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SPECIALTY SECTION  
This article was submitted to  
Plant Metabolism and Chemodiversity,  
a section of the journal  
Frontiers in Plant Science

RECEIVED 29 June 2022  
ACCEPTED 02 August 2022  
PUBLISHED 02 September 2022

CITATION  
Zhang X-X, Zuo J-Q, Wang Y-T, Duan H-Y,  
Yuan J-H and Hu Y-H (2022) Paeoniflorin in  
Paeoniaceae: Distribution, influencing  
factors, and biosynthesis.  
*Front. Plant Sci.* 13:980854.  
doi: 10.3389/fpls.2022.980854

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# Paeoniflorin in Paeoniaceae: Distribution, influencing factors, and biosynthesis

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Paeoniflorin, a monoterpene glucoside, is increasingly used in the clinical treatment of many diseases because it has a variety of pharmacological activities. Besides, paeoniflorin has been considered the characteristic chemical constituent of Paeoniaceae plants since it was first reported in 1963. Therefore, how to better develop and utilize paeoniflorin in Paeoniaceae has always been a research hotspot. We reviewed the current knowledge on paeoniflorin in Paeoniaceae, with particular emphasis on its distribution and influencing factors. Moreover, the limited understanding of the biosynthesis pathway has restricted the production of paeoniflorin by synthetic biology. This review provides insights into the post-modification pathway of paeoniflorin biosynthesis and proposes directions for further analysis in the future.

## KEYWORDS

Paeoniaceae, Paeoniflorin, influencing factors, biosynthesis, post-modification enzyme, Monoterpene

## Introduction

Paeoniaceae is a single genus family, and *Paeonia* consists of 34 species, comprising shrubs and perennial herbs mainly distributed in temperate Eurasia, northwest Africa, and western North American (Hong, 2010, 2021). The shrubs, called tree peonies, belong to Section *Moutan* DC., and the perennials, called herbaceous peonies, are classified into Sect. *Paeonia* or Sect. *Onaepia* Lindley (Hong, 2011). China has the most concentrated distribution of Paeoniaceae plants, and it is also one of the centers of differentiation and development of existing Paeoniaceae primitive groups (Zhou et al., 2021). Since the Eastern Han Dynasty (25–220 A.D.), Paeoniaceae plants have been widely cultivated and used in China because of their special medicinal effects (Li et al., 2011). Currently, there are three traditional Chinese medicines in China, Mudanpi, Baishao, and Chishao, which are processed from the roots of Paeoniaceae plants (Chinese Pharmacopoeia Committee, 2020). These three medicines have the functions of clearing heat, cooling blood, promoting blood circulation, and removing blood stasis (Tan et al., 2020; Wu et al., 2020).

Although there are many active components in Paeoniaceae plants, paeoniflorin, a monoterpene bicyclic glycoside, is considered the most important medicinal ingredient

(Xiu et al., 2021). Modern medical studies have shown that paeoniflorin has immunoregulatory, antidepressant, anti-arthritis, antithrombosis, anti-tumor, hepatoprotective, cerebral ischemic injury protective, and neuroprotective effects (Zhang et al., 2019a; Zhang, 2020). Moreover, paeoniflorin, found only in Paeoniaceae plants in nature, is considered the characteristic constituent of Paeoniaceae (Yu and Xiao, 1987). Therefore, numerous studies have focused on the distribution of paeoniflorin in Paeoniaceae to better develop and utilize these plants for medicinal purposes (He, 2010; Yang et al., 2020; Yan et al., 2021). As a secondary metabolite, the content of paeoniflorin is affected by many factors, including biological and abiotic factors (Chen et al., 2009; Feng et al., 2012; Shen et al., 2019; Zhang and Wei, 2020). The influence of these factors on the content of paeoniflorin has become a topic of research interest. Our research group has also carried out several studies on paeoniflorin in Paeoniaceae plants (Zhang et al., 2019b). Thus, it is necessary to systematically review this research and establish expectations for future studies.

Currently, paeoniflorin is mainly extracted from the roots of Paeoniaceae plants, but this has many disadvantages, such as low yield, difficulty in separation of extracts, and wastage of plant resources (Nie et al., 2005). Although paeoniflorin has been synthesized by chemical methods for a long time, it is not used in actual production because of its complicated process, high production cost, and contaminated production process (Corey and Wu, 1993; Hatakeyama et al., 1994). As an active natural product with important medicinal value, paeoniflorin is increasingly required for drug preparation; for this reason, it is important to realize its large-scale production economically. The biosynthesis of natural products is one of the most important research directions in synthetic biology. Compared with the two abovementioned methods, biosynthesis has many advantages, such as a single product, high yield, an environmentally friendly process, and no restriction of raw materials (Sun et al., 2017). However, the biosynthesis pathway of paeoniflorin has not been fully elucidated, especially the modification stage that determines its structural and functional specificity. There are few studies on the enzymes involved in this stage, which fundamentally limit the production of paeoniflorin by synthetic biology.

In this review, we summarized the distribution characteristics of paeoniflorin and the effects of different factors on paeoniflorin content in Paeoniaceae. More importantly, we predicted the post-modification pathway of paeoniflorin biosynthesis and analyzed the paeoniflorin biosynthesis pathway.

## Paeoniflorin in Paeoniaceae

### Characteristic constituents of Paeoniaceae

The secondary metabolites in Paeoniaceae are complex, with more than 300 chemical components identified, mainly including monoterpene glycosides, triterpenoids, flavonoids, tannins,

phenolic acids, sugars, steroids, and volatile oils (He et al., 2010). Paeoniflorin was first isolated from the roots of *Paeonia albiflora* and named by Shibata et al. (1963), who pointed out that paeoniflorin was D-glucoside with a benzoylated C<sub>10</sub>-compound (C<sub>10</sub>H<sub>14</sub>O<sub>5</sub>). Further studies showed that paeoniflorin is a monoterpene glucoside whose basic skeleton is a pinane derivative (Shibata et al., 1964; Aimi et al., 1969; Kaneda et al., 1972). Subsequently, an increasing number of species belonging to Paeoniaceae have been reported to contain paeoniflorin in their roots (Egon and Cooper, 1969). Paeoniflorin has always only been found in Paeoniaceae plants (Yu and Xiao, 1987). Therefore, paeoniflorin was naturally considered to be the characteristic constituent of Paeoniaceae until 2008, when it was isolated from the water fern *Salvinia molesta* (Choudhary et al., 2008), which was the first report that paeoniflorin occurred in plants other than Paeoniaceae. In our previous study (Zhang et al., 2019b), we did not detect paeoniflorin in *S. molesta* by UPLC-MS, nor did we detect it in any of the 16 plants from different families. In addition to the roots of Paeoniaceae plants, other organs, including flowers, stems, leaves, fruits, seeds, and rhizomes, have also been investigated, and the results show that paeoniflorin is distributed throughout the plant. These results have thus confirmed that paeoniflorin is a characteristic constituent of Paeoniaceae without tissue specificity.

Although paeoniflorin occurs widely in the roots of species belonging to Paeoniaceae, its contents were quite different (Table 1; Supplementary Table 1). He et al. (1980) determined the paeoniflorin content in the roots of 11 species and varieties, and the content ranged from 0.09 to 10.72%. Yu and Xiao (1985, 1987) showed that the paeoniflorin content of 19 species and 6 varieties ranged from 0.40 to 4.36%, and a study by Jin et al. (1989) on 13 species and varieties found paeoniflorin contents ranging from 0.76 to 7.78%. Recently, our study included 16 species that exhibited a wide range of paeoniflorin content, from 0.22 to 5.12% (Zhang et al., 2019b). Many studies with a few samples (Han et al., 2008; Yang et al., 2020; Yan et al., 2021; Li et al., 2021a) that were not representative have shown that the paeoniflorin content in the roots of different species differs. In all the above studies, the roots varied by sampling time and location, and the detection methods of paeoniflorin were different; therefore, there is no agreement on which species have the highest and lowest paeoniflorin content. However, the consistent conclusion was that the paeoniflorin content of herbaceous peony roots was higher than that of tree peony roots. Furthermore, most studies have concluded that the paeoniflorin content in the roots of species collected from the wild habitat was higher than that in the roots of species collected from the cultivated habitat (Hu et al., 2000; Guo et al., 2002; Zhou et al., 2003; Wang et al., 2012).

