

Genome-wide identification and characterization of 3-Dehydroquinate Dehydratase/ Shikimate Dehydrogenases (DHD/SDH) family genes in *Rubus chingii* Hu

Xueli An

Zhejiang Sci-Tech University

Yuezhen Li

Zhejiang Sci-Tech University

Yating Gong

Zhejiang Sci-Tech University

Nana Feng

Zhejiang Sci-Tech University

Bowei He

Dongfeng Yang

Zhejiang Sci-Tech University

Hongfa Li

Jianhong Chen

Zhuoni Hou

houzhuoni@zstu.edu.cn

Zhejiang Sci-Tech University

Zongsuo Liang

Zhejiang Sci-Tech University

Research Article

Keywords: *Rubus chingii* Hu, DHD/SDH, gene expression analysis, bioinformatics analysis, gallic acid

Posted Date: December 9th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-8132531/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Objective

In the plant shikimate pathway, the dual-function enzyme 3-Dehydroquinate dehydratase/shikimate dehydrogenase (DHD/SDH) stands alone as the sole catalyst responsible for generating both shikimate and gallic acid. This enzyme is instrumental in secondary metabolism, with particular significance in the metabolic route leading to gallic acid production.

Methods

This work pinpointed members of the DHD/SDH gene family in *Rubus chingii* Hu through bioinformatic methods and then undertook a thorough examination of their physicochemical properties, predicted subcellular localization, chromosomal distribution, phylogenetic relationships, and promoter cis-acting elements. Additionally, quantitative real-time PCR was used to map the expression patterns of these genes across different tissues, at various fruit developmental stages, and in response to external hormone treatments. In parallel, high-performance liquid chromatography (HPLC) quantified gallic acid and ellagic acid levels in multiple tissues and developmental stages of fruit, offering a comprehensive view of the *DHD/SDH* gene family's characteristics and expression dynamics in *Rubus chingii* Hu.

Results

Results revealed four DHD/SDH family members in *Rubus palmatus*, evenly distributed across two chromosomes, encoding protein sequences ranging from 440 to 1325 amino acids. Phylogenetic analysis indicated that RcDHD/SDH proteins belong to the fourth subfamily. Subcellular localization predictions showed these proteins reside in the cell nucleus and all contain the conserved DHD/SDH functional domains. Promoter analysis revealed that RcDHD/SDH genes contain multiple cis-acting elements, including hormone-responsive, light-responsive, and cold-responsive elements. Expression pattern analysis demonstrated significant differences in the expression of the RcDHD/SDH gene family across various tissues and organs in *Rubus palmatus*. Specifically, RcDHD/SDH1 to RcDHD/SDH4 exhibit relatively similar expression levels in roots, while RcDHD/SDH4 shows high abundance in stems and leaves. During fruit development, the overall expression level of the RcDHD/SDH gene family peaks during the early fruit stage and subsequently declines. This suggests that this gene family may play a crucial role in the early regulation of fruit growth and development.

Conclusion

This research marks the first in-depth analysis of the *DHD/SDH* gene family in *R. chingii*, offering paving the way for subsequent functional investigations and insights into their involvement in shikimate and gallic acid biosynthesis pathway.

1. Introduction

Rubus chingii Hu (Fu-Pen-Zi in China), a Rosaceae family member, serves as both a traditional Chinese medicinal and edible plant, valued for its nutritional and therapeutic properties. The fully ripened fruits of this plant are prized as

premium fresh produce, whereas the dried unripe fruits are used in traditional Chinese medicine (TCM) to tonify the liver and kidneys and treat urinary disorders^[1]. *R. chingii* is rich in diverse bioactive compounds, encompassing flavonoids, phenolic acids, terpenoids, alkaloids, organic acids, and tannins^[2]. Chief among these is, gallic acid (GA), a phenolic compound in the fruit, that demonstrates a wide range of biological effects including anti-inflammatory, antiviral, antibacterial, antiallergic, antitumor, and antimelanogenic activities^[3].

