

MicroRNA-mediated regulation of ginsenoside biosynthesis in *Panax ginseng* and its biotechnological implications

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Abstract

Panax ginseng, renowned for its therapeutic properties, derives much of its medicinal value from ginsenosides, a group of bioactive triterpenoid saponins. The biosynthesis of ginsenosides is regulated by various mechanisms, including microRNAs (miRNAs), which play key roles in gene regulation. Recent studies have identified numerous miRNAs in *P. ginseng* and other plants, highlighting their potential to influence triterpenoid biosynthesis by targeting key genes in the pathway. This mini-review explores the current understanding of miRNA-mediated regulation in *P. ginseng* and discusses the potential for controlling ginsenoside production through miRNA manipulation. Although miRNA research in *P. ginseng* is still primitive, ongoing studies suggest its potential for promising applications in agriculture and medicine. Further functional studies on these miRNAs could provide valuable insights into optimizing ginsenoside biosynthesis and enhancing medicinal properties.

Keywords

Panax ginseng, ginsenoside biosynthesis, microRNAs, gene regulation, posttranscriptional gene silencing

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Introduction

Panax ginseng Meyer, often referred to as the “king of oriental medicine,” has been utilized for centuries in East Asia, particularly in Korea and China, for its health-promoting and longevity-enhancing properties.¹ The *Panax* genus, a vital member of the Araliaceae family, holds significant medicinal and economic value, with *P. ginseng* being particularly esteemed for its rich diversity of bioactive constituents.² Among these, triterpenoid saponins—commonly known as ginsenosides—are the primary pharmacologically active compounds. To date, more than 150 ginsenosides have been identified, contributing to the extensive therapeutic potential of *P. ginseng* and underscoring the importance of further research to explore its full range of applications in medicine and biotechnology.^{1,3}

The biosynthesis of ginsenosides begins with the formation of squalene, a linear C30 molecule synthesized via the condensation of two farnesyl diphosphate molecules derived from isopentenyl diphosphate and dimethylallyl diphosphate.^{4,5} This process is followed by enzymatic steps that produce (S)-2,3-oxidosqualene, which is cyclized by oxidosqualene cyclases to form the dammarenyl cation, a key intermediate in ginsenoside biosynthesis.^{6–8} The structures of ginsenosides are then further diversified by subsequent oxidation by cytochrome P450 monooxygenases and glycosylation.⁹ Despite significant progress in elucidating the ginsenoside biosynthetic pathway, its key enzymatic steps and regulatory mechanisms are not yet fully understood.

MicroRNAs (miRNAs) are small (~20–24 nucleotides), highly conserved noncoding RNAs that regulate gene expression posttranscriptionally by guiding the RNA-induced silencing complex to target mRNAs, leading to mRNA degradation or translational repression.¹⁰ This regulatory mechanism is essential for various biological processes in plants, including developmental timing, cell differentiation, and tissue proliferation. Recent advances in molecular biology, genomics, and metabolomics have provided unprecedented insights into the intricate regulatory networks governing miRNA-mediated control of secondary metabolite biosynthesis and accumulation, suggesting that miRNAs may serve as the key regulators of these processes.^{11–13}

In this mini-review, we summarize the current understanding of miRNAs in *P. ginseng* and explore their potential role in the miRNA-mediated regulation of ginsenoside biosynthesis. Although miRNA research in *P. ginseng* is still in its early stages compared to other plant species, this review discusses the emerging opportunities in enhancing both the yield and structural diversity of ginsenosides through targeted miRNA manipulation.

miRNAs in *P. ginseng*

With the advent of next-generation sequencing technologies and sophisticated bioinformatics tools, significant progress has been made in identifying miRNAs from expressed sequence tags (ESTs) and small RNA sequencing datasets in *P. ginseng*. A landmark study in 2012 identified 73 conserved miRNAs classified into 33 families, as well as 28 non-conserved miRNAs from nine families, by analyzing a small RNA library derived from pooled RNAs isolated from the roots, stems, leaves, and flowers of *P. ginseng*.¹⁴ Additionally, a subsequent study identified 69 miRNAs across 44 families using ESTs

generated by Illumina sequencing.¹⁵ These miRNAs were predicted to target 346 genes primarily involved in key cellular processes, such as transporter activity, kinase signaling, transcriptional regulation, and protein interactions. Building on these efforts, subsequent research has employed more advanced strategies to uncover novel miRNAs and elucidate their roles in gene regulation. Notably, a comprehensive analysis of small RNA sequencing data from *P. ginseng* roots collected in Changbai Mountain, China, led to the identification of 3798 miRNAs, including 298 known and 3500 putative novel miRNAs.¹⁶ Computational target prediction and pathway enrichment analyses have associated these miRNAs with vital biological pathways, predominantly those related to plant development and metabolic processes.