### Factors influencing paeoniflorin content in Paeoniaceae

#### Plant ages

Some studies have suggested that older *Paeonia lactiflora* plants have a higher paeoniflorin content in their roots (Huang

TABLE 1 Paeoniflorin content in the roots of different species belonging to Paeoniaceae.

Species	Method	Content (mg/g)	References
<i>Paeonia suffruticosa</i>	TLC	9.0–25.0*	He et al., 1980; Yu and Xiao, 1987
<i>P. suffruticosa</i>	HPLC	14.1–25.6*	Guo et al., 2002
<i>Paeonia ostii</i>	HPLC	17.4*	Guo et al., 2002
<i>P. ostii</i>	HPLC-MS	5.8–7.6	Zhang et al., 2019b; Yan et al., 2021
<i>Paeonia qiui</i>	HPLC-MS	14.0–24.2	Zhang et al., 2019b; Yan et al., 2021
<i>Paeonia rockii</i>	HPLC	13.7–46.8*	Guo et al., 2002
<i>P. rockii</i>	HPLC-MS	6.0–8.7	Zhang et al., 2019b; Yan et al., 2021
<i>Paeonia jishanensis</i>	HPLC-MS	17.2–19.6	Zhang et al., 2019b; Yan et al., 2021
<i>Paeonia decomposita</i>	HPLC	43.4–48.2*	Guo et al., 2002
<i>P. decomposita</i>	HPLC-MS	12.3–14.0	Zhang et al., 2019b; Yan et al., 2021
<i>Paeonia rotundiloba</i>	HPLC-MS	9.8	Yan et al., 2021
<i>Paeonia szechuanica</i>	TLC	7.9–18.8*	Yu and Xiao, 1987
<i>Paeonia delavayi</i>	TLC	7.8–19.3*	He et al., 1980; Yu and Xiao, 1987
<i>P. delavayi</i>	HPLC-MS	1.9–16.2	Zhang et al., 2019b; Yan et al., 2021
<i>P. delavayi</i> var. <i>lutea</i>	TLC	14.2–25.2*	He et al., 1980; Yu and Xiao, 1987
<i>P. delavayi</i> var. <i>lutea</i>	RT-HPLC	7.6–18.2*	Jin et al., 1989
<i>Paeonia ludlowii</i>	HPLC-MS	0.9–2.3	Zhang et al., 2019b; Yan et al., 2021
<i>Paeonia lactiflora</i>	TLC	28.1–107.2*	He et al., 1980; Yu and Xiao, 1987
<i>P. lactiflora</i>	RT-HPLC	23.3–75.5*	Jin et al., 1989
<i>P. lactiflora</i>	HPLC-MS	22.2–92.3	Zhang et al., 2019b; Yang et al., 2020; Yan et al., 2021
<i>P. lactiflora</i> var. <i>trichocarpa</i>	TLC	49.6–57.0*	He et al., 1980
<i>P. lactiflora</i> var. <i>trichocarpa</i>	RT-HPLC	64.5–77.8*	Jin et al., 1989
<i>Paeonia veitchii</i>	TLC	13.4–57.6*	He et al., 1980; Yu and Xiao, 1987
<i>P. veitchii</i>	RT-HPLC	41.3–52.1*	Jin et al., 1989
<i>P. veitchii</i>	HPLC-MS	12.1–90.6	Yuan et al., 2020; Yang et al., 2020
<i>P. veitchii</i> var. <i>uniflora</i>	TLC	34.7*	Yu and Xiao, 1987
<i>P. veitchii</i> var. <i>woodwardii</i>	TLC	9.6*	Yu and Xiao, 1987
<i>P. veitchii</i> var. <i>woodwardii</i>	RT-HPLC	33.8*	Jin et al., 1989
<i>Paeonia obovata</i>	TLC	2.7–24.6*	He et al., 1980; Yu and Xiao, 1987
<i>P. obovata</i>	RT-HPLC	14.7*	Jin et al., 1989
<i>P. obovata</i>	HPLC-MS	0.5–33.5	Zhang et al., 2019b; Yang et al., 2020; Yan et al., 2021
<i>P. obovata</i> var. <i>willmottiae</i>	TLC	0.9–25.4*	He et al., 1980
<i>P. obovata</i> var. <i>willmottiae</i>	RT-HPLC	38.2*	Jin et al., 1989
<i>P. obovata</i> ssp. <i>willmottiae</i>	HPLC-MS	6.9–21.6	Zhang et al., 2019b; Yang et al., 2020; Yan et al., 2021
<i>Paeonia emodi</i>	TLC	5.6*	Yu and Xiao, 1987
<i>P. emodi</i>	HPLC-MS	24.5–51.7	Zhang et al., 2019b; Yang et al., 2020; Yan et al., 2021
<i>Paeonia sterniana</i>	HPLC-MS	24.7–53.3	Zhang et al., 2019b; Yang et al., 2020; Yan et al., 2021
<i>Paeonia intermedia</i>	HPLC-MS	3.1–18.9	Zhang et al., 2019b; Yang et al., 2020; Yan et al., 2021
<i>Paeonia mairei</i>	TLC	26.6–27.2*	He et al., 1980; Yu and Xiao, 1987
<i>P. mairei</i>	RT-HPLC	14.0–30.7*	Jin et al., 1989
<i>P. mairei</i>	HPLC-MS	14.5–29.5	Zhang et al., 2019b; Yang et al., 2020; Yan et al., 2021
<i>Paeonia anomala</i>	TLC	13.0*	Yu and Xiao, 1987
<i>P. anomala</i>	RT-HPLC	26.2*	Jin et al., 1989
<i>P. anomala</i>	HPLC-MS	6.9–64.7	Zhang et al., 2019b; Yang et al., 2020; Yan et al., 2021
<i>P. anomala</i> var. <i>intermedia</i>	TLC	13.9–24.0*	He et al., 1980; Yu and Xiao, 1987
<i>P. anomala</i> var. <i>intermedia</i>	RT-HPLC	22.1*	Jin et al., 1989
<i>Paeonia sinkiangensis</i>	TLC	13.3–14.8*	He et al., 1980; Yu and Xiao, 1987
<i>P. sinkiangensis</i>	RT-HPLC	29.5*	Jin et al., 1989
<i>Paeonia japonica</i>	RT-HPLC	11.1*	Jin et al., 1989
<i>Paeonia officinalis</i>	TLC	16.1–25.7*	Yu and Xiao, 1987

(Continued)

TABLE 1 Continued

Species	Method	Content (mg/g)	References
<i>Paeonia decora</i>	TLC	17.8–21.6*	Yu and Xiao, 1987
<i>Paeonia tenuifolia</i>	TLC	23.1–42.5*	Yu and Xiao, 1987
<i>Paeonia bakeri</i>	TLC	16.2*	Yu and Xiao, 1987
<i>Paeonia paradoxa</i>	TLC	6.8*	Yu and Xiao, 1987
<i>Paeonia humilis</i> var. <i>villosa</i>	TLC	14.4*	Yu and Xiao, 1987
<i>Paeonia banatica</i>	TLC	14*	Yu and Xiao, 1987
<i>Paeonia corallina</i>	TLC	8.3*	Yu and Xiao, 1987
<i>P. coriacea</i> var. <i>atlantica</i>	TLC	4*	Yu and Xiao, 1987

\*The content in the original study was expressed as the percentage.

et al., 2000; Zhang, 2000; Jian et al., 2010). However, if the roots are more than 5 years old, the paeoniflorin content decreases (Jian et al., 2010; Kang, 2011). Other studies have reported contrasting results, that is, the paeoniflorin content in the roots is negatively correlated with plant age (Xue et al., 1992; Li et al., 2008; Yan et al., 2019). The reason for this may be that the starch in the roots increases with the growth of the plant, and paeoniflorin decomposes *in vivo* (Xue et al., 1992). Our studies have shown that there is no significant correlation between paeoniflorin content and plant age in either the roots of *Paeonia ostii* or in the roots of *P. lactiflora* (Zhang et al., 2019b). In conclusion, plant ages affect paeoniflorin content in the roots (Supplementary Table 2), but there is no unified conclusion on this effect.

