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Research Article

Age-induced Changes in Ginsenoside Accumulation and Primary Metabolic Characteristics of Panax Ginseng in Transplantation Mode

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ABSTRACT

Background: Ginseng (*Panax ginseng* Mayer) is an important natural medicine. However, a long culture period and challenging quality control requirements limit its further use. Although artificial cultivation can yield a sustainable medicinal supply, research on the association between the transplantation and chaining of metabolic networks, especially the regulation of ginsenoside biosynthetic pathways, is limited.

Methods: Herein, we performed Liquid chromatography tandem mass spectrometry based metabolomic measurements to evaluate ginsenoside accumulation and categorise differentially abundant metabolites (DAMs). Transcriptome measurements using an Illumina Platform were then conducted to probe the landscape of genetic alterations in ginseng at various ages in transplantation mode. Using pathway data and crosstalk DAMs obtained by MapMan, we constructed a metabolic profile of transplantation Ginseng.

Results: Accumulation of active ingredients was not obvious during the first 4 years (in the field), but following transplantation, the ginsenoside content increased significantly from 6–8 years (in the wild). Glycerolipid metabolism and Glycerophospholipid metabolism were the most significant metabolic pathways, as Lipids and lipid-like molecule affected the yield of ginsenosides. Starch and sucrose were the most active metabolic pathways during transplantation Ginseng growth.

Conclusion: This study expands our understanding of metabolic network features and the accumulation of specific compounds during different growth stages of this perennial herbaceous plant when growing in transplantation mode. The findings provide a basis for selecting the optimal transplanting time.

1. Introduction

Panax ginseng (Ginseng; Araliaceae family), a perennial herbaceous plant that has been widely utilised as a medical herb for >2000 years, is mainly produced in Korea, Japan and northeast China [1]. Ginseng studies have mainly focused on the composition and pharmacology of active ingredients [2–4], and the pharmacological/bioactive constituents in Ginseng tend to increase with cultivation age [5]. However, research on Ginseng cultivation mode relative to ginsenoside accumulation is scarce.

As a typical perennial herbaceous plant, Ginseng is usually harvested after 5–15 years of cultivation, depending on the cultivation mode [6]. In China, the most common cultivation mode is field planting (garden Ginseng; G-GS), wild planting (under forest Ginseng; F-GS) and transplanted planting (transplantation Ginseng; T-GS). G-GS is the fastest-growing mode, with a harvesting period of 5–6 years. F-GS involves seeding in forests and growing in wild environments without artificial intervention [7], followed by harvesting after >15 years of

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growth. T-GS involves culturing in the field for 2−4 years, transplanting to wild environments such as mountains and forests, and growing for another 4−10 years [8]. T-GS meets both pharmacological requirements and commercial demand. However, the mechanisms underlying transplantation operations in T-GS remain unclear, limiting its application in the Ginseng planting industry. A series spatiotemporal analyses are needed that assess ginsenoside accumulation, metabolite patterns, and changes in gene expression during the process of T-GS growth, especially before/after transplantation. Herein, we performed metabolomic measurement of Ginseng at various ages in transplantation mode to evaluate ginsenoside accumulation in T-GS. Additionally, we categorised differentially abundant metabolites (DAMs) according to the metabolomic profile of T-GS at various ages, and screened differentially expressed genes (DEGs) during different growth stages of T-GS. Using pathway data generated by MapMan and crosstalk DEMs, we constructed a metabolic profile of T-GS.

The objectives of the present study were (1) to characterise the accumulation of active ingredients in Ginseng at various ages in transplantation mode, and (2) to assess the primary metabolic properties and hub DAMs (hub-DAMs) during Ginseng growth. This study expands our understanding of the metabolic network features and the accumulation of specific compounds in relation to age during perennial herbaceous plant growth in transplantation mode. The findings provide a basis for selecting the optimal transplanting time.

2. Materials and methods

2.1. Sources of materials

Ginseng roots utilised in this research were randomly acquired from planting bases in KuanDian, Liaoning province, China (N40°43′46.80″, E124°46′40.55″) in 2021. We collected 2- and 4-year-old Ginseng from the field, and 6- and 8-year-old Ginseng from mountain bases. Regarding soil composition, enzyme activity in rhizosphere soil and the microbial content of Ginseng rhizosphere soil in field and mountain bases are shown in Tables S1−S3. Samples of Ginseng (each group included of six replicates) were briefly washed five times with distilled water to remove dust on the surface, and all 24 samples were placed into dry ice immediately for transport.

2.2. Metabolic analysis of ginseng at various ages

Liquid chromatography tandem mass spectrometry (LC-MS/MS) was utilised for metabolomic analyses. Metabolomics data were converted to mzXML files using MSConvert Version 2.1 (ProteoWizard), and metabolic peak extraction and quality control analysis of mzXML data were conducted by XCMS R-3.3.3 software (http://metlin.scripps.edu/download/). CAMERA was used to annotate adduct and product ion peaks, and for metabolite identification and quantification, and screening of DAMs was conducted using metaX software (http://metax.genomics.cn/). Fold-change (FC) analysis and t-tests were used as univariate analysis methods, and *p*-values were corrected using the Bonferroni Holm (BH) procedure to yield *q*-values. Variable importance in projection (VIP) values were evaluated by multivariate statistical analysis, and PLS-DA was carried out using Simca-P + V12.0 (Umetrix, Sweden). Ions with (1) ratio ≥2 or ratio ≤1/2, (2) *q*-value ≤0.05, and (3) VIP ≥1 were considered differentially abundant metabolite ions.