In flora and microorganisms life form, the shikimate pathway serves as a production line for fundamental building blocks of aromatic amino acids and a diverse selection of secondary metabolites, such as tannins and those hydrolyzable polyphenols. The DHD/SDH (3-dehydroquinate dehydratase/shikimate dehydrogenase) family represents a key branch point in the polyphenols manufacturing process. Unlike most microbial enzymes that specialize in one function, DHD/SDH in plants is a rare example of a bifunctional protein that bucked the evolutionary trend. While microorganisms typically keep these two activities, DHD and SDH in separate genes, plants have managed to combine them into a single *AroDE* gene, a striking case of functional divergence from their microbial counterparts^[4]. This bifunctional enzyme the transformation of 3-dehydroquinate (DHQ) into dehydroshikimate (DHS), which serves as a precursor that can be channeled toward either shikimic acid (SA) or oxidized to gallic acid (GA)^[5]. Within plant systems, this pathway stands as the principal mechanism for gallic acid production^[6], rendering DHD/SDH a pivotal control point in the biosynthetic route to GA^[6]. Gallic acid also acts as the building block for ellagic acid creation, which comes about through a series of chemical reactions and molecular joining processes that require several enzyme-catalyzed stages. Because different plant species have developed unique biological and evolutionary characteristics, the quantity and role of their DHD/SDH genes can differ quite a bit from one type of plant to another. In The tea plant (*Camellia sinensis*), has had three *DHD/SDH* genes identified with their expression patterns getting the once-over from researchers^[4]. In grapevine (*Vitis vinifera*), four isoforms of DHD/SDH have been reported^[8]. Similarly, Rida et al^[9] struck similar gold in pomegranate (*Punica granatum*), where they uncovered four DHD/SDH isoforms. Meanwhile, other model plants like *Arabidopsis thaliana*^[10] and tomato (*Solanum lycopersicum*)^[11], have played it close to the vest, with just a single gene encoding what appears to be a DHD/SDH protein sporting a predicted plastid-targeting peptide having been cloned. Tobacco, however, breaks the mold by sporting two distinct genes encoding plastidial and cytosolic DHD/SDH enzymes^[12]. The DHD/SDH gene family across various plant species such as grapevine^[8], pomegranate^[9], and tea^[13], typically comprises 4 to 6 members, with some of encoding bifunctional DHD/SDH enzymes, and others operating as specialized quinate dehydrogenases (QDH)^[14]. Despite sharing over half of their amino acid sequence, these isoforms exhibit subtle differences in active site residues and three-dimensional configurations, which may influence their catalytic properties and substrate specificities^{[15]–[16]}. To date, researchers have yet to conduct a comprehensive analysis of the *DHD/SDH* gene family in *R. chingii*. Understanding the composition, structural features, and expression dynamics of these genes is crucial for clarifying their involvement in gallic acid biosynthesis and other secondary metabolic pathways.

To get to the bottom of the biological roles played by the *DHD/SDH* gene family in fruit development and polyphenol biosynthesis with in *Rubus chingii*, researchers conducted a comprehensive genome-wide analysis and characterization of *RcDHD/SDH* genes leveraging the chromosome-level reference genome of *R. chingii*^[17]. A suite of bioinformatics tools was brought to bear to document the physicochemical properties of these proteins, conserved motifs, determine their phylogenetic placement, map cis-regulatory motifs, and pin down their chromosomal plocations. Moreover, the team measured transcript abundance across different tissue types, throughout defined key developmental stages of fruit, and under the influence of externally applied phytohormone including methyl jasmonate (MeJA), abscisic acid (ABA), and indole-3-acetic acid (IAA). This investigation sheds new light on the

molecular mechanisms underlying gallic acid metabolism in *R. chingii* fruits and sets the stage for further functional exploration of *DHD/SDH* genes in the broader context of polyphenol biosynthesis.

2. Materials and Methods

2.1 Plant materials

R. chingii samples were collected from a farm-raised population situated in Linqi village, with Chun'an county, Hangzhou, China (29°50'N, 119°5'E). Over the April to May 2025 period, multiple plant tissues were harvested, including roots, stems, leaves, flowers, and fruits at various ripeness stages (young green fruit, mature green fruit, yellow fruit, and red fruit) (Fig. 1). All samples were flash frozen in liquid nitrogen right away and stored at -80°C pending analysis. Uniform, healthy one-year-old seedlings which free of pests or infection were selected for hormone treatments. The adaxial and abaxial surfaces of the leaves were sprayed with solutions containing 100 $\mu\text{mol}\cdot\text{L}^{-1}$ concentrations solutions of methyl jasmonate (MeJA), abscisic acid (ABA), and indole-3-acetic acid (IAA), respectively. As a baseline for comparison, plants given distilled water served as the control group (CK). Each treatment involved three biological replicates, with three plants in each set. Spraying was performed once every other days for a total of three applications. Seven days after the initial treatment, leaves from the second node (counting from the apex) of the main stem were gathered from each plant. These samples were flash-frozen in liquid nitrogen, and stored at -80°C for subsequent examination.

2.2 Identification of *RcDHD/SDH* Gene Family Members in *Rubus chingii*

The published *Arabidopsis thaliana* DHD/SDH protein sequence was retrieved from the TAIR database (www.arabidopsis.org/) and used as a query to perform BLAST searches against the *Rubus chingii* genome for homologous sequences. Candidate sequences were further screened and validated using the NCBI CD-Search tool (www.ncbi.nlm.nih.gov/) to verify that the presence and integrity of the conserved domains.

In addition, the conserved domain of DHD/SDH (PF08501) was downloaded from the Pfam database (<https://pfam.xfam.org/>) and used for HMM-based validation in TBtools software^[18]. Four DHD/SDH homologous genes were identified and named *RcDHD/SDH1* to *RcDHD/SDH4* according to chromosomal locations.

2.3 Physicochemical Properties, Chromosomal Localization, and Synteny Analysis of *RcDHD/SDH* Gene Family Members

The ExPASy ProtParam tool (<http://cn.expasy.org/tools>)^[19] was employed to evaluate the physicochemical properties of *RcDHD/SDH* proteins, encompassing parameters such as amino acid length, molecular weight, theoretical isoelectric point (pI), instability index, aliphatic index, and grand average of hydropathicity (GRAVY). To determine their potential cellular destinations the Plant-PLoc server (www.csbio.sjtu.edu.cn/bioinf/plant-multi) was utilized for subcellular localization predictions. Meanwhile, chromosomal positions of the *RcDHD/SDH* genes were derived from the GFF annotation file, with their spatial arrangement subsequently illustrated through TBtools software.

