

REVIEW

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# A review of integrative omic approaches for understanding the biosynthesis of triterpenoid saponins in *Panax japonicus*

Yuxin Zhuang<sup>1</sup>, Yihui Fang<sup>1</sup>, Zeqing Xin<sup>1</sup>, Huanhuan Qi<sup>2</sup>, Dengxiang Du<sup>2</sup>, Mengmeng Ren<sup>1</sup> and Shaopeng Zhang<sup>1\*</sup> 

## Abstract

*Panax japonicus* C.A.Meyer, also known as Zhujieshen, is a classic *Panax* herb of the Araliaceae family. It has significant effects for the treatment of Qi stagnation and blood stasis in traditional Chinese medicine, and is used as an important substitute for *P. ginseng* and *P. notoginseng* by minority ethnic groups in China. Accordingly, many studies have been conducted to explore the pharmacological benefits of *P. japonicus*. However, the exploitation of *P. japonicus* saponins is hampered by the absence of genomic data and the limited availability of functional genes. Therefore, the recent advances in omics approaches, such as genomics, transcriptomics, proteomics and metabolomics, have enabled the characterization of molecular biosynthetic mechanism of *P. japonicus* saponins. This review highlights the emerging breakthrough in multi-omics in identifying novel bioactive compounds and their biosynthetic genes in *P. japonicus*. In addition, the review also summarizes the biosynthetic pathways of triterpenoid saponins and developmental mechanisms of *P. japonicus* that can be helpful to realize the intrinsic metabolite differences among *Panax* species. Particularly, this review unravels the whole picture of saponin biosynthesis in *P. japonicus* on molecular aspects and provides a comprehensive understanding of multi-omics for the application value of *P. japonicus* in medicine.

**Keywords** *Panax japonicus*, Omics, Functional genes, Triterpenoid saponins

## Introduction

The *Panax* L. (Araliaceae), as one of the most important medicinal plants, has been used for medical therapeutics for thousands of years [1]. Triterpenoid saponins are the major active components in *Panax* species and exhibit a wide range of pharmacological activities, such as anti-inflammatory, anti-cancer, anti-microbial and anti-viral activities [2]. These saponins in *Panax* species are generally named as ginsenosides, which can be classified into

three styles based on the differences of aglycones, including dammarane-type, oleanane (OA)-type, and ocotillol (OCT)-type saponins [3]. Dammarane-type saponins can be further divided into two groups based on their aglycone structures: protopanaxadiol (PPD) and protopanaxatriol (PPT) [3].

The genus *Panax* consists of about 20 species mainly distributed in East Asia and North America [4]. Of these 20 *Panax* species, seven species (*P. ginseng*, *P. quinquefolius*, *P. notoginseng*, *P. japonicus*, *P. stipuleanatus*, *P. pseudoginseng* and *P. trifolius*) and one species complex (*P. bipinnatifidus*) have been clearly defined according to their geographic distributions and phylogenetic relationships [5].

As one of the *Panax* species with a long history of medicinal use, *P. japonicus* C.A.Meyer, known as “Zhujieshen” in China due to its bamboo-like rhizomes, grows

\*Correspondence:

Shaopeng Zhang  
shaopeng@whpu.edu.cn

<sup>1</sup> Center for Se-Rich Agricultural Products Processing, School of Modern Industry for Selenium Science and Engineering, National R&D, Wuhan Polytechnic University, Wuhan 430023, China

<sup>2</sup> School of Life Science and Technology, Wuhan Polytechnic University, Wuhan 430023, China

naturally in Japan, Korea and southwest China, which has been recorded in the Chinese Pharmacopoeia [6] (Fig. 1). *P. japonicus* var. *major*, the main variety of *P. japonicus*, is commonly known as “Zhuzishen” in China owing to its bead-like rhizomes and is also officially listed in the Chinese Pharmacopoeia (Fig. 1). In the common medicinal practice, *P. japonicus* var. *major* (Zhuzishen) was often used interchangeably with *P. japonicus* (Zhujieshen) due to their similar composition. According to traditional Chinese medicine (TCM) theory, Zhujieshen and Zhuzishen all had the sweet and bitter taste, and showed the function of regulating the immune system, relieving pain and removing blood stasis. But, Zhuzishen was slightly colder than Zhujieshen in nature, which showed additional efficacy in nourishing the lung [7].

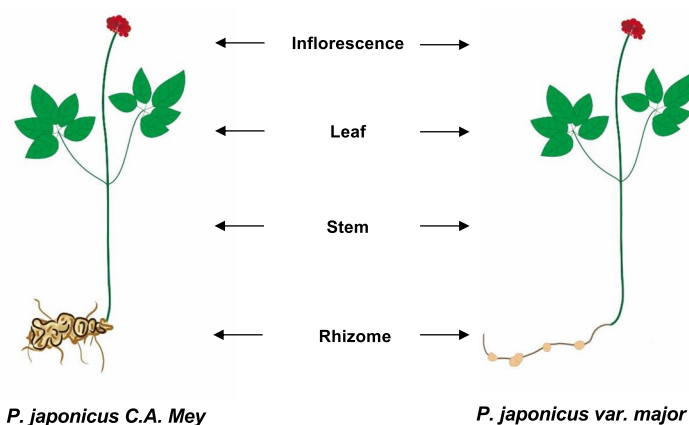
In the history of traditional medicinal use, the roots and rhizomes of *Panax* species were mainly used as herbal medicine in Asian countries. Many modern studies have also shown that *P. japonicus* rhizomes have been used as the tonic and hemostatic agent for the treatment of physical weakness and traumatic injury [8–10]. *P. japonicus* rhizomes were always considered to have combined effects of both *P. ginseng*’s “conserving vitality” activities and *P. notoginseng*’s “replenishing blood” activities [6]. Therefore, *P. japonicus* rhizome had been widely used as a substitute for the root of *P. ginseng* and *P. notoginseng* in China, and was even named as “the king of herbs”, especially in the Tujia and Hmong medicine.

As a medically important *Panax* species, *P. japonicus* ginsenosides were the most abundant active constituents in the rhizome, which was found to be threefold higher than that of *P. quinquefolius*, and sevenfold higher than that of *P. ginseng* [11]. These ginsenosides from *P. japonicus* mainly contain oleanane-type pentacyclic triterpenes. Oleanane-type ginsenosides are considered as important biological substances with various pharmacological

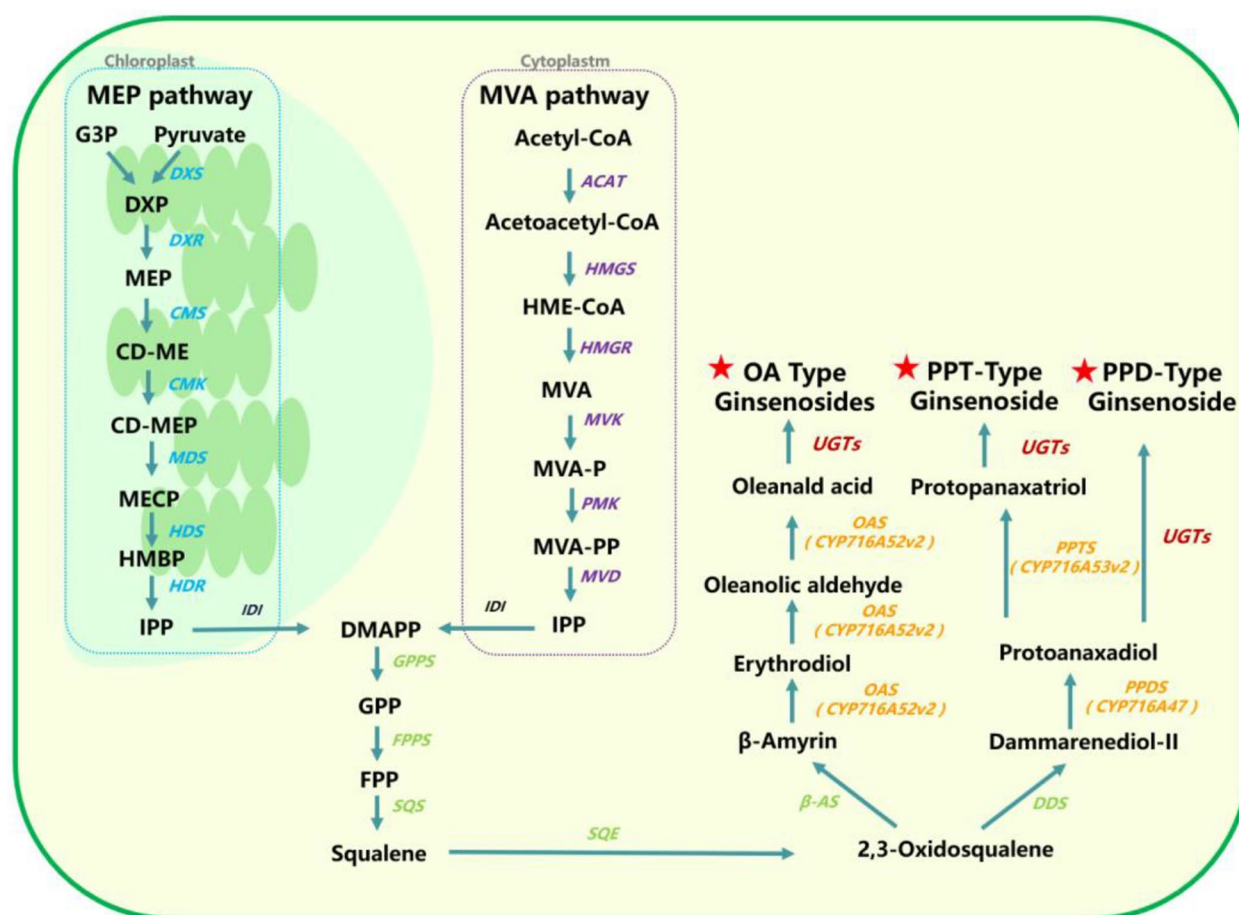
activities [12]. Chikusetsusaponin IV, V, and IVa are found to be three typical oleanane-type ginsenosides in *P. japonicus* [13].