2.2.1. Active ingredient accumulation in ginseng at different ages

Using meta-X software, we matched the fingerprint of ginsenosides K, F1, F3, Rb1, Rb2, Rb3, Rc, Re, Rf, Rg1, Rg2, Rg3 (S-form), Rg5, Rg6, Rh1, Rh2 (S-form), and Ro to the metabolite results, resulting in ginsenoside content at different ages. Firstly, we plotted ginsenoside concentrations at different ages using the R package ggplot2. Secondly, heatmaps of *p*-values and VIP values were generated using the TBtools package Heatmap [9]. In VIP value heatmaps red represents upregulation and green represents downregulation.

2.2.2. Metabolomic characteristics of ginseng at different ages

Principal component analysis (PCA) of metabolomics data was performed using the R package FactoMineR and factoextra, and 3-D (including PC1, PC2 and PC3) scatter PCA plots were used to visualise similarities between samples. We constructed bar charts of identifiable and unidentifiable DAMs using ggplot, and constructed Venn diagrams (generated by the R packages grid, futile.logger and VennDiagram) for 2 vs 4, 4 vs 6, and 6 vs 8-years-old Ginseng comparisons to identify important DAMs during the transplanting operation. To determine changes in DAMs at different ages, we generated a VIP value heatmap (using the TBtools package Heatmap) based on DAMs classification, where red represents upregulation and green represents downregulation. Finally, Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) functional enrichment analyses of DEMs were performed using DAVID (https://david.ncifcrf.gov/).

2.3. Transcription analysis of ginseng at different ages

2.3.1. mRNA library construction and sequencing

Total RNA was isolated and purified using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Poly (A) RNA was purified from 1 μg total RNA using Dynabeads Oligo (dT)25-61005 (Thermo Fisher, Waltham, Massachusetts, USA). The cleaved RNA fragments were reverse-transcribed to generate cDNA using SuperScript II Reverse Transcriptase (cat. 1896649; Invitrogen, USA), and these were used to synthesise U-labelled second-stranded DNAs using *Escherichia coli* DNA polymerase I (cat.m0209; NEB, USA), RNase H (cat.m0297; NEB) and dUTP Solution (cat. R0133; Thermo Fisher). After treatment of the U-labelled second-stranded DNAs with heat-labile UDG enzyme (cat.m0280; NEB), the ligated products were amplified by PCR using the following conditions: initial denaturation at 95°C for 3 min, followed by 8 cycles of denaturation at 98°C for 15 s, annealing at 60°C for 15 s, and extension at 72°C for 30 s, and a final extension at 72°C for 5 min. Finally, we performed 2 × 150 bp paired-end sequencing (PE150) on an Illumina Novaseq 6000 platform (LC-Bio Technology Co., Ltd., Hangzhou, China) following the manufacturer's recommended protocol.

2.3.2. PCA analysis

FPKM values (transformed from fastq data) were taken as inputs for PCA and DEG analyses. To explore changes in transcription in Ginseng at different ages, we generated scatter PCA plots using the R packages FactoMineR and Factoextra based on the FPKM data.

2.3.3. Screening differentially expressed genes (DEGs)

To identify general trends in hub genes in Ginseng at different ages, we firstly screened DEGs using $p < 0.05$ and $|\text{Log2FC}| > 1$ (FC > 2 or FC < 0.5) as cutoff criteria. Next, we plotted bar charts using upregulated and downregulated DEGs selected from 2 vs 4, 4 vs 6, and 6 vs 8-year-old Ginseng comparisons. Finally, a volcano plot of DEGs for 2 vs 4, 4 vs 6, and 6 vs 8-year-old Ginseng comparisons was constructed using R packages ggplot2, ggrepel, ggthemes and gridExtra, where red represents upregulation and green represents downregulation DEGs.

2.4. Primary metabolic pathway construction

2.4.1. Gene annotation

Since Ginseng is a non-model organism, in order to access the metabolic functions of the identified DEGs, we conducted gene annotation using MapMan (https://mapman.gabipd.org) [10]. We uploaded the original fastq data to Mercator (https://mapman.gabipd.org/app/mercator), sequences were aligned, and protein domain prediction was performed based on model organisms, yielding bining (BIN) codes for genes.

2.4.2. Primary metabolic pathway construction

Using BIN codes for Ginseng, we constructed a primary metabolic network based on RPKM values. DEG annotations obtained using Mercator were imported into MapMan 3.6.0RC1, and mapping libraries were uploaded for primary metabolism, including starch and sucrose, lipid biosynthesis, the tricarboxylic acid (TCA) cycle, the Calvin cycle, and photorespiration metabolic pathways in plants, yielding BIN information and expression levels of hub-DEGs in different metabolic pathways. Using crosstalk metabolites, we linked these metabolic pathways and plotted primary metabolic maps using Adobe Illustrator CC2015 software. We also noted correlations between DEGs and metabolites for 2 vs 4, 4 vs 6, and 6 vs 8-year-old Ginseng comparisons.

3. Results

3.1. Active ingredient accumulation in ginseng at different ages

Firstly, we assessed changes in active ingredients in Ginseng at different ages according to metabolomic data (peak area ratio), and generated a series of scatter plots for 16 ginsenosides (Fig. 1A–P). We then assessed p and VIP values using heatmaps (Fig. 1R) to compare different ages. As shown in Fig. 1R, although there were statistically significant differences between 2- and 4-year-old Ginseng (2 vs 4) for some ginsenosides ($p < 0.05$), VIP values between groups were low, except for ginsenoside Ro. Clearly, in the first 4 years, ginsenoside accumulation in Ginseng was limited, without significant increases or decreases.