## Development stages

Paeoniaceae are perennial herbs or woody shrubs, with flower buds and leaf buds sprouting in early spring and falling into dormancy in autumn. Their development stages can be divided into budding, leaf unfolding, buds, flowering, fruiting, and withering (Li et al., 2011). Numerous studies have confirmed that the paeoniflorin contents in the roots at different developmental stages are significantly different (Supplementary Table 3). With the development of plants, the paeoniflorin content of the roots first increased and then decreased. The highest content was usually observed in spring (budding or flowering stages; He et al., 1980; Yu and Xiao, 1985; Jin et al., 1991, 2010; Huang et al., 2000; Fu et al., 2020). It seems best to harvest roots in spring to produce Mudanpi, Baishao, and Chishao. However, the roots are often harvested in autumn during the actual production in consideration of the dry matter accumulation. In addition, the level of paeoniflorin in the roots also varies throughout the day, increasing with temperature (Chen et al., 2002). This founding shows that it is better to harvest the roots at noon. Unlike the change of paeoniflorin in the roots, paeoniflorin in the leaves regularly decreased with plant development (Jian et al., 2010; Zhang et al., 2019b; Fu et al., 2020). The roots of Paeoniaceae plants usually can be used as medicines after four to five years of cultivation. However, the leaves can be harvested every year, so they can be developed as a new resource of paeoniflorin. Besides, removing some leaves at an early stage can reduce the incidence of plant

disease, which is also much more conducive to the healthy growth of Paeoniaceae plants.

## Organs

Paeoniflorin has been widely found in various organs of Paeoniaceae plants, but its contents were significantly different (Supplementary Table 4). The paeoniflorin content in *P. lactiflora*, from high to low, was in the following order: rhizomes, roots, leaves, stems, and flowers (Hu et al., 2000). Yang et al. (2001) determined that the paeoniflorin content in *Paeonia rockii* was in the following order: branch bark, root phloem, fibrous root, leaf, and petiole. However, among the organs of *Paeonia delavayi* var. *lutea*, the highest paeoniflorin content is found in the fruit, followed by the leaf, cork, cortex, stem, and xylem (Feng et al., 2012). The paeoniflorin distribution in *P. ostii* differs from these results, with the highest paeoniflorin content in the leaves, followed by stems, petioles, petals, xylem, and phloem (Zhang et al., 2019b). During the production of Mudanpi, Baishao, and Chishao, only the roots are harvested, while the other organs of Paeoniaceae plants are discarded. These studies indicate that the other organs also have potential medicinal value due to the considerable amounts of paeoniflorin.

Paeoniflorin content was also different in different root tissues. The paeoniflorin content in the rhizome of *P. lactiflora* is close to or even higher than that in the root, and the content in the branch roots and fine roots is higher than that in the main roots and thick roots, respectively (Hu et al., 2000; Chen et al., 2002; Kang, 2011; Ji et al., 2014). The paeoniflorin content in the phloem (2.69%) of *P. lactiflora* is similar to that in the xylem (2.90%), but it is significantly lower than that in the cork periderm (4.65%; Wu and Zhao, 2005). Based on high mass resolution matrix-assisted laser desorption/ionization MS imaging, Li et al. (2021b) reported that paeoniflorin was observed mainly in the cork and phloem region of *P. suffruticosa* and in the xylem rays of *P. lactiflora*, showing a great contrast with wedge-shaped xylem patches. The distribution of paeoniflorin in the roots of most wild species belonging to Sect. *Moutan* is similar to that of *P. suffruticosa*. In contrast, paeoniflorin is mainly distributed in the xylem of *P. ostii*, regardless of whether the roots are from plants grown in wild or

cultivated habitats (Zhang et al., 2019b). This finding also indicates that the root xylem should be fully utilized for medicinal purposes instead of being discarded.

### Abiotic factors

In addition to biological factors, some abiotic factors, including the annual average temperature, annual precipitation, annual sunshine duration, annual total solar radiation, and soil type of the plant habitat, also affected the paeoniflorin content (Hu et al., 2000; Han et al., 2008; Yuan et al., 2020; Zhang, 2020). There is a significant positive correlation between the paeoniflorin content in the roots and annual precipitation (Zhou et al., 2007), but a significant negative correlation has been observed between the paeoniflorin content in the roots and the annual average temperature (Yuan et al., 2020). Moreover, the elements zinc and potassium in the soil also play positive roles in paeoniflorin synthesis (Chen et al., 2009). These results reveal that the ecological and environmental factors of habitat should be carefully considered in order to produce the traditional Chinese medicines with high paeoniflorin content. Boiling and drying are usually necessary steps in the production of Mudanpi, Baishao, and Chishao, and these measures are considered to have effects on paeoniflorin content. High temperatures and long-term heating account for the decrease in paeoniflorin content due to its poor thermostability (Jin et al., 2015; Huo et al., 2017; Shen et al., 2019; Zhang et al., 2019b), and thermostability is negatively correlated with the water content of the roots (Yan et al., 2003). These studies show that the freeze-drying is better suited for increasing paeoniflorin contents in the production of traditional Chinese medicines.

## Paeoniflorin biosynthesis in Paeoniaceae

### Characterization of the complete pathway

Paeoniflorin is a monoterpene glycoside, and its biosynthesis pathway can be divided into three stages (Figure 1). The universal precursor, isopentenyl pyrophosphate (IPP), and its isomer, dimethylallyl diphosphate (DMAPP), are synthesized in the first stage. Plants have two pathways for producing IPP and DMAPP. One is the mevalonate (MVA) pathway located in the cytoplasm with acetyl CoA as the raw material (Bekkering et al., 2018), and the other is the 2-C-methyl-D-erythritol4-phosphate (MEP) pathway, or 1-deoxy-D-xylulose5-phosphate (DXP) pathway, which is located in plastids and uses pyruvate and glyceraldehyde-3-phosphate as raw materials (Shi et al., 2019). These two pathways do not exist in isolation, and there is a universal interaction between them (Laule et al., 2003). The paeoniflorin content has been determined in different organs of *P. lactiflora* (Yuan et al., 2013), and the relative expression of 24 genes encoding enzymes involved in MEP/DXP and MVA

pathways has been revealed. Correlation analysis indicates that hydroxymethylglutaryl-CoA synthase and phosphomevalonate kinase in the MVA pathway play important roles in the biosynthesis of paeoniflorin. In the second stage, geranyl diphosphate (GPP), the monoterpene precursor, is formed by head-to-tail condensation of one IPP and one DMAPP catalyzed by geranyl diphosphate synthase in the plastids (Zebec et al., 2016).