All ginsenosides from *Panax* species are initially generated from 2, 3-oxidosqualene, which could be synthesized from the cytoplasmic-derived mevalonate (MVA) pathway and plastidial methylerythritol 4-phosphate (MEP) pathway (Fig. 2). Generally, the MVA pathway contributes predominantly to ginsenoside biosynthesis compared to the MEP pathway [3]. 2,3-oxidosqualene is the key precursor for the formation of oleanane- and dammarane-type triterpene skeletons by different oxidosqualene cyclases (OSCs). These OSCs are mainly classified into dammarenediol synthase (DS) and  $\beta$ -amyrin synthase ( $\beta$ -AS). In the subsequent biosynthetic steps of ginsenoside modification, cytochrome P450-dependent monooxygenases (P450s) and UDP-glucosyltransferases (UGTs) are major enzymes to catalyze the oxidation and glycosylation of triterpene scaffolds, respectively. The functions of these genes in the triterpenoid saponin biosynthesis pathway are presented in Table 1. A large number of key genes involved in the biosynthesis of different triterpene skeletons have been identified, especially in *P. ginseng*, *P. notoginseng* and *P. quinquefolius* [14]. However, the specific genes governing key processes in *P. japonicus*, such as *P. japonicus* saponin biosynthesis, growth regulation, and stress adaptation, remain largely unknown [15]

With the development of system biology and multi-omics technologies (genomics, transcriptomics, metabolomics, and proteomics) over the past two decades, the herbal research was gradually transformed from traditional experiment-based approaches to the omics-based approaches [16]. Using omics technologies, researchers can not only explore key genes related to core metabolites from multiple perspectives but also advance from



**Fig. 1** The typical morphological characteristics of *P. japonicus* plant and its variety



**Fig. 2** Ginsenoside biosynthetic pathway in *Panax* species. Genes involved in the ginsenoside biosynthesis pathway are denoted in italics. Blue (RGB(0, 176, 240), #00b0f0) represents genes from the MEP pathway, purple (RGB(112, 48, 160), #7030a0) indicates genes from the MVA pathway, and green (RGB(146, 208, 80), #92d050) signifies other genes within the ginsenoside biosynthetic pathway. Genes belonging to the CYP family are marked in orange (RGB(255, 153, 0), #ff9900), while UGT family genes are highlighted in red (RGB(192, 0, 0), #c00000). Abbreviations are as follows: G3P: Glyceraldehyde-3-phosphate, DXP: 1-Deoxy-D-xylulose-5-phosphate, MEP: 2-C-Methyl-D-erythritol 4-phosphate, CD-ME: 4-(Cytidine 5'-diphospho)-2-C-methyl-D-erythritol, CD-MEP: 4-(Cytidine 5'-diphospho)-2-C-methyl-D-erythritol 2-phosphate, MECP: 2-C-Methyl-D-erythritol 2,4-cyclodiphosphate, HMBP: (E)-4-Hydroxy-3-methylbut-2-en-1-yl diphosphate, IPP: Isopentenyl diphosphate, DMAPP: Dimethylallyl diphosphate, HMG-CoA: 3-Hydroxy-3-methylglutaryl-CoA, MVA: Mevalonic acid, MVA-P: Mevalonate-5-phosphate, MVA-PP: Mevalonate-5-diphosphate, GPP: Geranyl diphosphate, FPP: Farnesyl diphosphate, *DXS*: 1-deoxy-D-xylulose-5-phosphate synthase, *DXR*: 1-deoxy-D-xylulose-5-phosphate reductoisomerase, *CMS*: 2-C-methyl-D-erythritol 4-phosphate cytidyltransferase, *CMK*: 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase, *MDS*: 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase, *HDS*: 4-hydroxy-3-methylbut-2-enyl-diphosphate synthase, *HDR*: 4-hydroxy-3-methylbut-2-enyl-diphosphate reductase, *ACAT*: Acetyl-CoA acetyltransferase, *HMGS*: 3-hydroxy-3-methylglutaryl-CoA synthase, *HMGR*: 3-hydroxy-3-methylglutaryl-CoA reductase, *MVK*: Mevalonate kinase, *PMK*: Phosphomevalonate kinase, *MVD*: Mevalonate-5-pyrophosphate decarboxylase, *IDI*: Isopentenyl-diphosphate Delta-isomerase, *GGPS*: Geranylgeranyl diphosphate synthase, *FPS*: Farnesyl diphosphate synthase, *SQS*: Squalene synthase, *SQE*: Squalene epoxidase,  *$\beta$ -AS*:  $\beta$ -amyrin synthase, *DDS*: Dammaradiol-II synthase, *OAS*: Oleanolic acid synthase, *PPDS*: Protopanaxadiol Synthase, *PPTS*: Protopanaxatriol Synthase, *UGT*: UDP-glycosyltransferase

trait exploration to variety improvement. Compared with *P. ginseng*, *P. quinquefolius* and *P. notoginseng*, *P. japonicus* and *P. japonicus* var. *major* have received far less literature support, particularly for the field of omics-based research. For *P. japonicus*, a perennial and genetically complex polyploid, the application of omics technologies is crucial. The integrative omic approaches enable research into its genetic, metabolic, and molecular

mechanisms of saponin biosynthesis and support the conservation of its germplasm resources.

In this review, we concentrated on multi-omics analysis in applications to gain a better understanding of the *P. japonicus* evolution, the characterization of ginsenoside biosynthetic genes and their transcriptional regulation in *P. japonicus*. We believe that this review would provide

**Table 1** Genes Involved in Ginsenoside Biosynthesis in *Panax* Species

| Gene (Enzyme) | Full Name                                                | Metabolic Pathway               | Function                                                                                                                                                                                                                            |
|---------------|----------------------------------------------------------|---------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>DXS</i>    | 1-deoxy-D-xylulose-5-phosphate synthase                  | MEP pathway                     | Catalyzes the condensation of pyruvate + Glyceraldehyde-3-phosphate (G3P) to form 1-Deoxy-D-xylulose-5-phosphate (DXP). It is the first rate-limiting enzyme of the MEP pathway, determining the carbon flow rate into this pathway |
| <i>DXR</i>    | 1-deoxy-D-xylulose-5-phosphate reductoisomerase          | MEP pathway                     | Catalyzes the reductive isomerization of DXP to Generate 2-C-methyl-D-erythritol 4-phosphate (MEP)                                                                                                                                  |
| <i>CMS</i>    | 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase | MEP pathway                     | Catalyzes the reaction of MEP with CTP to form 4-(Cytidine 5'-diphospho)-2-C-methyl-D-erythritol (CD-ME)                                                                                                                            |
| <i>CMK</i>    | 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase        | MEP pathway                     | Catalyzes the phosphorylation of CD-ME to generate 4-(Cytidine 5'-diphospho)-2-C-methyl-D-erythritol 2-phosphate (CD-MEP)                                                                                                           |
| <i>MDS</i>    | 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase    | MEP pathway                     | Catalyzes the cyclization of CD-MEP to form 2-C-Methyl-D-erythritol 2,4-cyclodiphosphate (MECP)                                                                                                                                     |
| <i>HDS</i>    | 4-hydroxy-3-methylbut-2-enyl-diphosphate synthase        | MEP pathway                     | Catalyzes the conversion of MECP to (E)-4-Hydroxy-3-methylbut-2-en-1-yl diphosphate (HMBP)                                                                                                                                          |
| <i>HDR</i>    | 4-hydroxy-3-methylbut-2-enyl-diphosphate reductase       | MEP pathway                     | Catalyzes the reduction of HMBP to generate Isopentenyl diphosphate (IPP) and Dimethylallyl diphosphate (DMAPP); this is the final step of the MEP pathway                                                                          |
| <i>ACAT</i>   | Acetyl-CoA acetyltransferase                             | MVA pathway                     | Catalyzes the condensation of 2 molecules of Acetyl-CoA to form Acetoacetyl-CoA                                                                                                                                                     |
| <i>HMGS</i>   | 3-hydroxy-3-methylglutaryl-CoA synthase                  | MVA pathway                     | Catalyzes the condensation of Acetoacetyl-CoA with acetyl-CoA to form 3-Hydroxy-3-methylglutaryl-CoA (HMG-CoA)                                                                                                                      |
| <i>HMGR</i>   | 3-hydroxy-3-methylglutaryl-CoA reductase                 | MVA pathway                     | Catalyzes the reduction of HMG-CoA to generate Mevalonic acid (MVA). It is the key rate-limiting enzyme of the MVA pathway, and this irreversible reaction determines the rate of cytosolic terpenoid synthesis                     |
| <i>MVK</i>    | Mevalonate kinase                                        | MVA pathway                     | Catalyzes the phosphorylation of MVA to form Mevalonate-5-phosphate (MVA-P);                                                                                                                                                        |
| <i>PMK</i>    | Phosphomevalonate kinase                                 | MVA pathway                     | Catalyzes the further phosphorylation of MVA-P to generate Mevalonate-5-diphosphate (MVA-PP)                                                                                                                                        |
| <i>MVD</i>    | Mevalonate-5-pyrophosphate decarboxylase                 | MVA pathway                     | Catalyzes the decarboxylation of MVA-PP to form Isopentenyl diphosphate (IPP)                                                                                                                                                       |
| <i>IDI</i>    | Isopentenyl-diphosphate Delta-isomerase                  | Triterpenoid skeleton synthesis | Catalyzes the interconversion of IPP and DMAPP                                                                                                                                                                                      |
| <i>GGPS</i>   | Geranylgeranyl diphosphate synthase                      | Triterpenoid skeleton synthesis | Catalyzes the condensation of IPP and DMAPP to form Geranylgeranyl diphosphate (GPP)                                                                                                                                                |
| <i>FPS</i>    | Farnesyl diphosphate synthase                            | Triterpenoid skeleton synthesis | Catalyzes the condensation of IPP and DMAPP to form Farnesyl diphosphate (FPP)                                                                                                                                                      |
| <i>SQS</i>    | Squalene synthase                                        | Triterpenoid skeleton synthesis | Catalyzes the condensation of 2 molecules of FPP to form squalene                                                                                                                                                                   |
| <i>SQE</i>    | Squalene epoxidase                                       | Triterpenoid skeleton synthesis | Catalyzes the oxidation of squalene to form 2,3-oxidosqualene, which is a key precursor for triterpenoid synthesis                                                                                                                  |
| $\beta$ -AS   | $\beta$ -amyrin synthase                                 | Triterpenoid skeleton synthesis | Catalyzes the cyclization of 2,3-oxidosqualene to form $\beta$ -amyrin, which is the skeleton precursor of oleanane-type triterpenoids                                                                                              |
| <i>DDS</i>    | Dammarenediol-II synthase                                | Triterpenoid skeleton synthesis | Catalyzes the cyclization of 2,3-oxidosqualene to form dammarenediol-II, which is the skeleton precursor of dammarane-type triterpenoids                                                                                            |