3.2. Evaluation and identification of DAMs in ginseng at different ages

Based on the metabolomic data, we identified 9376 metabolites in the four different age groups. PCA results (Fig. 2A) indicated an overall consistent metabolite composition in the initial 4-year period, but after 6 and 8 years there were significant differences. As shown in Fig. 2B, the numbers of DAMs in 2 vs 4, 4 vs 6, and 6 vs 8 comparisons were 85/1952, 81/3310, and 136/5160, respectively, where the former value represents metabolites identified and the later represents unidentified metabolites. The proportion of DAMs in Ginseng clearly increased over time. As shown in Figs. 2C and 136 metabolites were identified in 24 samples; the 4 vs 6 and 6 vs 8 comparisons yielded the most similar DAMs, hence 6- and 8-year-old Ginseng had similar distributions of DAMs after transplantation at 4 years (Fig. S1).

We categorised the identifiable DAMs in different comparisons (Fig. 2D). Lipids and lipid-like molecules (e.g., ginsenosides) were the most abundant, especially in the 6 vs 8 comparison. Similarly, organic nitrogen compounds changed slightly in the 2 vs 4 comparison, and varied dramatically from 4 and 8 years. However, benzenoids, organic acids and derivatives differed more from 2 to 6 years, and less from 6 to 8 years. These results suggest that these two classes are important for ginsenoside synthesis in the early stages, but ginsenoside accumulation remained more stable in the latter stages. Changes in specific DAMs are shown in Fig. 2E. The results of functional enrichment analysis of DEGs from different comparisons are shown in Figure S2. DAMs were found to be involved in various processes including the valproic acid metabolism pathway, phospholipid biosynthesis, and butyrate metabolism (Fig. S3).

3.3. Evaluation and identification of DEGs in ginseng at different ages

Based on the transcriptome data, we identified 59,353 genes in the four different age groups. Firstly, we analysed gene expression in different age groups (Fig. 3A). The points representing the results of PCA of 2 vs 8 years were the most dispersed; by contrast, all points for 2 vs 4 years were scattered in the same quadrant. Meanwhile, compared with 2 vs 4 years, a more similar mRNA distribution was observed for 4 and 6 years.

Next, we evaluated and identified DEGs during the culture period (Fig. 3B). Fig. 3C–E and Tables S4–S6 show the distributions of DEGs in 2 vs 4, 4 vs 6, and 6 vs 8 comparisons. Obviously, during the first 4 years, Ginseng in garden exhibited a similar biological and metabolic characteristic, however, it was not associated with accumulation of active ingredients.

From 4 to 6 years, both DEGs and DAMs increased dramatically. However, ginsenosides varied, and some displayed a declining trend (Fig. 1). Remarkably, compared with 2, 4, and 6 years, gene expression in Ginseng at 8 years was mostly condensed within the Q3 quadrant (Fig. 3A). Therefore, it can be speculated that gene expression in Ginseng reached a steady state by this stage, which caused significant ginsenoside accumulation after 8 years.

3.4. Metabolic map of ginseng at different ages

We assessed DEGs in Ginseng at different ages using MapMan software, transformed the resulting data into BIN code (Tables S7–S9), and identified the metabolic signatures according to these BIN codes. Overall, year 4 vs. year 6 yielded the most primary DAMs, involving 822 metabolic signatures annotated by 63 BIN codes; in the year 2 vs. year 4 comparison, we only identified 454 metabolic signatures annotated by 42 BIN codes, and 45 BIN codes were screened in the year 6 vs. year 8 comparison, with 525 metabolic signatures annotated. These results imply that, from year 2 to year 4, metabolic pathways in Ginseng were relatively stable, but transplantation in year 4 perturbed metabolic pathways, and changes in metabolites occurred later than changes in metabolic pathways; therefore, although year 4 and year 6 shared a similar metabolome composition (Fig. S1B), samples from these two years exhibited significant differences in primary metabolism. By contrast, the year 6 and year 8 comparison yielded more DAMs than the year 4 and year 6 comparison, but exhibited stable primary metabolism characteristics (Fig. S1B). These results indicate that the stability of primary metabolism was negatively correlated with DAMs. This implies that transplantation in year 4 triggers direct metabolic changes. Using MapMan data (BIN codes) annotated based on DEGs profiles, we built a primary metabolic map of Ginseng (Fig. 4).

4. Discussion

The traditional Ginseng cultivation belt spans East Asia, covering China, the Korean Peninsula, Japan and the far-east of Russia [11,12]. With a long-term planting practice and the culture characteristics of Ginseng well established, T-GS is considered the best practice for commercial applications. This cultivation strategy is widely utilised in KuanDian (124.78° longitude, 40.73° latitude), one of the major ginseng cultivation regions in China. However, there exists limited research on changes in metabolites, especially active ingredients, before and after Ginseng transplantation, and this should be addressed because transplantation time selection and T-GS quality evaluation are important.

Previous Ginseng genetic studies investigated various aspects of ginsenoside biosynthesis such as enzymatic function, epigenetics and signalling pathways [13,14]. Herein, using DAMs annotated by DEGs, we estimated changes in primary metabolites in T-GS throughout the cultivation period, and evaluated associations between primary metabolites and the main active ingredients in T-GS during the production cycle.