Syntenic relationships between *R. chingii* and *Glycine max* (soybean) and *Oryza sativa* (rice) were identified using MCScanX software^[20]. The synteny relationships between *R. chingii* and soybean were further visualized using the Dual Synteny Plot module in TBtools.

2.4 Phylogenetic Analysis of *RcDHD/SDH* Family Members

Previously reported DHD/SDH protein sequences from *Arabidopsis thaliana*, *Oryza sativa*, *Glycine max*, *Solanum tuberosum*, *Vitis vinifera*, *Prunus avium*, *Eucalyptus grandis*, *Olea europaea*, *Pistacia vera*, *Zea mays*, and *Capsicum annuum* were accessed from the Phytozome database (<http://www.phytozome.net>). These sequences, along with the *R. chingii* *RcDHD/SDH* proteins, were aligned using MEGA11 software (<https://www.megasoftware.net/>)^[21] for multiple sequence alignment. Next, a phylogenetic tree was constructed using the neighbor-joining (NJ) method with 2000 bootstrap replications, and default parameters. The resulting tree was visualized and annotated using the iTOL platform (<https://itol.embl.de/>).

2.5 Gene Structure and Conserved Motif Analysis of *RcDHD/SDH* Genes

Exon-intron structures of *RcDHD/SDH* genes were obtained from GFF annotation files and visualized using the Gene Structure Shown module in TBtools. Conserved motifs of *RcDHD/SDH* proteins were identified using the MEME Suite (<https://meme-suite.org>)^[22] with the maximum number of motifs set to 10 and all other parameters at default settings. Both the number and distribution of motifs were visualized using TBtools.

2.6 Secondary and Tertiary Structure Prediction of *RcDHD/SDH* Proteins

The secondary structures of *RcDHD/SDH* proteins—comprising α -helices, β -strands, β -turns, and random coils—were forecasted using SOPMA (<https://npsa-prabi.ibcp.fr>). Tertiary structures were modeled and rendered visible with the aid of the SWISS-MODEL online tool (<http://swissmodel.expasy.org>)^[23].

2.7 Cis-Acting Regulatory Element Analysis of *RcDHD/SDH* Promoters

Promoter sequences (2000 bp upstream of the start codon) of *RcDHD/SDH1* to *RcDHD/SDH4* were analyzed for cis-acting regulatory elements using the PlantCARE database (<https://bioinformatics.psb.ugent.be/webtools/plantcare/html/>)^[24]. Elements associated with plant growth and development, hormone responsiveness, and stress responses were identified and visualized using TBtools.

2.8 Expression Analysis of *RcDHD/SDH* Gene Family in Different Tissues, Fruit Developmental Stages, and Hormone Treatments

Total RNA was isolated from various tissues of *R. chingii* (root, stem, leaf, flower, and fruit), as well as from fruits at different developmental stages (young green fruit, mature green fruit, yellow fruit, and red fruit), and from leaves subjected to treatment with MeJA, IAA, or ABA. The extraction was performed using the Plant Polysaccharide & Polyphenol Total RNA Extraction Kit (RC411, Vazyme Biotech, Nanjing, China)^[25]. RNA quantity and purity were evaluated with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). Subsequently, first-strand cDNA was synthesized utilizing the HiScript III All-in-One RT SuperMix (R333, Vazyme Biotech, Nanjing, China), which then served as the template for assessing the expression patterns of *RcDHD/SDH1* to *RcDHD/SDH4* genes.

Gene-specific primers targeting *RcDHD/SDH1* to *RcDHD/SDH4* were designed using Primer Premier 5.0 software (Table 1), which then synthesized by Youkang Biotech Co., Ltd. (Zhejiang, China). The *RuEEF-1A* gene from *R. chingii* served as the internal reference^[26]. For quantitative real-time PCR (qRT-PCR), cDNA was diluted to a working

concentration of 50 ng·μL⁻¹. The reactions eactions were performed on an ABI 7500 Real-Time PCR System (Applied Biosystems, USA) with using ChamQ Universal SYBR qPCR Master Mix (Q711, Vazyme Biotech, Nanjing, China), strictly following the manufacturer’s instructions.Each 20 μL reaction contained 10 μL SYBR Green Master Mix, 1 μL forward primer, 1 μL reverse primer, 1 μL cDNA template (50 ng/μL), and 7 μL ddH₂O. The qPCR program included an initial denaturation step at 95°C for 30 s, followed by 40 cycles of 95°C for 10 s and 60°C for 30 s. Relative gene expression levels were calculated using the 2^{- $\Delta\Delta C_T$} method [27]. Each sample included was tested in triplicate three biological replicates, And Sstatistical differences among samples were analyzed assessed using using Duncan’s multiple range test (P < 0.05) in SPSS software.