**Table 1** (continued)

| Gene (Enzyme)         | Full Name                 | Metabolic Pathway                | Function                                                                                                                                   |
|-----------------------|---------------------------|----------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------|
| OAS<br>(CYP716A52v2)  | Oleanolic acid synthase   | Formation of sugar donors        | Catalyzes the oxidation of $\beta$ -amyrin to form oleanolic acid, which is the direct precursor of OA-type ginsenosides                   |
| PPDS<br>(CYP716A47)   | Protopanaxadiol Synthase  | Formation of sugar donors        | Catalyzes the hydroxylation of dammarenediol-II to form protopanaxadiol, which is the direct precursor of PPD-type ginsenosides            |
| PPTS<br>(CYP716A53v2) | Protopanaxatriol Synthase | Formation of sugar donors        | Catalyzes the further hydroxylation of protopanaxadiol to form protopanaxatriol, which is the direct precursor of PPT-type ginsenosides    |
| UGT                   | UDP-glycosyltransferase   | Modification of saponin skeleton | Catalyzes the transfer of glycosyl groups (e.g., glucose) to the hydroxyl groups of triterpenoid precursors to form the final ginsenosides |

new insights for understanding the specificity and complexity of *P. japonicus*.

### The Genome Analysis in *P. japonicus*

The whole genome analysis can shed light on the evolutionary complexity of *Panax* species and the biochemical diversity of secondary metabolites, and provide a holistic view of ginsenoside biosynthesis in *Panax* plants. Most members in *Panax* genus generally have large and heterozygous genomes. For example, the whole genome sequences were 3.45 Gb for *P. ginseng* [17, 18], 2.41 Gb for *P. notogineng* [19, 20], 3.6 Gb for *P. quinquefolius* [21] and 3.0 Gb for *P. vietnamensis* [22], respectively (Table 2).

*P. japonicus* is also an allotetraploid like ginseng, with the increased genetic background. Due to the short domestication history of *P. japonicus*, its genetic background is usually scrambled, making its genome mining challenging. The first and currently only de novo assembled genome of *P. japonicus* was published in 2022 by Wang's group [21]. These *P. japonicus* samples ( $4x=48$ ) were collected from Hokkaido, Japan. This genome assembly possessed a small genome (2.09 Gb) with a high level of heterozygosity (heterozygous rate = 1.07%) (Table 2). Although these *Panax* species varied dramatically in genome size, they actually showed high collinearity across orthologous chromosomes [21]. In the *P.*

*japonicus* genome, the total length of contigs was 2.09 Gb with 74,307 protein-coding genes. The overall GC content of *P. japonicus* genome is 33.93%. Almost more than 50% of the *P. japonicus* genome was comprised of repetitive sequences, including long-terminal repeats (LTRs) and retrotransposons (RTs). The expansion of *Copia*, *Gypsy*, and other unknown retrotransposons was observed in the *P. japonicus* genome [21]. And, the tandem duplication was found to increase the copy number of triterpene saponin synthesis genes, suggesting the crucial roles of tandem duplication in the evolution of triterpene saponin biosynthesis [18]. Many important triterpene OSC (oxidosqualene cyclases) genes, such as lanosterol synthase (*LAS*) and cycloartenol synthase (*CAS*) genes, were also produced by tandem duplication in *Panax* plants [4]. These features suggested that broad-scale chromosomal rearrangements might result in extensive diversification of genome structure in *P. japonicus* and many genes related to the phenylpropanoid and triterpenoid biosynthesis pathways, such as the cytochrome P450 and *UGT* genes, were found to be significantly expanded in *P. japonicus* during the polyploidization processes. These findings indicated that gene duplication had enriched the biosynthetic genes for important metabolites in *P. japonicus*. Thus, these studies would considerably improve our

**Table 2** Genome Assembly Statistics of Different *Panax* Species

| Items                   | <i>P. japonicus</i> | <i>P. ginseng</i> | <i>P. notogineng</i> | <i>P. quinquefolius</i> | <i>P. vietnamensis</i> |
|-------------------------|---------------------|-------------------|----------------------|-------------------------|------------------------|
| Genome size (Gb)        | 2.09                | 3.45              | 2.41                 | 3.60                    | 3.00                   |
| GC content (%)          | 33.93               | 34.71             | 34.43                | 34.51                   | 35.11                  |
| N50 contig length (Mb)  | 1.22                | 147.37            | 1.45                 | 0.87                    | 0.57                   |
| Number of genes         | 74,307              | 77,266            | 47,870               | 64,247                  | 79,364                 |
| Genome BUSCO (%)        | 93.00               | 99.3              | 95.40                | 93.93                   | 92.4                   |
| LTR Assembly Index, LAI | 16.24               | 8.71              | 12.44                | 7.99                    | -                      |
| Reference               | [21]                | [18]              | [19]                 | [21]                    | [22]                   |

GC content is the percentage of guanine (G) and cytosine (C) nucleotides within the DNA sequence

understanding of the *P. japonicus* genome and genes coding for ginsenoside biosynthesis.

### The Transcriptome Analysis in *P. japonicus*

With the development of next-generation sequencing (NGS), transcriptomic characterization has been progressively conducted in *P. japonicus*, enhancing our understanding of triterpenoid pathways and their regulatory mechanisms [23] (Table 3).

Our prior study employed Illumina RNA sequencing to generate a transcriptomic map of *P. japonicus*, establishing the first transcriptome database for this species [24]. The sequencing was conducted on rhizomes of a 6-year-old *P. japonicus* cultivar collected from Enshi of China, resulting in the identification of 553,294 transcripts with lengths exceeding 200 bp. This dataset included 81 single genes implicated in triterpene saponin biosynthesis, 29 key genes associated with triterpene saponin backbone

synthesis, and an additional 34 cytochrome P450 genes and 18 glycosyltransferase genes predicted to participate in saponin backbone modification. This foundational study paved the way for subsequent research on the *P. japonicus* transcriptome. In 2016, a new transcriptome assembly was constructed for five tissues (flower, leaf, secondary root, young rhizome, and old rhizome) of *P. japonicus* growing in the medicinal plant garden of Health sciences university of Hokkaido in Japan, generating 2.4 Gbps of reads [11]. Among these, 28 genes related to the MVA pathway of triterpene saponin synthesis and 24 CYP450 and 48 UGTs associated with ginsenoside synthesis were identified. These homologous genes exhibited high expression levels, especially in the rhizomes of *P. japonicus*, and these genes were also found to be conserved in the rhizomes of other *Panax* species, including *P. ginseng*, *P. notoginseng*, and *P. quinquefolius*.

