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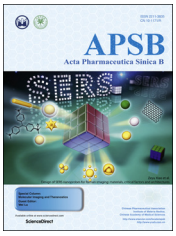

ORIGINAL ARTICLE

Integrated metabolomic and transcriptomic analyses revealed the distribution of saponins in *Panax notoginseng*

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KEY WORDS

Panax notoginseng;
Saponin;
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Abstract *Panax notoginseng* is famous for its important therapeutic effects. Saponins are bioactive compounds found in different parts and developmental stages of *P. notoginseng* plants. Thus, it is urgently to study saponins distribution in different parts and growth ages of *P. notoginseng* plants. In this study, potential biomarkers were found, and their chemical characteristic differences were revealed through metabolomic analysis. High-performance liquid chromatography data indicated the higher content of saponins (*i.e.*, Rg1, Re, Rd, and Rb1) in the underground parts than that in the aerial parts. 20(*S*)-Protopanaxadiol saponins were mainly distributed in the aerial parts. Additionally, the total saponin content in the 3-year-old *P. notoginseng* plant (188.0 mg/g) was 1.4-fold higher than that in 2-year-old plant (130.5 mg/g). The transcriptomic analysis indicated the tissue-specific transcription expression of

Abbreviations: AACT, acetoacetyl-CoA acyltransferase; DS, dammarenediol-II synthase; DXPS, 1-deoxy-D-xylulose 5-phosphate synthase; DXPR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; FPS, farnesyl pyrophosphate synthase; FPP, farnesyl diphosphate; GDPS, gerenyl diphosphatesynthase; HDS, 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate synthase; HMGS, 3-hydroxy-3-methylglutaryl-CoA synthase; HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase; HPLC–UV, high-performance liquid chromatography-ultraviolet detection; IPP, isoprenyl diphosphate; IPPI, isopentenyl pyrophosphate isomerase; ISPD, 2-*C*-methylerythritol 4-phosphatecytidyl transferase; ISPE, 4-(cytidine-5′-diphospho)-2-*C*-methylerythritol kinase; ISPH, 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate reductase; MECPS, 2-*C*-methylerythritol-2,4-cyclophosphate synthase; MEP, 2-*C*-methyl-D-erythritol-4-phosphate; MVA, mevalonate; MVDD, mevalonate diphosphate decarboxylase; MVK, mevalonate kinase; OPLS-DA, orthogonal partial least-squares discrimination analysis; P450, P450-monooxygenase; PCA, principal component analysis; PDS, 20(*S*)-protopanaxadiol saponins; PMK, phosphomevalonate kinase; PTS, 20(*S*)-protopanaxatriol saponins; SE, squalene epoxidase; SS, squalene synthase; UGTs, UDP-glycosyltransferases; UPLC–MS, ultrahigh-performance liquid chromatography–mass spectrometry; VIP, variable importance in projection

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genes, namely, *PnFPS*, *PnSS*, *PnSE1*, *PnSE2*, and *PnDS*, which encoded critical synthases in saponin biosyntheses. These genes showed similar expression patterns among the parts of *P. notoginseng* plants. The expression levels of these genes in the flowers and leaves were 5.2fold higher than that in the roots and fibrils. These results suggested that saponins might be actively synthesized in the aerial parts and transformed to the underground parts. This study provides insights into the chemical and genetic characteristics of *P. notoginseng* to facilitate the synthesis of its secondary metabolites and a scientific basis for appropriate collection and rational use of this plant.

1. Introduction

Panax notoginseng (Burk) F.H. Chen is a perennial plant famous for its important therapeutic effects, such as antidepressant, estrogen-like activity, anti-tumor, anti-oxidant, hepatoprotective, and anti-inflammation[1–4]. *P. notoginseng* is commonly used as a single or major ingredient in capsules, tablets, pills, and medicinal liquors. *P. notoginseng* is widely consumed in the health food market[5]. The major active components of this plant are protopanaxadriol and protopanaxatriol saponins, whose pharmacological effects are related to the different plant parts and developmental stages[6,7]. Saponins from the leaves and flowers of *P. notoginseng* exert anti-tumor and hepatoprotective effects[8,9]. Additionally, the saponin contents of *P. notoginseng* can be easily influenced by region of origin, resource[10], harvest time[11], and processing[12]. Mature *P. notoginseng* are generally more expensive than the younger ones because of their higher quality[5]. 3-Year-old *P. notoginseng* plants mainly serve as medicinal materials in the market, and 2-year-old samples are also sold in the market. The parts and developmental stages of *P. notoginseng* plants can influence the safe use of medicinal materials. There are fewer studies to clarify the chemical characteristics in different parts and growth stages of *P. notoginseng* plants. Metabolomics is a scientific study that reveals metabolite differences for authentication of species[13–17] differentiation of cultivation regions[18,19], age discrimination[20,21], and discrimination of raw and processed samples[22]. Thus, the chemical characteristics of saponins in the aerial and underground parts of 2- and 3-year-old *P. notoginseng* plants could be analyzed.