Several alternative methods have been proposed to determine the age of ginseng, including the histochemical staining method to analyze the number of secretory duct layers in dyed samples of cut roots using electron microscopy, the annual ring method for counting the annual rings in the cut root, the ginsenoside analysis method to assess specific saponin contents for each age group, the metabolomics approach for age discrimination, and the analysis of telomere length and telomerase activity.^{17–19} Moreover, a degradome sequencing analysis has revealed differential miRNA expression profiles in ginsengs of varying ages (e.g. 15-year ginseng vs. 6-year ginseng). This underscores the potential of miRNAs as biomarkers for determining the age of ginseng.²⁰ Nevertheless, miRNA expression patterns are also influenced by other factors, such as geographical origin and environmental conditions. Therefore, a comprehensive and systematic analysis of specific miRNAs is crucial to confirm their reliability as age-specific markers for ginsengs. Additionally, the regulatory roles and functions of these miRNAs in *P. ginseng* could offer further insights into their age-dependent expression profiles and biological significance.

Field cultivation of *P. ginseng* typically takes 4 to 6 years and requires stringent quality control, as ginseng growth is highly sensitive to environmental factors, such as soil composition, climate, shading, pathogens, and pests. To overcome these challenges, cell and tissue culture methods have been extensively explored to accelerate and improve the yield of ginsenoside production.^{8,21} Among these approaches, the adventitious root cultures have emerged as a promising strategy, resulting in high ginsenoside yields and improved stability under various physical and chemical conditions.⁸ While the hairy root cultures also show significant potential due to their rapid growth and suitability for genetic transformation, adventitious root cultures are more favored for large-scale production.^{8,21,22} Comparative profiling of miRNA abundance between the adventitious and hairy roots of ginseng revealed differential miRNA expression profiles. Notably, miR156, miR396, miR166, and miR399 exhibited significantly elevated expression in the hairy roots, whereas miR172 and miR157 were upregulated in adventitious roots.²³ These findings underscore the potential role of specific miRNAs in modulating root-type-specific developmental pathways in ginseng. In *Arabidopsis*, overexpression of miR156 significantly promotes lateral root formation, while its suppression results in a marked reduction in lateral root development.²⁴ By regulating the expression of growth-regulating factor (GRF), miR396 plays a pivotal role in mediating the transition of stem cells to transit-amplifying cells in the root meristem.²⁵ Additionally,

overexpression of miR166 influences primary root growth by downregulating the HD-ZIP III transcripts.²⁶ Taken together, these findings suggest that miRNAs are key regulators of root architecture and development, offering valuable insights into miRNA-mediated pathways for improving the growth and differentiation of adventitious and hairy roots of ginseng. This could pave the way for optimizing the root-based biomass production of ginseng.

