widely consumed in the Far East, particularly in Korea, China, and other countries for longevity (Kim et al. 2018a). The *Panax* genus belongs to the Araliaceae family and most members of this genus have medicinal properties. There are 17 species of the *Panax* genus, including *Panax ginseng*, *Panax quinquefolius*, and *Panax notoginseng*. *Panax vietnamensis* has been widely cultivated in Korea, China, USA, Canada, and Vietnam (Shim et al. 2021). The pharmacological benefits of ginseng include the treatment of inflammation (Hofseth and Wargovich 2007; Saba et al. 2018; Yang et al. 2022), diabetics, cancer (Jin et al. 2016; Wang et al. 2016; Ahuja et al. 2018; Kim et al 2021a), neurological ailments (Mohanan et al. 2018; Kim et al. 2018a; Huang et al. 2019; Li et al. 2021), influenza A (Yoo et al. 2012; Lee et al. 2014), and even coronavirus disease 2019 (Ratan et al. 2021; Yi 2022). Among the various metabolites in ginseng, triterpenoid saponins are the key active components responsible for the medicinal properties of ginseng, and more than 150 types of ginsenoside saponins have been characterized (Mohanan et al. 2018).

Ginsenosides are divided into dammarane and oleanane types based on the structure of the aglycones. Dammarane-type ginsenosides are further classified into protopanaxadiol (PPD) and protopanaxatriol (PPT), whereas ocotillol-type ginsenosides are derived from oleanolic acid precursors (Shin et al. 2015). As several cytochrome P450 genes (CYPs) and uridine glycosyl transferases (UGTs) modify aglycone moieties, PPD- and PPT-type ginsenosides are further classified with chromatographic mobility according to the presence of sugar moieties. The PPD-type ginsenosides are Ra1, Ra2, Ra3, Rb1, Rb2, Rb3, Rc, Rd, quinquenosides

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(R1, Rs1, Rs2, and Rs3), and malonyl ginsenosides (mRb1, mRb2, mRc, and mRd) (Kim et al. 2015a, b; Shin et al. 2015). Each includes an aglycone with a dammarane structure and sugar moieties connected to the -OH at the C-3 and/or C-2 positions. The PPT-type ginsenosides are Re, Rf, Rg1, Rg2, Rh1, F1, and notoginsenosides (R1 and R2). The ocotillol-type ginsenosides are pentacyclic terpenoid compounds, which are the only characterized members of the ocotillol group in minor quantities in *P. ginseng*, while abundant in *P. quinquefolius*, *P. japonicus*, and *P. vietnamensis* (Christensen 2009).

Ginseng requires specific climatic and soil conditions for cultivation; therefore, only a few countries cultivate it. Ginseng cultivation is widely practiced in Korea, China, and North America, but there are considerable differences among these countries in the ginseng species they grow. *P. ginseng* has been cultivated in Korea, and North America (USA and Canada) has cultivated *P. quinquefolius*. China is a major producer of ginseng, cultivating various varieties such as *P. ginseng*, *P. quinquefolius*, and *P. notoginseng* (known as Chinese ginseng) (Baeg et al. 2013). With the high demand for ginseng-based medicinal materials and the increasing loss of mountain/wild ginseng, cultivated ginseng has become a major source. There is a considerable difference in morphology (Fig. 1) and ginsenoside contents between mountain-grown ginseng and cultivated ginseng (Suh et al. 2010; Chen et al. 2020). Korean breeding is systematic with diverse landraces, cultivars, and breeding lines. In China, a cultivation method in which ginseng populations are mixed is used to produce ginseng, with a focus on the ginsenoside content. The North American ginseng breeding style is often found in mountain ranges or privately owned landscapes in dense forests.

Fig. 1 The morphological characteristics of 10-year-old mountain ginseng plants and 6-year-old cultivated *Panax ginseng* and *Panax quinquefolius* grown in Korea. Mountain ginseng was obtained from a local breeder, while *P. ginseng* and *P. quinquefolius* in the picture were grown for breeding

Unlike the pharmacological efficacy of ginseng known to date, there is a gap in the physiological aspects of ginseng that have not been widely researched. In recent decades, efforts have been made to update the various features of ginseng plant characteristics, biosynthesis of ginsenosides (Kim et al. 2015a, b; Hou et al. 2021), tissue culture techniques (Qiang et al. 2020), ginseng evolution (Shim et al. 2021), and cultivar properties (Lee et al. 2015; Zhang et al. 2020; Kim et al. 2021b). In this review, we recapitulate the status of ginseng genome analysis in understanding the evolution of ginseng, the functional characterization of ginsenoside biosynthetic genes, and its regulation. We believe that this review would provide a one-stop solution for readers as well as encourage more research outcomes, particularly in the evolution of ginseng and ginsenoside biosynthesis.