**Table 3** Summary Statistics for the *P. japonicus* Transcriptome

| Sequencing Samples                                                                                           | Sequencing Platform | Data                                                  | Assemble Software            | Assemble                                             | References |
|--------------------------------------------------------------------------------------------------------------|---------------------|-------------------------------------------------------|------------------------------|------------------------------------------------------|------------|
| The rhizome of six-year-old <i>P. japonicus</i>                                                              | Illumina            | 65,100,458, 52,912,296 and 77,672,802 short reads,    | Trinity                      | 553,294 transcripts, 203283 unigenes                 | [24]       |
| The flowers, leaves, rhizomes, young rhizomes, and mature rhizomes of seven-year-old <i>P. japonicus</i>     | Illumina            | 2.4 Gbps reads                                        | Trinity                      | 206,610 contigs, 135,235 unigenes                    | [11]       |
| The young roots, lateral roots, and mature roots of the rhizome from five-year-old <i>Panax japonicus</i>    | Illumina, PacBio    | 62.2 G clean reads (NGS), 256,605 insert reads (SMRT) | CD-HIT, Quiver               | 136,063 non-redundant unique full-length transcripts | [25]       |
| The internodes and nodes of the rhizome from six-year-old <i>P. japonicus</i> var. <i>major</i>              | Illumina            | 424,112,448 reads                                     | Trinity                      | 979,833 transcripts, 392564 unigenes                 | [26]       |
| The rhizomes, leaves, stems, and lateral roots of 1-year-old, 2-year-old, and 3-year-old <i>P. japonicus</i> | PacBio              | NA                                                    | Trinity                      | 750,245 unigenes                                     | [27]       |
| Four-year-old <i>P. japonicus</i> , main roots, fibrous roots, stems, leaves, and flowers                    | Illumina            | NA                                                    | HISAT2, StringTie, Ballgohan | NA                                                   | [28]       |
| The periderm, cortex, and stele of the rhizome from three-year-old <i>P. japonicus</i>                       | Illumina            | 55 Gb                                                 | Trinity                      | 777,642 contigs, 119,882 unigenes                    | [29]       |
| Three distinct germination stages of <i>P. japonicus</i> seeds                                               | Illumina            | Total 120 M clean reads                               | Trinity                      | 117,204 unigenes                                     | [30]       |
| Three-year-old leaf, fruit, stem and root of <i>P. japonicus</i> var. <i>major</i>                           | Illumina            | 229 Gb                                                | Trinity                      | 216,108 unigenes                                     | [31]       |
| The roots, leaves, flowers, and fruits of three-year-old <i>P. japonicus</i> var. <i>major</i>               | Illumina            | NA                                                    | Trinity                      | 211,650 transcripts, 90,985 unigenes                 | [32]       |
| Five distinct color developmental stages of <i>P. japonicus</i> fruits                                       | Illumina            | 93.94 Gbp                                             | Trinity                      | 462,117 unigenes                                     | [33]       |

Xu and Yang et al. [25, 29] conducted a comprehensive transcriptomic analysis of different tissues (young rhizome, old rhizome, secondary root, rhizome periderm, rhizome cortex and rhizome stele) from 5-year-old *P. japonicus* in Enshi, China, which was based on NGS and single-molecule real-time sequencing (SMRT) technologies. A total of 2,087, 340, and 1,228 uniquely expressed transcripts were identified in the young rhizome, old rhizome, and secondary root, respectively. A total of 138 CYP genes were identified ( $FDR \leq 0.05$ ,  $FC \geq 2$ ). Among these, 43 (31.2%), 57 (41.3%), and 38 (27.5%) genes were predominantly expressed in the young rhizome, old rhizome, and secondary root, respectively. Additionally, 18 UGT genes were identified ( $FDR \leq 0.05$ ,  $FC \geq 2$ ), in which 13 (72.2%), 3 (16.7%) and 2 (11.1%) genes with the high expression levels were respectively observed in the young rhizome, old rhizome, and secondary root. As for the periderm, cortex, and stele of rhizome, 37 (69.8%), 7 (13.2%), and 9 (17.0%) CYP transcripts showed the highest expression levels, respectively. Among the UGT transcripts, 21 (60.0%), 8 (22.9%), and 6 (17.1%) exhibited peak expression in the young rhizome, old rhizome, and secondary root, respectively.

Their findings gave a concise explanation at the transcriptional level for saponin distribution and accumulation across the different tissues of *P. japonicus*. They suggested that the young rhizome and its periderm might be the most important medicinal tissues in the underground parts of *P. japonicus*.

For *P. japonicus* var. *major*, its rhizome was special, forming two different parts (node and internode). The rhizome node served as the primary site for oleanane-type ginsenosides in 6-year-old *P. japonicus* var. *major*, in which the content of oleanane-type ginsenosides was nearly four times that of the internode [26]. In order to elucidate the molecular mechanism underlying this different saponin accumulation in different tissues, Zhang et al. [26] investigated the transcriptomic differences between nodes and internodes of *P. japonicus* var. *major* rhizomes. 392,564 unigenes had been de novo assembled in this study, with an average length of 612.14 bp and an N50 length of 875 bp. Interestingly, among 25,112 differentially expressed unigenes, a set of 48 enriched unigenes associated with isoprenoid biosynthesis, including most MVA pathway genes, were all highly expressed in the nodes. This result indicated that the rhizome node was the main tissue for synthesizing the skeletons of triterpene saponins compared with the rhizome internode. In 2024, Luo et al. conducted the RNA-Seq analysis on four organs (rhizome, leaf, flower, and fruit) of *P. japonicus* var. *major* [32]. Differential expression analysis also indicated that rhizomes exhibited more upregulated genes compared to other organs, and contained the

highest number of organ-specific genes. Analysis of three transcription factor families (*WRKY*, *MYB*, and *bHLH*) revealed that *WRKY* genes were significantly highly expressed in the rhizomes *P. japonicus* var. *major*, suggesting their potential key role in triterpenoid saponin biosynthesis.

In recent years, combinatorial transcriptomics techniques have been receiving more attention, which enables us to better disclose potential genes associated with ginsenoside biosynthesis in *P. japonicus*. In 2023, a comprehensive analysis of the metabolites and transcriptome was conducted in four tissues (rhizomes, stems, leaves, and lateral roots) of 1-3 year-old *P. japonicus* from Enshi, Hubei of China, in which a total of 750,245 transcripts and 3,482 protein groups were identified in *P. japonicus* [27]. In this study, OA-type ginsenosides were found mainly distributed in rhizomes, PPD-type ginsenosides were mainly distributed in leaves, and PPT-type ginsenosides were mainly distributed in lateral roots. At least 45 candidate genes were found to show significant tissue-specific expression abundance, of which the order of their expression levels in different tissues was rhizomes, leaves, lateral roots and stems from high to low. And 29 CYPs and 92 UGTs genes related to ginsenoside biosynthesis were finally found through differential expression analysis and correlation analysis.

In 2024, the difference in flavonoid biosynthesis among five tissues (main roots, fibrous roots, stems, leaves and flowers) of 4-year-old *P. japonicus* was analyzed by combining broad metabolomics and transcriptomics [28]. In this study, metabolomic analysis detected 133 flavonoid metabolites annotated to the flavonoid biosynthesis pathway. The major flavonoid metabolites significantly accumulated in aboveground parts, particularly in flowers and leaves, followed by stems, but less in underground parts. This accumulation pattern of flavonoids in *P. japonicus* was closely associated with the expression of multiple structural genes (*PjC4H*, *Pj4CL*, *PjCHS*, *PjCHI*, *PjF3H*, *PjF3'H*, *PjCYP*, *PjPAL*, and *PjUGT*) as well as several transcription factors (*PjbHLH*, *PjMYB*, *PjWRKY*, *PjWD40*, *PjbZIP*, *PjbHLH*, *PjGRAS*, and *PjAP2*).

In addition, *P. japonicus* seeds were usually difficult to germinate in natural conditions. The molecular events occurring in the seed germination were largely unknown. In 2024, Zhou et al. firstly investigated the molecular mechanisms underlying seed germination in *P. japonicus* [30]. Fresh *P. japonicus* were harvested from Xintang Township, Enshi, Hubei Province of China. Three developmental stages of seeds were investigated, including stage I (no sand storage), stage II (sand storage for 60 days), and stage III (sand storage for 120 days). This study compared the seeds in different germination stages in terms of morphological features, antioxidant enzyme

activities and transcriptomics. Functional annotation revealed that the seed germination was primarily regulated by a series of genes related to pathways including ROS (Reactive Oxygen Species) scavenging metabolism, plant hormone signal transduction, starch and sucrose metabolism, energy supply and phenylpropanoid biosynthesis. Many key transcription factors, such as *bHLHs*, *MYBs*, *WRKYs*, and *bZIPs*, also played crucial roles in the seed germination process.

The development of *P. japonicus* fruits was also special, in which only the apical capitulum could develop into the normal fruit with distinctive color trait changes. In 2025, Huang et al. established the first gene expression resource for fruit development in *P. japonicus* [33]. *P. japonicus* fruits were collected from Anshun City, Guizhou Province of China. Five different color change periods of the fruit were investigated, including the green fruit period, green fruit turning period, red fruit period, black fruit period and fruit mature period. They revealed the correlation between color trait changes, pigment contents and secondary metabolite contents of *P. japonicus* fruits. The total number of differentially expressed genes generated by intercomparison of the five periods was 17,895, of which 9,435 genes were up-regulated and 8,460 genes were down-regulated. The whole flavonoid biosynthesis metabolism pathway was found to be critically involved in the promotion of color trait changes in *P. japonicus* fruits, in which 81 candidate genes were obtained in this flavonoid biosynthesis pathway. Similar findings have also been observed in studies on *P. ginseng*. Bian et al. (2025) conducted a study on the mechanism of *P. ginseng* fruit ripening using integrated metabolomic and transcriptomic analyses, revealing that genes in the flavonoid biosynthesis pathway were systematically upregulated during fruit development [34].

### The Metabolome and Proteome Analysis in *P. japonicus*

Triterpene saponins have made significant contributions to the biological activities of *Panax* genus, also including *P. japonicus*, which are mainly responsible for the prevention of liver fibrosis, neuroprotection and cardioprotective activities [35, 36]. Different from the other ginseng species, the oleanolic acid-type saponins are the main saponin components in *P. japonicus* [37]. In addition to triterpenoid saponins, polysaccharides, amino acids and microelements are also found to contribute to their multiple bioactivities in *P. japonicus* herbs [3].

As a powerful and effective tool, the metabolome could systematically elucidate the chemical characteristics at a systems level, which played an important role in the molecular networks connecting genotype to phenotype [38]. Commonly used metabolomics techniques included

nuclear magnetic resonance (NMR), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS). And, data analysis methods primarily included principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) [39].