Table 1
Primer sequences used in this study

Primer name	Forward primer (5'→3')	Reverse primer (5'→3')
RuEEF-1A	CCTTTCGCGCTCAGCCTTGA	AAGTCGACCACCACGGGTCA
RcDHD/SDH1	GTGTTCGGTATAATTGGAAAGC	CTATTGACTTGGCAACTGGAT
RcDHD/SDH2	GATCCGGTTGCCAAGTCCATAG	GCAGAAATAGCCCCAAAATAGTCC
RcDHD/SDH3	TTGCCAACTCTTGTCACTTAC	AATATAATCTGCTCCCAATTCCAT
RcDHD/SDH4	GTCACAGCAAAAGTCCTCTA	TGGCAATGGAATCAATCTCA

DHD/SDH: 3-Dehydroquinate dehydratase/shikimate dehydrogenase.

2.9 Determination of Gallic Acid and Ellagic Acid Contents in Different Tissues and Fruit Developmental Stages of *Rubus chingii*

Fresh samples of diverse plant tissues and fruits at various developmental stages were collected, and approximately 0.5 g of finely powdered raspberry material (sifted through a No. 4 sieve) was accurately weighed and transferred into a stoppered Erlenmeyer flask. Precisely 50 mL of 70% methanol was added and the total weight recorded. The mixture was refluxed in a water bath at 60°C for 1 h, then allowed to return to room temperature before being weighed again. Any weight loss was compensated with 70% methanol, followed by thorough mixing and subsequent filtration. An aliquot of 1 mL of the successive filtrate was accurately measured, which was then diluted to 5 mL with 70% methanol in a volumetric flask. After ensuring proper mixing, this solution was filtered through a 0.22 μm membrane filter to remove any remaining particulates. The resulting extract was stored in 1.5 mL amber vials for HPLC analysis.

High-performance liquid chromatography (HPLC) was performed on a Thermo ODS C18 column (250 mm × 4.6 mm, 5 μm). The mobile phase consisted of acetonitrile (A) and 0.2% phosphoric acid aqueous solution (B) with the following gradient program: 0–8 min, 15% A; 8–23 min, 15%→33% A; 23–39 min, 33%→42% A; 39–45 min, 42%→28% A; 45–60 min, 28%→15% A. The analysis was conducted at a flow rate of 1.0 mL min⁻¹ while keeping the column temperature at steady 30°C. For detection, the wavelengths were set at 254 nm for ellagic acid and 271 nm for gallic acid, with each injection delivering 10 μL of sample into the system.

2.10 Data Analysis

Data processing and figure generation were carried out with GraphPad Prism 8. Statistical significance was analyzed with SPSS software employing the least significant difference (LSD) test, with differences considered significant at P < 0.05. Figures were finalized and refined using Adobe Illustrator 2021.

3. Results and Analysis

3.1 Identification and Physicochemical Properties of the RcDHD/SDH Gene Family in *Rubus chingii*

Through bioinformatics approaches, a total of four *DHD/SDH* family members were pinpointed from the *R. chingii* genome. These genes were labeled *RcDHD/SDH1* to *RcDHD/SDH4* based on their chromosomal locations. Analysis of their physicochemical properties revealed that the RcDHD/SDH proteins consist of between 440 and 1325 amino acids, averaging out at around 893.75. RcDHD/SDH3 was the largest member of the group. Their estimated molecular weights ranged from 47.55 to 145.90 kDa, with an average around 99.03 kDa. The isoelectric points (pI) varied from 5.60 to 9.06, with an median value of 6.73, with RcDHD/SDH3 possessed the highest pI value. The aliphatic index fell between 78.16 and 97.36. Most of the RcDHD/SDH proteins were found to be quite stable, with instability indices below 40. However, an outlier, RcDHD/SDH4, was classified as unstable (Table 2).

The grand average of hydropathicity (GRAVY) scores indicated that RcDHD/SDH1 had a hydrophobic character, with the remaining proteins exhibiting hydrophilic properties. Subcellular localization prediction positioned RcDHD/SDH1 ~ RcDHD/SDH4 appeared to be located in the nucleus, indicating functional divergence among the family members.

Table 2
Physicochemical properties of *RcDHD/SDH* family proteins in *R. chingii*

Protein name	Number of amino acids	Molecular weight/kDa	Isoelectric point	Instability index	Aliphatic index	Grand average of hydropathicity	Subcellular localization
RcDHD/SDH1	440	47.55	6.38	27.87	94.18	0.02	nucleus
RcDHD/SDH2	537	58.08	5.89	26.38	97.36	-0.01	nucleus
RcDHD/SDH3	1325	145.90	9.06	36.46	78.16	-0.43	nucleus
RcDHD/SDH4	1273	144.61	5.6	42.33	89.84	-0.48	nucleus

3.2 Chromosomal Localization and Synteny Analysis of the RcDHD/SDH Gene Family in *Rubus chingii*

Based on the chromosome-level genome annotation of *R. chingii*, a chromosomal distribution map of the *RcDHD/SDH* gene family was meticulously charted (Fig. 2-A). The findings revealed that all four *RcDHD/SDH* genes were evenly distributed on two chromosomes: LG01 and LG06. Specifically, *RcDHD/SDH1* and *RcDHD/SDH2* were found on LG01, while *RcDHD/SDH3* and *RcDHD/SDH4* were located on LG06. The genes on each chromosome appeared to be clustered, hinting that family members sharing the same chromosome might have similar functions. In addition, a collinearity analysis within *R. chingii* indicated that no tandem duplication events occurred among *RcDHD/SDH* family members (Fig. 2-B).