Current Status of the Ginseng Genome

Advancements in sequencing technologies have had a greater impact on strengthening our knowledge of plant genomes. Genome profiling tools such as GenomeScope2 and Sumdgeplot have facilitated the reference-free assembly of the polyploidy genome of wild crop plants (Kress et al. 2022). Moreover, an increase in organelle genome studies in ginseng has shed light on the diversity, evolution, and authentication of breeding lines and ginseng materials. The whole genome provides information on the complexity of the ginseng genome, evolutionary diversity, and functional understanding of ginseng genes. Genome studies in Korean ginseng are more highly developed and advanced than those in American ginseng, although both are phylogenetically related species.

Intracellular Organellar Genome Advances the Diversity and Authentication of Ginseng

Chloroplasts (CPs) and mitochondria (MT) are intracellular organelles present in plants, contain their own genome, and are maternally inherited (Greiner et al. 2015). The CP genome is a quadripartite structure with two inverted repeat regions and a large and small copy region (Park et al. 2021). The CP genome comparison among nine Korean ginseng cultivars (Chunpoong, Yunpoong, Cheongsun, Gopoong, Gumpoong, Sunone, Sunpoong, Sunun, and Sunhyang), two landraces (Jakyung and Hwangsook), and *P. ginseng* collections in China (Damaya, Ermaya, and Gaolisen) has shown 14 single nucleotide polymorphisms (SNPs), suggesting a high homogeneity among ginseng plants between these countries (Kim et al. 2015b; Nguyen et al. 2020).

Notably, the CP genomes of ginseng collected in China and the Korean ginseng landrace Jakyung are identical. In contrast, the ginseng cultivar Sunhyang has a unique polymorphism among all accessions (Kim et al. 2015b). Chinese ginseng collections Damaya, Ermaya, Gaolisen, and Yeshanshen share identical CP genomes. Notably, even the positions of minor allelic variations among these collections are shared, indicating that these alleles are subjected to selection pressure due to changing environments (Zhao et al. 2014a). The CP genomes of *Panax* species have highly conserved structures (Kim et al. 2016a). Nucleotide variations and variation hotspots are abundant in the intergenic sequence (IGS) regions *trnE-trnT*, *trnT-psbD*, *ndhF-rpl32*, and *rpl14-rpl16* and are useful for understanding the diversity of *P. ginseng* along with other *Panax* species. A large insertion in the *rps2-rpoC2* IGS was found between *P. ginseng* and *P. quinquefolius*, and not in the ginseng cultivars. It is estimated that *P. quinquefolius* diverged from *P. ginseng* 0.29 million years ago (MYA), and the diploid ginseng, *P. notoginseng* diverged 1.30 MYA from the common ancestor (Kim et al. 2013), thereby providing vital insight into the evolution of *Panax* species in Asia.

Unlike CP genomes with conserved structures, the MT genome is large, ranging from 200 to 2000 kbp, and complex because most genes are shared or integrated with other genomes of plant cells (Morley and Nielsen 2017). Based on several MT genome datasets, the mtDNA structure in plants is predicted to be circular, with two pairs of large and small circles and linear arrays (Gualberto et al. 2013). Although diverse and polymorphic, the mechanism by which the MT genome reproduces its pattern in many species for several generations remains elusive. Recently, the MT genome of the Gumpoong cultivar was successfully assembled in a master circle using nanopore long-read sequencing data. The analysis showed that the MT genome is 464.7 kbp in size with 72 unique genes and 10.42% of mitogenome sequences of plastid origin (MTPT). In addition, this MTPT region is a mutational hotspot containing 74.5% variation, which is atypical in plants. Significantly, Kompetitive allele-specific PCR from SNP regions excluding the MTPT sites showed greater variation among Korean ginseng cultivars (12 cultivars and 47 breeding lines), whereas no variation was observed among the 10 American ginseng populations (Jang et al. 2020). These indicate that *P. quinquefolius*, introduced for breeding in Korea, could be derived from the same germplasm, while there is a wider diversity among cultivars and Korean ginseng breeding lines within the MT genome. In another study on the Korean ginseng landrace Jakyung, phylogenetic analysis using MT protein-coding genes revealed a close relationship with *Daucus carota* in the Apiales family (Jang et al. 2021), thereby revealing the evolutionary signatures of *P. ginseng* in Korea. Unlike the CP genome, which is conserved among Korean ginseng cultivars with minor variations, the MT genome provides deeper knowledge about its diversity and MTPT regions among Korean cultivars, which could be used for cultivar authentication.

