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**Growth-year-dependent multi-omics remodeling of triterpenoid saponin accumulation
and rhizosphere microbiota in *Clematis florida* var. *Plena***

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Abstract

Clematis florida var. *plena*, known as Shiershichen in She ethnopharmacy, is an important medicinal plant in Fujian Province, China, yet the biological basis of its growth-year-dependent quality formation remains unclear. Here, we integrated root metabolomics, transcriptomics, and rhizosphere microbiome profiling to compare one-year-old and three-year-old cultivated plants. HPLC analysis showed that three-year-old roots accumulated significantly higher levels of total saponins and oleanolic acid, whereas total flavonoids showed no significant difference. Untargeted LC-MS/MS detected 7,477 metabolites, including 102 pentacyclic triterpenoids and 21 oleanolic-acid-skeleton compounds, with most differentially accumulated triterpenoids and terpenoid glycosides enriched in three-year-old plants. Transcriptome assembly generated 36,981 unigenes and identified 4,043 differentially expressed genes between growth years, with enrichment in terpenoid backbone, flavonoid, phenylpropanoid, monoterpene, and diterpene biosynthesis. Growth-year-associated differences in CYP450, UGT, and hormone-signaling genes provide transcriptomic evidence that triterpenoid scaffold modification, glycosylation, and stress-responsive signaling are candidate processes associated with chemical maturation. Rhizosphere bacterial communities were also reshaped with plant age, and multi-omics networks linked selected microbial taxa with saponin-associated metabolites and gene modules. Together, these findings establish a plant gene expression-bioactive metabolite-rhizosphere microbiome association framework for *C. florida* var. *plena* and provide evidence for harvest-year selection, cultivation optimization, and quality-marker development.

Keywords: *Clematis florida* var. *plena*; triterpenoid saponins; oleanolic acid; multi-omics; rhizosphere microbiome

1.Introduction

Clematis florida var. *plena*, a perennial member of the Ranunculaceae, is a characteristic medicinal plant used in She ethnopharmacy in Fujian Province, China. Its roots and rhizomes, known locally as Shiershichen, have traditionally been used for anti-inflammatory, antirheumatic, analgesic, and related therapeutic purposes[1-3]. Phytochemical and pharmacological studies have identified triterpenoid saponins, oleanolic-acid-type compounds, alkaloids, and other bioactive secondary metabolites in this species[2,3], providing a chemical basis for its medicinal value. However, how these pharmacologically relevant metabolites accumulate during cultivation remains poorly defined. This limitation is not only mechanistic but also practical, because harvest age directly affects medicinal-plant quality, yet the molecular and ecological criteria for determining the optimal harvest year of *C. florida* var. *plena* remain unresolved.

Growth duration is a major determinant of medicinal quality because it integrates root maturation, biomass allocation, carbon partitioning, defense metabolism, and plant-soil interactions. Studies in perennial medicinal plants show that growth years can substantially alter marker-compound accumulation, biomass production, metabolite composition, and biological activity, thereby influencing the optimal harvest period and quality-control strategy [4,5]. In root- or rhizome-derived medicinal plants, the transition from early establishment to mature storage-organ development is often accompanied by reprogramming of primary and secondary metabolism; integrated metabolomic and transcriptomic studies in *Panax notoginseng* and *Polygonatum odoratum* have shown that growth stage or growth year reshapes saponin-, polysaccharide-, and precursor-related pathways [6,7]. For *C. florida* var. *plena*, the key question is therefore not simply whether older plants differ from younger plants, but whether chemical maturation reflects coordinated changes in metabolite accumulation, biosynthetic gene expression, and rhizosphere microbial assembly. This integrative

perspective is supported by recent work showing that plant secondary metabolites and plant microbiomes can reciprocally influence one another, and that root exudates and medicinal-plant rhizosphere metabolites can shape microbial community structure[8,9]. Establishing these connections is essential for moving harvest-year selection from empirical practice toward biologically informed quality control.

Triterpenoid saponins are central to the medicinal quality of *C. florida* var. *plena*. Their biosynthesis depends on isoprenoid precursor supply from the mevalonate and methylerythritol phosphate pathways, which generate isopentenyl diphosphate and dimethylallyl diphosphate[10,11]. These precursors are converted into prenyl diphosphates and then farnesyl diphosphate, which is condensed by squalene synthase and oxidized by squalene epoxidase to form 2,3-oxidosqualene[12-14]. Oxidosqualene cyclases, including β -amyrin synthase, generate the triterpenoid scaffold, whereas cytochrome P450 monooxygenases and UDP-glycosyltransferases subsequently catalyze scaffold oxidation and glycosylation, producing structurally diverse oleanane-type saponins [15-18]. Because these downstream modifications influence compound diversity, solubility, stability, and bioactivity, CYP450 and UGT families represent key candidate gene layers through which growth duration may shape saponin composition and quality.

Flavonoids provide a second major axis of secondary metabolism in medicinal plants. They arise from phenylpropanoid and flavonoid pathways involving phenylalanine ammonia-lyase, cinnamate 4-hydroxylase, 4-coumarate CoA ligase, chalcone synthase, chalcone isomerase, flavone synthase, isoflavone synthase, and glycosyltransferases [19-21]. These compounds may contribute to antioxidant capacity, stress adaptation, and pharmacological activity. However, flavonoid accumulation may not parallel triterpenoid saponin accumulation during plant maturation. A rigorous assessment of quality formation in *C. florida* var. *plena* therefore requires simultaneous evaluation of total flavonoids, individual flavonoid and

98 flavonoid-glycoside metabolites, and the expression of pathway genes to determine whether
99 flavonoid metabolism responds to growth year in concert with, or independently from,
100 triterpenoid metabolism.

101 Medicinal quality is also shaped by the rhizosphere, where roots interact continuously with
102 microorganisms through exudation, nutrient exchange, immune signaling, and microbial
103 elicitation. Rhizosphere microorganisms can influence plant growth, phenology, nutrient
104 acquisition, stress tolerance, and secondary-metabolite biosynthesis[22-26]. In turn, plant
105 developmental stage, root physiology, and metabolite secretion can selectively restructure
106 microbial communities. This reciprocal relationship creates a plant-microbe-metabolite
107 feedback system in which growth duration may affect medicinal quality not only through
108 intrinsic transcriptional regulation but also through age-dependent microbial recruitment. For
109 medicinal plants, such interactions are particularly relevant because specific microbial taxa
110 may be associated with the accumulation, transformation, or stabilization of
111 pharmacologically active compounds.

112 Multi-omics integration provides a direct strategy for resolving the interconnected processes
113 that shape medicinal-plant quality. Metabolomics captures the chemical phenotype that
114 underlies medicinal value and quality control, whereas transcriptomics identifies growth- or
115 condition-responsive biosynthetic genes, regulatory modules, and candidate pathways,
116 particularly in non-model medicinal plants with limited genomic resources[27-29].
117 Rhizosphere microbiome profiling, commonly based on 16S rRNA amplicon sequencing,
118 further defines microbial restructuring around the root system and allows plant metabolic
119 variation to be interpreted in an ecological context[8]. Joint analysis of these layers can reveal
120 candidate relationships among bioactive metabolite accumulation, plant gene-expression
121 modules, and microbial abundance, as shown in recent studies integrating transcriptome,
122 metabolome, and microbiome data in medicinal plants such as *Psammosilene tunicoides*,

Codonopsis Radix, and root-associated plant systems[30,31]. This framework is especially important for non-model medicinal plants such as *C. florida* var. *plena*, for which reference genomic resources, experimentally validated pathway genes, and cultivation-based quality markers remain limited.

Here, we compared one-year-old and three-year-old cultivated *C. florida* var. *plena* using integrated root metabolomic, root transcriptomic, and rhizosphere microbiome analyses. We asked whether major active components, particularly total saponins, oleanolic acid, and flavonoids, differ between growth years; whether triterpenoid saponin and flavonoid accumulation is associated with coordinated changes in biosynthetic, modification, transport, and hormone-signaling genes; and whether growth-year-dependent rhizosphere restructuring is linked to saponin-associated metabolites and gene modules. By integrating these layers, we aimed to establish a gene expression, bioactive metabolite, rhizosphere microecology framework for explaining quality formation in *C. florida* var. *plena* and for guiding standardized cultivation, harvest-year selection, quality improvement, and medicinal-resource development.

2. Materials and methods

2.1 Plant and rhizosphere soil materials

Plant materials (roots of artificially cultivated *Clematis florida* var. *Plena*) and corresponding rhizosphere soil samples were collected in October 2022 from the She-medicine ecological garden of Fu'an Lvya She-medicine Planting Professional Cooperative, Longxin Village, Xibing Town, Fu'an City, Ningde, Fujian Province, China. Written permission for sample collection was obtained from the land-owner, Fu'an Lvya She-medicine Planting Professional Cooperative, prior to sampling. One-year-old plants were designated as GY, and three-year-old plants were designated as GS. The two growth-year groups were cultivated in separate fields within Longxin Village. Species identity was confirmed after sampling. A total of 39 plants

were selected for the study. Fresh roots were excised from each plant and divided into two portions. One portion was immediately frozen in liquid nitrogen and stored at -80°C for RNA extraction. Rhizosphere soil tightly adhering to the roots was collected aseptically for microbial DNA extraction. The remaining root material was placed in airtight containers and stored for metabolite analysis.

2.2 Total RNA extraction, library preparation, and transcriptome sequencing

Total RNA was extracted from frozen root tissues of GY and GS plants. Root samples were ground to a fine powder in liquid nitrogen, and RNA was isolated using TRIzol® Reagent (TaKaRa Bio. Inc., Dalian, China) according to the manufacturer's protocol. RNA purity and concentration were assessed with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, MA, USA). RNA quality was examined by agarose gel electrophoresis, and RNA integrity was evaluated using an Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA). Poly(A) RNA was enriched using oligo(dT) primers and used for cDNA library construction with the TruSeq™ RNA Sample Preparation Kit (Illumina, San Diego, USA). Library concentration was measured with a TBS380 fluorometer (Promega, WI, USA). Paired-end sequencing was performed on an Illumina HiSeq platform to generate 150 bp reads.

2.3 Transcriptome processing, assembly, and functional annotation

Raw reads in FASTQ format were filtered using Trimmomatic to remove adapter sequences, reads containing poly-N regions, and low-quality reads. The remaining clean reads were assembled de novo with Trinity v2.4 using the paired-end assembly mode. Contigs were further clustered and assembled into transcripts, and the longest representative sequence was retained as a unigene for downstream analysis. Unigenes were functionally annotated by alignment against the NCBI non-redundant protein database, Swiss-Prot, eggNOG, and KOG databases using DIAMOND, with an e-value cutoff of 1×10^{-5} . Functional assignments were based on the best-matched protein hit. KEGG pathway annotation was performed by mapping

unigenes to the Kyoto Encyclopedia of Genes and Genomes database. Gene Ontology annotations were assigned using Swiss-Prot-to-GO mapping. Clean reads from each sample were mapped back to the assembled unigenes using Bowtie2. Gene expression levels were calculated as fragments per kilobase of transcript per million mapped reads using eXpress . Differentially expressed genes between GY and GS were identified with DESeq2. Genes with an adjusted P value < 0.05 and $|\log_2 \text{fold change}| > 1$ were considered differentially expressed. Hierarchical clustering of differentially expressed genes was performed in R (v3.2.0). GO and KEGG enrichment analyses were conducted using a hypergeometric test, and significant enrichment results were visualized as bar plots and bubble plots in R (v3.2.0).