Ginsenoside biosynthesis and miRNAs

Despite extensive efforts to enhance ginsenoside production through tissue and cell cultures, the overall productivity remains suboptimal. Metabolic engineering has emerged as a promising strategy, with genetic modifications of *Panax* species to target key genes in ginsenoside biosynthesis.²⁷ For higher plants, numerous studies have shown that miRNAs play a crucial role in the biosynthesis of secondary metabolites, including flavonoids, terpenoids, and alkaloids.^{11,22} Compared to advances in other plant species, miRNA research in *P. ginseng* is still in its infancy. For the present review, we used Target Finder (version 1.0) and psRNATarget to identify 37 putative miRNAs from the 2432 miRNAs reported by Liu et al.,²³ which target 25 genes involved in ginsenoside biosynthesis (Table 1 and Supplemental Table 1). As shown in Supplemental Figure 1, farnesyl diphosphate synthase (FPPS), a key enzyme in the isoprenoid pathway, catalyzes the sequential condensation of dimethylallyl diphosphate and isopentenyl diphosphate to produce geranyl diphosphate (C10) and farnesyl diphosphate (FPP; C15).²⁸ As a crucial branching point in isoprene metabolism, FPP serves as a precursor for various bioactive compounds, including sesquiterpenoids, phytosterols, triterpenoids, abscisic acid, plastoquinone, coenzyme Q, and farnesylated proteins.²⁸ The importance of FPPS in ginsenoside biosynthesis has been demonstrated by overexpressing *PgFPPS* (*Pg_S0304.36*) in the transgenic ginseng hairy roots, which led to a 2.4-fold increase in the ginsenoside content.²⁹ Two *PgFPPS* genes have been identified in the *P. ginseng* genome, with a ginseng-specific miRNA (*pgi-novel-1899*) specifically targeting these genes (Table 1).^{23,27} Squalene epoxidase (SQE) catalyzes the oxidation of C30-squalene to 2,3-oxidosqualene, a rate-limiting step in triterpenoid and phytosterol biosynthesis. Silencing *PgSQE1* in ginseng adventitious roots has been shown to result in decreased ginsenoside production and increased phytosterol accumulation; the overexpression of *PgSQE1* enhanced the levels of both metabolites.^{7,30} The observed increase in phytosterols in *PgSQE1*-RNAi roots is likely mediated by functional redundancy, as the silencing of *PgSQE1* significantly upregulated *PgSQE2*.⁷ Functionally redundant duplicate genes are believed to provide a backup for critical functions in the presence of severe mutations.³¹ Notably, *SQE* is highly duplicated in plants, with six copies in *Arabidopsis*, five in *Glycyrrhiza* species, and 12 in *P. ginseng*.²⁷ miRNA target databases have revealed that a single miRNA can regulate multiple genes, while a single gene can be targeted by numerous miRNAs.³² As shown in Table 1, *pgi-novel-2095* is predicted to target various *PgSQE* genes, underscoring the potential of *pgi-novel-2095* as a key regulator for mitigating the functional redundancy among *PgSQEs*.

Table 1. The miRNA-targeted genes involved in the isoprenoid and ginsenoside biosynthesis pathways in *Panax ginseng*.

Pathway	Genes	Gene IDs ^a	miRNA IDs ^b
MVA (mevalonate pathway)	AACT (acetoacetyl-CoA thiolase)	Pg_S6240.3	pgi-novel-592
		Pg_S0022.46	pgi-novel-1319
	HMGS (hydroxymethylglutaryl-CoA synthase)	Pg_S0849.33	pgi-novel-1022
		Pg_S6647.3	pgi-novel-1022
		Pg_S6896.2	pgi-novel-1022
	PMK (phosphomevalonate kinase)	Pg_S4594.4	pgi-novel-171
		Pg_S3098.25	pgi-novel-965a, pgi-novel-965b
		Pg_S5751.1	pgi-novel-224, pgi-novel-743, pgi-novel-2328
		Pg_S1168.2	pgi-novel-2124
		Pg_S7337.2	pgi-novel-2124
MEP (methylerythritol 4-phosphate)	IDI (isopentenyl-diphosphate delta-isomerase)	Pg_S1214.23	pgi-novel-1337
	DXS (1-deoxy-D-xylulose-5-phosphate synthase)	Pg_S0148.25	pgi-novel-1451a, pgi-novel-1451b, pgi-novel-1692, pgi-novel-1904, pgi-novel-2126
	MEP-CT (2-C-methyl-D-erythritol 4-phosphate cytidyltransferase)		
	MECDPS	Pg_S7699.3	pgi-novel-1706, pgi-novel-2172
	((E)-4-hydroxy-3-methylbut-2-enyl-diphosphate synthase)		
Downstream pathway (triterpenoid/ginsenoside pathway)	FPPS (farnesyl diphosphate synthase)	Pg_S0304.36	pgi-novel-1210
	SQE (squalene epoxidase)	Pg_S8325.1	pgi-novel-1210
		Pg_S1693.31	pgi-novel-335, pgi-novel-1799
	β-AS (β-amyrin synthase)	Pg_S3767.15	pgi-novel-2282
		Pg_S4651.2	pgi-novel-2095
		Pg_S6081.2	pgi-novel-2095
		Pg_S6152.1	pgi-novel-2095
		Pg_S2939.4	pgi-novel-1527a, pgi-novel-1527b
		Pg_S0034.2	pgi-novel-452, pgi-novel-539,

(continued)

Table 1. Continued.