Cross-species collinearity analysis comparing the *R. chingii* with the dicotyledonous plant soybean (*Glycine max*) and the monocotyledonous plant rice (*Oryza sativa*) revealed syntenic relationships for the *RcDHD/SDH* genes (Fig. 2-C, D). Whereas collinear gene pairs turned up between *R. chingii* and rice, five syntenic gene pairs were detected between *R. chingii* and soybean. Notably, all five collinear gene pairs of these collinear genes were situated on LG06 of *R. chingii*, which raises the possibility that these genes may have gone through segmental duplication and

subsequently diverged evolutionarily. These results suggest a closer evolutionary relationship between *R. chingii* and other dicotyledons compared to monocotyledons.

3.3 Phylogenetic Analysis of the *RcDHD/SDH* Gene Family

To delve into the evolutionary relationships of RcDHD/SDH proteins, multiple sequence alignment was performed using RcDHD/SDH protein sequences from various plant species. A phylogenetic tree was constructed using MEGA software based on 47 DHD/SDH protein sequences from *Arabidopsis thaliana*, *Oryza sativa*, *Glycine max*, *Solanum tuberosum*, *Vitis vinifera*, *Prunus avium*, *Eucalyptus spp.*, *Olea europaea*, *Pistacia vera*, *Zea mays*, and *Capsicum annuum* (Fig. 3). These proteins were categorized into five subgroups (Group I to V), containing 10, 6, 8, 6, and 17 members, respectively. Subsequently, the *RcDHD/SDH* genes from *R. chingii* were mapped into these groups to explore their evolutionary affiliations.

The results revealed that all four *RcDHD/SDH* members clustered within Group IV, together with their counterparts *GmDHD/SDH4* from soybean and *PvDHD/SDH4* from pistachio, both of which are dicot species. This indicates that these *RcDHD/SDH* genes share a closer evolutionary relationship with those from dicotyledons, with both their DNA sequence and potentially their function having remained relatively conserved during evolution. Conversely, *RcDHD/SDH* genes failed to group with their monocot counterparts from plants like *O. sativa* and *Z. mays*, which were grouped into more distantly related clades. This supports the hypothesis that monocots and dicots have undergone largely parallel evolutionary trajectories, leading to the formation of distinct gene lineages.

3.4 Gene Structure, Sequence Alignment, and Motif Analysis of the *RcDHD/SDH* Gene Family in *Rubus chingii*

Based on the meticulous genome annotation of *R. chingii*, the intricate gene structures of *RcDHD/SDH* family members were visualized (Fig. 4-A). The *RcDHD/SDH* genes exhibited a size range from 1,323 to 3,978 bp, with an average length of 2,684.25 bp. The member from *RcDHD/SDH1* to *RcDHD/SDH4* harbor between 9 and 14 exons of varying lengths: *RcDHD/SDH1* had 9 exons, *RcDHD/SDH2* holds 11 exons, and *RcDHD/SDH3* and *RcDHD/SDH4* each carry 14 exons.

Ten conserved motifs were identified within the *RcDHD/SDH* family members (Fig. 4-B). Motif 3 and motif 9 corresponded to the N-terminal domains of RcDHD/SDH proteins, while motif 6 represented the C-terminal domain. Each of the four RcDHD/SDH proteins boast 6 to 9 conserved motifs and all shared motifs 1, 2, 4, 5, and 7, indicating a high degree of conservation among the RcDHD/SDH protein family members.

Comparative analysis of the *DHD/SDH* amino-acid sequences in *Rubus chingii* revealed a high degree of identity between *RcDHD/SDH2* and *RcDHD/SDH3*, whereas *RcDHD/SDH2* and *RcDHD/SDH4* exhibited marked divergence (Fig. 4-D). These findings indicate that the *RcDHD/SDH* gene family retains both conserved core domains and lineage-specific variability.

3.5 Secondary and Tertiary Structure Analysis of RcDHD/SDH Proteins in *Rubus chingii*

The secondary structures of RcDHD/SDH proteins were predicted using the SOPMA tool (Table 3). All RcDHD/SDH family members comprised α -helices, random coils, and extended strands, while β -sheets were absent. The average proportions were 46.17%, 40.17%, and 13.66%, respectively. Notably, *RcDHD/SDH4* exhibited the highest α -helix

content (64.96%) and the lowest extended strand proportion (9.19%), whereas RcDHD/SDH1 displayed the highest extended strand content (17.27%).

Tertiary structure modeling conducted via SWISS-MODEL revealed conserved DHD/SDH domains across all RcDHD/SDH proteins (Fig. 5). The predicted tertiary conformations were consistent with the secondary structure compositions, supporting the structural conservation within this gene family.