2.4 Microbial DNA extraction and 16S rRNA gene sequencing

Genomic DNA was extracted from rhizosphere soil using the MagPure Soil DNA LQ Kit (Magen Biotechnology, Guangzhou, China) according to the manufacturer's instructions. DNA concentration and purity were measured with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, MA, USA), and DNA integrity was checked by agarose gel electrophoresis. Extracted DNA was stored at -20°C until PCR amplification. The V3-V4 region of the bacterial 16S rRNA gene was amplified using barcoded primers 343F (5'-TACGGRAGGCAGCAG-3') and 798R (5'-AGGGTATCTAATCCT-3'). PCR amplification was performed using Takara Ex Taq DNA polymerase (TaKaRa Bio. Inc., Dalian, China). Amplicons were purified, pooled, and sequenced for bacterial community profiling.

2.5 Microbial sequence processing and community analysis

Raw paired-end reads were processed in FASTQ format. Adapter sequences were removed with Cutadapt, and low-quality reads were filtered. Denoising, paired-read merging, chimera removal, and amplicon sequence variant inference were performed with DADA2 in QIIME2 using default parameters. Representative ASV sequences and an ASV abundance table were

generated for downstream analysis. Taxonomic classification was performed against the SILVA database v138 using the q2-feature-classifier plugin. Alpha diversity was evaluated using the Chao1 and Shannon indices. Beta diversity was assessed using unweighted UniFrac distances, followed by principal coordinates analysis. Differences in microbial diversity and taxonomic abundance between groups were tested in R using the Wilcoxon rank-sum test. Differentially enriched taxa were further identified using linear discriminant analysis effect size analysis.

2.6 Metabolite extraction

Root samples stored at -80°C were thawed at room temperature before extraction. For each sample, 60 mg of tissue was mixed with 600 µL of methanol containing L-2-chlorophenylalanine as an internal standard at 0.06 mg/mL. The mixture was vortexed for 10 s, extracted by ultrasonication in an ice-water bath for 10 min, and incubated at -20°C for 30 min. Samples were then centrifuged at 13,000 rpm for 10 min at 4°C. The supernatant was collected, filtered through a 0.22-µm membrane, transferred to LC vials, and stored at -80°C until LC-MS analysis. A pooled quality-control sample was prepared by mixing equal aliquots from all samples.

2.7 LC-MS/MS metabolomic profiling

Metabolomic profiling was performed using an ACQUITY UPLC I-Class Plus system coupled to a Q-Exactive mass spectrometer equipped with a heated electrospray ionization source (Thermo Fisher Scientific, MA, USA). Samples were analyzed in both positive and negative ionization modes. Chromatographic separation was carried out on an ACQUITY UPLC HSS T3 column (1.8 µm, 2.1 × 100 mm) (Waters, MA, USA). The mobile phases consisted of water with 0.1% formic acid as solvent A and acetonitrile with 0.1% formic acid as solvent B. The gradient program was as follows: 5% B at 0.01 min, held for 2 min; 30% B at 4 min; 50% B at 8 min; 80% B at 10 min; 100% B at 14 min, held until 15 min; and

returned to 5% B at 15.1 min, followed by equilibration until 16 min. The flow rate was 0.35 mL/min, the column temperature was maintained at 45°C, the autosampler temperature was set at 10°C, and the injection volume was 2 µL.

2.8 Statistical analysis

Statistical analyses were conducted for phytochemical, metabolomic, transcriptomic, and microbial datasets before multi-omics integration. Differences between one-year-old and three-year-old plants were tested using Student's t-test or the Wilcoxon rank-sum test, with significance set at $P < 0.05$. Differentially accumulated metabolites were identified using VIP > 1.0 and $P < 0.05$. Differentially expressed genes were detected with DESeq2 using adjusted $P < 0.05$ and $|\log_2 \text{fold change}| > 1$. GO and KEGG enrichment analyses were performed using a hypergeometric test. Microbial alpha diversity was compared using the Wilcoxon rank-sum test, and beta diversity was assessed using unweighted UniFrac distances and principal coordinates analysis. Differential taxa were identified using LEfSe. Multi-omics associations among metabolites, gene modules, and microbial taxa were evaluated using correlation-based network analysis and WGCNA. Correlation results were interpreted as associations rather than evidence of causality.

3. Results

3.1 Integrated profiling strategy and active-component accumulation in *C. florida* var. *plena*

To examine how cultivation age shapes quality formation in *Clematis florida* var. *plena*, we collected roots and matched rhizosphere soils from one-year-old (GY) and three-year-old (GS) cultivated plants. The study combined targeted and untargeted metabolite profiling of roots with root RNA sequencing and 16S rRNA profiling of rhizosphere bacterial communities. This framework enabled parallel assessment of medically relevant metabolite accumulation,

biosynthetic gene expression, and age-associated shifts in the rhizosphere microbiome (Figure 1).

HPLC analysis first established the targeted phytochemical differences between growth years.

Compared with GY roots, GS roots accumulated significantly higher levels of total saponins (t-test, $P < 0.01$; Figure 2A) and oleanolic acid (t-test, $P < 0.01$; Figure 2B). In contrast, total flavonoid content did not differ significantly between GY and GS roots (Figure S1).

Untargeted LC-MS/MS profiling further resolved the broader metabolic differences underlying this targeted phytochemical pattern. Multivariate analysis separated GY and GS root samples, indicating distinct growth-year-associated metabolic profiles (Figure 2C).

Consistently, volcano analysis showed extensive metabolite-level variation between the two groups (Figure 2D). Across all samples, 7,477 metabolites were detected and assigned to 20 major categories (Table S1), including lipids and lipid-like molecules, phenylpropanoids and polyketides, organic acids and derivatives, organic heterocyclic compounds, organic oxygen compounds, alkaloids and derivatives, nucleosides and analogs, lignans and related compounds, and other metabolite classes.

Classification of metabolite abundance showed that amino acids, peptides and analogs, triterpenoids and terpene glycosides, carbohydrates and carbohydrate conjugates, and fatty acids and conjugates represented major detected classes (Figure 2E). Differential metabolite screening using $VIP > 1.00$ and $P < 0.05$ identified 452 differentially accumulated metabolites across 102 metabolite classes (Table S5), including 36 flavonoids and flavonoid glycosides, 31 triterpenoids and terpenoid glycosides, 35 amino acids, peptides and analogs, 41 carbohydrates and conjugates, 23 fatty acids and conjugates, and additional compound groups. Most differentially accumulated triterpenoids and terpenoid glycosides showed higher relative abundance in GS than in GY roots (Figure 2F). Further structural classification identified 102

pentacyclic triterpenoids (Table S3), including 21 compounds with an oleanolic-acid core skeleton (Figure 2G; Table S4).

3.2 Transcriptome assembly and growth-year-responsive genes

To test whether growth-year-dependent metabolite accumulation was accompanied by transcriptional reprogramming, we sequenced, assembled, and annotated root transcriptomes from GY and GS plants. De novo assembly yielded 36,981 unigenes with a total length of 44,667,931 bp, a mean length of 1,207.83 bp, and an N50 of 1,757 bp. The assembly included 28,591 predicted coding sequences and 7,562 simple sequence repeats (Table S7). Functional annotation assigned 61.86%, 46.57%, 13.46%, 35.96%, 39.76%, 56.81%, and 42.74% of the unigenes to the NR, Swiss-Prot, KEGG, KOG, GO, eggNOG, and Pfam databases, respectively (Table S8). Among the annotated sequences, 4,976 unigenes were mapped to KEGG pathways, including 3,964 assigned to metabolism. Notably, 194 unigenes, representing 3.90% of KEGG-annotated unigenes, were associated with terpenoid and polyketide metabolism. GO classification further placed 8,215 unigenes in the metabolic-process category, accounting for 55.87% of GO-annotated assignments (Figure S2B). Comparative expression analysis identified 4,043 differentially expressed genes between GY and GS roots, with 1,312 upregulated and 2,731 downregulated genes. Principal component analysis and hierarchical clustering separated the two growth-year groups (Figure 3A, 3B). Enrichment analysis linked these DEGs to secondary-metabolism pathways, including terpenoid backbone biosynthesis, monoterpene and diterpene biosynthesis, flavonoid biosynthesis, and phenylpropanoid biosynthesis (Figure 3C, 3D).

3.3 CYP450, UGT, and hormone-signaling genes define candidate regulatory layers

Because triterpenoid and flavonoid diversity is largely generated through scaffold oxidation and glycosylation, we next examined differentially expressed genes encoding CYP450 and UGT enzymes. Genes assigned to terpenoid and polyketide metabolism showed clear

growth-year-specific expression patterns (Figure 3E). Among the DEGs, 32 CYP450 genes differed between GY and GS roots, including five annotated in terpenoid and polyketide metabolism (Figure 3F). These CYP450s were associated with carotenoid biosynthesis, brassinosteroid biosynthesis, and zeatin biosynthesis. CYP450 genes linked to carotenoid biosynthesis were more highly expressed in GY, whereas one CYP450 associated with flavonoid and phenylpropanoid biosynthesis was more highly expressed in GS. UGT-related DEGs, mainly assigned to flavone, flavonol, and phenylpropanoid metabolism, also displayed growth-year-dependent expression patterns (Figure 3G). Mapped expression profiles of triterpenoid and flavonoid biosynthetic pathways provided additional evidence of growth-year-dependent pathway remodeling (Figure 4A, 4B). In parallel, genes associated with jasmonic acid, abscisic acid, cytokinin, auxin, gibberellin, and salicylic acid signaling differed between GY and GS roots. Several signaling components, including MYC2, AHP, A-ARR, GH3/SAUR, GID1/2, and TGA, showed higher expression in GS.