Pathway	Genes	Gene IDs ^a	miRNA IDs ^b
DDS (dammarenediol II synthase)		Pg_S3517.9	pgi-novel-564, pgi-novel-1073, pgi-novel-2029
			pgi-novel-266a to 266c, pgi-novel-787, pgi-novel-2080, pgi-novel-2015,
		Pg_S3586.1	pgi-novel-669
PPTS (protopanaxatriol synthase)		Pg_S0325.7	pgi-novel-2196

^aGene list has been provided by Mohanan et al.²⁷

^bmiRNA IDs have been generated by Liu et al.²³

PgMEP-CT (2-C-methyl-D-erythritol 4-phosphate cytidyltransferase, Pg_S0148.25), *Pg β -AS* (β -amyrin synthase, Pg_S0034.2), and *PgDDS* (dammarene-11-ol synthase, Pg_S3517.9) are each targeted by more than five miRNAs (Table 1). The spatiotemporal expression of genes is regulated by a complex network of transcription factors and epigenetic regulators, including miRNAs.³³ The involvement of multiple miRNAs in targeting a single gene further refines this regulation by enabling nuanced control across different temporal, spatial, and physiological conditions. In addition, multiple miRNAs can also target different regions of the same mRNA, influencing various aspects of gene expression, such as mRNA stability, translation efficiency, and subcellular localization, leading to multifaceted regulation.^{34,35} Overall, these multi-target miRNAs provide a robust and adaptable system for fine-tuning gene expression. RNAi-mediated silencing of *PgDDS* in ginseng adventitious roots has been shown to result in a substantial reduction in the production of dammarane-type ginsenoside, with levels dropping by up to 84.5%.³⁶ Conversely, silencing *Pg β -AS* has been found to decrease the level of oleanane-type ginsenoside while increasing the accumulation of dammarane-type ginsenoside in ginseng hairy roots.³⁷ As DDS and β -AS compete for the same precursor, these results suggest that the gene silencing approach shifts the metabolic flux, enhancing the biosynthesis of either dammarane-type or oleanane-type ginsenosides depending on the gene targeted. Gene silencing serves as a critical mechanism for attenuating gene expression in plant systems, predominantly through two complementary pathways: (1) transcriptional gene silencing, which involves the repression of transcription, and (2) posttranscriptional gene silencing (PTGS), characterized by mRNA degradation at the posttranscriptional level.³⁸ Current gene-silencing methodologies including RNAi operate primarily through PTGS, underscoring the significance of this regulatory process in modern genetic engineering and functional genomics.³⁸ In addition to these methodologies, a recent study has demonstrated that exogenous miRNAs can induce PTGS in plants. In *Arabidopsis*, plants overexpressing miR156 modulate the expression of squamosa-promoter binding protein-like transcription factor genes in wild-type (WT) plants.³⁹ Similarly, in rice, the miR399-overexpressing plants influence the expression of phosphate 2 in WT plants.³⁹ This indicates that miRNAs are released to the surrounding environment, enabling neighboring plants to absorb them and revealing a previously unrecognized mechanism of inter-plant communication driven by miRNAs. This inter-plant signaling pathway suggests a promising strategy for controlling ginsenoside production by leveraging miRNA-mediated interactions among plants, particularly through the hydroponic cultivation of WT ginseng alongside other plant species that overexpress specific ginseng miRNAs.