Table 3

Analysis of secondary structure of RcDHD/SDH family proteins in *R. chingii*

Protein name	Alpha helix	Extended strand	Beta turn	Random coil
RcDHD/SDH1	43.41	17.27	0.00	39.32
RcDHD/SDH2	45.44	16.01	0.00	38.55
RcDHD/SDH3	30.87	12.15	0.00	56.98
RcDHD/SDH4	64.96	9.19	0.00	25.84

RcDHD/SDH1 RcDHD/SDH2 RcDHD/SDH3 RcDHD/SDH4

3.6 Cis-Acting Element Analysis of *RcDHD/SDH* Gene Promoters in *Rubus chingii*

To explore the potential regulatory mechanisms of *RcDHD/SDH* genes, the promoter regions (2,000 bp upstream of the start codon) were analyzed using the PlantCARE database. A total of 26 types of cis-acting elements were identified and classified into three functional categories: plant growth and development, hormone responsiveness, and stress responsiveness (Fig. 6). Each *RcDHD/SDH* promoter contained 18 to 23 cis-elements. *RcDHD/SDH4* harbored the highest number, while the other three genes displayed comparable numbers of regulatory elements. Hormone-responsive elements were the most abundant, particularly abscisic acid-responsive elements (ABRE, rendered in forest green) and jasmonic acid-responsive elements (MYC, highlighted in orange), suggesting that *RcDHD/SDH* gene expression may be regulated by phytohormones and potentially involved in the biosynthesis of phenolic compounds. Additionally, elements responsive to light, low temperature, drought, and anaerobic conditions were also detected. These findings indicate that *RcDHD/SDH* genes may play significant roles in the growth and development of *R. chingii*, as well as in its responses to various environmental stresses.

3.7 Expression Patterns of *RcDHD/SDH* Genes in Different Tissues of *Rubus chingii*

To investigate the tissue-specific expression profiles of *RcDHD/SDH* genes, quantitative real-time PCR (qRT-PCR) was performed on five tissues: root, stem, leaf, flower, and riped fruit (red fruit). The expression levels of *RcDHD/SDH1* to *RcDHD/SDH4* were quantified and normalized using a reference gene, and relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method. As shown in Fig. 7, all four *RcDHD/SDH* genes were expressed across the five examined tissues, but exhibited distinct tissue-specific expression patterns and differential expression levels among gene members. *RcDHD/SDH1* to *RcDHD/SDH4* showed relatively consistent expression in roots, while their expression in flowers remained low. Notably, *RcDHD/SDH4* exhibited the highest expression levels in stems and leaves, which were significantly higher than those in other tissues ($P < 0.05$).

These results suggest that *RcDHD/SDH* genes may have diverse biological functions in different tissues, with *RcDHD/SDH4* potentially playing a crucial role in stem and leaf physiology.

3.8 Expression Patterns of *RcDHD/SDH* Genes During Fruit Maturation in *Rubus chingii*

To investigate the potential roles of *RcDHD/SDH* gene family members during fruit development in *Rubus chingii*, their expression patterns were analyzed at four developmental stages: young green fruit, mature green fruit, yellow fruit, and red fruit (Fig. 8). All four *RcDHD/SDH* genes were expressed throughout the fruit maturation process but exhibited significantly higher expression at the young green fruit stage, followed by a gradual decrease as the fruit matured. Among them, *RcDHD/SDH4* showed generally low expression levels across all stages, whereas *RcDHD/SDH3* exhibited relatively high expression throughout development. *RcDHD/SDH1* and *RcDHD/SDH2* displayed peak expression at the young green fruit stage, followed by a sharp decline during ripening, suggesting their possible involvement in early metabolic regulation during fruit development. In contrast, *RcDHD/SDH3* and *RcDHD/SDH4* showed a consistent downward trend in expression, with *RcDHD/SDH4* showing the most pronounced and sustained decrease, potentially indicating its role in the negative regulation of ripening-associated metabolic pathways.

Overall, *RcDHD/SDH* genes were more actively expressed in the early stages of fruit development, implying their critical roles in the early regulatory networks of fruit growth and maturation in *Rubus chingii*.

3.9 Expression Profiles of *RcDHD/SDH* Genes Under Exogenous Hormone Treatments in *Rubus chingii*

To validate the results of the cis-acting element analysis, qRT-PCR was performed to examine the expression patterns of *RcDHD/SDH* genes in response to exogenous treatments with methyl jasmonate (MeJA), indole-3-acetic acid (IAA), and abscisic acid (ABA) (Fig. 9). The results revealed that all four *RcDHD/SDH* genes were significantly upregulated by MeJA treatment. Notably, *RcDHD/SDH3* and *RcDHD/SDH4* exhibited dramatic increases in transcript levels—approximately 8-fold and 7-fold, respectively—indicating strong MeJA-specific induction. *RcDHD/SDH1* and *RcDHD/SDH2* showed broader hormonal responsiveness, with significant upregulation not only under MeJA but also under IAA and ABA treatments. Among these, *RcDHD/SDH2* displayed the most robust response to IAA, with expression levels increasing by nearly 10-fold. In contrast, *RcDHD/SDH4* expression was suppressed by ABA, while the remaining genes were positively regulated.