Base on the DAM results obtained by non-targeted GC-MS (Fig. 1), there was no significant ginsenoside accumulation in the first 4 years, but there was a dramatic increase in the last 4 years. Transplantation is beneficial to the generation of active ingredients, which accelerates ginsenoside accumulation. Previous studies indicated that plants exhibit a stable metabolite composition in terms of primary metabolite accumulation in the first few years, thereby providing sufficient energy for germination and growth [15,16]. However, biosynthesis of plant secondary metabolites is spatially and temporally regulated; plants adapt their metabolism in response to the generation of specific secondary

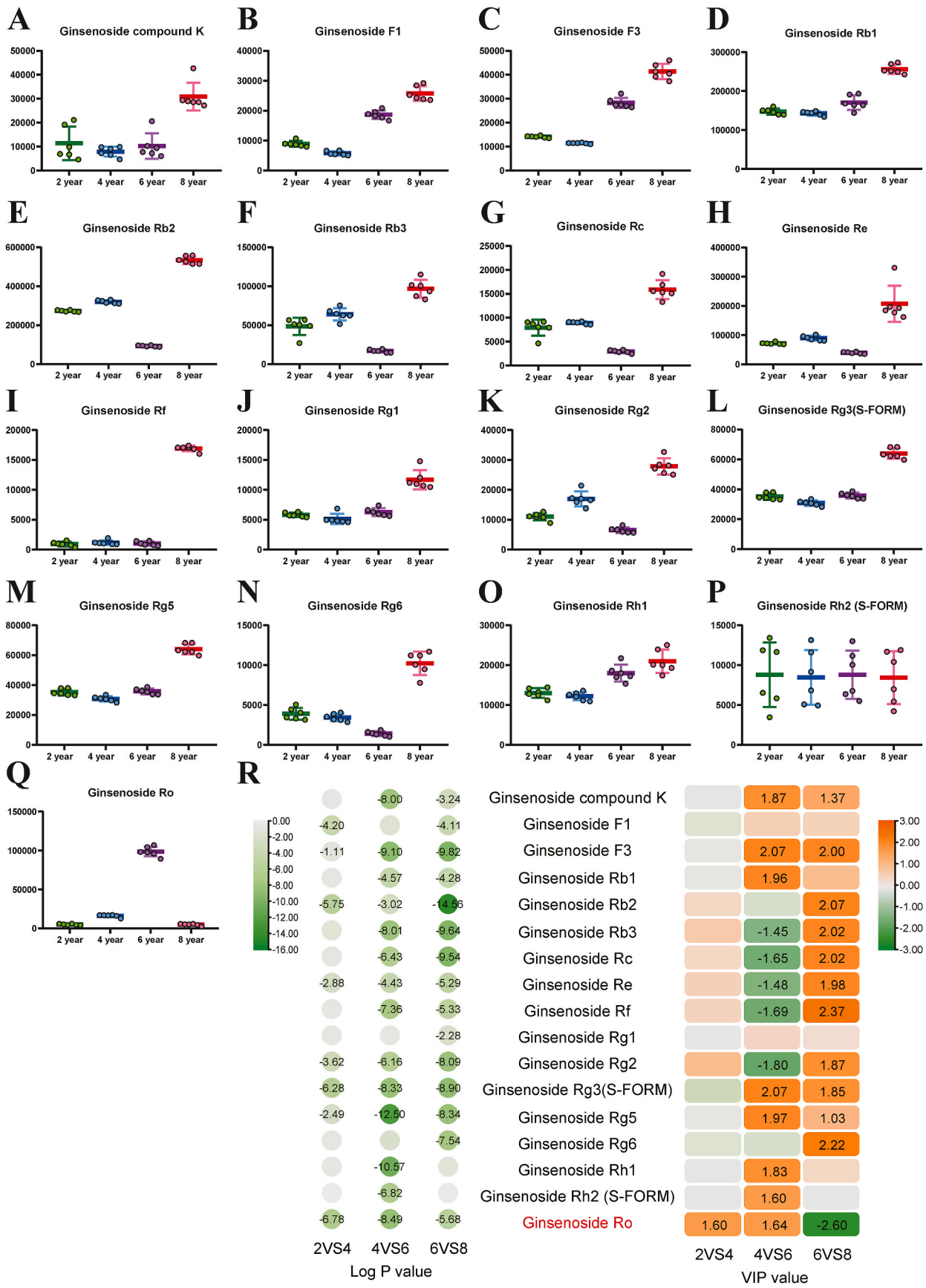

Fig. 1. The variation of active ingredient in Ginseng at various ages according to the metabolomic data output by peak area ratio and generated a series scatter plot of 16 ginsenosides screened (A-P). Then, we visualized the statistical results, including p-value and VIP value, by a heatmap (R) between different years groups.