Ginseng Draft Genome

The functional genomics of ginseng began with the characterization of genes and transcriptomics of adventitious roots after methyl jasmonate (MeJA) elicitor treatment. Advancements in genome assembly methods have led to progress in the assembly of the ginseng genome for the identification of the ginsenoside biosynthesis pathway, which was initially attempted by a Chinese group (Xu et al. 2017). The first de novo genome assembly of the ginseng cultivar Chunpoong was published by a Korean group (Kim et al. 2018b). These studies have considerably improved our understanding of the ginseng genome, genes coding for ginsenoside biosynthesis, and other functional genomics of ginseng. The draft ginseng genome is predicted to be 3.6 GB in size with 59,352 protein-coding genes (Kim et al. 2018b). The abundant genes in the ginseng genome are the result of two rounds of whole genome duplication events at 2.2 MYA. A primitive genome duplication event occurs at 28 MYA, known as Pg-β, and a recent event occurred 2.2 MYA, known as *Pg-α* (Waminal et al. 2021). Essentially, 60% of the ginseng genome is covered by repeat sequences, including long-terminal repeat retrotransposons (LTR-RTs) belonging to LTR/Gypsy (49%) and *PgDel* (30%). The ginseng genome also provides information on the ginsenoside pathway, including paralogs (Table 1). The multiple copy numbers present in mevalonate pathway genes and triterpenoid biosynthesis genes suggest the importance of these genes in the ginsenoside pathway. The identified transcription factors, regulators, and protein kinase genes in the ginseng genome are twice as abundant as those in most plant genomes (Kim et al. 2018b). The roles of these factors in ginseng development, perennial growth, and metabolite biosynthesis need to be investigated. In addition, the pseudo-chromosome level assembly of the ginseng genome could advance our understanding of genome arrangement, gene structure, and metabolic gene clusters in ginseng. Moreover, synteny-based analyses among *Panax* species might provide insights into genome evolution as well as speciation.

Genes Involved in Ginsenoside Biosynthesis

Ginsenosides are found in various organs of ginseng, such as the leaves, stems, roots, berries, and seeds (Christensen 2009). They are synthesized from the cytoplasmic-derived mevalonate (MVA) and plastid-derived 2-C-Methyl-D-erythritol 4-phosphate (MEP) pathways. Several studies

Table 1 List of predicted genes involved in isoprenoid and ginsenoside biosynthesis in *P. ginseng*

Pathway	Genes	Genes IDs	End product
MVA (Mevalonate Pathway)	*AACT* (*Acetoacetyl-CoA thiolase*)	Pg_S1068.4, Pg_S6240.3, Pg_S0022.46	Acetoacetyl-CoA
	HMGS (*Hydroxymethylglutaryl-CoA synthase*)	Pg_S0849.33, Pg_S6647.3, Pg_S6896.2,Pg_S4594.4, Pg_S2776.2	HMG-CoA
	HMGR (*Hydroxymethylglutaryl-CoA reductase*)	Pg_S0126.10,Pg_S0913.16, Pg_S1295.30, Pg_S3959.3, Pg_S6083.2, Pg_S6137.4	Mevalonate
	MVK (*Mevalonate Kinase*)	Pg_S0573.2, Pg_S1114.3	Mevalonate-phospahte
	PMK (*Phosphomevalonate Kinase*)	Pg_S3098.25, Pg_S3321.6, Pg_S5751.1, Pg_S2636.15	Mevalonate-pyrophospahte
	MVD (*Diphosphomevalonate decarboxylase*)	Pg_S1430.1, Pg_S3074.4	Isopentyl diphospahte
	IDI (*Isopentenyl-diphosphate Delta-isomerase*)	Pg_S1168.2, Pg_S7337.2, Pg_S6728.1	DMAPP
MEP (Methylerythritol 4-phosphate)	*DXS* (*1-deoxy-D-xylulose-5-phosphate synthase*)	Pg_S0859.7, Pg_S1214.23, Pg_S1675.7, Pg_S1908.21, Pg_S2229.10, Pg_S2533.15, Pg_S3759.10, Pg_S5599.2	DXP (1-deoxy-D-xylulose 5-phosphate)
	DXR (*1-deoxy-D-xylulose-5-phosphate reductoisomerase*)	Pg_S0166.7, Pg_S4650.8, Pg_S3733.18, Pg_S1373.8	MEP (2-C-methyl-D-erythritol 4-phosphate)
	MEP-CT (*2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase*)	Pg_S0148.25, Pg_S0544.32	CDP-ME (4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol)
	CDP-MEK (*4-diphosphocytidyl-2-C-methyl-D-erythritol kinase*)	Pg_S0550.8, Pg_S2198.2	CDP-ME2P (2-Phospho-4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol)
	MECDPS (*(E)-4-hydroxy-3-methylbut-2-enyl-diphosphate synthase*)	Pg_S0285.3, Pg_S1005.15, Pg_S4369.6, Pg_S7699.3	ME-2,4cPP (2-C-methyl-D-erythritol 2,4-cyclodiphosphate)
	HMBPPS (*1-hydroxy-2-methyl-2-butenyl 4-diphosphate synthase*)	Pg_S0247.51, Pg_S0833.9, Pg_S1135.32, Pg_S5991.6	HMBPP (1-hydroxy-2-methyl-2-butenyl 4-diphosphate)
	HMBPPR (*4-hydroxy-3-methylbut-2-en-1-yl diphosphate reductase*)	Pg_S0447.5, Pg_S3713.7, Pg_S4375.11, Pg_S4375.10, Pg_S4604.8, Pg_S4604.9	IPP (Isopentenyl diphosphate)
	GPS (*Geranyl diphosphate synthase*)	Pg_S7299.1, Pg_S3016.11	GPP (Geranyl diphosphate)