These findings are consistent with the predicted hormone-responsive elements in the gene promoters, suggesting that *RcDHD/SDH* family members may act in coordination during jasmonate-mediated defense responses and the crosstalk between growth and stress signaling pathways.

3.10 Analysis of Gallic Acid and Ellagic Acid Contents in Different Tissues and Fruit Developmental Stages of *Rubus chingii*

High-performance liquid chromatography (HPLC) was employed to determine the dynamic changes in gallic acid and ellagic acid contents across various tissues and fruit developmental stages of *R. chingii*. The results revealed significant spatial and temporal differences in the accumulation of these phenolic compounds. During fruit development, both gallic acid and ellagic acid exhibited their highest concentrations at the LG, which were markedly greater than those observed at the BG, YE, and RE stages. Their contents gradually declined and stabilized as the fruit matured, indicating that the young fruit stage represents a critical period for the biosynthesis of these metabolites.

In terms of tissue distribution, gallic acid was predominantly enriched in the roots, with levels significantly higher than those in stems, leaves, flowers, and regenerated tissues, whereas ellagic acid accumulated mainly in leaves and flowers, displaying concentrations well above those in roots. These findings demonstrate that gallic acid and ellagic acid exhibit distinct tissue-specific and developmental accumulation patterns, suggesting that their metabolic regulation may be closely related to plant defense, reproductive development, and environmental adaptation. This provides valuable theoretical guidance for the medicinal utilization and efficient exploitation of bioactive components in *R. chingii*.

4. Discussion

The shikimate pathway serves as a crucial metabolic bridge between primary and secondary metabolism. *DHD/SDH* genes encode the only bifunctional enzyme in this pathway, capable of catalyzing both the formation of shikimate and the biosynthesis of gallic acid ^[5]. *Rubus chingii*, a dual-purpose medicinal and edible plant recorded in the *Pharmacopoeia of the People's Republic of China (2020 Edition)* ^[28], is rich in secondary metabolites such as flavonoids, phenolic acids, and polysaccharides ^[2], which not only regulate plant growth but are also modulated by structural genes and transcription factors.

In this study, a systematic identification and characterization of the *DHD/SDH* gene family in *R. chingii* was performed, revealing insights into their structural features, evolutionary relationships, and potential functions. A total of four *RcDHD/SDH* gene family members were identified in the *R. chingii* genome, which is comparable to the number reported in other species—such as one member in *Arabidopsis thaliana* ^[10] and *Solanum lycopersicum* ^[11], two in *Nicotiana tabacum* ^[12], and four to six members in species such as grapevine ^[8], pomegranate ^[9], and tea plant ^[13]. This may reflect the high demand for gallic acid accumulation during the growth and development of *R. chingii*.

Gene duplication analysis revealed the presence of tandem duplications within the *RcDHD/SDH* family, suggesting that variation in the number of *DHD/SDH* genes across species could be attributed to tandem and segmental duplication events. Physicochemical property analysis indicated that, except for *RcDHD/SDH4*, all *RcDHD/SDH* proteins are stable, exhibiting varying degrees of hydrophobicity and differences in subcellular localization—implying that they may play diverse roles in cellular metabolic pathways.

Chromosomal localization and collinearity analyses revealed that the *RcDHD/SDH* genes are clustered on LG01 and LG06, exhibiting strong synteny with soybean but not with rice, further supporting the notion that this gene family is highly conserved among dicotyledonous species. Phylogenetic tree analysis also showed that *RcDHD/SDH* members cluster with homologs from soybean and pistachio in Group IV, whereas they did not form a clade with genes from monocots. This suggests that the gene family may have undergone lineage-specific expansion within dicots during evolution, maintaining structural and functional conservation.

Gene structure and motif analysis further confirmed that all *RcDHD/SDH* members share similar exon–intron organizations and conserved motif compositions. They commonly possess typical conserved motifs of the short-chain dehydrogenase/reductase (SDR) family, and tertiary structure prediction also revealed the presence of characteristic DHD/SDH domains. These findings provide molecular evidence supporting the functional consistency and evolutionary stability of this gene family.

Promoter analysis identified extensive enrichment of hormone-responsive cis-elements, including those responsive to abscisic acid (ABA), methyl jasmonate (MeJA), and indoleacetic acid (IAA), as well as stress-responsive elements related to light, low temperature, and drought. This suggests that *RcDHD/SDH* genes may be involved in complex regulatory networks modulating developmental and environmental responses.

To investigate the relationship between *RcDHD/SDH* genes and the biosynthesis of gallic acid and ellagic acid, a combined analysis of qRT-PCR and HPLC was performed, revealing a close association between *RcDHD/SDH* family members and phenolic compound accumulation in *Rubus chingii*. HPLC results showed that gallic acid and ellagic acid reached their highest levels during the young fruit stage, a pattern that closely paralleled the generally elevated expression of *RcDHD/SDH* genes at the same stage. In particular, *RcDHD/SDH1* and *RcDHD/SDH2* exhibited strong expression in young fruit but were significantly downregulated at maturity, suggesting their involvement in early gallic acid biosynthesis or related secondary metabolic regulation. In contrast, the continuously decreasing expression of *RcDHD/SDH3* and *RcDHD/SDH4* throughout fruit development implies a role in the dynamic regulation of ellagic acid or downstream flavonoid pathways.