Study has reported the triterpene saponins synthesized through 2-*C*-methyl-D-erythritol-4-phosphate and mevalonate pathways[23]. Putative cytochrome P450-dependent monoxygenases and UDP-glycosyltransferases involved in triterpene saponin biosynthesis were discovered[24]. In this process, the main enzymes involved are farnesyl pyrophosphate synthase (FPS), squalene synthase (SS), squalene epoxidase (SE), and dammarenediol-II synthase (DS). Niu et al.[25] cloned genes (*i.e.*, *PnFPS*, *PnSS*, *PnSEs*, and *PnDS*) that encoded these enzymes and analyzed their tissue-specific expression pattern by using mature 4-year-old *P. notoginseng* plants. However, such expression patterns have not been reported in different parts of 2- and 3-year-old *P. notoginseng* plants. Further analysis of the expression patterns of these genes could contribute to reveal saponin distributions within *P. notoginseng*.

In this study, we investigated the characteristic chemical markers of saponins in different parts of 2- and 3-year-old *P. notoginseng* plants through ultrahigh-performance liquid chromatography–mass spectrometry (UPLC–MS) analysis. Saponin content was further determined through high-performance liquid chromatography (HPLC–UV) analysis to define their distribution. The tissue-specific expression patterns of genes related to saponin synthesis were analyzed to reveal their molecular characteristics. Our findings provide a scientific basis for appropriate collection and application of *P. notoginseng*, and help for resolving the issue of drug safety usage.

2. Materials and methods

2.1. Materials and chemicals

Six batches of whole 2- and 3-year-old *P. notoginseng* plants in their flowering stage were collected from Wenshan, Yunnan Province, China (N 23.5° and E 104°). Each plant was separated into five different parts including the root, fibril, stem, leaf, and flower (Fig. 1). All voucher specimens were carefully washed, cut into small pieces, and immediately deposited at −80 °C for further processing.

Six standards of saponins (*i.e.*, Rg1, Re, Rd, Rb1, Rb2, and Rc) and a standard of notoginsenoside R1 with 98.0% purity were purchased from Shanghai Tauto Biotech Company (Shanghai, China). The standard stock solutions were dissolved in methanol (Fair Lawn, NJ, USA) and stored at −20 °C prior to use. Other chemicals and solvents were of analytical grade.

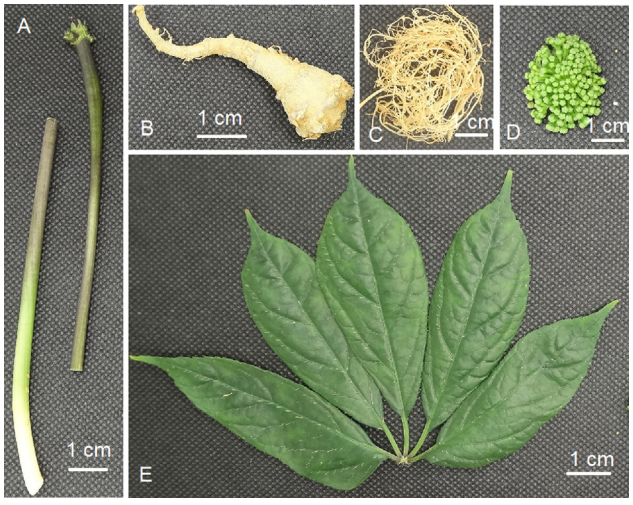

Figure 1 Plant traits of *P. notoginseng*. (A) Stem; (B) root; (C) fibril; (D) flower; and (E) leaf.

2.2. HPLC–MS conditions

All samples were crushed, and 0.1 g of the powdered sample was weighed and mixed with 1.0 mL of pure methanol containing 0.1% formic acid under vortex for 10 s. The mixture was sonicated for 10 min, frozen at −20 °C for 1 h, and centrifuged at 10,000 rpm (Cetrifuge, Sigma 3-30KS, Germany) for 10 min. The upper layer was collected, filtered through 0.22 μm filter, and transferred to a sample vial. The vial was injected into the column for UPLC–quadruple time-of-flight (QTOF)/MS analysis.