3.4 Rhizosphere microbial communities are reshaped with growth year

To assess whether cultivation age was accompanied by changes in the rhizosphere microbiome, we performed 16S rRNA sequencing on rhizosphere soil from GY and GS plants. The dataset comprised 2,522,004 high-quality reads from 34 rhizosphere samples and resolved 10,475 OTUs. Alpha-diversity analysis showed no significant difference in microbial richness between the two growth-year groups (Figure 5A). Core-taxon analysis further indicated that a large fraction of the dominant microbiome was shared across samples, with shared dominant taxa accounting for approximately 71.43% at the genus level and 97.96% at the phylum level (Figure 5B). The dominant genera included *Candidatus Solibacter*, *Bryobacter*, *Sphingomonas*, *Gaiella*, *Gemmatimonas*, and *Dongia* (Figure 5C). At the phylum level, Actinobacteriota, Proteobacteria, Acidobacteriota, Gemmatimonadota, Bacteroidota,

Myxococcota, Nitrospirota, Verrucomicrobiota, Firmicutes, Chloroflexi, and several lower-abundance phyla formed the major rhizosphere community fraction (Figure 5D). Despite this broadly conserved core community, several phyla differed significantly between GY and GS rhizospheres (Figure 5E). Actinobacteriota and Bacteroidota were more abundant in GY, whereas RCP2-54, Entothaeonellaeota, Myxococcota, Acidobacteriota, Verrucomicrobiota, and Nitrospirota were lower in GY than in GS ($P < 0.05$; Figure 5E). Community-level ordination also separated the two growth-year groups (Figure 6A). At finer taxonomic resolution, *Kribbella* sp., *Streptomyces durhamensis*, *Marmoricola* sp., *Hamadaea tsunoensis*, *Nocardioidea* sp., *Arthrobacter* sp., *Micromonospora echinospora*, *Flavisolibacter* sp., *Sinomonas atrocyanea*, and *Streptomyces griseorubiginosus* showed marked abundance differences (Figure 6B, 6C). LEfSe analysis identified additional discriminant lineages, including Bacteroidia, Nocardioideae, Propionibacteriales, Micromonosporaceae, Micromonosporales, Actinobacteria, and Actinobacteriota (Figure 6D, 6E).

3.5 Microbe-metabolite and microbe-gene networks identify candidate regulatory associations

To examine whether the age-associated shift in rhizosphere composition was linked to root metabolic variation, we constructed microbe-metabolite correlation networks. At the species level, the strongest associations involved members of the uncultured Myxococcales OM27 clade, *Agromyces*, *Rhizocola*, *Streptomyces griseorubiginosus*, and *Actinophytocola*, together with metabolites such as 18-carboxy-19,20-dinor-leukotriene E4, 4-hydroxybutyric acid, bisindolylmaleimide IV, cadaverine, and D-maltose (Figure 7A-C). A genus-level network further connected 23 bacterial taxa with 81 metabolites. Prominent genera in this network included *Streptococcus*, *Lysobacter*, *Absconditabacteriales* (SR1), *Flavitalea*, and *Adhaeribacter*, whereas associated metabolite features included Niazimin A, prazosin, serotonin, dihydrouracil, poriolide, malvidin 3-(6"-acetylglucoside)-5-glucoside, D-maltose,

and Melilotoside D (Figure 7D-F). A more stringent network, constructed using $|r| > 0.9$ and $P < 0.01$, linked 17 microbial taxa with 24 significantly altered metabolites (Figure 7G). *Prevotella*, *Kribbella*, *Flavitalea*, and other taxa were associated with metabolites including 4-hydroxybutyric acid, 3-[3-(sulfooxy)phenyl]propanoic acid, 10-deacetyl-7-xylosyltaxol C, bisphenol A monomethyl ether, and diisodityrosine.

Gene-microbe correlation analysis provided an additional layer of evidence for coordinated changes in root transcriptional activity and rhizosphere composition. The strongest pairwise associations included *Aeromicrobium* with *TRINITY_DN18384_c0_g1_i2_3*, *Flavobacterium* with *TRINITY_DN22825_c0_g1_i1_1*, and *Galbitalea* with *TRINITY_DN18671_c0_g2_i1_1*, *TRINITY_DN17494_c0_g1_i8_1*, and *TRINITY_DN4027_c0_g1_i1_1* (Figure 8A). Network analysis highlighted *Galbitalea*, *Ohtaekwangia*, *Flavobacterium*, *Aeromicrobium*, and JG36-GS-52 as prominent microbial nodes, whereas *TRINITY_DN1557_c0_g1_i1_1*, *TRINITY_DN24360_c0_g1_i1_1*, *TRINITY_DN1409_c0_g1_i1_1*, *TRINITY_DN23377_c0_g1_i1_1*, and *TRINITY_DN16979_c0_g1_i1_1* represented highly connected gene nodes (Figure 8B, 8C). A broader co-occurrence network contained 160 nodes and 1,093 edges, dominated by positive associations (1,089 positive and four negative edges; Figure 8D). Highly connected genera included JG36-GS-52, *Aeromonas*, *Novosphingobium*, *Caulobacter*, *Altererythrobacter*, *Actinoplanes*, *Flavobacterium*, *Agromyces*, *Mycobacterium*, *Dinghuibacter*, *Lapillicoccus*, and *Nocardioides*.

3.6 Gene and microbial modules associated with saponin accumulation

We next used WGCNA to identify gene co-expression and microbial co-abundance modules associated with saponin accumulation. The analysis resolved 31 gene modules and four microbial modules (Figure 9A, 9D). Among them, 16 gene modules and one microbial module showed significant positive or negative correlations with saponin-related metabolite features (Figure 9B, 9E). The module analysis focused on eight oleanane-type or

saponin-associated features oleanolic acid 3-[rhamnosyl-(1→4)-glucosyl-(1→6)-glucoside], oleanolic acid, beta-hederin, a 28-[glucosyl-(1→6)-glucosyl]-annotated derivative, oleanolic acid 3-arabinoside, Pitheduloside B, oleanolic acid 3-[glucosyl-(1→4)-xyloside], and Pitheduloside F. The Oleanolic acid 3-[rhamnosyl-(1→4)-glucosyl-(1→6)-glucoside] was associated with 11 gene modules, oleanolic acid with seven, beta-hederin with eight, oleanolic acid 3-arabinoside with four, Pitheduloside B with six, oleanolic acid 3-[glucosyl-(1→4)-xyloside] with six gene modules and one microbial module, and Pitheduloside F with five modules. Several gene modules, including salmon2, chocolate3, darkturquoise, brown2, blue, coral3, coral, and darkseagreen2, together with the microbial blue module, showed broad positive associations with most saponin-related features. Additional positive correlations were observed for the darkorange, lightcyan, plum4, navajowhite1, lavenderblush1, coral2, and skyblue modules. Other modules were negatively correlated with saponin content, indicating that saponin accumulation is linked to both activated and repressed co-expression programs (Figure 9C, 9F). Across the saponin-associated modules, 45 hub genes were identified in the blue3, brown2, coral2, coral3, darkseagreen2, lavenderblush1, lightcyan, plum4, and salmon2 modules, whereas five central microorganisms were detected in the turquoise microbial module (Fig. 9G).

Discussion

This study provides a multi-layered framework for understanding growth-year-dependent quality formation in *C. florida* var. *plena*, a medicinal plant used in She ethnopharmacy. The most consistent chemical signature was the enrichment of total saponins and oleanolic acid in three-year-old roots, whereas total flavonoid content remained comparatively stable. This distinction is biologically important because it suggests that cultivation duration does not uniformly increase all specialized metabolites. Instead, root maturation appears to preferentially strengthen the triterpenoid saponin branch, which is closely aligned with the

known pharmacological properties of Clematis species[2,3,32,33]. The combined metabolomic, transcriptomic, and rhizosphere microbiome data therefore support a model in which medicinal quality emerges from the interaction between developmental maturation, secondary-metabolic capacity, compound modification, and plant-microbe associations.

The increase in total saponins and oleanolic acid in GS roots has direct implications for medicinal quality assessment. The enrichment of saponins in three-year-old roots is consistent with previous pharmacological studies on *C. florida* and *C. florida* var. *plena*. Total saponins from plena clematis have been reported to inhibit the growth of several human tumor cell lines, including EC9706, KB, BGC-823, and HepG-2 cells[2]. In addition, triterpene saponins isolated from *C. florida* showed anti-inflammatory activity in both in vitro and in vivo assays [32]. More recently, topical application of *C. florida* was investigated in rheumatoid arthritis-related models, where treatment reduced inflammatory indicators, including IL-6, COX-2, TNF- α , IL-1 β , and rheumatoid factor, and alleviated inflammatory or arthritic phenotypes[34]. These studies strengthen the biological relevance of the present findings: the metabolites enriched in mature roots belong to the same chemical class most directly associated with the reported anti-inflammatory, antirheumatic, and cytotoxic activities of *C. florida*.

The present metabolomic analysis extends earlier phytochemical work by resolving the triterpenoid pool in greater detail. The detection of 102 pentacyclic triterpenoids, including 21 oleanolic-acid-skeleton compounds, expands the chemical framework for evaluating *C. florida* var. *plena*. More importantly, most differentially accumulated triterpenoids and terpenoid glycosides were enriched in GS, supporting the interpretation that the third cultivation year represents a more chemically mature stage for saponin accumulation. This distinction matters because different saponin derivatives may differ substantially in polarity, membrane activity, stability, pharmacokinetics, and bioactivity. Related studies in Clematis

chinensis have identified multiple triterpene saponins from roots and rhizomes and linked them to potential anti-inflammatory activity[35]. Other work on *C. chinensis* also showed that several triterpenoid saponins inhibited TNF- α secretion in lipopolysaccharide-stimulated murine macrophages, suggesting possible relevance to autoimmune or inflammatory disorders [36]. Cytotoxic triterpenoid saponins have also been reported from *Clematis tangutica*, where individual saponins showed activity against human cancer cell lines [37]. These comparisons indicate that the medicinal relevance of *Clematis* saponins is determined not only by total abundance but also by the structural composition of the saponin pool.

Similar age-dependent shifts have been reported in perennial medicinal plants such as *Polygala tenuifolia*, *Panax* species, and *Dendrobium*, where plant age influences the balance between primary growth, storage metabolism, and accumulation of pharmacologically active secondary metabolites[38-41]. This does not mean that older material is always superior. Rather, each medicinal plant has a species-specific temporal window in which biomass, metabolite concentration, and compound composition jointly determine harvest value. For *C. florida* var. *plena*, the comparison between one-year-old and three-year-old plants indicates that the third cultivation year represents a more advanced stage for oleanolic-acid-type saponin accumulation. Whether this stage is optimal for harvest requires additional growth years, because saponin accumulation may increase gradually, plateau, or decline after reaching a developmental peak.

The transcriptomic results suggest that chemical maturation in *C. florida* var. *plena* is unlikely to be controlled by a single upstream biosynthetic step. Differentially expressed genes were enriched in terpenoid backbone biosynthesis, monoterpenoid and diterpenoid biosynthesis, flavonoid biosynthesis, and phenylpropanoid biosynthesis, indicating broad reorganization of secondary metabolism during root maturation. Genes involved in precursor supply, including those in the MVA and MEP pathways, may influence the availability of IPP and DMAPP, the

core isoprenoid building blocks for triterpenoid biosynthesis [42-44]. However, the expression pattern of upstream genes such as DXS alone does not explain the higher saponin content in GS. This point is mechanistically important because oleanane-type saponin accumulation depends not only on precursor flux and scaffold formation, but also on downstream oxidation, glycosylation, transport, and storage[45,46]. The observed growth-year-associated changes in CYP450 and UGT genes therefore provide a more plausible transcriptional layer for explaining saponin diversification. CYP450 enzymes introduce oxidative modifications on triterpenoid scaffolds, whereas UGTs determine glycosylation patterns that affect solubility, stability, cellular compartmentalization, and biological activity[45,47]. Thus, saponin enrichment in mature roots may reflect coordinated changes in pathway flux and compound decoration rather than simple activation of a single biosynthetic gene.