Future directions

The regulation of ginsenoside biosynthesis by miRNAs represents a compelling frontier in plant biotechnology, with profound implications for both fundamental research and industrial applications. While significant strides have been made in understanding the molecular mechanisms underlying ginsenoside production, the miRNA-mediated regulation of key biosynthetic enzymes remains incompletely characterized. Future studies

should prioritize identifying specific miRNAs targeting critical biosynthetic genes and elucidating their precise molecular interactions. In addition, analyzing expression patterns, such as tissue-specific and stress-induced expression, is helpful for understanding the correlation between miRNAs and ginsenoside biosynthesis. Experimental validation of miRNA-target interactions is crucial for advancing their practical application. Techniques such as transient expression and transfection offer powerful tools for this purpose.^{40,41} In *P. ginseng*, well-documented methodologies, including protoplast isolation and transient expression systems,^{42–44} provide a robust platform for investigating miRNA functionality and downstream regulatory effects. These approaches will be instrumental in deciphering the roles of miRNAs in secondary metabolism and verifying bioinformatics predictions. Moreover, exploring the interplay between miRNAs and other regulatory elements, such as transcription factors and protein kinases, could deepen our understanding of metabolic flux optimization. Such insights are essential for overcoming current challenges associated with large-scale ginsenoside production and enhancing the metabolic engineering of ginseng. By integrating miRNA-based regulation, genetic modification, and synthetic biology, it is possible to develop sustainable, cost-effective strategies for ginsenoside biosynthesis, with significant implications for agricultural and biopharmaceutical industries.

One innovative approach involves using CRISPR/Cas9 technology to modulate miRNA expression. Genome editing via non-homologous end joining facilitates the introduction of insertions or deletions (indels) in pre-miRNA processing sites, thereby disrupting miRNA biogenesis and maturation.^{45,46} For example, in *Arabidopsis*, miR858 indel mutations increased the accumulation of flavonoids and anthocyanins by altering the expression of phenylpropanoid pathway-related genes.⁴⁷ Similar strategies, including homology- and recombination-directed repair and homology-directed repair, have enabled the targeted deletion of miRNA genes or their promoters, as demonstrated in *Glycine max*, where CRISPR/Cas9 effectively edited miR1509 and miR1514.⁴⁸ However, editing miRNAs poses unique challenges due to their small size and frequent intronic encoding, complicating the design of effective guide RNAs.^{48,49} Despite these limitations, the precision and versatility of CRISPR/Cas9 make it a powerful tool for tailoring secondary metabolic pathways in plants.⁵⁰ Continued advancements in this area could pave the way for enhanced ginsenoside yield, improved crop resilience, and innovative therapeutic applications.

Recent studies have underscored the emerging role of plant-derived miRNAs in cross-kingdom gene regulation, particularly their ability to modulate human gene expression through dietary intake or medicinal applications.^{51–55} Bioinformatics analyses have predicted that ginseng miRNAs target an extensive array of human genes—specifically, 2868 miRNAs have been shown to interact with a total of 50,992 human genes implicated in various diseases, including cancer, immune system disorders, and neurological conditions.¹⁶ This suggests that ginseng miRNAs, in addition to the well-known ginsenosides, may contribute to its therapeutic effects. However, the cross-kingdom function of miRNAs in medicinal plants, including ginseng, remains based solely on computational predictions, which lack experimental validation and reliability.^{54,55} To advance the therapeutic potential of ginseng miRNAs, future research should focus on validating their

stability, absorption, and functional impact in human systems. Understanding the molecular mechanisms governing their action could pave the way for miRNA-based phytotherapeutics, offering novel approaches for disease prevention and treatment.

Conclusion

In conclusion, miRNAs should play a critical role in the regulation of ginsenoside biosynthesis in *P. ginseng*, influencing key enzymes and metabolic pathways. Despite significant progress, much remains to be understood about the specific miRNAs involved, their targets, and their interplay with other regulatory elements. Moving forward, integrating multi-omics approaches and advanced genome editing technologies will be key to unlocking the full potential of miRNAs in the biosynthesis of valuable natural products like ginsenosides.

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Statements and declarations

Ethical considerations

Ethical approval is not applicable to this article.

Author contributions/CRedit

All authors contributed to the study's conception and design. SHE and TKH conceptualized and supervised the work. SHE prepared the outline. All authors contributed to the drafting of the manuscript and were involved in the writing, reviewing, and editing of the manuscript.

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Conflicting interests

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Data availability

It is not applicable to this article as no new data were created or analyzed in this study.

Supplemental material

Supplemental material for this article is available online.

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