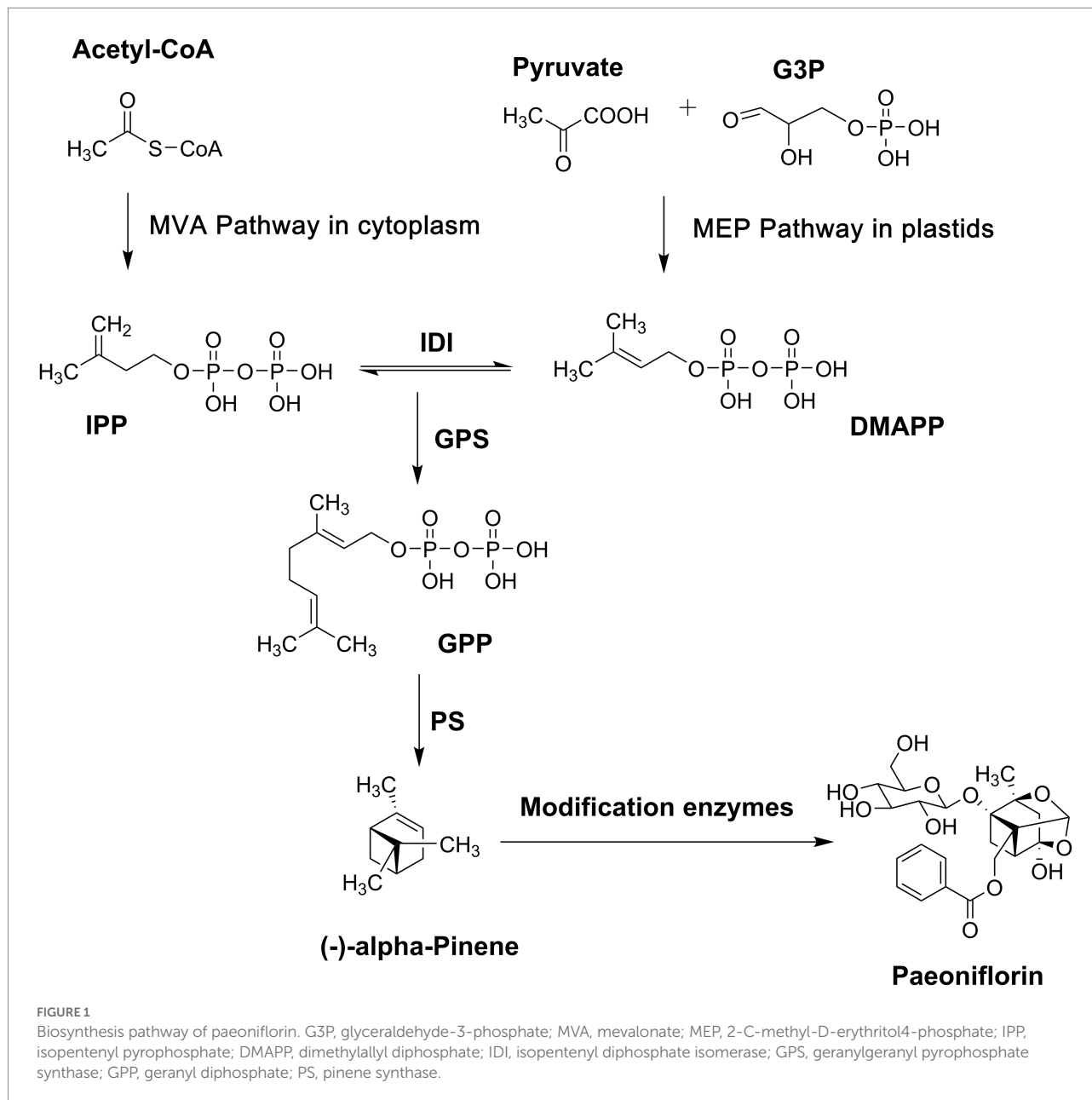
Then, the formation and post-modification of the basic pinene skeleton occur in the final stage. Terpenoid synthase (TPS), located at the branch point of the isoprenoid pathway, catalyzes GPP to form the monoterpene skeleton directly. Therefore, TPS is not only the key enzyme for terpenoid synthesis but also the main inducer for the structural diversity of terpenoids (Chen et al., 2011). A TPS gene named *PIPIN*, which encodes  $\alpha$ -pinene synthase, was isolated from *P. lactiflora* (Ma et al., 2016). An *in vitro* enzyme assay showed that PIPIN converted GPP into  $\alpha$ -pinene as a single product. Terpenoids in plants are often not the direct products obtained by the one-step catalysis of TPS, and most of them must be modified by special groups, even if their skeleton structures are rearranged. These post-modifications, including glycosylation, acylation, epoxidation, hydroxylation, halogenation, and addition and reduction reactions, substantially increase the variety of terpenoids and their structural diversity (Rivas et al., 2013; Zhao et al., 2014; Li et al., 2015). Unfortunately, no studies have addressed the post-modification of paeoniflorin biosynthesis.

### Hypothesized pathways of post-modification

The basic skeleton of paeoniflorin is  $\alpha$ -pinane, which is also connected to the benzoyl group,  $\beta$ -D-glucopyranosyl group, and hemiketal-acetal linkage. Based on previous literature (Chedgy et al., 2015; Xu et al., 2016), we speculated that the  $\beta$ -D-glucopyranosyl group is connected to the skeleton through glycosylation, which is catalyzed by glycosyltransferase, while the benzoyl group is connected to the skeleton through acylation, which is catalyzed by benzoyltransferase (Figure 2).

Unfortunately, there is a lack of sufficient understanding of the biosynthetic mechanism of the hemiketal-acetal linkage. Based on the compounds with structures similar to paeoniflorin in Paeoniaceae (He et al., 2010; Wu et al., 2010), the biosynthesis pathway of hemiketal-acetal linkage is proposed for the first time in the present study (Figure 3). First, the C-4 of  $\alpha$ -pinene is hydroxylated by hydroxylase and then oxidized to the ketone group by dehydrogenase. Second, C-2 and C-9 are both hydroxylated by hydroxylases, and then, the two hydroxyl groups dehydrate to form a cyclic ether linkage. Finally, C-9 continues to be added to the hydroxyl group under the catalysis of hydroxylase, at which time the hemiacetal linkage is formed. Then, the hydroxyl group of C-9 reacts with the ketone group to form the hemiketal, while the hemiacetal is converted into