The UPLC–MS analysis was performed using a UPLC system (Waters, UK) coupled to an electrospray ionization-QTOF/MS (Waters, UK). A C18 reversed phase column (50 mm × 2.1 mm, i.d. 1.7 μm, Acquity HPLC BEH, Waters, UK) was used for UPLC separation, and the sample injection volume was 10 μL. The column temperature was kept at 35 °C, and the flow rate was kept at 0.4 mL/min. The gradient was composed of water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B). The linear gradient was set as follows: 0–2 min for 1% B to 20% B, 2–3 min for 20% B to 50% B, 3–7 min for 50% B to 80% B, 7–7.5 min for 80% B to 99% B, 7.5–9 min for 99% B, 9–9.1 min for 99% B to 1% B, and 9.1–10 min for 1% B.

High-accuracy MS data were recorded using MassLynx 4.1 software (Waters, UK). The capillary voltage was set at 3.0 kV for positive and negative modes, and the cone voltage was set at 40 V for both modes. The source temperature was set at 120 °C with a cone gas flow of 50 L/h. The desolvation temperature was set at 400 °C with desolvation gas flow of 800 L/h. MS^E data were acquired in continuous mode by using ramp collision energy in two scan functions. The conditions for low-energy mode are scan range of 50–2000 Da, scan time of 0.2 s, and collision energy of 6 V. The conditions for high-energy mode are scan range of 50–2000 Da, scan time of 0.2 s, and collision energy ramp of 10–40 V.

2.3. HPLC–UV conditions

Agilent HPLC 1260 series system (Agilent, USA) equipped with a quaternary pump, an autosampler, a column compartment, and a VWD was used for HPLC analysis. A C18 reversed phase column (250 mm × 4.6 mm, i.d. 5 μm, Eclipse XDB; Agilent, USA) was used for separation, and the sample injection volume was set as 10 μL. The column temperature was kept at 25 °C, the flow rate at 1.0 mL/min, and wavelength at 203 nm. The Gradient was composed of water (A) and acetonitrile (B), and the linear gradient was set as follows: 0–12 min for 19% B and 12–60 min for 19% B to 36% B.

2.4. Gene expression conditions

Total RNA was isolated from the root, fibril, stem, leaf, and flower by using a plant RNA Isolation Mini Kit (BioTeke, Beijing, China). Concentration and quality of the extracted RNA were tested using NanoDrop 2000 spectrophotometer and 1% agarose gel electrophoresis before cDNA synthesis.

Approximately 800 ng of DNase I-treated total RNA was converted into single-stranded cDNA by using FastQuant RT Kit (With gDNase) (TIANGEN, Beijing, China). The cDNA products were diluted 10fold with deionized water and used as a template for real-time PCR. All primers used in this study were synthesized based on the description of Niu et al.[25]. The quantitative reaction was conducted using an SYBR®Green Realtime PCR Master Mix (Toyobo, Japan). The reaction mixture contained 10 μL of SYBR Green qPCR Master Mix, 0.8 μL of 10 μmol/L each of the forward and reverse primers, and 2 μL of the cDNA template and added with sterile water to obtain a final volume of 30 μL. Each reaction was repeated three times. PCR amplification was conducted under the following conditions: 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, 55 °C for 15 s, and 72 °C for 15 s. The expression levels of the key genes were calculated by comparing the cycle threshold value (C_t) for each gene to the C_t of the reference gene 18S rRNA[24].

2.5. Data analysis

LC–MS raw data were transformed into MassLynx 4.1 Software (Waters, USA) to obtain the molecular features of the samples for non-targeted metabolic analysis of the extracted features. Multivariate data analysis was conducted with SIMCA-P software. Principal component analysis (PCA) was performed to obtain an overview of the sample distribution and observe possible outliers. Orthogonal partial least-squares discrimination analysis (OPLS-DA) was performed to identify the metabolites that significantly contributed to clustering and discrimination. S-plot and variable importance in projection (VIP) were used to evaluate the variable contribution. Variables with VIP >1 were deemed as potential biomarkers. HPLC and gene expression data were managed by SPSS 17.0 software for analysis of variance. Mean values were set as significant or non-significant by paired t-tests ($P<0.05$).