Table 1 (continued)

Pathway	Genes	Genes IDs	End product
Downstream pathway (Triterpenoid / Ginsenoside pathway)	*FPPS* (*Farnesyl diphosphate synthase*)	Pg_S0304.36, Pg_S8325.1	FPP (Farnesyl diphosphate)
	SS (*Squalene Synthase*)	Pg_S0992.8, Pg_S1637.7, Pg_S1678.33, Pg_S2014.27	(SQ) Squalene
	SQE (*Squalene Epoxidase*)	Pg_S0129.28, Pg_S1693.31, Pg_S2606.7, Pg_S2606.8, Pg_S2840.6, Pg_S3767.15, Pg_S6308.10, Pg_S4651.2, Pg_S1672.1, Pg_S6081.2, Pg_S6152.1, Pg_S3064.5	SE (2,3-oxidosqualene)
	Beta-AS (*β-amyrin synthase*)	Pg_S0888.6, Pg_S2492.7, Pg_S2801.2, Pg_S0034.9, Pg_S2939.4, Pg_S0034.2, Pg_S4815.4,Pg_S0361.30	BAS(β-amyrin)
	DDS (*Dammarenediol II synthase*)	Pg_S3318.3, Pg_S4166.7, Pg_S3517.9,Pg_S3586.1	DDS (Dammarenediol)
	PPDS (*Protopanaxadiol synthase*)	Pg_S4733.5, Pg_S3293.6	PPD (Protopanaxadiol)
	PPTS (*Protopanaxatriol synthase*)	Pg_S1770.12, Pg_S0325.7	PPT (Protopanaxatriol)

have proven that the cytosolic MVA pathway contributes predominantly to ginsenoside biosynthesis than the plastid-derived MEP pathway (Xue et al. 2019; Hou et al. 2021). The MVA pathway begins with the condensation of three units of Acetyl-CoA to form 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) by acetyl-CoA acetyltransferase and HMG-CoA synthase (HMGS). In ginseng, there are two functionally characterized HMGR paralogs that have distinct expression patterns (Kim et al. 2014a). *PgHMGR1* is widely expressed in the roots of adult ginseng plants (3-year-old and 6-year-old), whereas *PgHMGR2* expression gradually increases throughout ginseng development, suggesting that these expression patterns may contribute to the biosynthesis of ginsenosides. Intriguingly, MeJA treatment enriched the ginsenoside contents and specifically enhanced the expression of *PgHMGR1* rather than *PgHMGR2* (Kim et al. 2014a). IPP is produced from MVA by a sequential phosphorylation reaction catalyzed by mevalonate kinase and phospho-mevalonate kinase, and a decarboxylation reaction catalyzed by mevalonate diphosphate decarboxylase (MVD). Notably, the overexpression of *PgMVD* enhanced the accumulation of phytosterols, such as campesterol, stigmasterol, and β-sitosterol, up to 4.4-fold compared to ginsenosides (Kim et al. 2014b). Recently, Xue et al. showed the transcriptomic profiling of various ginseng tissues and age-dependent expression pattern changes in the MEP and MVA pathways. They found that transcripts in the MEP pathway are also abundant in ginseng root tissues, similar to the MVA pathway (Xue et al. 2019). However, the transcripts of 2-C-methyl-D-erythritol 4-phosphate cytidylyl transferase (IspD, MEP pathway) and MVA pathway genes, such as *PgMVK*, *PgPMK*, and *PgMVD*, are also transcribed at a low level (Xue et al. 2019). Based on these analyses, it could be assumed that ginsenoside biosynthesis could occur in roots in addition to the source-to-sink transport as proposed by Kim et al. (2016b). Several studies on monoterpenoids, sesquiterpenoids, isoprenoids, non-isoprenoids, and biosynthetic mechanisms have demonstrated a contribution of both MEP and MVA pathways in the biosynthesis of secondary metabolites (Hampel et al. 2005, 2007; Opitz et al. 2014; Wölwer-Rieck et al. 2014). However, if one pathway is inhibited, another pathway can compensate for biosynthesis (Zhao et al. 2014a, b). Consequently, based on these findings in ginseng, the MVA pathway seems to be a major contributor to the common precursor IPP.