Hormone signaling provides another mechanistic bridge between developmental age and secondary metabolism. Jasmonic acid and salicylic acid are widely implicated in defense-associated specialized metabolism, whereas abscisic acid, auxin, cytokinin, and gibberellin coordinate root development, stress adaptation, and source-sink balance [49-53]. The higher expression of genes associated with MYC2, TGA, GH3/SAUR, AHP, A-ARR, and GID1/2 in three-year-old roots suggests that mature roots may maintain a signaling environment more favorable to specialized metabolite accumulation. This interpretation is consistent with studies in *Glycyrrhiza*, *Panax*, and other medicinal plants, where jasmonate or salicylate treatments enhance triterpenoid saponin production[52,53]. Nevertheless, the present evidence remains correlative. Hormone-treatment experiments, inhibitor assays, time-course sampling, and targeted quantification of downstream saponins are needed to determine whether these signaling components directly regulate saponin biosynthetic genes in *C. florida* var. *plena*.

469 The rhizosphere microbiome adds an ecological layer to this quality-formation model. The
 470 absence of a major alpha-diversity difference indicates that cultivation age did not simply
 471 increase or decrease bacterial richness. Instead, the principal difference was taxonomic
 472 composition. This pattern is biologically plausible because root maturation changes exudate
 473 chemistry, nutrient demand, tissue turnover, and immune signaling, all of which can alter
 474 microbial recruitment[22-26,57]. The persistence of dominant genera such as *Candidatus*
 475 *Solibacter*, *Bryobacter*, *Sphingomonas*, *Gaiella*, *Gemmatimonas*, and *Dongia* suggests a
 476 shared microbial background across cultivation years, whereas differentially abundant taxa
 477 indicate age-associated restructuring. The mature rhizosphere should therefore be interpreted
 478 as compositionally reconfigured, not simply richer or poorer.

479 The microbe-metabolite and gene-microbe networks further suggest that rhizosphere
 480 restructuring is linked to the metabolic state of the root. Taxa such as *Lysobacter*, *Flavitalea*,
 481 *Adhaeribacter*, *Kribbella*, *Flavobacterium*, *Aeromicrobium*, *Galbitalea*, and *Dinghuibacter*
 482 were associated with metabolite features or gene modules connected to secondary metabolism.
 483 These associations are not evidence of direct microbial regulation, but they are biologically
 484 credible. Rhizosphere bacteria can affect secondary metabolism through nutrient mobilization,
 485 phytohormone production, immune elicitation, and modulation of root
 486 exudation[22,24,25,34,54-56]. Conversely, saponins, flavonoids, and phenylpropanoid
 487 derivatives may act as selective chemical filters that shape microbial community composition.
 488 The observed networks may therefore reflect reciprocal coupling: mature roots accumulate
 489 more triterpenoid saponins, while their altered chemical environment recruits or maintains a
 490 distinct microbial community that may further influence root metabolism.

491 The WGCNA results are particularly useful because they move the analysis from individual
 492 correlations to coordinated gene and microbial modules. Similar module-based approaches
 493 have been used in saponin-producing medicinal plants to identify gene modules and hub

genes associated with triterpenoid saponin accumulation, including candidate CYP450s, UGTs, transcription factors, and other pathway genes[42,58-59]. In the present study, multiple gene modules were associated with oleanolic acid, beta-hederin, Pitheduloside B, Pitheduloside F, and oleanolic-acid glycosides, indicating that saponin accumulation is modular and compound-specific. This interpretation fits the biochemical architecture of triterpenoid saponin biosynthesis, in which different derivatives are generated through distinct combinations of oxidosqualene cyclases, CYP450s, UGTs, transporters, and regulatory factors [42,47,60]. The association of a microbial module with oleanolic acid 3-[glucosyl-(1→4)-xyloside] is especially interesting because plant secondary metabolites and rhizosphere microbiomes can reciprocally shape one another, and recent studies have shown that root metabolites and rhizosphere microbial communities are tightly linked in medicinal or root-associated plant systems [8,61-62].

Despite these constraints, this study advances a biologically coherent model for quality formation in *C. florida var. plena*. Three-year cultivation is associated with higher triterpenoid saponin and oleanolic acid accumulation, together with coordinated changes in secondary-metabolism genes, CYP450 and UGT families, hormone-signaling components, and rhizosphere microbial networks. These findings support the use of growth year as an important quality determinant and provide molecular and microbial candidates for improving standardized cultivation. More broadly, the work illustrates that medicinal-plant quality is not solely a product of plant age or metabolite biosynthesis in isolation; it emerges from the interaction between developmental state, biosynthetic capacity, compound modification, and rhizosphere ecology.

Conclusion

This study demonstrates that growth year is a major determinant of medicinal quality in *C. florida var. plena*. Three-year-old plants accumulated significantly higher total saponins and

oleanolic acid than one-year-old plants, whereas total flavonoid content showed no significant difference. Integrated metabolomic and transcriptomic analyses indicated that this chemical maturation is associated with coordinated changes in triterpenoid- and flavonoid-related pathways, including CYP450, UGT, and hormone-signaling genes. Rhizosphere microbial communities were also reshaped with cultivation duration, and specific bacterial taxa and microbial modules were correlated with triterpenoid-related metabolites and saponin-associated gene modules. These findings support a plant gene expression-bioactive metabolite-rhizosphere microecology model for explaining quality formation in *C. florida* var. *plena*. The results provide theoretical and practical support for standardized cultivation, harvest-year selection, quality improvement, and medicinal-resource development of this Fujian She ethnomedicinal plant.

Declarations

Abbreviations

DEGs, differentially expressed genes; GO, Gene Ontology; DADA2, Deficiency of Adenosine Deaminase 2; LC-MS, Liquid Chromatography–Mass Spectrometry; UPLC, Ultra Performance Liquid Chromatography; HPLC, High-Performance Liquid Chromatography; OPLS-DA, Orthogonal Partial Least Squares Discriminant Analysis; KOG, Eukaryotic Ortholog Groups; KEGG, Kyoto Encyclopedia of Genes and Genomes; PCoA, Principal Coordinates Analysis; TOF-MS, Time-of-Flight Mass Spectrometry

Ethics approval and consent to participate

This study did not involve human participants or animal experiments. The plant and rhizosphere soil samples used in this study were collected from privately-managed cultivated fields. Written sampling permission was formally granted by the land-owner, Fu'an Lvya She-medicine Planting Professional Cooperative (dated 18 August 2022) before sample collection.

Consent for publication

Not applicable, as this manuscript does not contain any data from individual persons.

Availability of data and materials

All data generated and analysed during this study are deposited in publicly accessible repositories .

The transcriptome sequencing data are available in the NCBI BioProject database under accession PRJNA10610870.

The 16S rRNA gene amplicon sequencing data of root-associated microbiomes have been deposited in the Genome Sequence Archive (GSA) of the National Genomics Data Center (NGDC) under accession CRA049334.

The untargeted metabolomics data of *Clematis florida* var. *plena* roots are deposited in the NGDC OMIX database under accession OMIX020183.

All relevant raw data supporting the conclusions of this article are available via the above repository accessions. No restricted data are included in this study.

Competing Interest

The authors declare that they have no known financial or personal relationships that could have influenced the work reported in this paper.

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Authorship contribution

Haitao Zhang conceived the project and experiments; Xinrui Wang acquired the funding; Xinrui Wang and Gong Wang supervised the project; Haitao Zhang and Aizhu Lin analyzed data and performed LC-MS measurements; Haitao Zhang and Lu Chen wrote the manuscript. Xinrui Wang and Aizhu Lin revised the manuscript. Youxiong Zhong, Xudong Zhuang and Mingfeng Cai helped with sample collection.

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Figures and Figure legends

Figure 1

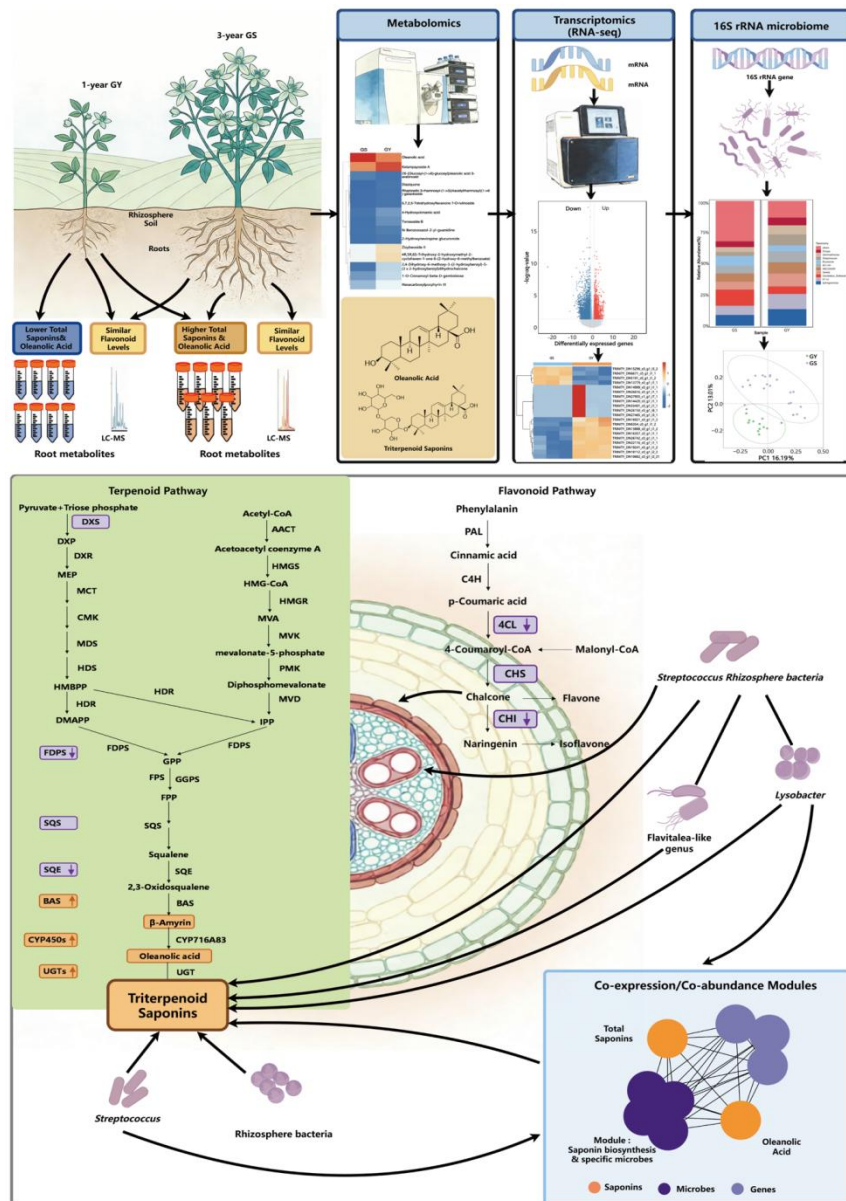


Figure 1. Integrated study design and working model for growth-year-dependent quality formation in *C. florida* var. *plena*. The workflow connects root metabolomics, transcriptomics, and rhizosphere microbiome profiling in one-year-old (GY) and three-year-old (GS) cultivated plants.