3. Results

3.1. Analysis of metabolic profiling in P. notoginseng plants

The chemical components in different parts and developmental stages of *P. notoginseng* plants were detected through UPLC–MS analysis (Supplementary Information Figs. S1 and S2). Saponin content was also detected in different parts and developmental stages of *P. notoginseng* (Fig. 2). In 2-year-old *P. notoginseng* plants, the root and fibril were clearly separated; however, the aerial parts (stem, leaf, and flower) overlapped each other and were not perfectly separated (Fig. 2A). The S-plot derived by OPLS-DA presents "S-form," and the upper-right and lower-left variables were available ions that contributed to the differences (Fig. 2C). The 24 potential biomarkers with VIP values >1 were presented in Supplementary Information Fig. S3A. The PCA results of 3-year-old *P. notoginseng* plant indicated that the five parts were separated from one another. PC1 revealed the differences between the underground parts (*i.e.*, root and fibril) and aerial parts (*i.e.*, stem, leaf, and flower) (Fig. 2B). Twenty-one potential biomarkers were identified based on the results of S-plot and VIP values (Fig. 2D and Supplementary Information Fig. S3B). The metabolic characteristics of saponin were compared in the same parts of 2- and 3-year-old *P. notoginseng* plants. The PCA results revealed that saponins differed in the same parts of 2- and 3-year *P. notoginseng* plants (Fig. 3A–E). OPLS–DA analysis showed potential markers in different growth stages of *P. notoginseng* plants. Ions that contributed to the separation were selected from the S-plot and regarded as potential markers (Fig. 3F–J). Potential markers that decided the differences were also identified by VIP values. A total of 13, 18, 14, 11, and 17 potential biomarkers (VIP>1) were identified in the roots, fibrils, stems, leaves, and flowers, respectively (Supplementary Information Fig. S4). These results indicated that

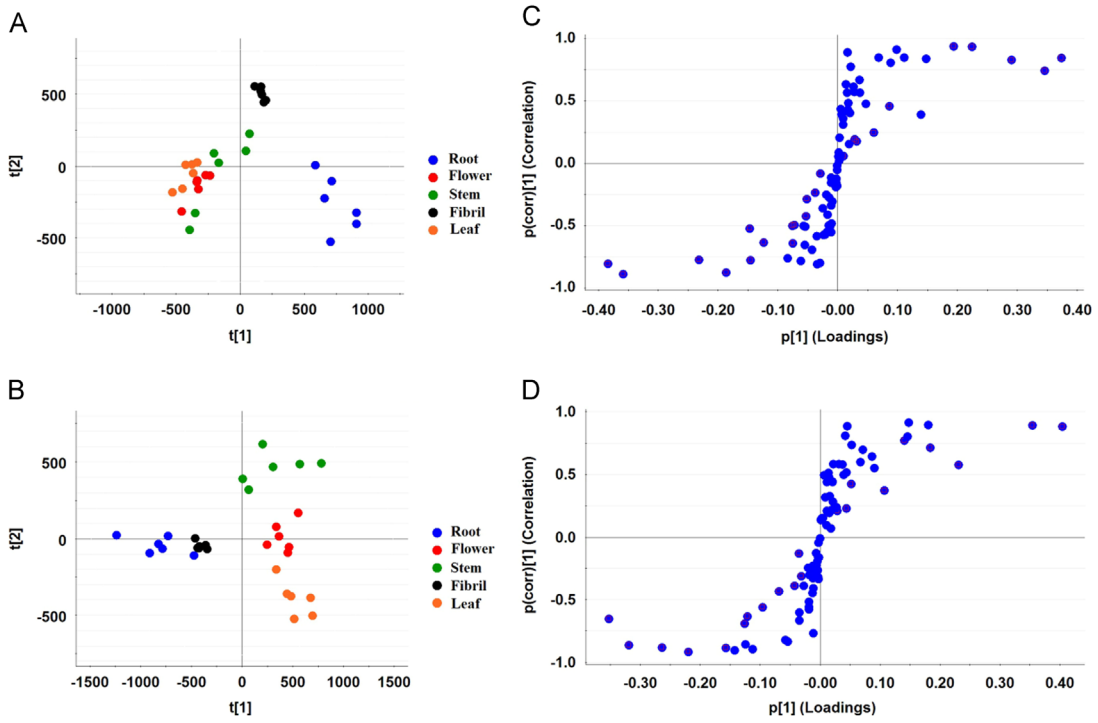

Figure 2 Metabolic analysis of saponins in different parts of *P. notoginseng*. 2- and 3-year-old *P. notoginseng* samples were used in saponin metabolite analysis by UPLC–MS. (A, C) PCA score plots and S-plot of the five parts in 2-year-old *P. notoginseng*; and (B, D) PCA score plots and S-plot of the five parts in 3-year-old *P. notoginseng*.

metabolites exhibited different distribution patterns in different parts and growth stages of *P. notoginseng* plants.