The skeleton of ginsenoside begins with the condensation of 10 carbon (C10) gernalypyrophosphate (GPP) with C5 IPP to yield C15-FPP catalyzed by farnesyl pyrophosphate synthase (FPS) (Karunanithi and Zerbe 2019). The contribution and importance of FPS were shown by the overexpression of *PgFPS* in ginseng, which showed a 2.4-fold increase in ginsenoside content in the transgenic hairy roots (Kim et al. 2014b). Two molecules of C15-FPP are condensed to

produce a C30-squalene molecule catalyzed by membrane-bound squalene synthase (SS) (Devarenne et al. 2002). This reaction is conserved among plants and humans; however, unlike humans with one copy of the *SS* gene, plants have multi-copy *SS* genes, suggesting their contribution to phytosterol and triterpenoid biosynthesis. Three *SS* genes were characterized in ginseng with biochemical activity characterized by complementation of the yeast *erg9* mutant (Kim et al. 2011). *PgSS1* is ubiquitously expressed in ginseng tissues, with enriched expression in the shoot apex and root; however, *PgSS2*, and *PgSS3* are uniquely expressed in specific organs (Lee et al. 2004). The enhanced expression of *PgSS1* either by overexpression and/or MeJA treatment promotes the expression of downstream genes, such as squalene epoxidase (*SE*), β-amyrin synthase (*BAS*), and accumulation of dammarane-type ginsenosides (Lee et al. 2004), suggesting that *PgSS1* is a key gene in regulating ginsenoside biosynthesis and phytosterol in ginseng. The C30-squalene is subsequently oxidized by squalene epoxidase (SQE) to form 2,3-oxidosqualene, which is considered the rate-limiting step in triterpenoid and phytosterol biosynthesis. SQE is present in high copy numbers in plants: six copies of SEs in *Arabidopsis* (Rasbery et al. 2007), five copies in *Glycyrrhiza* species (Manzoor et al. 2021), and 12 copies of SQEs in ginseng (Kim et al. 2018b). Although several paralogs of *PgSEQ* in ginseng have been functionally characterized, *PgSQE1* is abundantly expressed in all ginseng organs, whereas *PgSQE2* is expressed only in petioles and flower buds (Han et al. 2010). In situ hybridization revealed that *PgSE1* and *PgSE2* transcripts accumulate preferentially in petiole vascular bundles and resin ducts, raising the possibility that the role of paralogs in biosynthesis differs (Han et al. 2010). Specifically, MeJA treatment enhanced the accumulation of *PgSQE1* mRNA rather than suppressed *PgSQE2* (Han et al. 2010). Arabidopsis mutants lacking SQE show severe developmental defects, with a reduced root, hypocotyl elongation, and produced not viable seeds. Arabidopsis mutants have shown an accumulation of squalene, indicating the block in terpenoid biosynthesis (Rasbery et al. 2007). Likewise, a recent metabolomic study in ginseng seeds between non-dehiscence and dehiscence seeds shows the upregulation of squalene and phytosterol (stigmasterol branch) biosynthesis pathway (Min et al. 2022). These studies suggest that the squalene dynamics and composition of phytosterols could facilitate the germination of seeds. Moreover, RNAi analysis of *PgSQE1* in transgenic ginseng roots shows strong upregulation of *PgSQE2* and *PgCAS* and accumulation of phytosterols in transgenic roots (Han et al. 2010, 2020), suggesting that *PgSQE1* could be the primary enzyme responsible for ginsenoside biosynthesis. *PgSQE2* compensates *PgSQE1* for the accumulation of phytosterols but not for ginsenoside biosynthesis. In ginseng, the first committed step in ginsenoside biosynthesis is catalyzed by the dammarenediol-II synthase (DDS) enzyme, which cyclizes 2,3-oxidosqualene to synthesize a tetracyclic dammarenediol backbone (Tansakul et al. 2006), and there are four paralogs of *PgDDS* (Kim et al. 2018b). Han et al. showed that *PgDDS* is ubiquitously expressed in ginseng, such as in leaves, petioles, roots, and flower buds, and its expression is enhanced after treatment with MeJA (Han et al. 2006). Consequently, heterologous expression of *PgDDS* in *erg7* yeast mutants defective in lanosterol synthase and in tobacco cells could produce dammarenediol metabolites (Han et al. 2006; Liang et al. 2009). In addition, RNAi of *PgDDS* in ginseng adventitious roots has shown a significant reduction (up to 84.5%) in ginsenoside production (Han et al. 2006). DDS, BAS, and CAS share a common precursor, 2,3-oxidosqualene; thus, silencing of *PgCAS* shows an increase in *PgDDS* activity and in the accumulation of ginsenosides (up to 100%) (Liang et al. 2009). In addition to dammarane-type ginsenosides, oleanane-type pentacyclic minor ginsenosides are produced from β-amyrin by BAS with 65% similarity to PgDDS (Suzuki et al. 2006). Two *BAS* genes have been characterized in ginseng. *PgBAS1* is widely expressed in all tissues, whereas *PgBAS2* is exclusively expressed in flower buds and roots. Moreover, silencing of *PgDDS* enhanced the transcription of both *PgBAS* genes (Han et al. 2006). RNAi of *PgBAS* leads to reduced levels of β-amyrin and oleanane-type ginsenosides and upregulates dammarane-type ginsenoside levels (Zhao et al. 2015). Together with the dynamic expression pattern of OSCs in ginseng (*PgDDS, PgCAS*, and *PgBAS*), these findings suggest a strong interplay between transcripts and metabolite synthesis, contributing to ginsenoside biosynthesis. Phylogenetic analysis of OSCs revealed that they have a common ancestor with LAS in higher plants and have evolved as a result of tandem duplication and positive selection pressure (Xue et al. 2012).