Figure 2

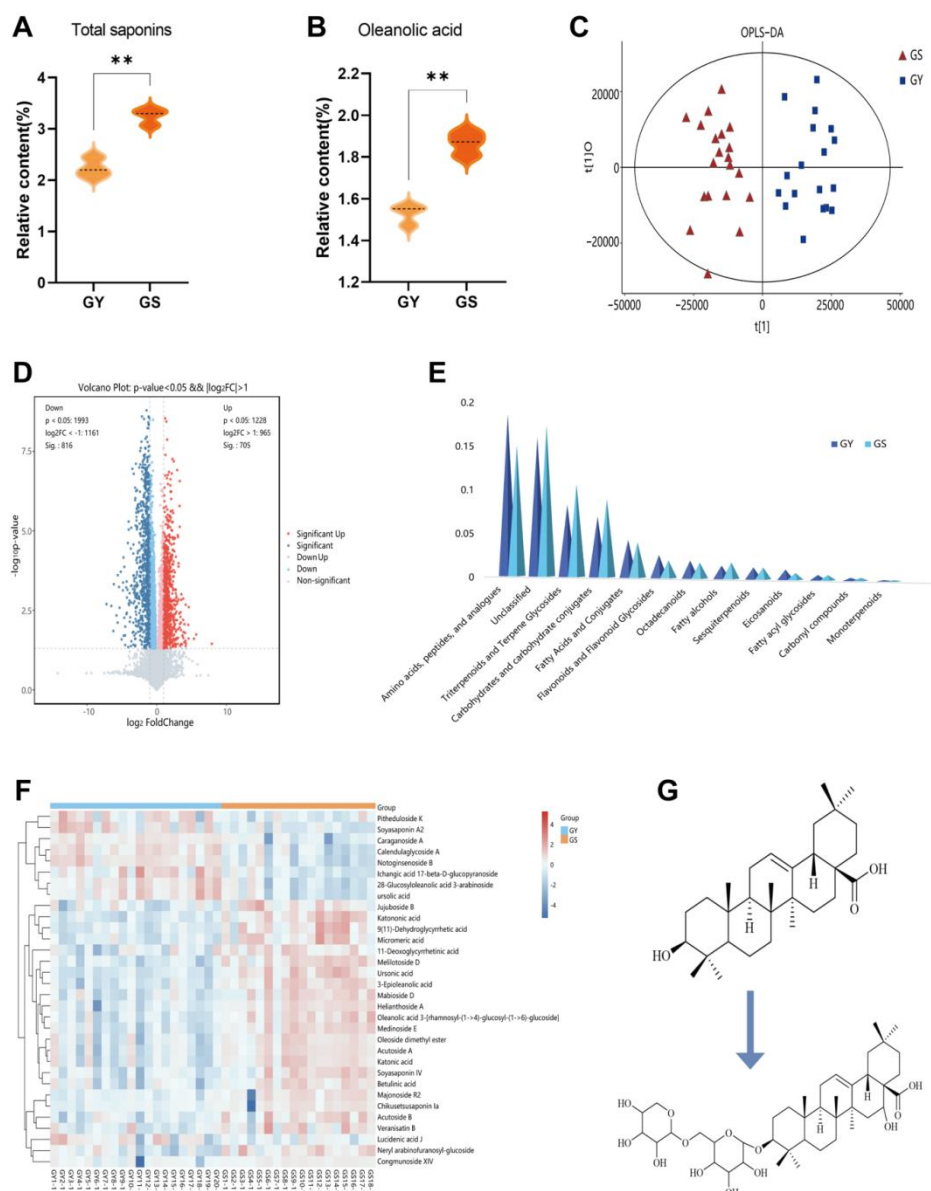


Figure 2. Growth-year-associated metabolite profiles in *C. florida* var. *plena* roots. (A) Total saponin content in GY and GS roots determined by HPLC. (B) Oleanolic acid content in GY and GS roots determined by HPLC. (C) Multivariate analysis of untargeted LC-MS/MS metabolomic. (D) Volcano plot showing differentially accumulated metabolites between GY and GS roots. (E) Relative abundance distribution of major metabolite classes. (F) Relative abundance patterns of differentially accumulated triterpenoids and terpenoid glycosides between GY and GS roots. (G) Structural classification of pentacyclic triterpenoids, including oleanolic-acid-skeleton compounds identified in the root metabolome. Data in panels A and B

are shown as mean $\pm$ standard error. Statistical significance was evaluated using Student's t-test; $P < 0.01$. Differentially accumulated metabolites were screened using $VIP > 1.0$ and $P < 0.05$. GY, one-year-old cultivated plants; GS, three-year-old cultivated plants.

Figure 3

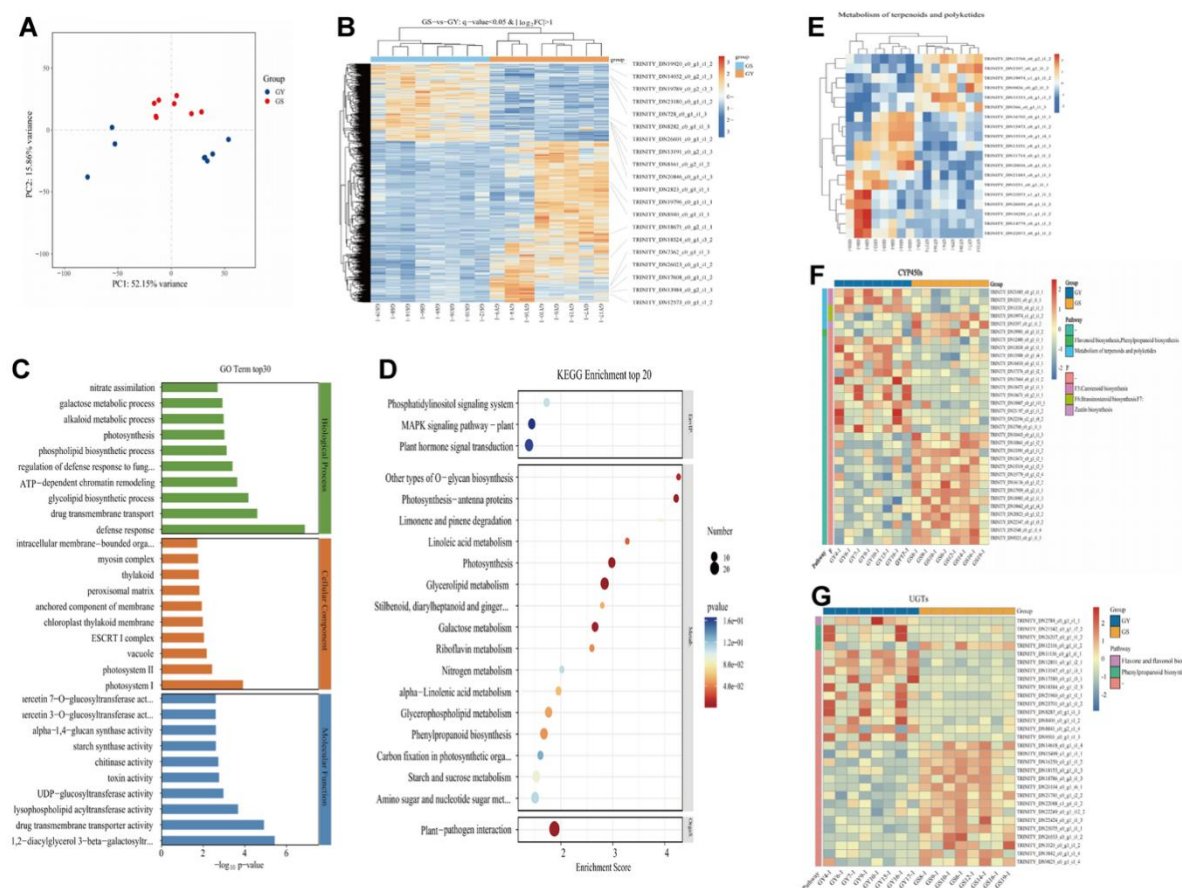


Figure3. Growth-year-associated transcriptomic remodeling and secondary-metabolism-related gene expression in *C. florida* var. *plena* roots. (A) Principal component analysis of root transcriptomes from GY and GS cultivated plants, showing separation of samples by growth year. (B) Hierarchical clustering heatmap of differentially expressed genes between GY and GS roots, with the side bar indicating genes upregulated or downregulated in the GS-versus-GY comparison. (C) Gene Ontology enrichment analysis of differentially expressed genes. Enriched terms are grouped into biological process, cellular component, and molecular function categories. (D) KEGG pathway enrichment analysis of differentially expressed genes, showing enrichment of pathways associated with secondary metabolism and plant metabolic regulation. (E) Expression patterns of differentially expressed genes assigned to terpenoid and polyketide metabolism. (F) Heatmap of differentially expressed cytochrome P450 genes associated with secondary-metabolism-related pathways. (G) Heatmap of differentially expressed UDP-glycosyltransferase genes associated with

secondary-metabolism-related pathways. Differentially expressed genes were identified using adjusted $P < 0.05$ and $|\log_2 \text{fold change}| > 1$. Heatmap colors indicate scaled relative expression levels across samples. GY, one-year-old cultivated plants; GS, three-year-old cultivated plants; CYP450, cytochrome P450 monooxygenase; UGT, UDP-glycosyltransferase.