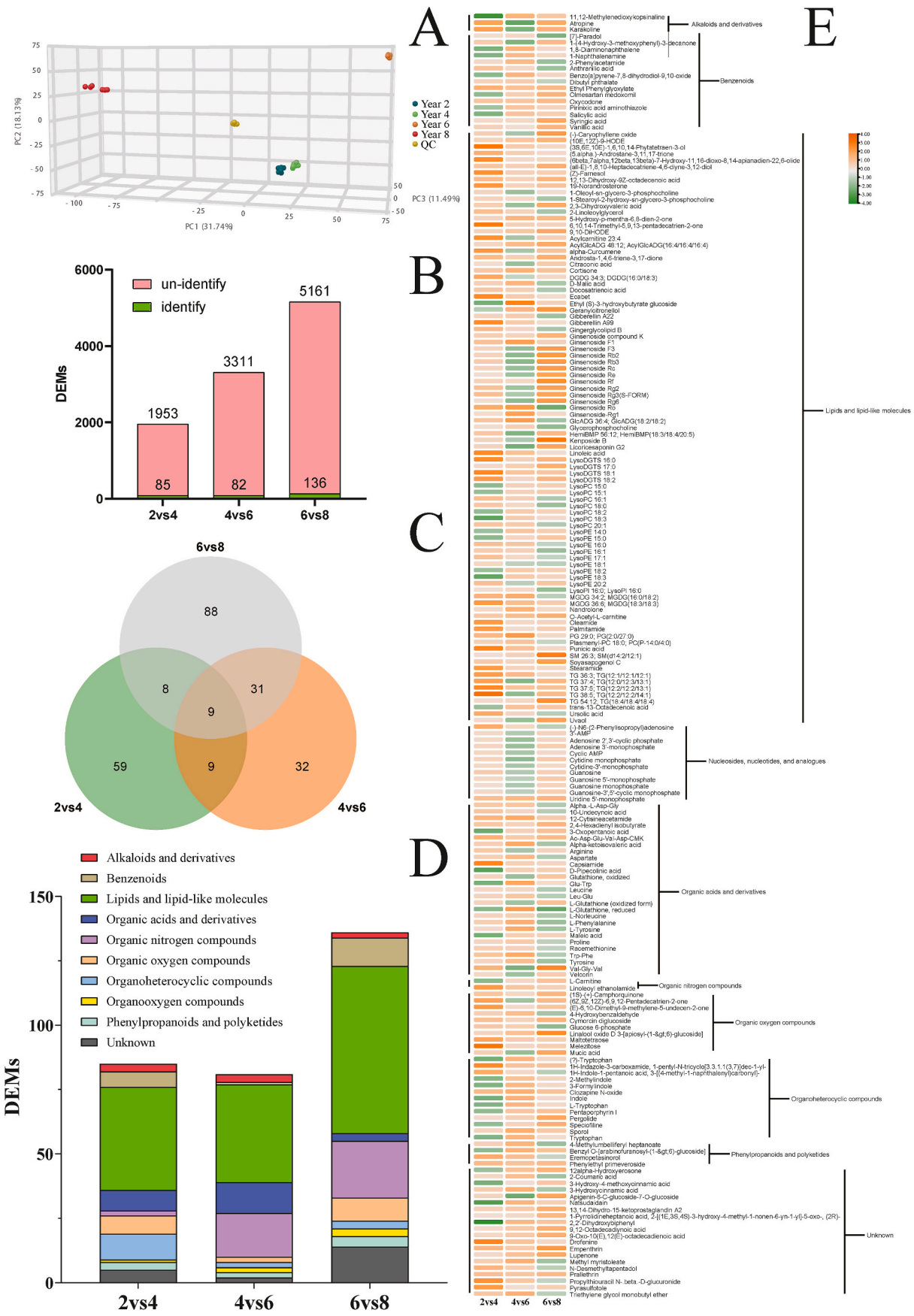

Fig. 2. The identification of DEMs in Ginseng at various ages,(A) represent the PCA result of Ginseng at various ages, a bar plot (B) was conducted to illustrate the DEMs distributed of Ginseng at various ages, (C) exhibited the identifiable metabolites and categorized them in (D). As (E) exhibited the trend of specific DEMs concentration change.