The Role of Glycosyl Transferases on the Diversity of Ginsenosides

The aglycone ginsenoside backbone is hydroxylated by two CYP enzymes, protopanaxadiol synthase (CYP716A47, hereafter PPDS) and protopanaxatriol synthase (CYP71653v2, hereafter PPTS), to produce PPD- and PPT-type ginsenosides (Han et al. 2011; Shin et al. 2015). Park et al. characterized *PgPPTS* genes using overexpression studies. The transgenic roots that accumulate *PgPPTS* transcripts have increased amounts of PPT-type ginsenosides (Rg1, Re, and Rf) and, conversely, contain lower levels of PPD-group ginsenosides (Park et al. 2016). The RNAi of *PgPPTS* genes showed a decrease in the expression of *PgPPTS* and PPT-group compounds and an increase in high levels of the PPD-group compound (Park et al. 2016). Likewise, CYPs appear to be involved in the conversion of

β-amyrin to oleanolic acid, an oleanane-type ginsenoside (ginsenoside Ro) precursor in ginseng. CYP716A52v2 (β-Amyrin 28-Oxidase), which belongs to the CYP714A family, significantly increases the concentration of ginsenoside Ro but does not increase the concentration of the dammarane-type ginsenoside (Han et al. 2013). Ectopic expression of PgBAS and CYP716A52v2 catalyzes the synthesis of β-amyrin to produce oleanolic acid in transgenic adventitious roots. These observations suggested that *CYP* genes are involved in the production of PPD-, PPT-, and oleanolic acid-type ginsenosides. Recently, CRISPR/CAS9 mediated editing of *PPTS* genes in ginseng reconfirmed the findings of RNAi studies. Knockout/knockdown of the *PgPPTS* gene in ginseng results in enhanced production of PPD-type ginsenosides (Choi et al. 2022). *CYP* genes involved in ginsenoside biosynthesis in *P. quinquefolius* were recently identified after MeJA elicitor treatment. Transcriptomic analysis showed that the mapped EST contigs contain 150 *CYP450* genes. It has been identified that 27 *CYP450* genes belong to the *CYP71* and *CYP85* superfamilies, which are abundant in the MeJA-treated transcriptome (Sun et al. 2010).

Glycosylation and biosynthesis of diverse ginsenosides are achieved by UDP-dependent glycosyltransferases (UGTs), which catalyze the transfer of sugar moieties (Hou et al. 2021). UGTs in *P. ginseng* catalyze the addition of monosaccharides to triterpene aglycones, primarily at C-3 and/or C-20 for PPD-type ginsenosides or at C-6 and/or C-20 for PPT-type ginsenosides (Rahimi et al. 2019). The transcriptome analysis, MeJA elicitor treatments, and gene mining following heterologous expression techniques have been used to identify candidate UGTs involved in ginsenoside biosynthesis and, most importantly, to produce pharmacologically valuable ginsenosides (Jung et al. 2014; Kang et al. 2018). Two UGTs, PgUGT74AE2 and PgUGT94Q2, have been identified in ginseng based on yeast transformation studies. PgUGT74AE2 transfers the glucose moiety from UDP-glucose (UDP-Glc) to the C3 hydroxyl groups of PPD and compound K to synthesize ginsenosides Rh2 and F2, respectively. In addition, PgUGT94Q2 transfers the glucose moiety from UDP-Glc to Rh2 and F2 to form Rg3 and Rd, respectively (Jung et al. 2014). The transcriptomic and metabolomic analyses after MeJA elicitor treatment of ginseng adventitious roots identified 11 candidate UGTs, which could be involved in ginsenoside glycosylation. Among these, three UGTs have been previously characterized in ginseng, whereas eight are new UGTs (Kang et al. 2018). Notably, MeJA treatment upregulated most of the PPD-type ginsenosides after 48 h, whereas PPT- and oleanane-type ginsenosides were unaffected. In particular, ginsenosides Rg3, Rd, and Rb3 exhibit dramatic accumulation (Kang et al. 2018). Similarly, MeJA treatment upregulated 11 UGTs and downregulated three UGTs in *P. quinquefolius* (Sun et al. 2010). In *P. ginseng*, no such downregulation of UGTs was observed after MeJA treatment, suggesting the complexity and uniqueness of the two ginseng species and their ginsenoside biosynthesis. A UGT from *P. quinquefolius* known as Pq3-O-UGT2, catalyzes the glycosylation of Rh2 and F2 to produce Rg3, and Rd has been identified (Lu et al. 2017). Phylogenetic analysis revealed that Pq3-O-UGT2 shares an evolutionary relationship with PgUGT94Q2. Notably, RNAi silencing of these respective UGTs in *P. ginseng* and *P. quinquefolius* revealed a reduction in ginsenoside Rd content, protopanaxadiol-type, and total ginsenoside levels. In addition, the expression of PPDS and PPTS is upregulated in *P. quinquefolius* (Lu et al. 2017). The ginsenoside Rd content was reduced in *P. quinquefolius,* whereas *P. ginseng* contains a high ratio of ginsenoside Rg1 to Rb1. Moreover, *PPDS* expression levels are higher in *P. quinquefolius* than in *P. ginseng*. These findings suggest the preserved biochemical function of UGT during evolutionary adaptation regarding transcriptional expression and regulation between the two ginseng species. Several discoveries related to UGTs and their possible roles in ginsenoside biosynthesis have been reported, although many are yet to be characterized. Dynamic changes in the gene expression of *CYPs* and *UGTs* observed in metabolic networks provide an opportunity for the metabolic engineering of ginsenosides.