Figure 4

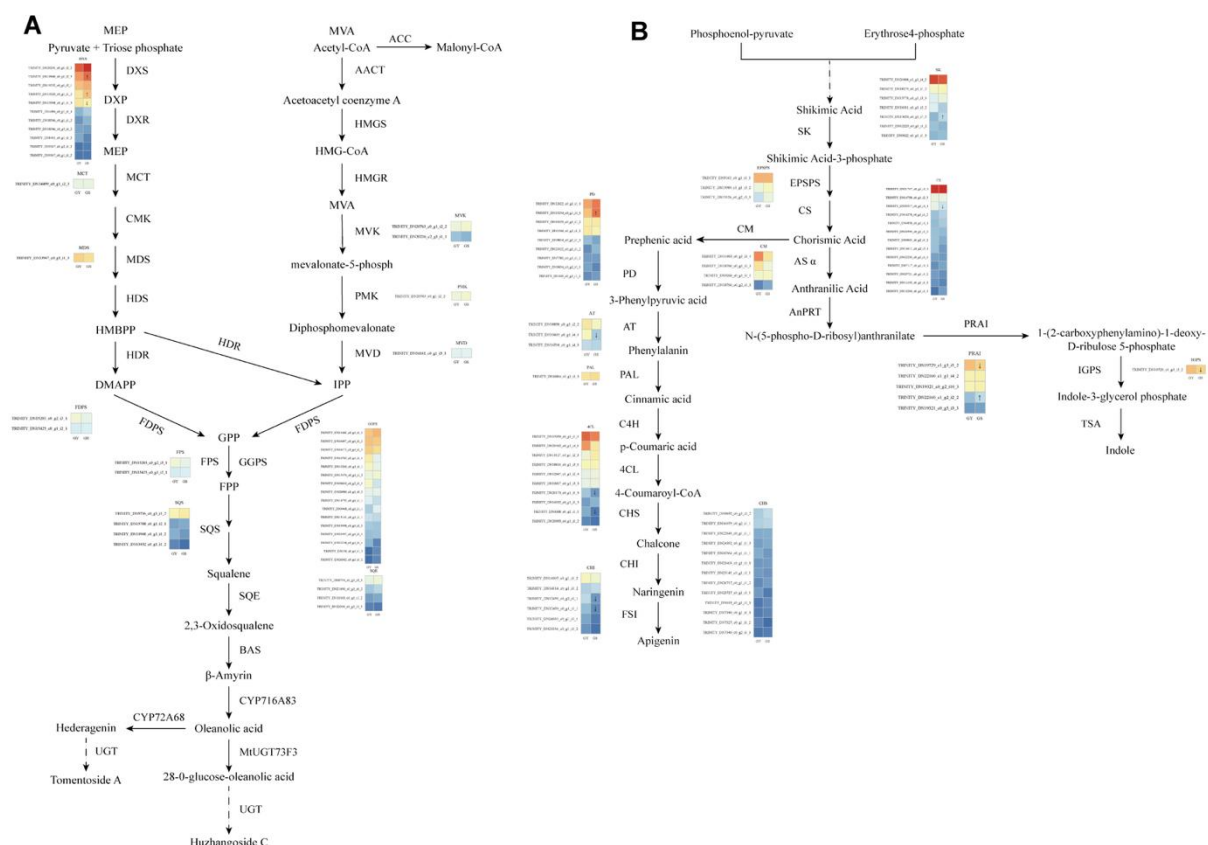


Figure 4. Growth-year-associated expression patterns of triterpenoid saponin and flavonoid biosynthetic pathways in *C. florida* var. *plena* roots. (A) Mapped expression profile of genes involved in triterpenoid saponin biosynthesis. (B) Mapped expression profile of genes involved in flavonoid and isoflavonoid biosynthesis. Heatmaps indicate growth-year-associated expression variation in genes assigned to these pathway steps. Colors indicate scaled relative expression levels according to the figure scale. GY, one-year-old cultivated plants; GS, three-year-old cultivated plants; MEP, methylerythritol phosphate pathway; MVA, mevalonate pathway; CYP450, cytochrome P450 monooxygenase; UGT, UDP-glycosyltransferase.

Figure 5

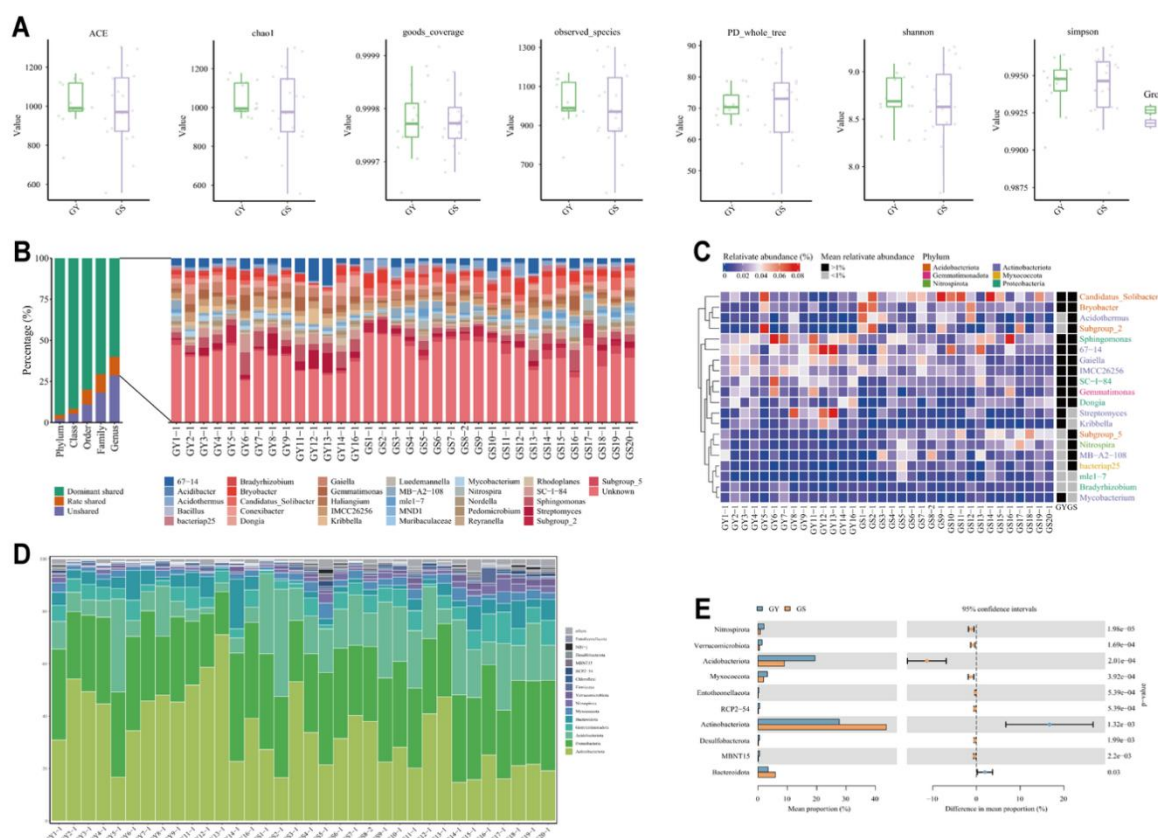


Figure 5. Rhizosphere bacterial diversity and taxonomic composition of one-year-old and three-year-old *C. florida* var. *plena* plants. (A) Alpha-diversity indices of rhizosphere bacterial communities in GY and GS plants. (B) Core and unique bacterial taxa in the rhizosphere microbiome. Stacked bars show the proportion of dominant core, rare core, conditionally rare, conditionally abundant, moderate, and sample-unique taxa across taxonomic levels. (C) Heatmap showing the relative abundance of dominant bacterial taxa across individual rhizosphere samples. (D) Genus-level bacterial community composition across rhizosphere samples. Each stacked bar represents one sample, and colors indicate the relative abundance of major bacterial genera. (E) Differential abundance analysis of dominant bacterial phyla between GY and GS rhizospheres. Bar plots show the relative abundance of selected phyla in each group, and the corresponding statistical comparison indicates phyla with significant growth-year-associated differences. Data are based on 16S rRNA sequencing of rhizosphere soil samples. GY, one-year-old cultivated plants; GS, three-year-old cultivated plants.

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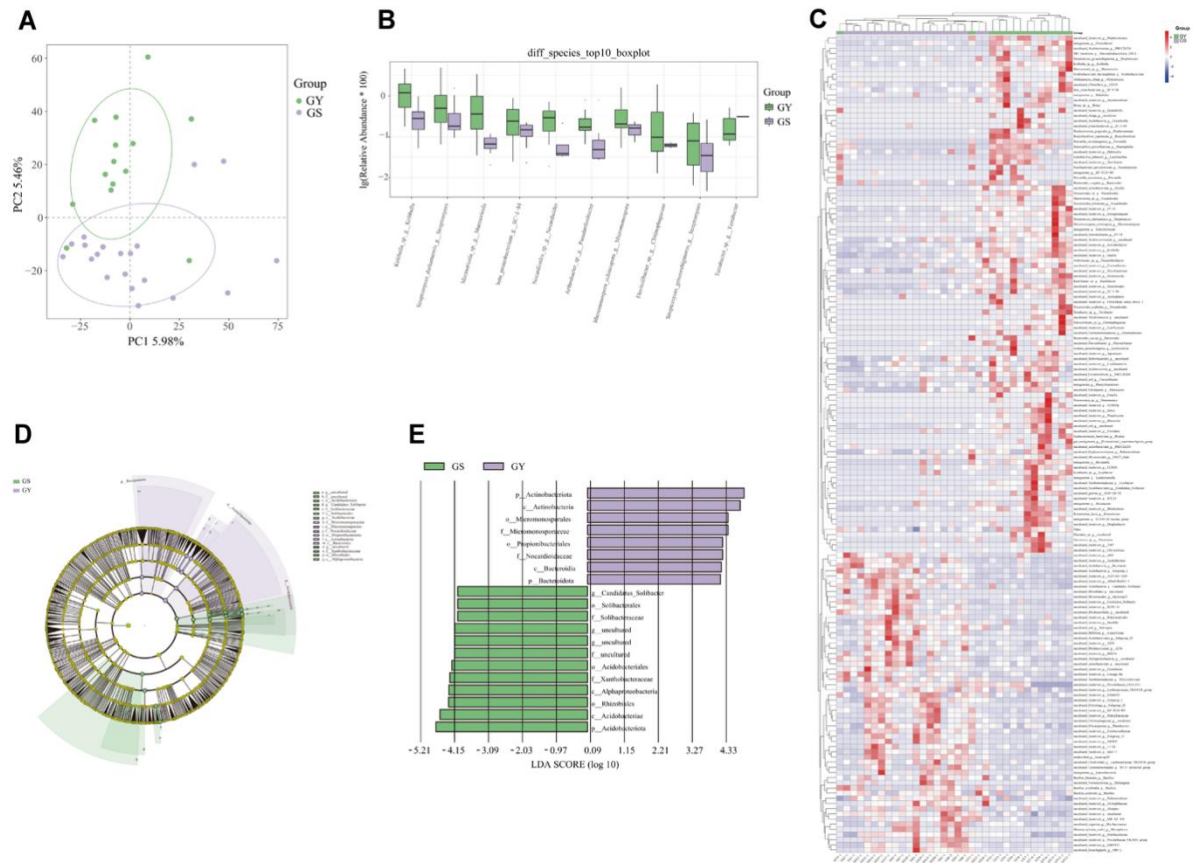
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917 **Figure 6**



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919 **Figure 6.** Growth-year-associated restructuring and discriminant taxa in the rhizosphere
 920 microbiome of *C. florida* var. *plena*. (A) Principal coordinates analysis of rhizosphere
 921 bacterial communities based on beta-diversity distances. Samples from GY and GS plants
 922 show separation in community structure. (B) Relative abundance of selected bacterial taxa
 923 that differed between GY and GS rhizospheres. Boxplots show abundance variation across
 924 samples for taxa with marked growth-year-associated differences. (C) Heatmap of
 925 differentially abundant bacterial taxa across individual rhizosphere samples. Rows represent
 926 bacterial taxa and columns represent samples; colors indicate relative abundance levels. (D)
 927 LefSe cladogram showing bacterial lineages with differential enrichment between GY and GS
 928 rhizospheres. Taxonomic nodes are colored according to the group in which they were
 929 enriched. (E) Linear discriminant analysis scores of discriminant bacterial taxa identified by
 930 LefSe. Positive and negative bars indicate taxa enriched in different growth-year groups. Data
 931 are based on 16S rRNA sequencing of rhizosphere soil samples. GY, one-year-old cultivated

932 plants; GS, three-year-old cultivated plants; LEfSe, linear discriminant analysis effect size;
933 LDA, linear discriminant analysis.