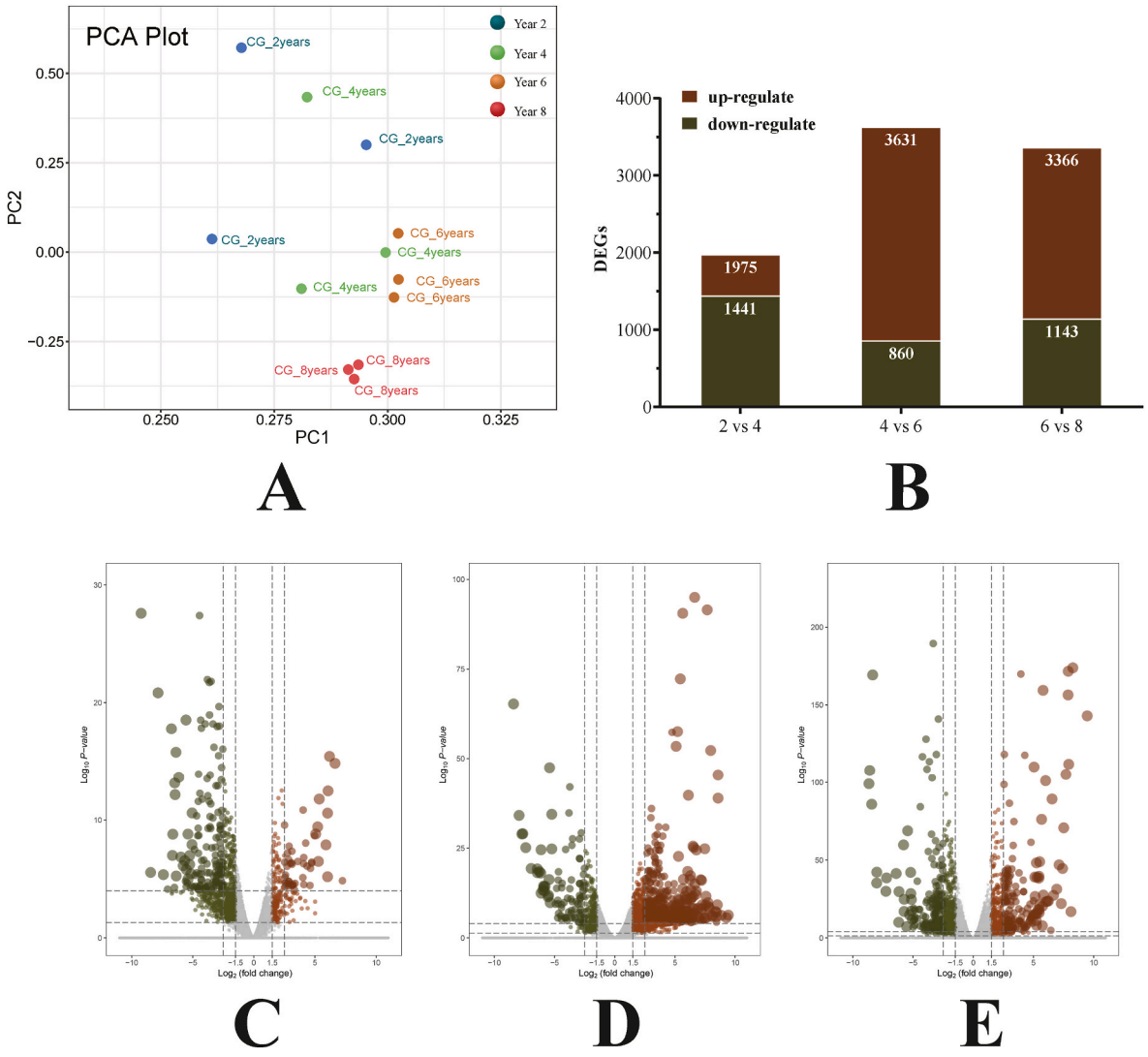

Fig. 3. The transcriptome feature of Ginseng during the annual growth, (A) exihibited the PCA results of gene expression in different years group, and (B) illustrated the DEGs changing during the culture period. (C-E) Exhibied the distribution of DEGs in 2vs4, 4vs6, 6vs8 years group, respectively.

metabolites over time, and this adaptive regulation can be observed in both annual and perennial plants [17,18].

We briefly summarise the major accumulation patterns of ginsenosides: (1) Ginsenosides K, F1, F3, Rb1, Rg1, Rg3,Rg5 and Rh1 exhibited a persistent increase from 4 to 8 years; (2) six ginsenosides (Rb2, Rb3, Rc, Re, Rg2 and Rg6) first decreased from 4 to 6 years, then increased from 6 to 8 years. Both accumulation patterns described above indicate that after transplantation and adaption, the new culture environment results in a new metabolic regulatory network, which influences the metabolic characteristics of Ginseng, especially the dramatic increase in active ingredients in year 8. It is worth noting that although the VIP values of some ginsenosides were not significantly different in different years (Fig. 1R), ginsenoside concentrations increased rapidly from 6 to 8 years. This may explain why T-GS involves transplanting in year 4 and harvesting in year 8 (when active ingredients have changed).

Regrettably, due to the clean-up operation for Ginseng in the field, we did not obtain Ginseng beyond 8 years old. In addition, according to information from local farmers, the wild environment is not suitable for Ginseng seeding and germination. There were no 0 to 4-year-old wild samples in the Ginseng growing base, therefore comparison of ginsenoside accumulation in gardens and wild conditions could not be performed. Consequently, it is difficult to evaluate the marginal effect of transplantation mode on ginsenosides accumulation, and the accumulation characteristics of ginsenosides in different cultivation environments caused by transplantation.

Regarding specific metabolite classification, lipids and lipid-like molecules exhibited the greatest changes among all categories (especially for year 4 to year 8). Lipid metabolism is closely correlated with the regulation of various physiological processes including the synthesis of plant cell membranes [19], storage of energy [20] and the synthesis of fatty acid derivatives for signal transduction [21]. Considering that ginsenosides are among the most important lipids and lipid-like molecules in Ginseng [22], it can be speculated that transplantation directly impacts lipid-like molecules in Ginseng during the cultivation process. Consistently, a similar trend was observed for organic nitrogen compounds; previous research indicated that the synthesis of undecyl pyrophosphate in plants is co-regulated by lipids and nitrogen metabolism [23,24], and the synthesis of ginsenosides is affected by the mevalonic acid pathway [25]. Herein, we report similar results showing that lipids and nitrogen metabolism provide the raw materials for ginsenoside formation (during the early stages) and accumulation (during the latter stages). However, our analysis was limited to major metabolite classification and it ignored the metabolic characteristics of specific metabolites in Ginseng. Importantly, based on the results, transplantation influences the metabolic landscape of Ginseng. Nevertheless, accounting for the huge metabolic datasets acquired in this study (we identified