Ginsenoside Biosynthesis and its Regulatory Mechanisms

To date, regulation of ginsenoside biosynthesis in *Panax* tetraploid and diploid species remains limited. Diverse regulatory controls of ginsenoside biosynthesis may be linked to the existence of multiple copies of metabolic genes and isoforms of biosynthetic enzymes. The diverse expression patterns of these isoforms in tissues and at different embryonic stages may be correlated with environmental and/or developmental cues (Kim et al. 2015a). The tissue- and organ-specific transcription of *PgHMGR*, *PgSS*, and *PgSE* might influence the differential accumulation of ginsenosides. For example, continuous dark treatment for 2 d induces the expression of *PgHMGR1* and increases the enzymatic activity of HMGR in 3-year-old ginseng plants (leaf and roots) with a subsequent increase in ginsenoside content. Additionally, the individual ginsenoside contents vary with dark treatment (Kim et al. 2014a). In tobacco, squalene is stored in the cytosol as a lipid particle and can be redirected toward sterol synthesis when needed. Inhibition of either SS or SE was found to trigger a several-fold increase in the enzymatic activity of HMGR, providing the first evidence for positive feedback regulation of this key enzyme in response to selective depletion of endogenous sterols (Wentzinger et al. 2002). Kim et al. proposed metabolic dynamics in ginseng according to the tissue and age of the ginseng plant

(Fig. 2). Total metabolite analysis from different organs and year of ginseng suggests that ginsenoside biosynthesis is active and maximum in juvenile ginseng (1–2-year-old) and starts to gradually decrease in the leaves of adult plants (3–5-year-old) (Kim et al. 2018c). It is proposed that the higher photosynthetic rate of adult ginseng could pave the way for the production of antioxidative metabolites such as osmoprotectants (Kim et al. 2018c). Owing to the oxidative metabolites in ginseng leaves, plants cannot allocate energy for ginsenoside metabolism. In American ginseng, leaflet removal has detrimental effects on root development. Leaf removal at the early stage of root development considerably affects the dry mass of the root, while removing 1–3 leaflets causes a noteworthy reduction in root weight during harvest. However, rhizome development is unaffected by this effect (Proctor 2008). These observations suggest that the leaf is the site of ginsenoside biosynthesis in ginseng plants, and similar to most plants, leaves are essential for the proper development of the root system. In addition to the functional characterization of genes involved in ginsenoside biosynthesis, several recent studies have identified that MYB, bHLH-MYC, and WRKY transcription factors are involved in the regulation of ginsenosides (Chu et al. 2018; Liu et al. 2019; Yao et al. 2020). The R2R3-MYB protein, PgMYB2, is expressed specifically in the roots, and its expression is rapidly induced after MeJA treatment in ginseng adventitious roots (Liu et al. 2019). DNA binding assays have shown that PgMYB2 binds to the *PgDDS* promoter and activates the expression of the *PgDDS* gene in the tobacco transient assay system (Liu et al. 2019). Similarly, a WRKY transcription factor, PgWRKY4X, positively regulates ginsenoside production and has been found in fungal elicitor-treated ginseng adventitious roots (Yao et al. 2020). *Chaetomium globosum*, an endophytic fungus isolated from *P. notoginseng*, elicits a 3.94-fold higher ginsenoside content in ginseng. *PgWRKY4X* was identified from positive correlation analysis of coupled transcriptomic and metabolomic data and showed a correlation with *PgHMGR* and *PgSE*. The binding assay with the W-box motif in *PgHMGR* and *PgSE* with *PgWRKY4X* has shown selective binding of the transcription factor to the *PgSE* promoter. Overexpression and RNAi transformation in ginseng showed a 4.47-fold upregulation of *PgSE* in *PgWRKY4X* overexpression lines compared to the control, whereas a 0.27-fold downregulation was shown in RNAi lines compared to the control. Furthermore, there was a significant upregulation of most ginsenoside biosynthesis genes, such as *PgHMGR PgGPS PgFPS, PgSS,* and *PgSE* in the overexpression lines. The total saponin content in these overexpression lines was 1.81-fold higher than that of the control. In addition, the contents of major ginsenosides Rb1, Rb2, Rd, Re, and Rg1 also increased in these lines (Yao et al. 2020). The total saponin contents in these overexpression lines are 1.81-fold higher than the control. In addition, the contents of major ginsenosides, Rb1, Rb2, Rd, Re, and Rg1, are also increased in these lines (Yao et al. 2020). These results suggest that the genes in the ginsenoside pathways could be uniquely targeted by various transcription factors and that their transcription abundance enhances ginsenoside biosynthesis. Although many genes have been functionally identified, little is known about the mechanism of regulating ginsenoside synthesis at the molecular level. Research related to this will be needed in the future.