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951 **Figure 7**

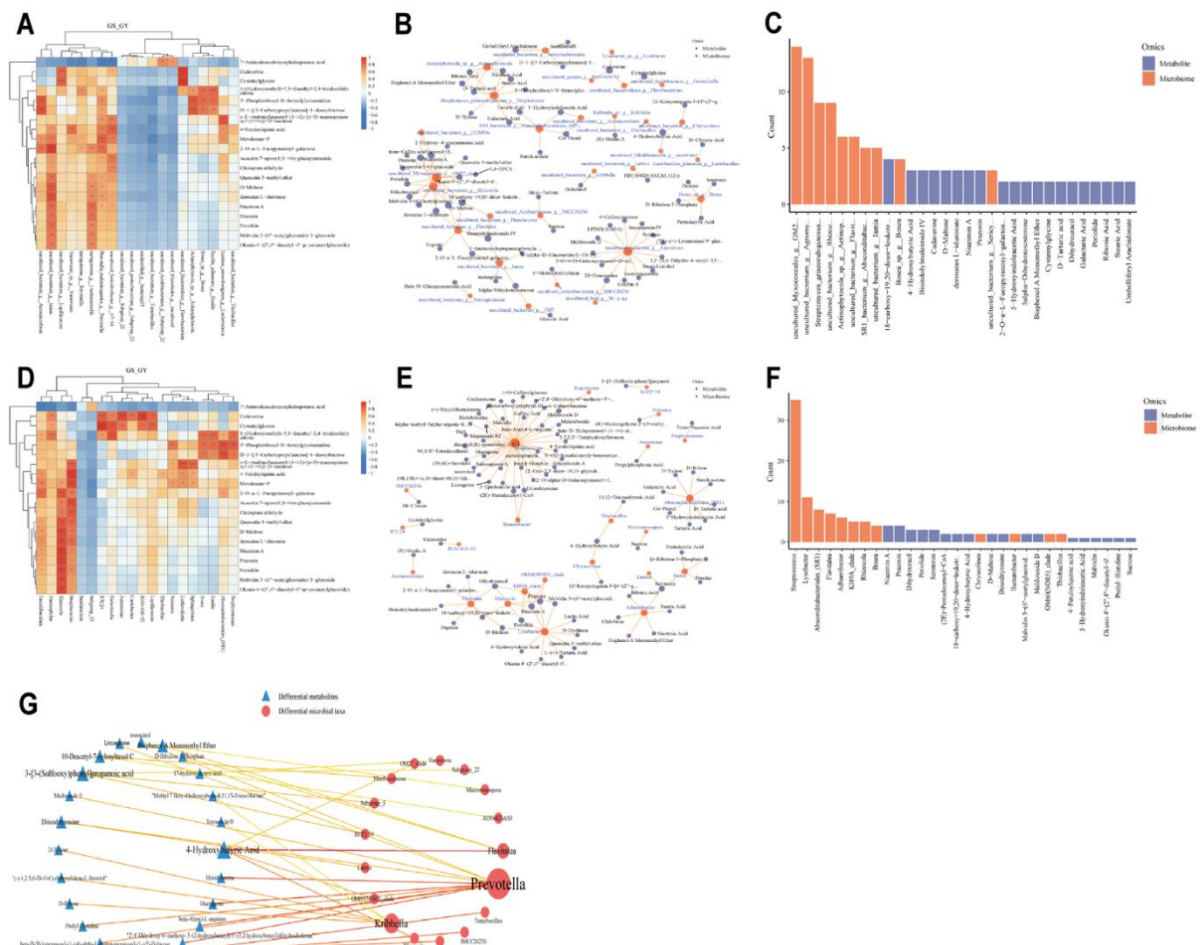
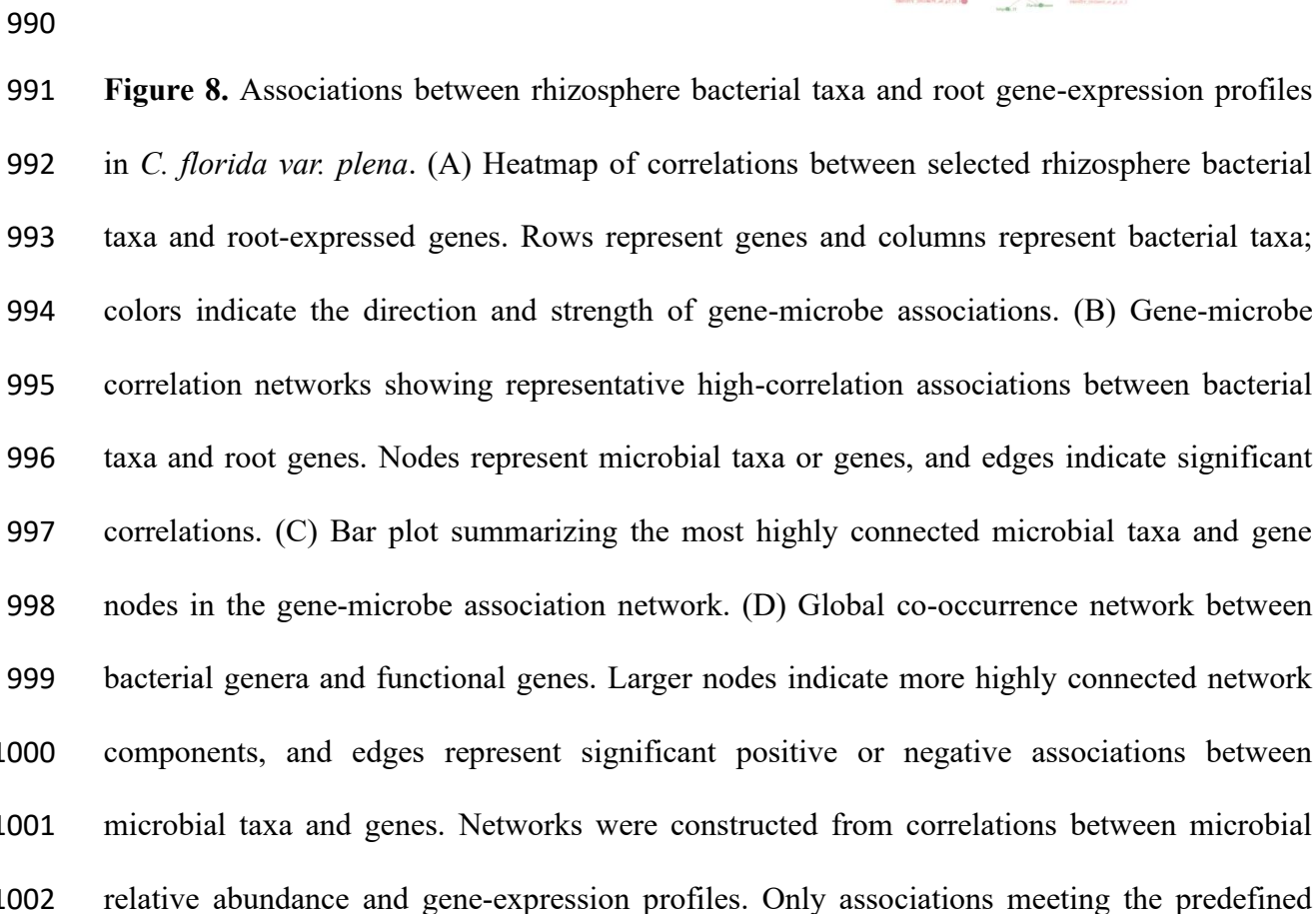


Figure 7. Microbe-metabolite association networks linking rhizosphere taxa with root metabolic profiles in *C. florida var. plena*. (A) Heatmap of species-level associations between rhizosphere bacterial taxa and metabolite features. Rows represent metabolites and columns represent bacterial taxa; colors indicate the direction and strength of correlations. (B) Species-level microbe-metabolite correlation network showing the strongest associations between bacterial taxa and metabolites. Nodes represent microbial taxa or metabolites, and edges represent significant correlations. (C) Bar plot showing the number of species-level microbe-metabolite associations for the dominant taxa or metabolite features in the network. (D) Heatmap of genus-level associations between rhizosphere bacterial genera and metabolite features. (E) Genus-level microbe-metabolite correlation network showing major associations between bacterial genera and root metabolites. (F) Bar plot summarizing the number of genus-level microbe-metabolite associations for dominant network components. (G) Stringent

microbe-metabolite network constructed using strongly correlated microbial taxa and significantly altered metabolites, highlighting candidate taxa linked to growth-year-associated metabolic variation. Networks were constructed from correlation analysis between microbial relative abundance and metabolite abundance. Only associations meeting the predefined correlation and significance thresholds were retained. Blue nodes represent microbial taxa, red nodes represent metabolites, and edges indicate significant microbe-metabolite correlations. Correlation-based associations do not establish direct causality.

Figure 8



correlation and significance thresholds were retained. Correlation-based links indicate statistical associations and should not be interpreted as direct regulatory interactions. GY, one-year-old cultivated plants; GS, three-year-old cultivated plants.