3.2. *Analysis of saponin content in P. notoginseng*

Saponin content was detected in different plant parts and developmental stages through HPLC analysis. The analysis method and the HPLC chromatogram of each compound were provided in Supplementary Information Table S1 and Fig. 4A. The linearity of all calibration curves were $R^2 > 0.999$. The precision, repeatability, and stability variations were 0.03%–1.12%, 0.19%–2.68%, and 1.21%–2.99%, respectively. The recovery ranged from 95.84% to 102.37%, with variations of 0.56%–2.87%. These results showed that the established HPLC method could be used for accurate and sensitive quantitative analyses of notoginsenoside R1 and six major saponins (*i.e.*, Rg1, Re, Rc, Rd, Rb1, and Rb2). The contents of main saponins significantly differed among different plant parts and developmental stages (Fig. 4B and C). In 2-year-old *P. notoginseng* plant, the highest levels of R1, Rg1, and Re were found in the roots (3.1, 46.1, and 1.8 mg/g, respectively), followed by fibrils (Fig. 4B). Moreover, Rb1 and Rd exhibited high levels in the roots and leaves. Rc and Rb2 were not detected in the underground parts (roots and fibrils) but were found at high levels in the leaves and flowers. In 3-year-old *P. notoginseng* plants, high levels of R1, Rg1, Re, Rb1, and Rd (4.1, 67.1, 4.0, 12.8, and 3.5 mg/g, respectively) were found in the roots. The distribution patterns of Rc and Rb2 were similar to those in 2-year-old *P. notoginseng* plant (Fig. 4C). In both 2- and 3-year old *P. notoginseng* plants, the contents of 20(*S*)-protopanaxatriol saponins (PTS), which included R1, Rg1, and Re, were high in the underground parts (i.e., roots and fibrils); meanwhile, the contents of 20(*S*)-protopanaxadiol saponins (PDS), which included Rb1, Rc, Rb2, and Rd, were high in the aerial parts (*i.e.*, leaves and flowers). The total saponin content in 3-year-old *P. notoginseng* (188.0 mg/g) was 1.4-fold higher than that in 2-year-old one (130.5 mg/g). These results indicated the high saponin contents (R1, Rg1, Re, Rb1, and Rd) were in the roots, while high Rb2 and Rc contents in the leaves and flowers. Hence, the contents and types of saponins were unevenly distributed in *P. notoginseng*.

3.3. *Gene expression profiling*

The tissue-specific expression patterns of five genes involved in triterpene saponin biosynthesis were evaluated through reverse transcriptional quantitative PCR. The relative expression of genes (*i.e.*, *PnFPS*, *PnSS*, *PnSE1*, *PnSE2*, and *PnDS*) demonstrated significantly similar expression patterns among the five parts (*i.e.*, roots, fibrils, stems, flowers, and leaves) at two developmental stages (Fig. 5). In 2-year-old *P. notoginseng*, the transcript levels of these genes were higher in the flowers and leaves than those in the roots and fibrils (Fig. 5A). The expression levels of *PnFPS*, *PnSS*, *PnSE1*, *PnSE2*, and *PnDS* in the flowers were 85.0-, 120.7-, 111.4-, 528.3-, and 49.0-fold higher than that in the roots, respectively. Compared with the 2-year-old *P. notoginseng* plant, the 3-year-old *P. notoginseng* plant showed similar gene expression profiling but slightly lower expression levels (Fig. 5B). Four genes (*i.e.*, *PnFPS*, *PnSS*, *PnSE1*, and *PnDS*) were highly expressed in the flowers, with 100.3-, 124.0-, 5.2-, and 42.7-fold higher expression level than those in the roots. The expression of *PnSE1* was the highest in the leaves and 7.1-fold higher than that in the roots. Additionally, the expression of the five genes exhibited significant correlations among the five parts ($P < 0.05$, Fig. 5C and D). Hence, the expression levels of *PnFPS*, *PnSS*, *PnSE1*, *PnSE2*, and *PnDS* in the flowers were significantly higher than that in the roots.