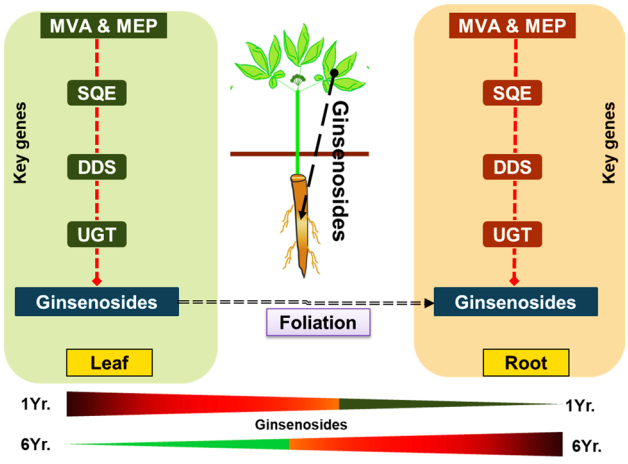

Fig. 2 Representative ginsenoside biosynthetic pathway in the leaves and root of *P. ginseng*. There could be a metabolic dynamics in ginseng from leaves to root during foliation and an age-dependent accumulation of ginsenosides in the roots (Kim et al. 2015a, 2018b). *MVA* mevalonate, *MEP* 2-C-Methyl-D-erythritol 4-phosphate, *IPP* isopentyl pyrophosphate, *FPS* Farnesyl pyrophosphate synthase, *SS* Squalene synthase, *SQE* Squalene epoxidase, *DDS* Dammaranediol synthase, *UGT* uridine glycosyl transferases

Conclusion and Future Perspectives

Ginsenoside triterpenoid saponins are pharmacologically valuable medicinal components that are exclusively present in the *Panax* species. Among these, *P. ginseng* and *P. quinquefolius* are the two most consumed medicinal plants worldwide because of their enriched ginsenoside content. To date, 150 ginsenoside metabolites have been identified in ginseng extracts, with varying sugar compositions in their dammarane or oleanolic acid saponin backbones. Research on ginseng has been actively conducted, focusing on the biosynthesis of ginsenoside metabolites, breeding of high-efficiency cultivars against stress, and analysis of relationships between ginsenoside content and root shape. The identification and functional characterization of ginsenoside biosynthetic isozymes and paralogous genes, such as *UGT*s and *CYP*s, still need to be elucidated. Compared to *P. ginseng*, research on *P. quinquefolius* remains limited and requires improvement to understand the complexity of

tetraploid ginseng. Findings from diploid *Panax* species focusing on physiology, development, and metabolic regulation could provide a vital lead for research on tetraploid *Panax* species. Thus, the diploid *Panax* species is another active research area that is yet to be elucidated. Molecular understanding of pharmacologically important metabolites from ginseng species provides the basis for molecular engineering of yeast and bacteria, or for molecular farming into tobacco or related species, which are easy to cultivate and share conserved metabolic pathways that will be efficient for the large-scale production of ginsenosides.

Despite its great potential in medical treatment, major ginsenosides are not well absorbed in blood circulation due to their large molecular weight, making them less bioavailable. On the other hand, minor ginsenosides have high cell membrane permeability due to their small molecular weight, showing higher pharmacological effects than major ginsenosides (Ke et al. 2022). Because minor ginsenosides are present in very low amounts in ginseng, they cannot fulfill the research and clinical needs. In addition, there is a limit to its use due to its low solubility in water and target accuracy. Therefore, research on the mass production of minor ginsenosides through plant transformation or modification from major ginsenosides and through the improvement of the solubility and target delivery using nanoparticles will be in the spotlight.

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Declarations

Conflict of interest The authors declare that they have no competing interests.

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