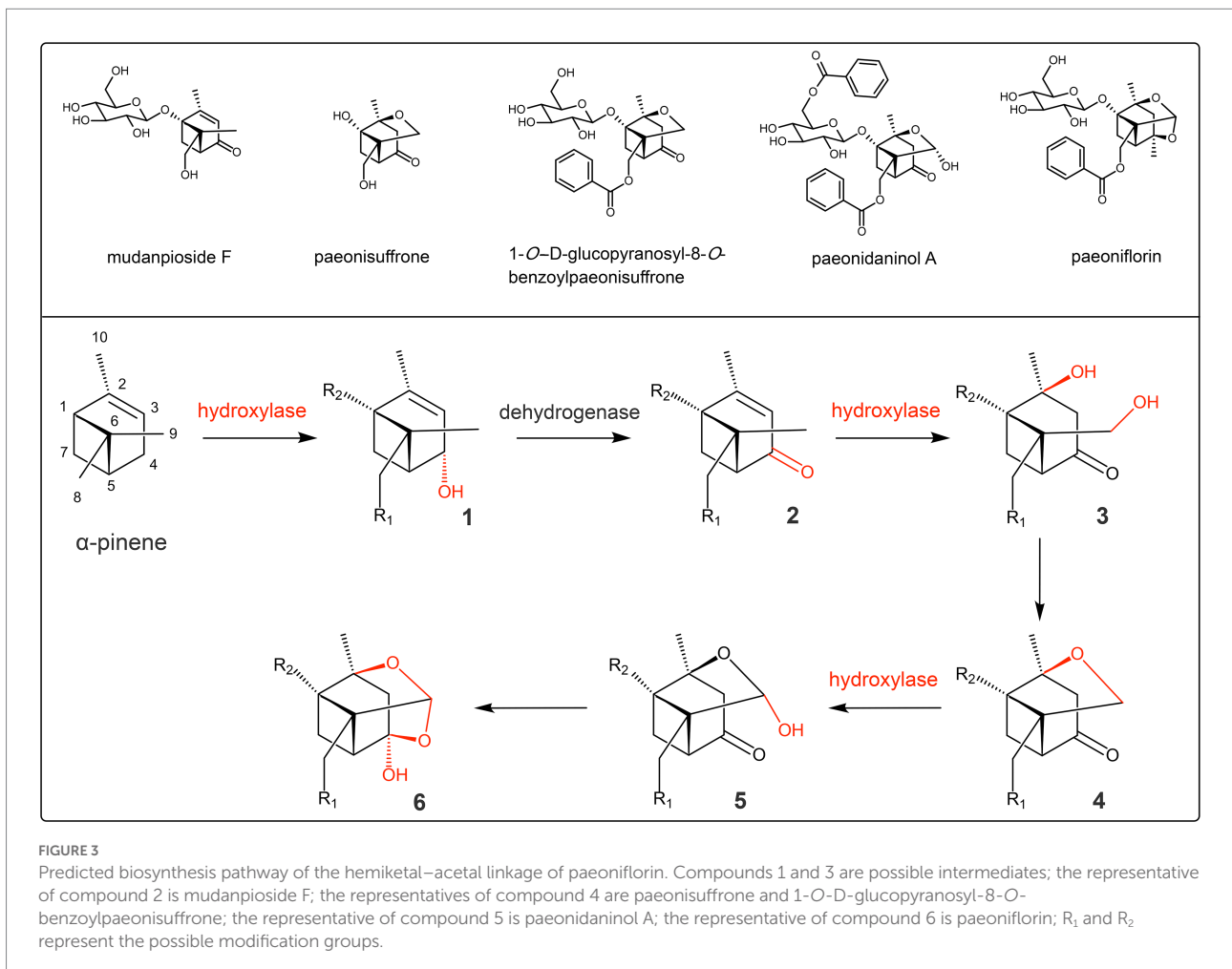
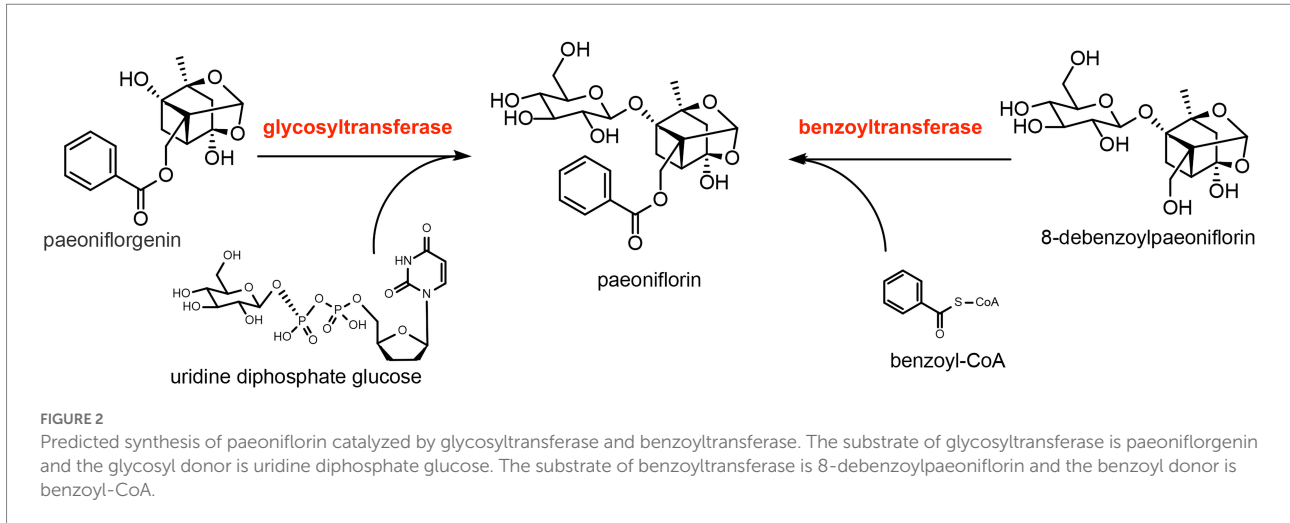


acetal, eventually forming the hemiketal–acetal linkage. In these reaction steps, the hydroxylation of C-2, C-4, and C-9 is the basis.

Combining the reaction process in Figure 2 and the structure of  $\alpha$ -pinene (Figure 3), it can be shown that the glycosylation modification is the reaction between the glycosyl donor (uridine diphosphate glucose) and the hydroxyl group at C-1, and the acylation modification is the reaction between the benzoyl donor (benzoyl-CoA) and the hydroxyl group at C-8. In these reaction steps, the hydroxylation of C-1 and C-8 is the basis. In summary, the hydroxylation of C1, C2, C4, C8, and C9 is the basic and necessary step in the modification stages of paeoniflorin biosynthesis, and hydroxylases play crucial roles in these stages.

## Discussion

High-throughput sequencing has been increasingly applied to the mining of key enzyme genes for natural product biosynthesis. By using plant tissues with large differences in natural product content as materials, many differentially expressed genes (DEGs) can be obtained by transcriptome sequencing, and the candidate enzyme genes can be further obtained based on a comparison with online databases, such as non-redundant protein sequences, Kyoto Encyclopedia of Genes and Genomes, and Gene Ontology. Plant materials are crucial throughout the sequencing process and directly determine the number of DEGs under the established analysis process. The plant materials for sequencing should preferably be based on the univariate principle, for example, the



same tissues with different developmental stages, and whether the same tissues experience hormone induction or adversity stress. *PIPIN*, the  $\alpha$ -pinene synthase gene, was isolated using the transcriptome data of *P. lactiflora* roots (Ma et al., 2016).

Paeoniflorin content in the leaves of *P. ostii* is significantly correlated with developmental stage, with the paeoniflorin content being higher at earlier developmental stages. These differences in paeoniflorin content make the leaf a more suitable sequencing

material for a detailed investigation of the biosynthesis pathway (Zhang et al., 2019b).

The functions of candidate genes obtained by transcriptome sequencing can be quickly identified by enzyme assays *in vitro*. However, for plant studies, it is usually insufficient to verify the functions of candidate genes through enzyme assays *in vitro*, and the functions of candidate genes in plants need to be further verified. Paeoniflorin is the characteristic constituent of Paeoniaceae; thus, it is almost impossible to verify the biological functions of candidate genes in model plants (e.g., *Arabidopsis* and *Petunia*). Additionally, at least at present, the long-life cycle of Paeoniaceae and the immature genetic transformation system make it difficult to overexpress candidate genes in herbaceous peony or tree peony to verify their biological functions. Fortunately, the virus-induced gene silencing system has been established in tree peony (Xie et al., 2019; Wang et al., 2020), which can be used to verify their biological functions by transiently silencing the candidate genes *in vivo*.