By combining DDA (Data Dependent Acquisition) and HDMSE (High Definition MSE) data acquisition methods, Zhang et al. comprehensively analyzed and characterized multiple components in *P. japonicus* using UHPLC/IM-QTOF-MS (Ultra-High Performance Liquid Chromatography/Ion Mobility Spectrometry-Quadrupole-Time of Flight Mass Spectrometry) technology. 76 OA-type ginsenosides (42.7% of the total), 15 PPD-type (8.4%), 33 PPT-type (18.5%) ginsenosides, 11 OCT-ginsenosides (6.2%) and 12 malonylginsenosides (6.7%) were characterized in *P. japonicus* [40]. Among them, 75 saponins might not have been isolated from other *Panax* genus.

In 2023, Jiang et al. systematically characterized ginsenosides in *P. japonicus* var. *major* using UHPLC/QTOF-MS and firstly revealed the spatial distribution of these ginsenosides in rhizome tissues by using DESI-MSI imaging [37]. A total of 272 ginsenosides were identified in *P. japonicus* var. *major*, including 138 ginsenosides that were possibly reported for the first time in the *Panax* genus. The identified ginsenosides were classified into seven categories: 75 OA-type ginsenosides (27.57%), 22 PPD-type (8.09%), 52 PPT-type ginsenosides (19.12%), 16 OCT-type ginsenosides (5.88%), 19 malonylated ginsenosides (6.99%), 35 ginsenosides with different C-17 side chains (12.87%), and 53 other types of ginsenosides (19.49%). The DESI-MSI experiments further revealed the predominant distribution of ginsenosides in the cork layer and phloem of the rhizome, and found that oleanane-type ginsenosides were more abundant in the nodes of *P. japonicus* var. *major*, which was consistent with the results characterized by UHPLC/QTOF-MS.

In 2024, Yasen et al. developed an offline two-dimensional liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry (HILIC×RP LC/QTOF-MS) method. They also constructed an internal database comprising 612 reported saponins and 228 predicted metabolites [41]. Using this method, 307 saponins were identified from *P. japonicus* rhizomes, of which 76 were newly identified in this species. These saponins primarily belong to the oleanane and dammarane types, with the dammarane type including PPD, PPT, OCT types, and various C17 side chain variants. Among them, 150 were oleanane-type ginsenosides and their derivatives, while 156 were dammarane-type ginsenosides comprising variants with diverse C17 side chains as well as esterified and acylated derivatives. Additionally, a class

of ginsenosides was identified as ursonic acid-type, which are isomers of oleanane-type ginsenosides.

Huang et al. first combined proteomic and metabolomic technologies to analyze the spatiotemporal distribution of ginsenoside biosynthesis and its associated genes in *P. japonicus* [27]. The content of ginsenosides was quantitatively analyzed using the UPLC-MRM-MS method. A positive correlation was observed between rhizome ginsenoside content and growth years. This correlation was largely attributed to the increasing proportion and content of OA-type ginsenosides. OA-type ginsenosides were predominantly distributed in the rhizomes. In lateral roots, the content of PPT-type ginsenosides was the highest, followed by PPD- and OA-type ginsenosides were the least. And, in leaves, ginsenosides were mainly composed of PPT- and PPD-type ginsenosides. In stems, ginsenoside composition was largely limited to PPT-type compounds, reflecting a relatively low chemical diversity. After the proteomic analysis, 3,482 proteins were identified in *P. japonicus*. Among these proteins, the expression levels of 10 CYP and 16 UGT proteins were strongly correlated with ginsenoside content (correlation coefficient > 0.8). These proteins, positively correlated with specific ginsenosides, might function in the biosynthesis of these compounds or their upstream precursors.

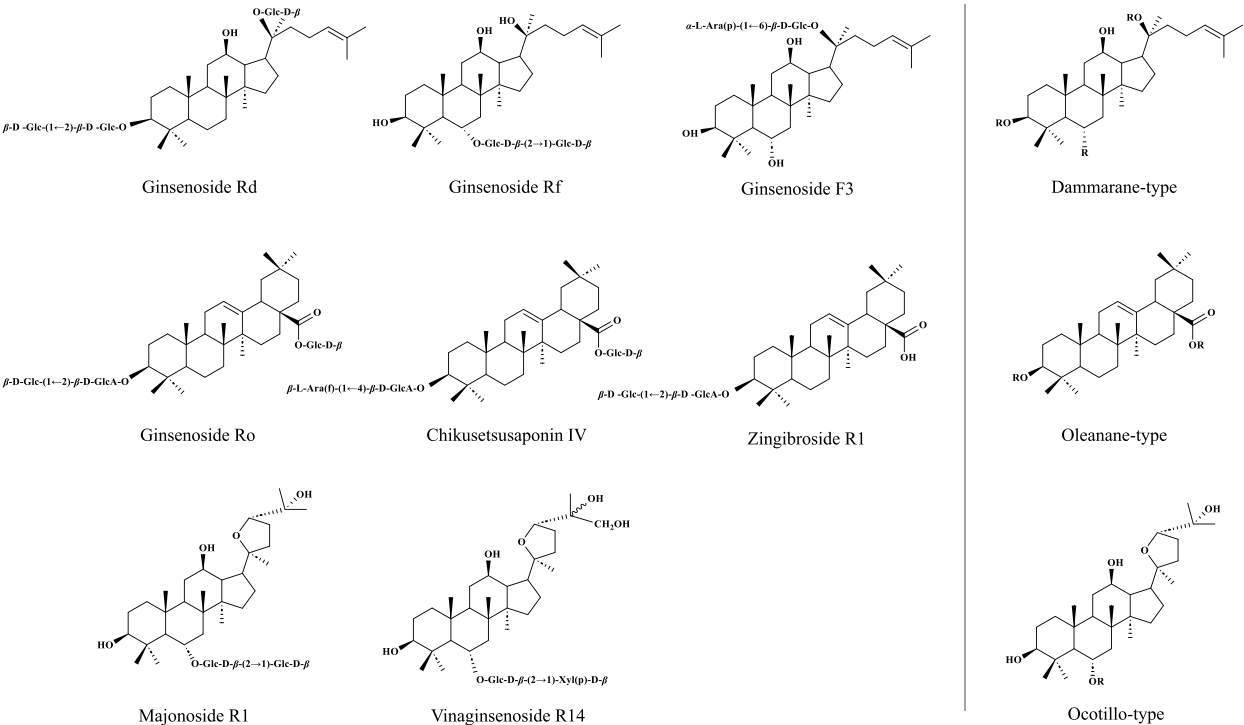
*P. japonicus* and *P. japonicus* var. *major* are two different ginseng herbs with similar bioactivities. However, there are few studies on the qualitative analysis of the chemical composition of the differences between these two similar *Panax* herbs. To comprehensively investigate the discrimination of these two *Panax* herbs, Du et al. (2018) first employed UHPLC-Q-Exactive/HRMS and HPLC-ESI-MS/MS methods to screen and identify the chemical compositions of *P. japonicus* and *P. japonicus* var. *major*. 22 representative ginsenosides (Rg1, Re, Rb1, Rc, Rb2, Rb3, Rf, Rd, Rg2, Rg3, F3, Rh1, Rh2, Ro, F1, F2, Notoginsenoside R1, pseudo F11, PPD, PPT, chikusetsusaponin IV and chikusetsusaponin IVa) were focused on and identified in this study [42]. Among these major ginsenosides, ginsenoside Ro, chikusetsusaponin IV and chikusetsusaponin IVa showed the highest content, especially in *P. japonicus* and *P. japonicus* var. *major*, which was about 20–70 times that of other ginsenosides. Subsequent studies have further confirmed this discovery, collectively demonstrating the abundance of OA-type compounds (e.g., chikusetsusaponin IV, IVa, and ginsenoside Ro) in both *P. japonicus* and *P. japonicus* var. *major* [7, 38, 41, 43–45]. Moreover, Du et al. [42] further found that the major difference between *P. japonicus* and *P. japonicus* var. *major* was in the content of these compounds (Rd, Rf, F3, Ro, and chikusetsusaponin IV) (Fig. 3). And, the average content of total ginsenosides

in *P. japonicus* was significantly higher than that in *P. japonicus* var. *major*, even though the PPD and PPT content in *P. japonicus* var. *major* was more abundant.