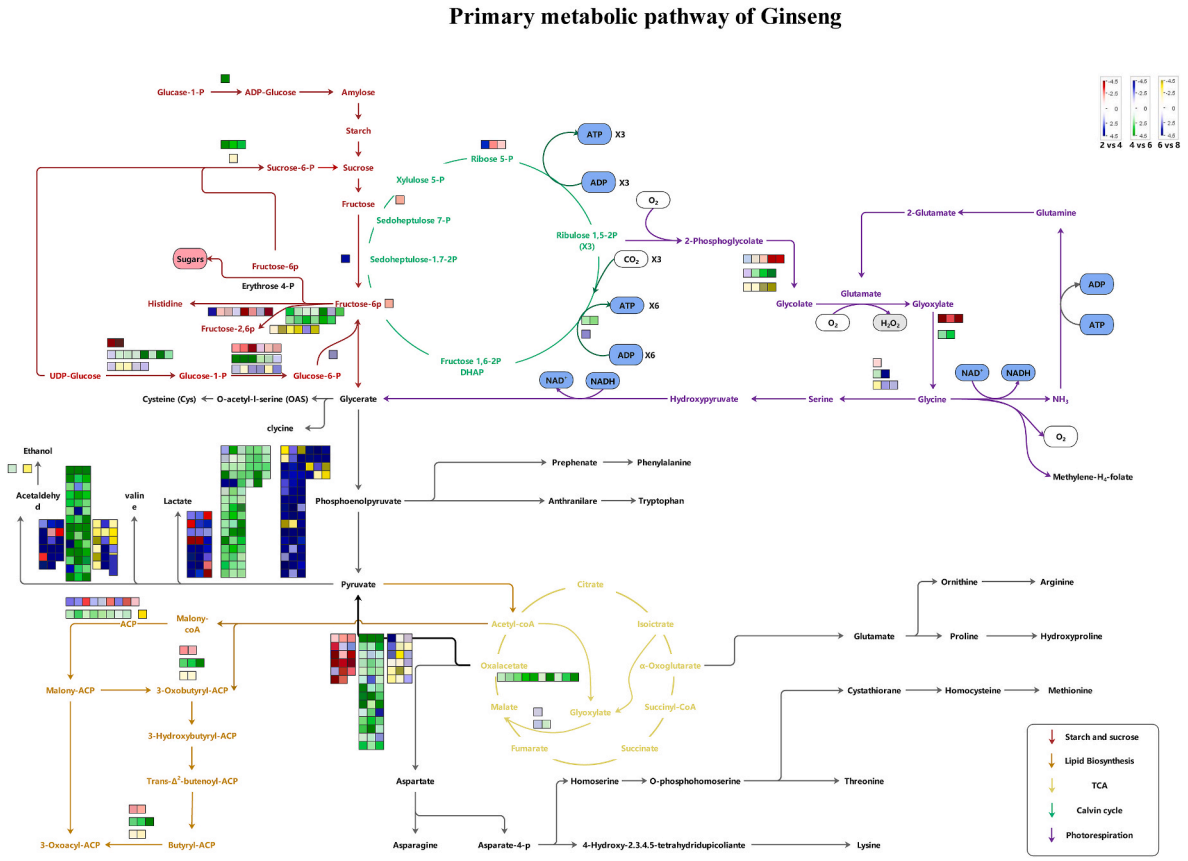

Fig. 4. The primary metabolic pathway of Ginseng, which including Starch and sucrose, Lipid Biosynthesis, TCA, Calvin cycle and Photorespiration pathways.

9376 metabolites in Ginseng), most of the metabolites could not be identified using available metabolite databases. To overcome these shortcomings, we performed a cross-validation to assess the metabolic pathways altered in Ginseng during the cultivation process by transcriptomics.

Firstly, the mRNA distributions of 2- and 4-year groups were similar and scattered, which reflected similar but unstable growth regulation in the early period. Nevertheless, from year 6 to year 8, after transplantation in year 4, mRNA levels in Ginseng tended to be stable thereafter. Thus, we presumed that transplantation might be of benefit in stabilising the biological characteristics of Ginseng. On the other hand, the 2 vs 4 comparison yielded fewer DEGs and DAMs, which also explains why ginsenoside accumulation was slow in the early stages. However, the huge differences in mRNA levels between year 4 and year 6 groups indicates that transplantation perturbed the mRNA distribution of Ginseng, thereby altering metabolic pathways. In summary, Ginseng exhibited stable expression (mRNA) and metabolic (metabolite) characteristics during the first 4 years in the field [26]. To further explore the changes in metabolic pathways, and identify metabolite signatures of Ginseng before and after transplantation, we built a metabolic pathways map based on the DEG profiles. Based on the MapMan mapping data and gene data annotated using the transcriptome results, we constructed a primary metabolic cycle containing five metabolic pathways including starch and sucrose, lipid biosynthesis, the TCA, the Calvin cycle, and photorespiration, which are linked by crosstalk metabolites.

The results revealed numerous DEGs involved in branched-chain metabolism related to glycolysis (pyruvate→lactate, pyruvate→acetaldehyde), which are hub metabolic nodes in TCA→starch and sucrose pathways. The glycolysis pathway mainly transfers free energy released from ATP hydrolysis [27]. Similar as Kim's research [16], hence we proved that glycolysis is a hub primary metabolism hub for ginsenoside accumulation, which is closely correlated with changes in culture environment.

Ginsenosides are mainly accumulated in roots [28]. Blocking of glycolysis and mitochondrial oxidative phosphorylation, the predominant sugar utilisation processes, would result in arrested or even completely blocked root growth [29]. On the other hand, appropriate exogenously supplied sugars stimulate main root elongation in Ginseng. Ginseng growth requires a substantial amount of sugars to maintain important physiological functions, involving an array of carbohydrate-active enzymes (CAZymes) that break down sugars and drive nutrition, thus providing energy precursors and compounds for Ginseng growth [30]. As shown in Fig. 4, the whole sugar metabolic cycle was activated, and hub links in the cycle included DP-glucose→-glucose-1-P→glucose-6-P (glycolytic pathway), and glucose-6-P→-fructose-6p→fructose-2, 6p (pentose phosphate pathway, PPP), with the former providing the initial energy for the latter [31].