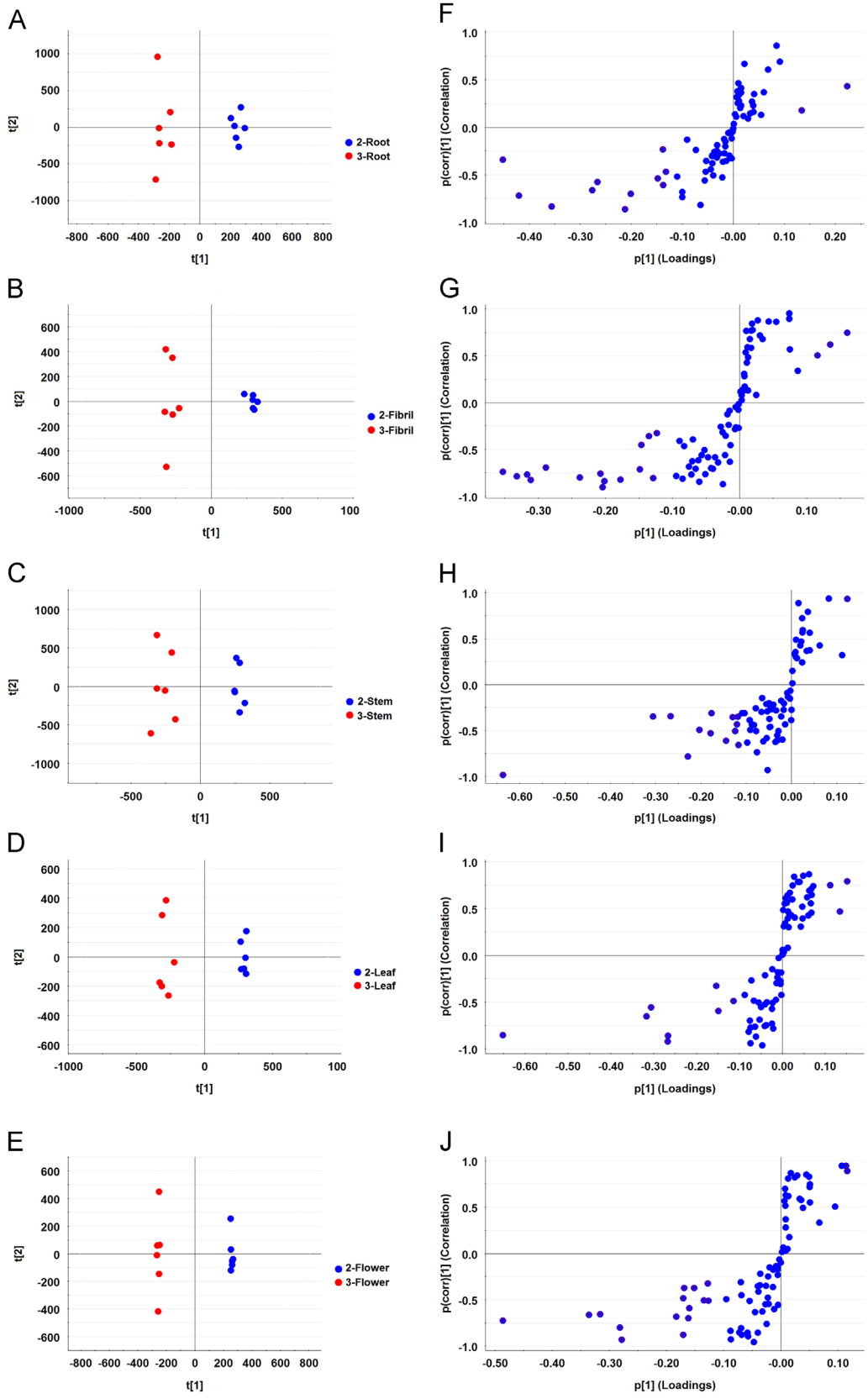

Figure 3 Metabolic analysis of saponins in different developmental stages. 2- and 3-Year-old of *P. notoginseng* samples were used in the saponin metabolite analysis by UPLC–MS. (A–E) PCA score plots of the roots, fibrils, stems, leaves, and flowers at two developmental stages, respectively; (F–J) S-plot of the roots, fibrils, stems, leaves, and flowers at two developmental stages, respectively.