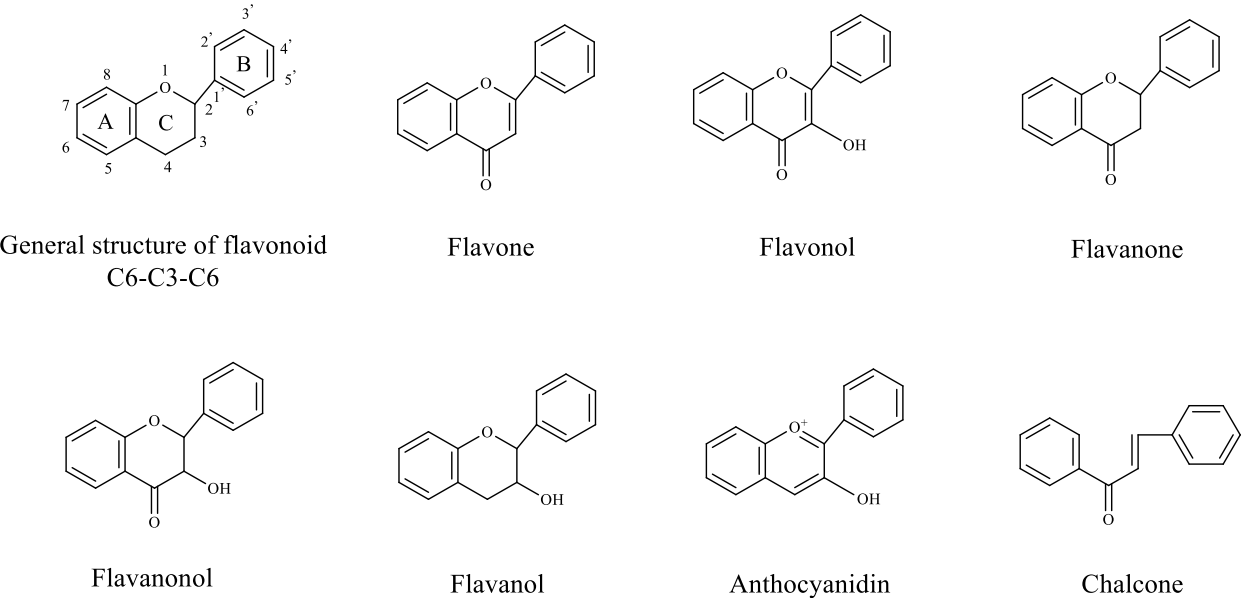
Furthermore, the differences in chemical composition among five *Panax* species (*P. ginseng*, *P. quinquefolius*, *P. notoginseng*, *P. japonicus*, and *P. japonicus* var. *major*) were evaluated through an integrated strategy combining untargeted metabolomics (UPLC-QTOF-MS profiling) with multivariate analysis (PCA, PLS-DA, and OPLS-DA) [7]. According to the screening results, there were some significant differences in chemical composition between these five *Panax* species, including saponins, amino acids, and fatty acids. The primary differences were still saponins, in which 12 important saponins (Zingibroside R1, Zingibroside R2, Zingibroside R4, American ginseng saponin-L11, Zingibroside Fa, Notoginsenoside-E, Majoroside R1, Majoroside R2, Ginsenoside Rd/Re, Ginsenoside Rb1, Chikusetsusaponin IVa, Chikusetsusaponin Ib) showed significant differences among these five *Panax* species. Although *P. japonicus* and *P. japonicus* var. *major* had a similar composition, several saponins were found to be significantly different between these two species, including zingibroside R1, majonoside R1, vinaginsenoside R14, ginsenoside Rd and Rf (Fig. 3). This result was partially consistent with Du's research in 2018 [31].

In addition to triterpene saponins, natural flavonoids, including flavones, isoflavones, flavanols, flavanones, flavanonols, chalcones, anthocyanidins and flavanols, are also important metabolites in *P. japonicus* (Fig. 4). Flavonoids play critical roles in diverse pharmacological effects and in plant adaptation to terrestrial environments. However, the distribution of flavonoids in different tissues of *P. japonicus* and the related genes involved in flavonoid accumulation have not yet been reported. Therefore, it is worthwhile to explore the flavonoid biosynthesis of secondary metabolites in *P. japonicus*.

In 2024, Chen et al. first investigated the changes in flavonoid contents in different tissues of four-year-old *P. japonicus* from Chongqing of China (main roots, fibrous roots, stems, leaves and flowers) by combining target metabolomics and transcriptomics. A total of 209 flavonoid metabolites in seven categories (flavones, flavonols, flavanones, flavanonols, flavanols, anthocyanidins and chalcones) were identified by using the UPLC-ESI-MS/MS system, in which flavonols and flavones were the most abundant in *P. japonicus*, comprising 106 (50.72%) and 63 (30.14%) metabolites, respectively [28]. The flavonoid contents in different tissues revealed significant differences, in which flavonoids, flavonols, flavanones and flavanonols predominantly accumulated in the aerial parts of the plant, particularly in the flowers and leaves. Moreover, the accumulation of 11 flavonoids in the ko00941 pathway (C10107, C00389, C09806, C09614,



**Fig. 3** Characteristic structure of triterpene saponins for differentiating between *P. japonicus* and *P. japonicus* var. *major*



**Fig. 4** Characteristic structure of flavonoids metabolites in *P. japonicus*

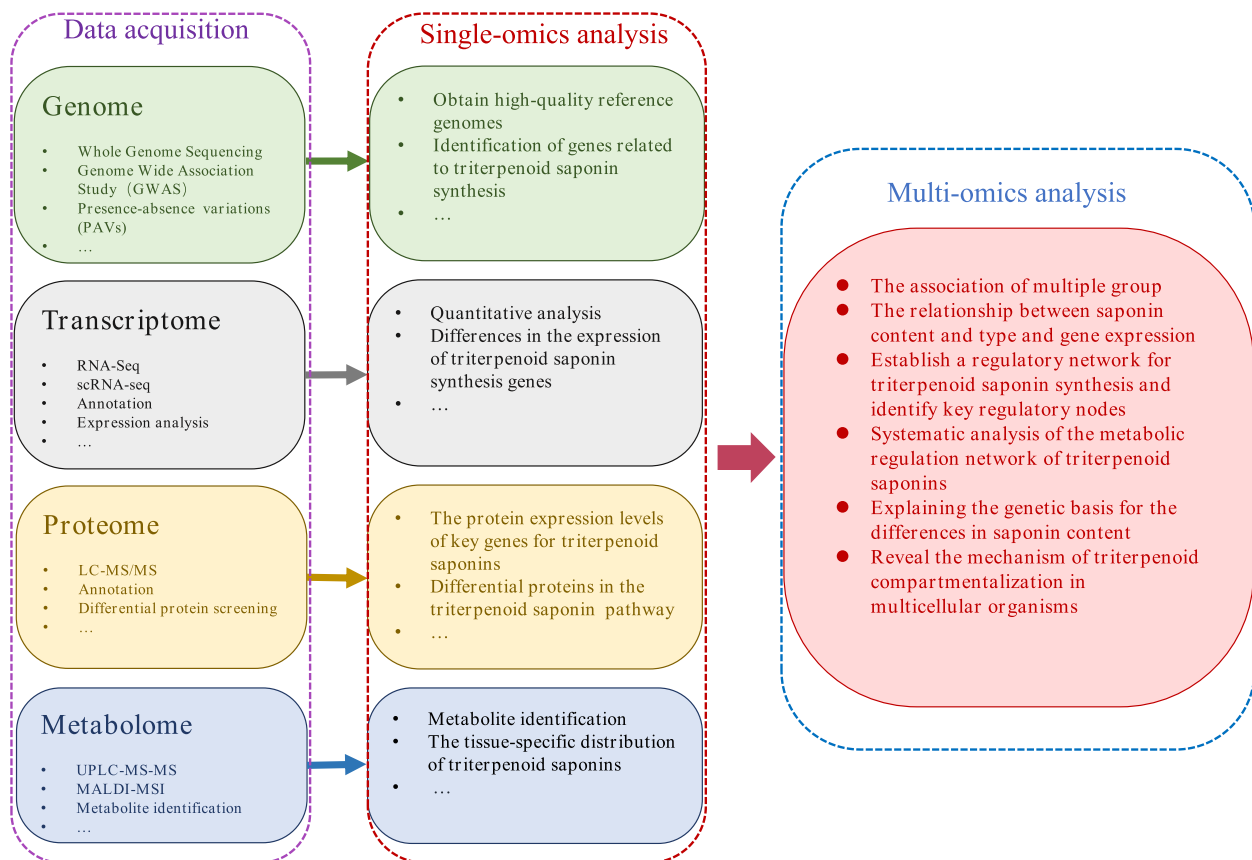
C09099, C09789, C16422, C16408, C01604, C06561 and C16406) was greatest in the flowers, followed by the leaves and stems, and was lowest in the main roots and fibrous roots.

**Integrative Multi-Omics Analysis in *P. japonicus***  
Integrating multi-omics analyses enable a comprehensive elucidation of key functional genes involved in triterpenoid saponin biosynthesis and their underlying

molecular mechanisms (Fig. 5). In *P. japonicus*, Huang et al. (2023) first employed a multi-omics approach to analyze the spatiotemporal distribution of ginsenoside biosynthesis and its regulatory genes by integrating transcriptomic, proteomic, and metabolomic data [27]. Two important conclusions could be drawn from this study. First, the variation in saponin content in *P. japonicus* originated from differences in the expression efficiency of upstream biosynthetic genes. Second, the specific expression of CYP450 genes regulated the ratio of protopanaxadiol (PPD) to protopanaxatriol (PPT). These findings were consistent with results of Tian et al. (2025) in their multi-omics study on *P. ginseng*, *P. quinquefolius*, and *P. notoginseng* [43]. In *P. japonicus* var. *major*, Yang et al. (2024) first conducted an integrated transcriptomic and metabolomic analysis of four tissues, including the root, stem, leaf, and fruit [44]. This study identified a total of 216,108 unigenes, exceeding the gene numbers documented in previous research. A total of 507 plant defense-related nucleotide binding site (NBS) genes

were identified, which showed that *P. japonicus* var. *major* had the highest number of NBS genes compared to other *Panax* species. Three *UGT* genes (*UGT94Q2*, *UGT74AE2*, and *UGT74M1*) showed a strong positive correlation with nine differentially accumulated saponins including chikusetsusaponin IVa, ginsenoside Rd, Ro, Rf, Rg2, Rg1, Rb2, Re, and Rb3. Two *CYP450* genes, *CYP716A47* and *CYP716A52V2*, also showed a significant positive correlation with all nine saponins.