Analysis of the paeoniflorin biosynthesis pathway is still at the preliminary stage, and many scientific questions are yet to be answered. At present, the most urgent task is the identification and functional analysis of key modification enzyme genes. There are several studies on the generation of  $\beta$ -D-glucopyranosyl and benzoyl groups in natural products, which can provide a reference for paeoniflorin biosynthesis. It is believed that the identification and functional analysis of glycosyltransferase and benzoyltransferase genes will be completed in a short time. Nevertheless, the biosynthesis of the hemiketal–acetal linkage has not yet been reported, and we speculate that there are several enzymes involved in this pathway, including multi-step reactions, which may be the key restricting the analysis of paeoniflorin biosynthesis. With the development of technology, some new techniques, such as a target identification strategy based on biosynthetic intermediate probes, have been successfully used to discover key enzymes (Gao et al., 2020). We speculate that a breakthrough will be achieved in the elucidation of paeoniflorin biosynthesis in the near future. This will not only provide technical support to produce paeoniflorin using synthetic biology but will also lay the theoretical foundation for the use of biotechnology to improve the quality of Paeoniaceae.

## Author contributions

X-XZ, Y-HH, and J-HY conceived and designed this review. X-XZ wrote the manuscript. J-QZ, Y-TW, and H-YD performed

the literature collection and made classification. J-HY and Y-HH edited and revised the manuscript. All authors contributed to the article and approved the submitted version.

## Funding

This work was supported by the National Natural Science Foundation of China (32000240), Key project at central government level, the ability establishment of sustainable use for valuable Chinese medicine resources (2060302), Chinese Universities Scientific Fund (2452020213), Open Project of Shanghai Key Laboratory of Plant Functional Genomics and Resources (PFGR202203), and Special Fund for Shanghai Landscaping Administration Bureau Program (G222415, G192418, G192419).

## Acknowledgments

We thank Yan-Long Zhang (Northwest A&F University) for supporting this work.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2022.980854/full#supplementary-material>

## References

- Aimi, N., Inaba, M., Watanabe, M., and Shibata, S. (1969). Chemical studies on the oriental plant drugs—XXIII: Paeoniflorin, a glucoside of Chinese peony root. *Tetrahedron* 25, 1825–1838. doi: 10.1016/S0040-4020(01)82804-0
- Bekkering, S., Arts, R. J., Novakovic, B., Kourtzelis, I., van der Heijden, C. D., Li, Y., et al. (2018). Metabolic induction of trained immunity through the mevalonate pathway. *Cell* 172, 135–146. doi: 10.1016/j.cell.2017.11.025
- Chedgy, R. J., Köllner, T. G., and Constabel, C. P. (2015). Functional characterization of two acyltransferases from *Populus trichocarpa* capable of synthesizing benzyl benzoate and salicyl benzoate, potential intermediates in salicinoid phenolic glycoside biosynthesis. *Phytochemistry* 113, 149–159. doi: 10.1016/j.phytochem.2014.10.018
- Chen, B. L., Hang, Y. Y., Zhou, Y. F., Shi, Y. Y., Guo, K. Y., and Chen, B. E. (2002). The dynamic change of paeoniaflorin content from *Paeonia lactiflora* pall root in