Figure 9

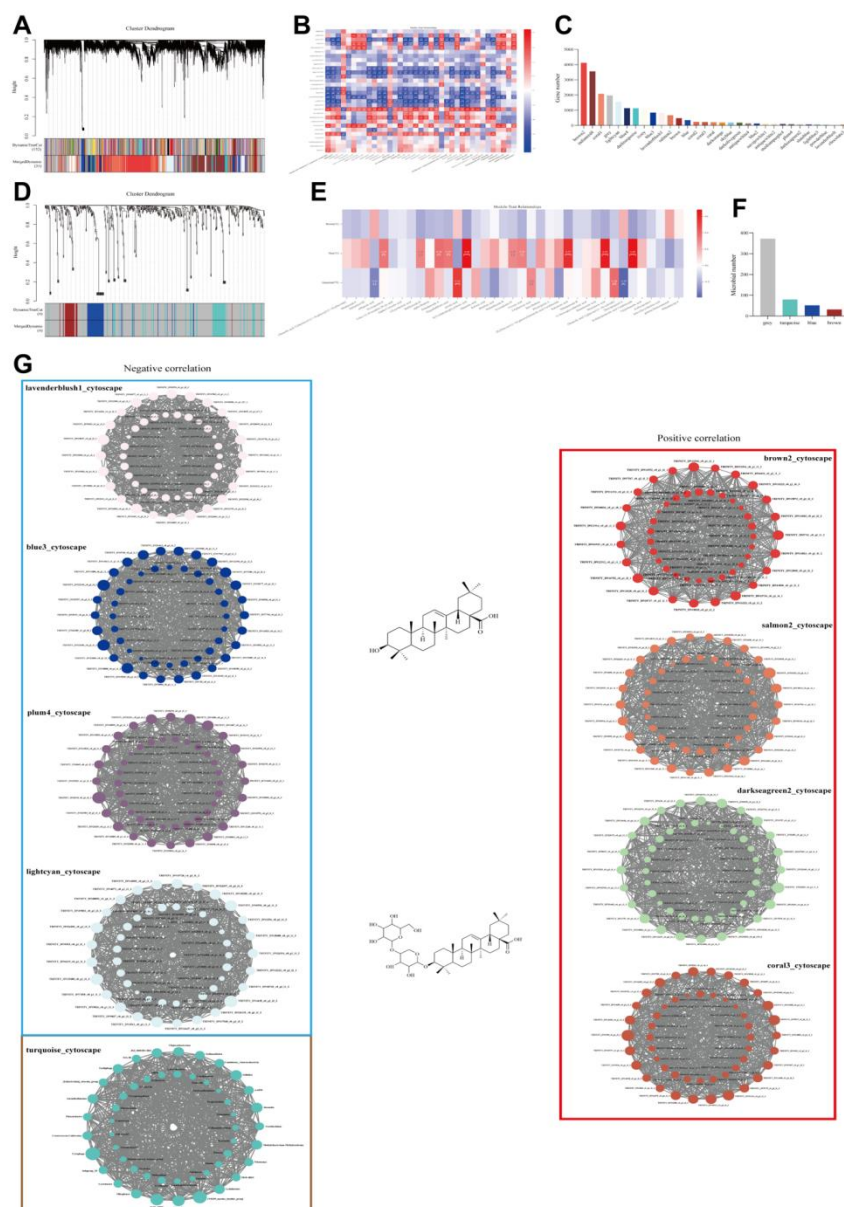


Figure 9. Co-expression and co-abundance modules associated with saponin-related metabolites in *C. florida* var. *plena*. (A) Weighted gene co-expression network analysis dendrogram showing gene module detection based on root transcriptome profiles. Branches represent clustered genes, and colors indicate assigned co-expression modules. (B) Heatmap showing correlations between gene co-expression modules and saponin-related metabolite features. Colors indicate positive or negative module–metabolite associations. (C) Bar plot summarizing the number of significant associations between gene modules and saponin-related metabolites. (D) Microbial co-abundance network analysis dendrogram

showing module detection based on rhizosphere bacterial abundance profiles. Colors indicate assigned microbial modules. (E) Heatmap showing correlations between microbial modules and saponin-related metabolite features. (F) Bar plot summarizing the number of significant associations between microbial modules and saponin-related metabolites. (G) Network visualization of saponin-associated gene and microbial modules. The left panel shows negatively correlated modules, and the right panel shows positively correlated modules. Nodes represent hub genes or microbial taxa within selected modules, and edges indicate module-based associations with saponin-related metabolite features. Chemical structures indicate representative saponin-related metabolites used in the module-association analysis. Only module-metabolite associations meeting the predefined significance threshold were retained. Correlations indicate statistical associations between gene-expression modules, microbial co-abundance modules, and saponin-related metabolite accumulation; they do not establish direct causality.

Supplemental information

**Growth-year-dependent multi-omics remodeling of triterpenoid saponin accumulation
and rhizosphere microbiota in *Clematis florida* var. *Plena***

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1085 (GY) and 3 years

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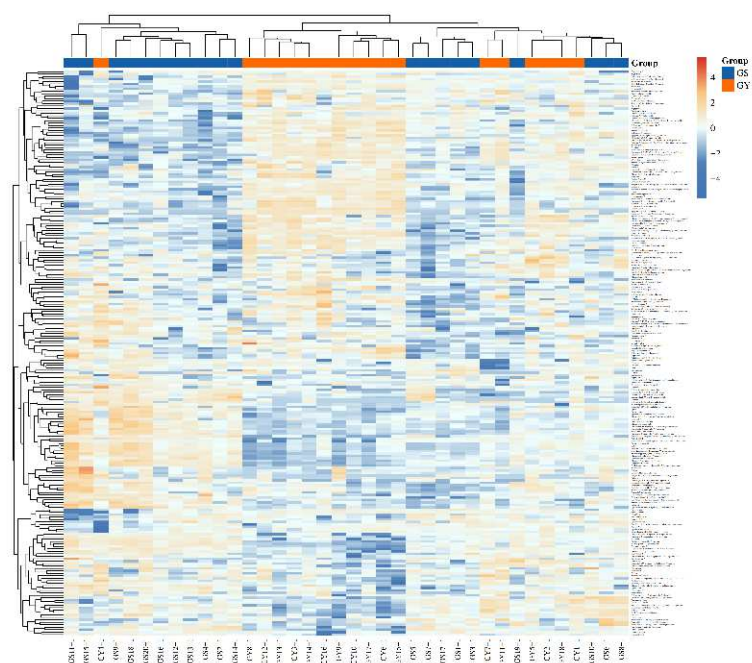
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1102 **Figure S2** All flavonoids compounds in *C. florida* var. *plena* cultivated for 1 year (GY) and 3
1103 years

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Figure S3

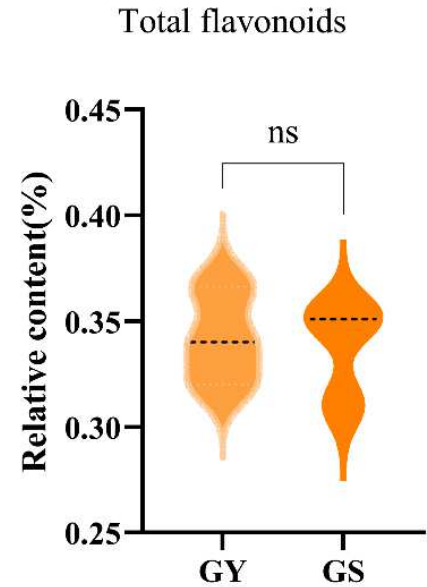
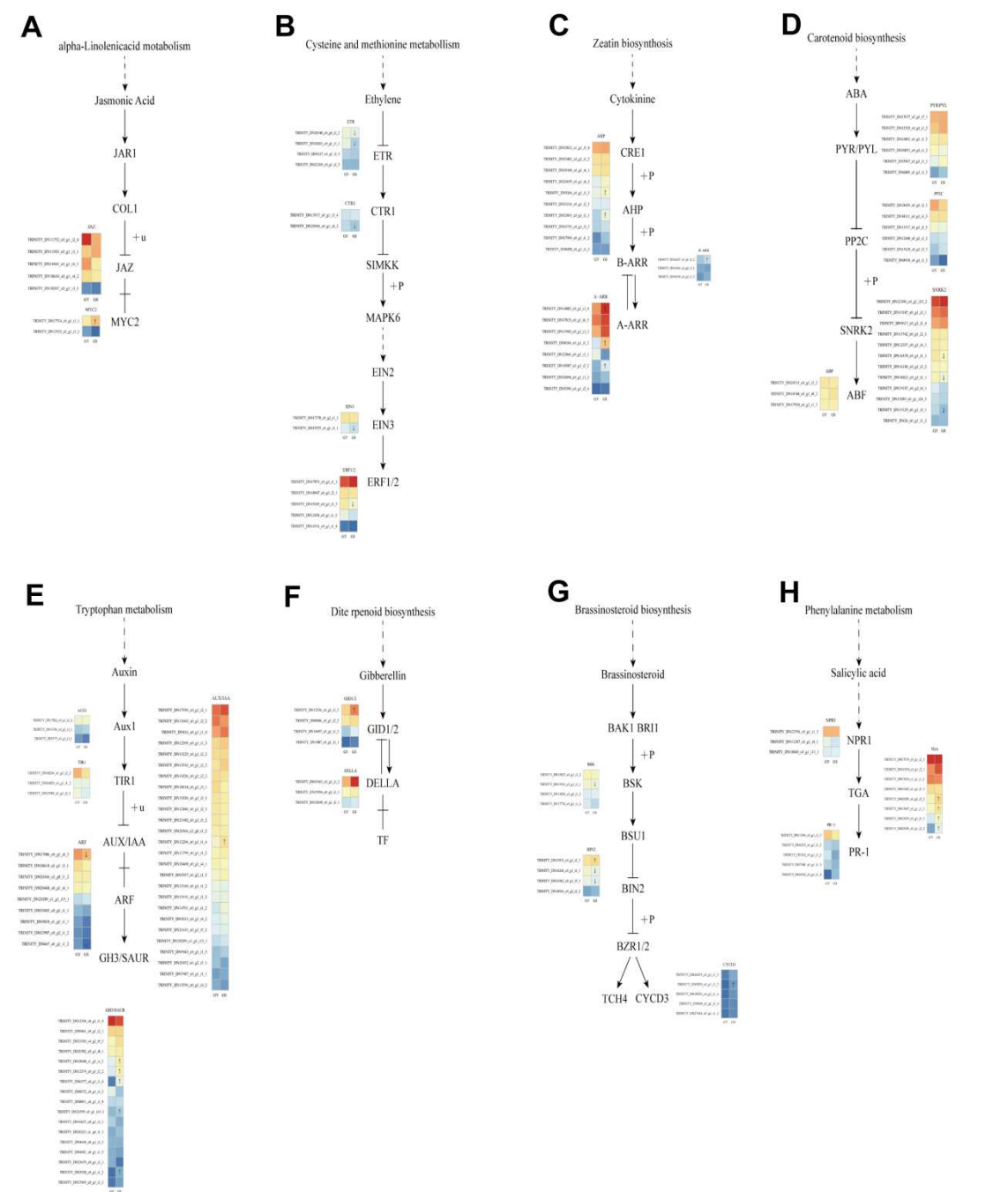


Figure S3 Comparative analysis of total flavonoids accumulation in *C. florida var. plena* cultivated for 1 year (GY) and 3 years

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Figure S4



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Figure S4 Comparative analysis of total flavonoids accumulation in *C. florida* var. *plena* cultivated for 1 year (GY) and 3 years

Supplementary Files

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- [SupplementalmaterialsTableS3S4S6S7.pdf](#)