Moreover, single-cell omics and spatial omics technologies have advanced rapidly in recent years, which allows high-throughput analysis of specific tissues at both single-cell and spatial resolution [44–46]. This shift has redirected research focus from merely identifying medicinal components to precisely determining their spatial distribution and understanding how different cells coordinate and collaborate within tissues. Wang et al. (2025) have integrated MALDI-MSI with single-cell RNA-seq (scRNA-seq) technology to establish a critical link between the spatial distribution of metabolites and cell type-specific gene



**Fig. 5** Schematic diagram of the multi-omics strategy for analyzing triterpenoid saponin biosynthesis. This diagram depicts the complete analytical strategy, including three key sections: (1) Data acquisition (2) Single-omics analysis; (3) Multi-omics analysis. RNA-Seq: RNA Sequencing; scRNA-seq: single-cell RNA Sequencing; LC-MS/MS: Liquid Chromatography-Tandem Mass Spectrometry; UPLC-MS-MS: Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry; MALDI-MSI: Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging; WGCNA: Weighted gene co-expression network analysis

expression patterns in *P. ginseng* [46]. The study revealed that genes involved in ginsenoside biosynthesis were highly expressed in guard cells and palisade mesophyll cells, the MVA pathway was mainly expressed in guard cells, and downstream genes were predominantly expressed in palisade mesophyll cells and phloem parenchyma cells. Yang et al. (2025) have performed a joint analysis of scRNA-seq and MALDI-MSI on the root tips of three *Panax* species (*P. notoginseng*, *P. ginseng* and *P. quinquefolium*) [47]. This study first revealed that the differentiation trajectories of root tip cells and the cell type-specific biosynthesis of saponins in three *Panax* species at single-cell resolution. However, there are currently no reports on single-cell or spatial omics research in *P. japonicus* and its varieties. Future research should focus on these novel omics technologies to elucidate the regulatory mechanisms of saponin biosynthesis in *P. japonicus*.

## Conclusion

*P. japonicus* has historically been regarded as the "king of herbs" in Tujia and Hmong traditional medicines in China, because it possesses both the conserving vitality activities of *P. ginseng* and the replenishing blood activities of *P. notoginseng* simultaneously. Also, it has been widely used in traditional healing for centuries to relieve sprains, hematemeses, cough, arthralgia and weakness after illness. Although numerous promising findings have been reported for *P. japonicus*, the molecular mechanisms underlying the variation in its active constituents are still poorly understood. With the rapid advances of integrative omics platforms including genomics, transcriptomics, metabolomics, and proteomic, the "systematic herbal-omics" term has been coined to holistically integrate the multi-layer omics datasets. These novel systematic research approaches fuel the identification of novel bioactive compounds as well as the decoding of their biosynthetic pathways in *P. japonicus*.

This review first addressed the latest research findings of *P. japonicus* in the field of combinatorial omics. Future omics research on *P. japonicus* should incorporate single-cell and spatial omics to elucidate the cell type-specific processes governing saponin biosynthesis within its rhizomes. Moreover, the multi-omics network should be constructed by incorporating genomic, transcriptomic, metabolic and proteomic data, enriching our multidimensional analysis of *P. japonicus*'s secondary metabolism.

Overall, this review provided novel perspectives to further understand the genetic background, morphological development, and ginsenoside biosynthesis of *P. japonicus*, which was helpful to foster the multifaceted development of *P. japonicus* industry and explore its full potential for human health and economic development.

## Acknowledgements

We sincerely thank the Institute of Chinese Herbal Medicines, Hubei Academy of Agricultural Sciences, for providing the essential resources.

## Authors' contributions

Yuxin Zhuang: Conceptualization, Methodology, Formal analysis, Writing-original draft; Yihui Fang and Zeqing Xin: Investigation. Huanhuan Qi, Dengxiang Du and Mengmeng Ren: Validation and Visualization. Shao-peng Zhang: Supervision, Project administration, Funding acquisition, Writing-review&editing.

## Funding

This work was financially supported by the key project at central government level: the ability establishment of sustainable use for valuable Chinese medicine resources (2060302).

## Data availability

The manuscript has no associated data.

## Declarations

### Ethics approval and consent to participate

None.

### Competing interests

The authors declare no conflict of interest.

Received: 4 November 2025 Revised: 31 January 2026 Accepted: 26 February 2026

Published online: 03 April 2026

## References

- Shi FX, Li MR, Li YL, Jiang P, Zhang C, Pan YZ, et al. The impacts of polyploidy, geographic and ecological isolations on the diversification of *panax* (araliaceae). BMC Plant Biol. 2015;15:297. <https://doi.org/10.1186/s12870-015-0669-0>.
- Fan W, Huang Y, Zheng H, Li S, Li Z, Yuan L, et al. Ginsenosides for the treatment of metabolic syndrome and cardiovascular diseases: pharmacology and mechanisms. Biomed Pharmacother. 2020;132:110915. <https://doi.org/10.1016/j.biopha.2020.110915>.
- Hou M, Wang R, Zhao S, Wang Z. Ginsenosides in *panax* genus and their biosynthesis. Acta Pharm Sin B. 2021;11(7):1813–34. <https://doi.org/10.1016/j.apsb.2020.12.017>.
- Zuo YJ, Wen J, Zhou SL. Intercontinental and intracontinental biogeography of the eastern Asian - eastern north American disjunct *panax* (the ginseng genus, araliaceae), emphasizing its diversification processes in eastern Asia. Mol Phylogenet Evol. 2017;117:60–74. <https://doi.org/10.1016/j.jmpev.2017.06.016>.
- Yang Z, Li X, Yang L, Peng S, Song W, Lin Y, et al. Comparative genomics reveals the diversification of triterpenoid biosynthesis and origin of ocotillo-type triterpenes in *panax*. Plant Commun. 2023;4(4):100591. <https://doi.org/10.1016/j.xplc.2023.100591>.
- Zhang S, Wang R, Zeng W, Zhu W, Zhang X, Wu C, et al. Resource investigation of traditional medicinal plant *panax japonicus* (T.nees) C.A. Mey and its varieties in China. J Ethnopharmacol. 2015;166:79–85. <https://doi.org/10.1016/j.jep.2015.02.051>.
- Ji R, Garran TA, Luo Y, Cheng M, Ren M, Zhou X. Untargeted metabolomic analysis and chemometrics to identify potential marker compounds for the chemical differentiation of *panax ginseng*, *P. quinquefolius*, *P. notoginseng*, *P. japonicus*, and *P. japonicus* var. *major*. Molecules (basel, Switzerland). 2023;28(6):2745. <https://doi.org/10.3390/molecules28062745>.
- Zhang H, Wang HF, Liu Y, Huang LJ, Wang ZF, Li Y. The haematopoietic effect of *panax japonicus* on blood deficiency model mice. J Ethnopharmacol. 2014;154(3):818–24. <https://doi.org/10.1016/j.jep.2014.05.008>.
- Gao Y, Wang R, Li L, He Y, Yuan D, Zhang Y, et al. Total saponins from *panax japonicus* reduce inflammation in adipocytes through the miR155/SOCS1/NFκB signaling pathway. Phytomedicine. 2023;115:154827. <https://doi.org/10.1016/j.phymed.2023.154827>.