- harvest time. *J. Plant Resour. Environ.* 11, 25–28. doi: 10.3969/j.issn.1674-7895.2002.02.006
- Chen, F., Tholl, D., Bohlmann, J., and Pichersky, E. (2011). The family of terpene synthases in plants: a mid-size family of genes for specialized metabolism that is highly diversified throughout the kingdom. *Plant J.* 66, 212–229. doi: 10.1111/j.1365-313x.2011.04520.x
- Chen, X., Zhang, X. Y., Zhang, R. R., and Wang, K. C. (2009). Effects of Mn, Fe, Zn and Cu on growth and paeoniflorin content of *Paeonia lactiflora*. *China J. Chin. Mater. Medica.* 34, 961–964.
- Chinese Pharmacopoeia Committee. (2020). *The Pharmacopoeia of the People's Republic of China*. Beijing: China Medical Science Press.
- Choudhary, M. I., Naheed, N., Abbaskhan, A., Musharraf, S. G., and Siddiqui, H. (2008). Phenolic and other constituents of fresh water fern *Salvinia molesta*. *Phytochemistry* 69, 1018–1023. doi: 10.1016/j.phytochem.2007.10.028
- Corey, E. J., and Wu, Y. J. (1993). Total synthesis of (±)-paeoniflorigenin and paeoniflorin. *J. Am. Chem. Soc.* 115, 8871–8872. doi: 10.1021/ja00072a063
- Egon, S., and Cooper, S. (1969). Untersuchungen über Inhaltsstoffe von *Paeonia*-Arten. *Arch. Pharm.* 302, 685–690. doi: 10.1002/ardp.19693020907
- Feng, Y., Wang, Y., Zhou, N., Jiang, B., and Yu, Y. (2012). Determination of paeoniflorin in different parts of *Paeonia delavayi* var. *lutea* by HPLC. *Chin. J. Exp. Tradit. Med. Form.* 18, 139–142. doi: 10.3969/j.issn.1005-9903.2012.09.041
- Fu, S. P., Shen, H. W., Wang, Q. B., Li, J. P., Wang, C., Guo, S. L., et al. (2020). Study on content determination of 6 active ingredients in *Paeonia lactiflora* during different harvesting periods and their variation. *China Pharm.* 31, 441–446. doi: 10.6039/j.issn.1001-0408.2020.04.10
- Gao, L., Su, C., Du, X., Wang, R., Chen, S., Zhou, Y., et al. (2020). FAD-dependent enzyme-catalysed intermolecular [4+2] cycloaddition in natural product biosynthesis. *Nat. Chem.* 12, 620–628. doi: 10.1038/s41557-020-0467-7
- Guo, B. L., Basang, D., Xiao, P. G., and Hong, D. Y. (2002). Research on the quality of original plants and material medicine of cortex *Paeoniae*. *China J. Chin. Mater. Medica.* 27, 654–657. doi: 10.3321/j.issn:1001-5302.2002.09.004
- Han, X. Y., Liu, Z. A., and Wang, L. S. (2008). Comparison of the content of effective components between tree peony wild species and main cultivars. *J. Chin. Med. Mater.* 31, 327–331. doi: 10.3321/j.issn:1001-4454.2008.03.002
- Hatakeyama, S., Kawamura, M., and Takano, S. (1994). Total synthesis of (–)-paeoniflorin. *J. Am. Chem. Soc.* 116, 4081–4082. doi: 10.1021/ja00088a055
- He, C. N., Peng, Y., Zhang, Y., Xu, L., Gu, J., and Xiao, P. (2010). Phytochemical and biological studies of *Paeoniaceae*. *Chem. Biodivers.* 7, 805–838. doi: 10.1002/cbdv.200800341
- He, C. N. (2010). *Study on the Pharmacophylogenetic Significance of the Paeonia in China*. Beijing: Chinese Academy of Medical Sciences and Peking Union Medical College.
- He, L. Y., Feng, R. Z., and Xiao, P. G. (1980). The occurrence of paeoniflorine in the genus *Paeonia*. *Acta Pharm. Sin.* 15, 429–433.
- Hong, D. Y. (2010). *Peonies of the World: Taxonomy and Phytogeography*. London: Royal Botanic Gardens Kew.
- Hong, D. Y. (2011). *Peonies of the World: Polymorphism and Diversity*. London: Royal Botanic Gardens Kew.
- Hong, D. Y. (2021). *Peonies of the World: Phylogeny and Evolution*. London: Royal Botanic Gardens Kew.
- Hu, S. L., Lan, G. L., Feng, X. F., Tang, X. J., and He, X. R. (2000). Determination of paeoniflorin content in different origins and parts of *Paeoniae lactiflora*. *China J. Chin. Mater. Medica.* 25, 714–416. doi: 10.3321/j.issn:1001-5302.2000.12.003
- Huang, M. Y., Wu, Z. W., and Zhang, X. G. (2000). Influence of harvesting periods and cultivation years on the quality of *Paeoniae lactiflora* cultivated in Zhongjiang. *J. Chin. Med. Mater.* 23, 435–436. doi: 10.3321/j.issn:1001-4454.2000.08.001
- Huo, X. G., Hu, X. T., Chen, L. X., Feng, X. C., Ding, L. Q., and Qiu, F. (2017). Study on the stability of paeoniflorin. *China Sci. Pap.* 12, 2092–2097. doi: 10.3969/j.issn.2095-2783.2017.18.010
- Ji, K. M., Fang, C. W., Guan, Y. Y., Guan, D. P., and Wu, J. H. (2014). Content comparison of the paeonol and paeoniflorin on different part of one Feng-cortex Moutan. *Chin. J. Exp. Tradit. Med. Form.* 20, 54–56. doi: 10.13422/j.cnki.syfx.2014180054
- Jian, Z. Y., Yu, J. B., and Wang, W. Q. (2010). RP-HPLC determination of main chemical components in different parts and different harvest periods of *Paeonia lactiflora*. *Acta Pharm. Sin.* 45, 489–493. doi: 10.16438/j.0513-4870.2010.04.009
- Jin, C. D., Xu, G. J., Jin, R. L., and Xu, L. S. (1989). Quantitative analysis of glucosides and benzoic acid in peony root from 13 species by RP-HPLC. *J. China Pharm. Univ.* 3, 139–142. doi: 10.3321/j.issn:1000-5048.1989.03.003
- Jin, C. D., Xu, G. J., Jin, R. L., Xu, L. S., and Cong, X. D. (1991). Quantitative analysis of the glucosides and benzoic acid in peony root collected in different seasons by RP-HPLC. *J. China Pharm. Univ.* 5, 279–281. doi: 10.3321/j.issn:1000-5048.1991.05.005
- Jin, C. S., Cai, Y. J., and Wu, D. L. (2010). Changes in the contents of paeoniflorin and total glycosides of paeony in *Paeoniae lactiflora* of Bozhou at different harvesting periods. *J. Chin. Med. Mater.* 33, 1548–1550. doi: 10.3969/j.issn.1001-1528.2000.04.012
- Jin, L., Zhao, W. S., Guo, Q. S., Zhang, W. S., and Ye, Z. L. (2015). Study on chemical components distribution in *Paeoniae radix Alba* and its processing methods. *China J. Chin. Mater. Medica.* 40, 1953–1959. doi: 10.4268/cjcm20151021
- Kaneda, M., Iitaka, Y., and Shibata, S. (1972). Chemical studies on the oriental plant drugs—XXXIII: the absolute structures of paeoniflorin, albiflorin, oxypaeoniflorin and benzoylpaeoniflorin isolated from chinese peony root. *Tetrahedron* 28, 4309–4317. doi: 10.1016/S0040-4020(01)88953-5
- Kang, X. F. (2011). *Study on Active and Nutritional Components of Paeonia lactiflora* Pall. Taian: Shandong Agricultural University. doi:10.7666/d.143830
- Laule, O., Fährholz, A., Chang, H. S., Zhu, T., Wang, X., Heifetz, P. B., et al. (2003). Crosstalk between cytosolic and plastidial pathways of isoprenoid biosynthesis in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* 100, 6866–6871. doi: 10.1073/pnas.1031755100
- Li, B., Ge, J. Y., Liu, W., Hu, D. J., and Li, P. (2021b). Unveiling spatial metabolome of *Paeonia suffruticosa* and *Paeonia lactiflora* roots using MALDI MS imaging. *New Phytol.* 231, 892–902. doi: 10.1111/nph.17393
- Li, J. J., Zhang, X. F., and Zhao, X. Q. (2011). *Tree Peony of China*. Beijing: Encyclopedia of China Publishing House.
- Li, R. R., Liu, H. X., Peng, P. H., and Hu, X. R. (2021a). Study on UPLC fingerprint of *Paeonia decomposita* cortex and simultaneous determination of four components. *China Meas. Test.* 47, 77–82. doi: 10.11857/j.issn.1674-5124.2021040089
- Li, W., Zhang, F., Chang, Y., Tao, Z., and Wang, G. (2015). Nicotinate O-Glucosylation is an evolutionarily metabolic trait important for seed germination under stress conditions in *Arabidopsis thaliana*. *Plant Cell* 27, 1907–1924. doi: 10.1105/tpc.15.00223
- Li, Y. F., Yan, X. K., Shen, F., and Li, T. L. (2008). Study on qualities of different growing time of *radix Paeoniae Alba*. *J. US-China Med. Sci.* 5, 23–25.
- Ma, X., Guo, J., Ma, Y., Jin, B., Zhan, Z., Yuan, Y., et al. (2016). Characterization of a monoterpene synthase from *Paeonia lactiflora* producing  $\alpha$ -pinene as its single product. *Biotechnol. Lett.* 38, 1213–1219. doi: 10.1007/s10529-016-2098-z
- Nie, X. Y., Shang, C., and Wang, Y. L. (2005). Study on separation of paeoniflorin from *Paeonia lactiflora* by macroporous adsorption resin. *Chin. Tradit. Pat. Med.* 27, 350–351. doi: 10.3969/j.issn.1001-1528.2005.03.037
- Rivas, F., Parra, A., Martinez, A., and Garcia-Granados, A. (2013). Enzymatic glycosylation of terpenoids. *Phytochem. Rev.* 12, 327–339. doi: 10.1007/s11101-013-9301-9
- Shen, M. L., Yan, B. J., and Qin, L. P. (2019). Research progress on different geographical areas, processing methods and collecting time of the effective compounds in *Paeoniae radix* (Shaoyao). *J. Zhejiang Chin. Med. Univ.* 43, 622–630. doi: 10.16466/j.issn1005-5509.2019.06.023
- Shi, M., Huang, F., Deng, C., Wang, Y., and Kai, G. (2019). Bioactivities, biosynthesis and biotechnological production of phenolic acids in *salvia miltiorrhiza*. *Crit. Rev. Food Sci.* 59, 953–964. doi: 10.1080/10408398.2018.1474170
- Shibata, S., Aimi, N., and Watanabe, M. (1964). Paeoniflorin, a monoterpene glucoside of Chinese peony root. *Tetrahedron Lett.* 5, 1991–1995. doi: 10.1016/S0040-4039(00)75129-X
- Shibata, S., Nakahara, M., and Aimi, N. (1963). Studies on the constituents of Japanese and Chinese crude drugs. VIII. Paeoniflorin, a glucoside of Chinese peony root. *Chem. Pharm. Bull.* 11, 372–378. doi: 10.1248/cpb.11.372
- Sun, L. C., Li, S. Y., Wang, F. Z., and Xin, F. J. (2017). Research progresses in the synthetic biology of terpenoids. *Biotechnol. Bull.* 33, 64–75. doi: 10.13560/j.cnki.biotech.bull.1985.2017.01.007
- Tan, Y. Q., Chen, H. W., Li, J., and Wu, Q. J. (2020). Efficacy, chemical constituents, and pharmacological actions of *radix Paeoniae Rubra* and *radix Paeoniae Alba*. *Front. Pharmacol.* 11, 1054. doi: 10.3389/fphar.2020.01054
- Wang, Q. L., Wang, W. Q., Wei, S. L., Yu, F. L., Peng, F., and Fang, Y. Q. (2012). Study on effect of different processing methods on seven main chemical components of wild and cultivated *Paeonia lactiflora*. *China J. Chin. Materia. Medica.* 37, 920–924. doi: 10.4268/cjcm20120711
- Wang, X., Li, J., Guo, J., Qiao, Q., Guo, X., and Ma, Y. (2020). The WRKY transcription factor PIWRKY65 enhances the resistance of *Paeonia lactiflora* (herbaceous peony) to *Alternaria tenuissima*. *Hortic. Res.* 7, 57. doi: 10.1038/s41438-020-0267-7
- Wu, C. C., Guo, X. H., Liu, X., Yang, M., and Wu, W. H. (2020). Advances in modern pharmaceutical action of Moutan cortex in recent five years. *Chin. J. New Drugs* 29, 281–284. doi: 10.3969/j.issn.1003-3734.2020.03.008
- Wu, J., and Zhao, L. (2005). Determination the change of paeoniflorin contents in different parts of cultivated *Paeonia lactiflora* before and after wilting by HPLC. *Chin. Tradit. Herb. Drug* 36, 122–123. doi: 10.7501/j.issn.0253-2670.2005.1.058