Roots of Ginseng are distributed deep in soil, resulting in an insufficient oxygen supply. However, it is remarkable that the DEGs involved in the glucose-1-P→glucose-6-P step exhibited similar expression in different age groups, and the number of DEGs involved in the fructose-6p→fructose-2,6p step was increased significantly in the 4 vs. 6 comparison compared with the other two comparisons. This suggests that transplantation may affect the metabolic characteristics of the PPP, and following activation of the PPP, accumulation of ginsenosides is accelerated significantly. Throughout the whole cultivation process of Ginseng, there were no significant DEGs related to the sugar metabolic pathway (glucase-1-P→ADP-glucose→amylose→starch→sucrose-6-P→sucrose→fructose), hence it can be inferred that the PPP in Ginseng roots is the main pathway affected by transplantation. Interestingly, despite it being the most important functional pathway for plant growth, there were no significant DEGs related to the TCA cycle, except for the malate→oxaloacetate step in the 4 vs. 6 comparison. Oxaloacetate is an important crosstalk metabolite linking the TCA cycle and the glycolysis

pathway, and it initiates the pyruvate metabolic pathway. We screened numerous DEGs in the 4 vs. 6 comparison correlated with oxaloacetate [32]. It was evident that Ginseng maintained stable TCA activity during the whole cultivation process, despite the environmental shift, but the glycolysis pathway was more sensitive to the change in environment, and its initiator is oxaloacetate in the TCA cycle.

In lipid metabolism, the most active step was ACP→malonyl-ACP, which is the initiator of fatty acid synthesis. In 2 vs. 4 and 4 vs. 6 comparisons, we screened more DEGs correlated with lipid metabolism than in the 6 vs. 8 comparison, indicating that fatty acid synthesis was more active in the early and middle stages of Ginseng development, but stable in the latter stages. The combined results of ginsenoside accumulation and lipid metabolism characteristics is similar as Han's study [33], which indicate that stable fatty acid synthesis is conducive to the formation of ginsenosides in the latter stages.

Unlike carbon and lipid metabolism, limited changes were observed in phospholipid metabolism; only a few DEGs identified in the 2 vs. 4 comparison were linked to the Calvin cycle. The main roles of the Calvin cycle are the fixation and assimilation of CO_2 [34], the synthesis of important intermediates [35], and maintaining energy and REDOX homeostasis [36]. There was a weak relationship between the Calvin cycle→photorespiration step in the early stages, but the Calvin cycle remained constant in the latter stages.

Overall, the PPP and energy metabolism serve as the most important regulatory links for ginsenoside accumulation, and the central steps in these two pathways are sensitive to changes in environmental/cultivation conditions, hence they can be considered hub metabolic links for T-GS cultivation. Furthermore, pyruvate, lactate and acetaldehyde are the hub-DAMs in the Ginseng transplantation process.

Interestingly, there were no significant amino acids and their intermediates screened during the whole process (asparagine and glutamate serve as the starting points). Nitrogen metabolism is closely correlated with photosynthesis in plants [37], and polyamine (spermine, spermidine and arginine) and urea (citrulline) metabolism regulate the photorespiration rates of plants, hence we assessed Ginseng roots in the present study, and identified a few DEGs that participate in nitrogen metabolism.

Previous studies utilised liquid chromatography-mass spectrometry [38], rapid-resolution liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry [39] and nuclear magnetic resonance spectroscopy (^{1}H NMR) [40] to analyse DAMs of Ginseng during cultivation, and they obtained key DEGs. However, the metabolites screened using metabolomics were complex, and it was difficult to evaluate the functional network of these DEGs using simple bioinformatics tools [41]. A specific, modular structure, splitting analysis could not establish links between DEGs and active ingredients in Ginseng. Meanwhile, using transcriptomics alone, it is only possible to speculate the vital physiological functions of Ginseng, since the metabolic landscape revealed is insufficient to illuminate the detailed metabolic processes in Ginseng during the cultivation process. Therefore, we conducted a combined metabolomics and transcriptomics analysis, thereby building up a panoramic metabolic landscape of T-GS from the early stages of field culture, through the middle stage of transplantation, and onto the latter stage of ginsenoside accumulation. Ultimately, we identified hub metabolic links and important DEGs. The findings provide a theoretical basis for the cultivation of T-GS. However, the current study was limited by the specificities of the samples, hence we did not evaluate the ginsenoside components and metabolic pathway features of transplanted/un-transplanted Ginseng and filed/mountain Ginseng. These limitations warrant follow-up work, we will further investigate the accumulative characteristics of the main constituents in ginseng, especially ginsenosides, in different culture conditions over different times. These experiments will disentangle the specific reasons for transplantation, and provide a basis for selection of transplantation time. Through analysis of soil components in field and mountain ginseng experiments, interactions between metabolic characteristics and different environments will be examined. We will then explore the relationships between individual soil components and metabolites during ginseng growth, and thereby provide a reference for transplantation mode, and further optimise the culture conditions. Compared with the other ginsenosides, ginsenoside Rh2 exhibited a flat accumulation trend; during the whole culture process, ginsenoside Rh2 did not present large fluctuations over time and space. This means that deeper analysis of the metabolic pathways and metabolic characteristics of ginsenoside Rh2 are needed in future ginsenoside accumulation research.

Contributions

(I) Conception and design: Wei Yuan; (II) Administrative support: Hui Zhang, Ting-guo Kang; (III) Provision of study materials or patients: Qing-feng Wang, Wen-han Pei, Si-yu Li (IV) Collection and assembly of data: Tian-min Wang, Dan Teng, Qing-feng Wang, Hui-peng Song; (V) Data analysis and interpretation: Wei Yuan, Qing-feng Wang; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Declaration of competing interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

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