10. Wang J, He L, Wang S, Zhao H, Chen J, Dong Y, et al. Therapeutic effect of the total saponin from *panax japonicus* on experimental autoimmune encephalomyelitis by attenuating inflammation and regulating gut microbiota in mice. *J Ethnopharmacol.* 2023;315:116681. <https://doi.org/10.1016/j.jep.2023.116681>.
11. Rai A, Yamazaki M, Takahashi H, Nakamura M, Kojoma M, Suzuki H, et al. RNA-seq transcriptome analysis of *panax japonicus*, and its comparison with other *panax* species to identify potential genes involved in the saponins biosynthesis. *Front Plant Sci.* 2016;7:481. <https://doi.org/10.3389/fpls.2016.00481>.
12. Augustin JM, Drok S, Shinoda T, Sanmiya K, Nielsen JK, Khakimov B, et al. UDP-glycosyltransferases from the UGT73C subfamily in *barbarea vulgaris* catalyze sapogenin 3-O-glucosylation in saponin-mediated insect resistance. *Plant Physiol.* 2012;160(4):1881–95. <https://doi.org/10.1104/pp.112.202747>.
13. He H, Xu J, Xu Y, Zhang C, Wang H, He Y, et al. Cardioprotective effects of saponins from *panax japonicus* on acute myocardial ischemia against oxidative stress-triggered damage and cardiac cell death in rats. *J Ethnopharmacol.* 2012;140(1):73–82. <https://doi.org/10.1016/j.jep.2011.12.024>.
14. Mohanan P, Yang TJ, Song YH. Genes and regulatory mechanisms for ginsenoside biosynthesis. *J Plant Biol.* 2023;66(1):87–97. <https://doi.org/10.1007/s12374-023-09384-7>.
15. Wang XJ, Xie Q, Liu Y, Jiang S, Li W, Li B, et al. *Panax japonicus* and chikusetsusaponins: a review of diverse biological activities and pharmacology mechanism. *Chin Herb Med.* 2021;13(1):64–77. <https://doi.org/10.1016/j.chmed.2020.12.003>.
16. Lin Y, Hao B, Lu YC, Dong Y, Li Y, Zhang GH, et al. PanaxGDB: a comprehensive platform for *panax*. *Front Plant Sci.* 2022;13:883818. <https://doi.org/10.3389/fpls.2022.883818>.
17. Xu J, Chu Y, Liao B, Xiao S, Yin Q, Bai R, et al. *Panax ginseng* genome examination for ginsenoside biosynthesis. *Gigascience.* 2017. <https://doi.org/10.1093/gigascience/gix093>.
18. Song Y, Zhang Y, Wang X, Yu X, Liao Y, Zhang H, et al. Telomere-to-telomere reference genome for *panax ginseng* highlights the evolution of saponin biosynthesis. *Hortic Res.* 2024;11(6):uhae107. <https://doi.org/10.1093/hr/uhae107>.
19. Yang Z, Liu G, Zhang G, Yan J, Dong Y, Lu Y, et al. The chromosome-scale high-quality genome assembly of *panax notoginseng* provides insight into dencichine biosynthesis. *Plant Biotechnol J.* 2021. <https://doi.org/10.1111/pbi.13558>.
20. Jiang Z, Tu L, Yang W, Zhang Y, Hu T, Ma B, et al. The chromosome-level reference genome assembly for *panax notoginseng* and insights into ginsenoside biosynthesis. *Plant Commun.* 2021. <https://doi.org/10.1016/j.xplc.2020.100113>.
21. Wang ZH, Wang XF, Lu T, Li MR, Jiang P, Zhao J, et al. Reshuffling of the ancestral core-eudicot genome shaped chromatin topology and epigenetic modification in *panax*. *Nat Commun.* 2022. <https://doi.org/10.1038/s41467-022-29561-5>.
22. Tien NQD, Ma X, Man LQ, Chi DTK, Huy NX, Nhut DT, et al. De novo whole-genome assembly and discovery of genes involved in triterpenoid saponin biosynthesis of vietnamese ginseng (*panax vietnamensis* ha et grushv.). *Physiol Mol Biol Plants.* 2021;27(10):2215–29. <https://doi.org/10.1007/s12298-021-01076-1>.
23. Guo J, Huang Z, Sun J, Cui X, Liu Y. Research progress and future development trends in medicinal plant transcriptomics. *Front Plant Sci.* 2021;12:691838. <https://doi.org/10.3389/fpls.2021.691838>.
24. Zhang S, Wu Y, Jin J, Hu B, Zeng W, Zhu W, et al. De novo characterization of *panax japonicus* C. A. Mey transcriptome and genes related to triterpenoid saponin biosynthesis. *Biochem Biophys Res Commun.* 2015;466(3):450–5. <https://doi.org/10.1016/j.bbrc.2015.09.048>.
25. Xu R, Zhang J, You J, Gao L, Li Y, Zhang S, et al. Full-length transcriptome sequencing and modular organization analysis of oleanolic acid- and dammarane-type saponins related gene expression patterns in *panax japonicus*. *Genomics.* 2020. <https://doi.org/10.1016/j.ygeno.2020.06.045>.
26. Zhang S, Wang G, Zuo T, Zhang X, Xu R, Zhu W, et al. Comparative transcriptome analysis of rhizome nodes and internodes in *panax japonicus* var. major reveals candidate genes involved in the biosynthesis of triterpenoid saponins. *Genomics.* 2020;112(2):1112–9. <https://doi.org/10.1016/j.ygeno.2019.06.025>.
27. Huang C, Li P, Yang X, Niu T, Zhao S, Yang L, et al. Integrated transcriptome and proteome analyses reveal candidate genes for ginsenoside biosynthesis in *panax japonicus* C. A. Meyer. *Frontiers in Plant Science.* 2022;13:1106145. <https://doi.org/10.3389/fpls.2022.1106145>.
28. Chen C, Wang P, Yan Y, Jiao Z, Xie S, Li Y, et al. Integrated metabolome and transcriptome analysis provide insight into the biosynthesis of flavonoids in *panax japonicus*. *Front Plant Sci.* 2024;15:1432563. <https://doi.org/10.3389/fpls.2024.1432563>.
29. Yang Q, Xiong C, Zhang J, Ming Y, Zhang S, Wang L, et al. Chemical and transcriptomic analyses provide new insights into key genes for ginsenoside biosynthesis in the rhizome of *panax japonicus* C. A. Meyer. *Molecules (Basel, Switzerland).* 2024;29(20):4936. <https://doi.org/10.3390/molecules29204936>.
30. Zhou W, Li X, Li D, Jiang X, Yang Y, You J, et al. Comparative transcriptome analysis provides novel insights into the seed germination of *panax japonicus*, an endangered species in China. *BMC Plant Biol.* 2024;24(1):1167. <https://doi.org/10.1186/s12870-024-05904-w>.
31. Xu X, Wang S, Wang H, Hu W, Han L, Chen B, et al. Simultaneous quantitative assays of 15 ginsenosides from 119 batches of ginseng samples representing 12 traditional Chinese medicines by ultra-high performance liquid chromatography coupled with charged aerosol detector. *J Chromatogr A.* 2021;1655:462504. <https://doi.org/10.1016/j.chroma.2021.462504>.
32. Luo X, Ye Z, Shi X, Hu Z, Shen J, You L, et al. Comparative transcriptomic analysis provides insights into the regulation of root-specific saponin production in *panax japonicus*. *Sci Rep.* 2024. <https://doi.org/10.1038/s41598-024-78720-9>.
33. Huang XL, Jin R, Tang TT, Liang E, Zhang L. Physiological, biochemical and transcriptomic analyses reveal the mechanism of variation in color traits in *panax japonicus* fruits. *Sci Rep.* 2025;15(1):13950. <https://doi.org/10.1038/s41598-025-89508-w>.
34. Bian X, Qiu Y, Zhao X, Wei H, Zhang Y, Zhang W, et al. Multi-omics dissection of metabolic and transcriptional regulation underlying fruit maturation in *panax ginseng*. *BMC Plant Biol.* 2025;25(1):1133. <https://doi.org/10.1186/s12870-025-07221-2>.
35. Yuan D, Xiang T, Huo Y, Liu C, Wang T, Zhou Z, et al. Preventive effects of total saponins of *panax japonicus* on fatty liver fibrosis in mice. *Arch Med Sci.* 2018;14(2):396–406. <https://doi.org/10.5114/aoms.2016.63260>.
36. Ling Y, Ouyang Y, Xue Y, Yu J, Liu W. In-depth excavation and screening of triterpene saponins from the rhizome of *panax japonicus* using high performance liquid chromatography coupled to electrospray ionization and quadrupole time-of-flight mass spectrometry. *J Chromatogr Sci.* 2023;61(10):972–9. <https://doi.org/10.1093/chromsci/bmad014>.
37. Jiang M, Li X, Zhao Y, Zou Y, Bai M, Yang Z, et al. Characterization of ginsenosides from *panax japonicus* var. *major* (zhu-zhi-shen) based on ultra-high performance liquid chromatography/quadrupole time-of-flight mass spectrometry and desorption electrospray ionization-mass spectrometry imaging. *Chin Med.* 2023;18(1):115. <https://doi.org/10.1186/s13020-023-00830-9>.
38. Dabbousy R, Rima M, Roufayel R, Rahal M, Legros C, Sabatier JM, et al. Plant metabolomics: the future of anticancer drug discovery. *Pharmaceuticals (Basel, Switzerland).* 2024;17(10):1307. <https://doi.org/10.3390/ph17101307>.
39. Zhang CX, Wang XY, Lin ZZ, Wang H, Qian YX, Li WW, et al. Highly selective monitoring of in-source fragmentation sapogenin product ions in positive mode enabling group-target ginsenosides profiling and simultaneous identification of seven *panax* herbal medicines. *J Chromatogr A.* 2020;1618:460850. <https://doi.org/10.1016/j.chroma.2020.460850>.
40. Zhang C, Zuo T, Wang X, Wang H, Hu Y, Li Z, et al. Integration of data-dependent acquisition (DDA) and data-independent high-definition MSE (HDMSE) for the comprehensive profiling and characterization of multi-components from *panax japonicus* by UHPLC/IM-QTOF-MS. *Molecules (basel, Switzerland).* 2019;24(15):2708. <https://doi.org/10.3390/molecules24152708>.
41. Yasen S, Li C, Wang S, Dong Y, Li H, Chen J, et al. Comprehensive characterization of triterpene saponins in rhizoma *panax japonici* by offline two-dimensional liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. *Molecules.* 2024;29(6):1295. <https://doi.org/10.3390/molecules29061295>.
42. Du Z, Li J, Zhang X, Pei J, Huang L. An integrated LC-MS-based strategy for the quality assessment and discrimination of three *panax* species. *Molecules.* 2018;23(11):2988. <https://doi.org/10.3390/molecules23112988>.
43. Tian Y, Wang ZL, Ye L, Wang Y, Di P, Wang H, et al. Comparative metabolome and transcriptome analyses reveal mechanisms for differences in ginsenoside profiles of herbal medicines derived from *panax* species. *Engineering.* 2025. <https://doi.org/10.1016/j.eng.2025.08.041>.

44. Yang L, Guo J, Wang P, Sun J, Liu M, Yang Y, et al. Integrative transcriptomic and metabolomic analysis reveals NBS resistance genes and metabolic pathways of *panax japonicus* var. major. *Ind Crops Prod.* 2024;216:118722. <https://doi.org/10.1016/j.indcrop.2024.118722>.
45. Chen C, Zhang X, Yue M. Spatial multi-omics in medicinal plants: from biosynthesis pathways to industrial applications. *Trends Plant Sci.* 2024. <https://doi.org/10.1016/j.tplants.2024.02.008>.
46. Wang R, Zhang L, Li X, Sun W, Wang Y, Huang L, et al. Single-cell sequencing and mass spectrometry imaging reveal the multicellular compartmentalization map of plant triterpenes: a ginsenoside in *panax ginseng* example. *Plant Biotechnol J.* 2025. <https://doi.org/10.1111/pbi.70198>.
47. Yang L, Yang Z, Liu M, Wang S, Wu H, Yang Q, et al. Integrated single-cell transcriptomics and spatial metabolomics unveil cellular differentiation and ginsenosides biosynthesis in *panax* root tips. *Hortic Res.* 2025. <https://doi.org/10.1093/hr/uhaf202>.

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