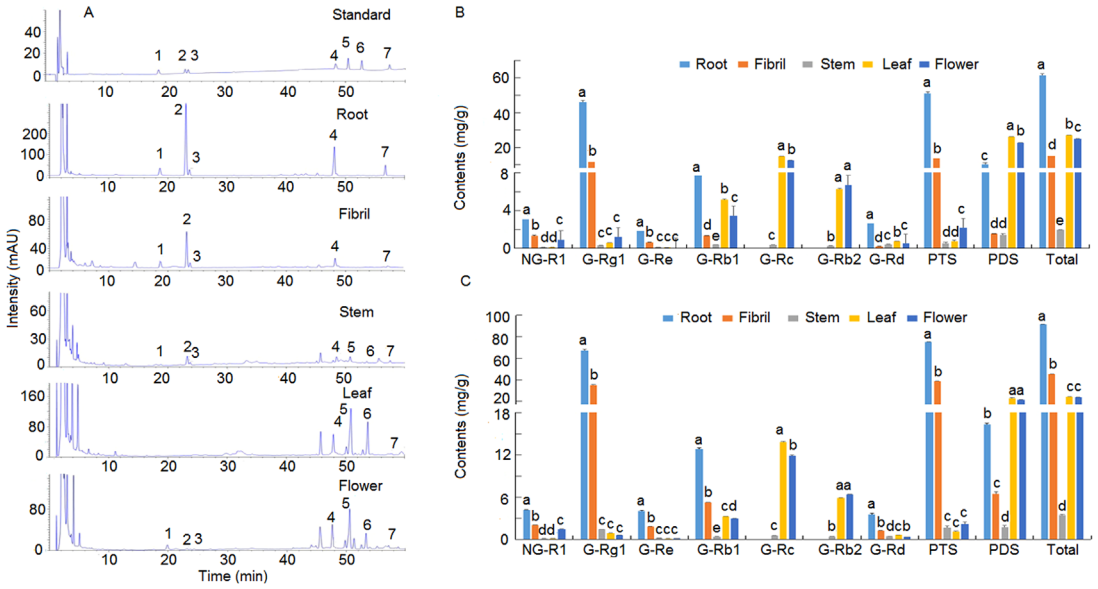

Figure 4 HPLC chromatograms and contents of saponins in *P. notoginseng*. (A) HPLC chromatogram profiles of *P. notoginseng* plants. 1. Notoginseng R1; 2. Ginsenoside Rg1; 3. Ginsenoside Re; 4. Ginsenoside Rb1; 5. Ginsenoside Rc; 6. Ginsenoside Rb2; 7. Ginsenoside Rd. (B) Contents of the seven saponins in 2-year-old *P. notoginseng*. (C) Contents of the seven saponins in 3-year-old *P. notoginseng*. NG: notoginsenoside; G: ginsenoside; PTS: total amount of 20(*S*)-protopanaxatriol saponins (PDS) including notoginsenoside R1 and ginsenosides Rg1 and Re; PDS: total amount of PDS including ginsenoside Rb1, Rc, Rb2, and Rd; Total: total amount of PTS and PDS including notoginsenoside R1 and ginsenosides Rg1, Re, Rb1, Rc, Rb2, and Rd. Different letters represent significant difference among the five parts at $P<0.05$.

4. Discussion

In this study, integrated metabolomic and transcriptomic analysis were used to determine the saponin distribution and the expression patterns of genes encoding key enzymes involved in saponin biosynthesis in *P. notoginseng* plants. Potential biomarkers were found in the aerial and underground parts of 2- and 3-year-old *P. notoginseng* plants. PTS and PDS showed tissue-specific distribution. The total saponin content was markedly higher in the 3-year-old *P. notoginseng* plant than that in the 2-year-old plant. Five genes (*i.e.*, *PnFPS*, *PnSS*, *PnSE1*, *PnSE2*, and *PnDS*) showed higher expression in the flowers and leaves than that in the underground parts.

Metabolite profiles have been applied to evaluate chemical characteristics in different root parts (*i.e.*, main roots, rhizomes, branch roots, and fibrous roots) of *P. notoginseng*[26]. In the present study, the PCA results revealed that saponins differed between the underground and aerial parts of *P. notoginseng* plants. The results of S-plot and VIP analyses indicated that these biomarkers were mainly PTS/PDS-type saponins, and metabolic differences in different plant parts and growth stages.

Triterpenoids possess important pharmacological effects, such as hypoglycemic activity (Rg1)[27], antioxidant activity (Re)[28], neuroprotection activity (Rb1)[29], improvement in learning and memory abilities (Rd)[30], immunity activity (Rb2)[31], and anti-inflammatory activity (Rc)[32]. Notoginsenoside R1 and ginsenoside (*i.e.*, Rg1, Re, Rb1, and Re) constitute up to 90% of the total *P. notoginseng* saponins[33]. Re and Rb1 are potential biomarkers in different parts of 3-year-old *P. notoginseng* plants. Thus, these saponins are the main bioactive components, and their distribution was further analyzed. The HPLC data showed that the contents of notoginsenoside R1 and saponins (*i.e.*, Rg1, Re, Rd, and Rb1) were the highest in the roots, followed by the fibrils. This finding is consistent with the *Chinese Pharmacopoeia* (2015)[34]. PTS was detected in the whole plant, and PDS was mainly distributed in the aerial parts; these results are similar to those reported in a previous study[35]. Hence, the aerial parts contained certain saponins and could be used to extract saponins for raw materials or health care products. The total saponin content in 3-year-old *P. notoginseng* (188.0 mg/g) was 1.4-fold higher than that in the 2-year-old plant (130.5 mg/g). This work provides references for rational use of different *P. notoginseng* parts.

Saponin biosynthesis is a complicated process that involves in various rate-limiting enzymes[36]. The biosynthesis of isoprenoid through both pathways is shown in Supplementary Information Fig. S5. Mevalonate-5-pyrophosphate decarboxylase, which is the last enzyme of the mevalonic pathway, produces isoprenyl diphosphate (IPP). Farnesyl diphosphate (FPP) is derived from IPP through the catalytic reaction of farnesyl diphosphate synthase (FPS)[37]. The two FPP molecules are placed into a C_{30} isopreniod squalene by SS[38]. The first oxygenation step in triterpenoid biosynthesis is performed by SE, which catalyzes the epoxidation of the double bond of squalene to form 2,3-oxidosqualene[23]. DS catalyzes the cyclization of 2,3-oxidosqualene to form various secondary metabolites[39]. The five genes (*i.e.*, *PnFPS*, *PnSS*, *PnSE1*, *PnSE2*, and *PnDS*) were confirmed to encode FPS, SS, SE, and DS in saponin biosyntheses[25]. In the present study, the transcript levels of *PnFPS*, *PnSS*, *PnSE1*, *PnSE2*, and *PnDS* were higher in the flowers and leaves than that those in the roots. The saponin contents (*i.e.*, Rg1, Re, Rd, and Rb1) were significantly higher in the underground parts than that in the aerial parts. Hence, saponins might be actively synthesized in the aerial parts and transformed to the underground parts; this finding is similar to previous reports[40]. In a previous study, the results of ^{13}C-labeling assays indicated that biosynthesized ginsenosides could be transported from ginseng leaves into roots[41]. Schramek et al.[42] reported that the products of photosynthetic metabolites (glucose and fructose) contributed to ginsenoside synthesis or movement into