- Wu, S. H., Wu, D. G., and Chen, Y. W. (2010). Chemical constituents and bioactivities of plants from the genus *Paeonia*. *Chem. Biodivers.* 7, 90–104. doi: 10.1002/cbdv.200800148
- Xie, L., Zhang, Q., Sun, D., Yang, W., Hu, J., Niu, L., et al. (2019). Virus-induced gene silencing in the perennial woody *Paeonia ostii*. *Peer J.* 7:e7001. doi: 10.7717/peerj.7001
- Xiu, J. X., Xu, J., Zhang, T. J., and Liu, C. X. (2021). Modern research progress of traditional Chinese medicine *Paeoniae radix Alba* and prediction of its Q-markers. *China J. Chin. Materia. Medica.* 46, 5486–5495. doi: 10.19540/j.cnki.cjcmm.20210818.201
- Xu, G., Cai, W., Gao, W., and Liu, C. (2016). A novel glucuronosyltransferase has an unprecedented ability to catalyze continuous two-step glucuronosylation of glycyrrhetic acid to yield glycyrrhizin. *New Phytol.* 212, 123–135. doi: 10.1111/nph.14039
- Xue, J. H., Xiao, T. H., Wang, X. H., and Hu, H. X. (1992). A study on the quality of Bozhou *radix Paeoniae Alba* in Anhui. *J. Chin. Med. Materia.* 15, 37–40. doi: 10.13863/j.issn1001-4454.1992.12.014
- Yan, A. D., Song, S., Sun, Y. H., and Xie, J. (2019). Characterization of the temporal changes in the content of paeonol and paeoniflorin components in Fengdan cortex. *J. Chin. Med. Materia.* 42, 541–544. doi: 10.13863/j.issn1001-4454.2019.03.012
- Yan, X. L., Zhang, J. J., Li, X. Y., Du, S. Y., and Li, W. (2003). Influence of temperature on the stability of paeoniflorin in *radix Paeonia*, extract and Qiankunqing granules. *Chin. Tradit. Herb. Drug* 34, 131–133.
- Yan, Z. G., Xie, L. H., Li, M. C., Yuan, M., Tian, Y., Sun, D. Y., et al. (2021). Phytochemical components and bioactivities of novel medicinal food–peony roots. *Food Res. Int.* 140:109902. doi: 10.1016/j.foodres.2020.109902
- Yang, S. S., Song, P. S., Luo, X. P., Zhang, B. C., and Mu, X. H. (2001). Analysis of paeonol and paeoniflorin in *Paeonia rockii* (S.G.haw et L.A.Laeuner) T.Hong et j.J.Li. *Northwest. Pharm. J.* 16, 108–109. doi: 10.3969/j.issn.1004-2407.2001.03.007
- Yang, Y., Li, S. S., Silva, J. A. T. D., Yu, X. N., and Wang, L. S. (2020). Characterization of phytochemicals in the roots of wild herbaceous peonies from China and screening for medicinal resources. *Phytochemistry* 174:112331. doi: 10.1016/j.phytochem.2020.112331
- Yu, J., and Xiao, P. G. (1985). Ontogenetic chemical changes of the active constituents in mudan (*Paeonia suffruticosa*) and shaoyao (*P. lactiflora*). *Acta Pharm. Sin.* 20, 782–784. doi: 10.16438/j.0513-4870.1985.10.011
- Yu, J., and Xiao, P. G. (1987). A preliminary study of the chemistry and systematics of Paeoniaceae. *Acta Pharm. Sin.* 25, 172–179.
- Yuan, M., Yan, Z. G., Sun, D. Y., Luo, X. N., Xie, L. H., Li, M. C., et al. (2020). New insights into the impact of ecological factor on bioactivities and phytochemical composition of *Paeonia veitchii*. *Chem. Biodivers.* 17:e2000813. doi: 10.1002/cbdv.202000813
- Yuan, Y., Yu, J., Jiang, C., Li, M., Lin, S., Wang, X., et al. (2013). Functional diversity of genes for the biosynthesis of paeoniflorin and its derivatives in *Paeonia*. *Int. J. Mol. Sci.* 14, 18502–18519. doi: 10.3390/ijms140918502
- Zebec, Z., Wilkes, J., Jervis, A. J., Scrutton, N. S., Takano, E., and Breitling, R. (2016). Towards synthesis of monoterpenes and derivatives using synthetic biology. *Curr. Opin. Chem. Biol.* 34, 37–43. doi: 10.1016/j.cbpa.2016.06.002
- Zhang, L. L., and Wei, W. (2020). Anti-inflammatory and immunoregulatory effects of paeoniflorin and total glucosides of paeony. *Pharmacol. Ther.* 207:107452. doi: 10.1016/j.pharmthera.2019.107452
- Zhang, M. (2020). *Study on Genetic Diversity of Medicinal Plant Paeoniae radix Rubra and Correlation Between Paeoniflorin and Climatic Factors*. Huhhot: Inner Mongolia University.
- Zhang, X. R. (2000). Determination of paeoniflorin in *Paeoniae lactiflora* at different growth ages by TCL. *Chin. Tradit. Herb. Drug* 31, 832–833. doi: 10.7501/j.issn.0253-2670.2000.11.2000011526
- Zhang, Y. G., Zhang, S. J., Bian, T. T., Si, X. L., Niu, J. T., Xin, E. D., et al. (2019a). New progress in pharmacological action of paeoniflorin. *Chin. Tradit. Herb. Drug* 50, 3735–3740. doi: 10.7501/j.issn.0253-2670.2019.15.033
- Zhang, X. X., Zhai, Y. H., Yuan, J. H., and Hu, Y. H. (2019b). New insights into Paeoniaceae used as medicinal plants in China. *Sci. Rep.* 9, 18469. doi: 10.1038/s41598-019-54863-y
- Zhao, Y. J., Cheng, Q. Q., Su, P., Chen, X., Wang, X. J., Gao, W., et al. (2014). Research progress relating to the role of cytochrome P450 in the biosynthesis of terpenoids in medicinal plants. *Appl. Microbiol. Biotechnol.* 98, 2371–2383. doi: 10.1007/s00253-013-5496-3
- Zhou, H. T., Luo, Y. Q., Hu, S. L., Li, R. K., Liu, H. W., and Feng, X. F. (2003). A comparative study on content of major constituents between *radix Paeoniae Rubra* and *radix Paeoniae Alba* by HPLC. *Chin. Pharm. J.* 38, 654–656. doi: 10.3321/j.issn:1001-2494.2003.09.005
- Zhou, S. L., Xu, C., Liu, J., Yu, Y., Wu, P., and Cheng, T. (2021). Out of the pan-Himalaya: evolutionary history of the Paeoniaceae revealed by phylogenomics. *J. Syst. Evol.* 59, 1170–1182. doi: 10.1111/jse.12688
- Zhou, Y. F., Tang, X. L., Hang, Y. Y., and Wu, B. C. (2007). Effects of producing area and growth age on paeoniaflorin content in root of *Paeonia lactiflora*. *Jiangsu Agr. Sci.* 1, 149–150. doi: 10.3969/j.issn.1002-1302.2007.01.052