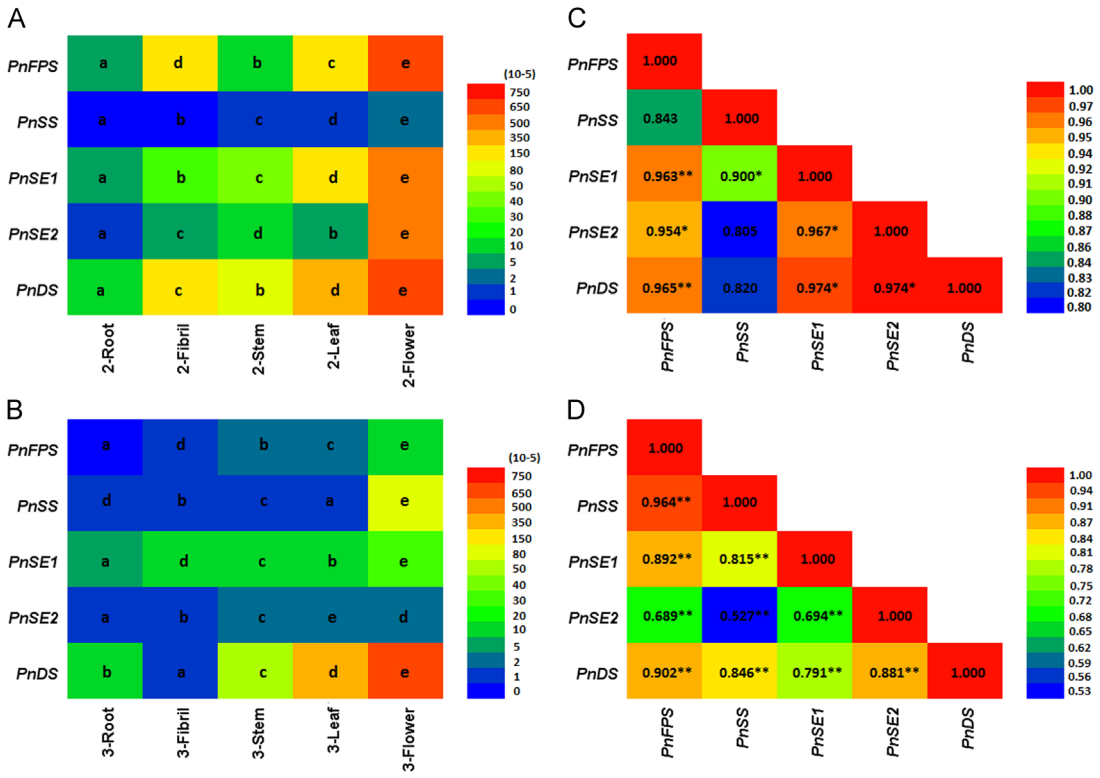

Figure 5 Expression profiling and Pearson correlation analysis of the five genes in *P. notoginseng*. Different letters represent significant differences. (A) Expression levels of the five genes in 2-year-old *P. notoginseng*. (B) Expression levels of the five genes in 3-year-old *P. notoginseng*. (C) Pearson correlation analysis of the five genes in 2-year-old *P. notoginseng*. (D) Pearson correlation analysis of the five genes in 3-year-old *P. notoginseng*. Different letters represent significant differences at $P<0.05$. The symbol * represents a significant correlation among the five genes at $P<0.05$, and the symbol ** represents a highly significant correlation between the five genes at $P<0.01$.

ginseng roots. In the present study, the high gene expression profiles in the flowers and leaves confirmed that saponins might be actively synthesized in the aerial parts and transformed to the underground parts.

This study elaborates the distribution of saponins and the expression patterns of related genes in different parts and developmental stages of *P. notoginseng* plants. Our data provide information for understanding the biochemical diversity of secondary metabolism and molecular characteristics of saponins. These results can guide the appropriate collection and rational use of *P. notoginseng* plants.

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.apsb.2017.12.010.

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