Tissue-specific analyses further revealed that *RcDHD/SDH4* showed the highest transcript levels in leaves and stems, consistent with the HPLC observation that ellagic acid accumulated mainly in leaves and flowers, indicating a possible role in phenolic compound accumulation related to leaf defense and photosynthesis. Gallic acid was most abundant in roots, and the relatively high expression of *RcDHD/SDH1* and *RcDHD/SDH2* in this tissue suggests a potential function in the cross-regulation of primary and secondary metabolism in roots.

As a key enzyme in the shikimate pathway, DHD/SDH has been demonstrated in multiple plant species to be closely associated with the biosynthesis of gallic acid, ellagic acid, and flavonoids. In tea (*Camellia sinensis*), downregulation of *DHD/SDH* expression significantly suppresses gallic acid production, thereby affecting catechin accumulation. Similarly, studies in pomegranate (*Punica granatum*) have reported a correlation between *DHQ/SDH* gene expression and gallic acid content in fruit ^[29], highlighting its potential role in gallic acid metabolism. Furthermore, alternative splicing of *DHD/SDH* under glyphosate stress in tea has been suggested to modulate gene function and influence stress responses ^[30]. Collectively, these findings indicate that *DHD/SDH* not only serves as a pivotal enzyme in the shikimate pathway but also acts as a critical regulatory node for the synthesis of key secondary metabolites such as gallic acid and ellagic acid in plants.

To further explore the potential relationship between *DHD/SDH* genes and the biosynthesis of metabolites such as gallic acid and ellagic acid, qRT-PCR analysis was conducted. Tissue-specific expression patterns revealed that all four *RcDHD/SDH* genes are expressed in roots, stems, leaves, and flowers, with apparent tissue preference. Notably, *RcDHD/SDH4* exhibited the highest expression in leaves and stems, suggesting a role in leaf metabolism or structural development. During fruit development, all four genes showed peak expression at the early fruit stage (small green fruit), followed by a gradual decline. *RcDHD/SDH1* and *RcDHD/SDH2* exhibited significant downregulation at the mature stage, implying roles in early-stage secondary metabolism or cell division. In contrast, the continuously decreasing expression of *RcDHD/SDH3* and *RcDHD/SDH4* suggests involvement in the dynamic regulation of phenolic or flavonoid biosynthetic pathways associated with fruit ripening.

Exogenous hormone treatments further validated the predicted functions of promoter cis-elements. Methyl jasmonate (MeJA) treatment significantly upregulated the expression of all *RcDHD/SDH* members, with *RcDHD/SDH3* and *RcDHD/SDH4* showing the most substantial increases—approximately 8-fold and 7-fold, respectively—indicating their high sensitivity to jasmonate signaling. In contrast, *RcDHD/SDH2* exhibited the strongest response to indoleacetic acid (IAA), with nearly a 10-fold increase in expression, suggesting a central role in auxin-mediated developmental regulation. Notably, abscisic acid (ABA) treatment suppressed *RcDHD/SDH4* expression, implying a distinct regulatory function for this gene under stress conditions. Collectively, these findings highlight the crucial role of the *RcDHD/SDH* gene family in hormone-mediated crosstalk between plant growth and defense. The genes may contribute to fruit development and environmental adaptation in *Rubus chingii* Hu by modulating the accumulation of downstream secondary metabolites.

In conclusion, this study comprehensively characterized the *RcDHD/SDH* gene family in *Rubus chingii* Hu, integrating gene structure, evolutionary relationships, expression profiles, and hormone responsiveness. The findings provide novel insights into the potential regulatory mechanisms of this gene family and offer a theoretical and molecular foundation for future functional studies and breeding of improved cultivars.

5. Conclusion

In this study, four members of the *RcDHD/SDH* gene family were systematically identified in *Rubus chingii* Hu, and their characteristics were comprehensively analyzed from multiple perspectives, including gene structure, chromosomal localization, phylogenetic relationships, conserved motifs, protein structure, promoter cis-elements, and expression patterns. Notably, *RcDHD/SDH4* exhibited high expression levels in stems and leaves, which is consistent with the HPLC results showing ellagic acid enrichment in leaves and flowers, suggesting a possible involvement in phenolic accumulation related to leaf defense and photosynthesis. During fruit development, both gallic acid and ellagic acid accumulated to relatively high levels at the early fruit stage. During fruit development, all four genes showed peak expression at the small green fruit stage, followed by a gradual decline, indicating their possible involvement in early fruit developmental processes. Hormone treatment experiments further demonstrated that the *RcDHD/SDH* genes are responsive to MeJA, IAA, and ABA, highlighting their roles in plant growth, development, and stress responses. Collectively, this study provides a theoretical foundation and valuable resources for the functional characterization of the *DHD/SDH* gene family in *R. chingii* and lays the groundwork for future applications in fruit quality improvement.

Abbreviations

RNA-Seq	RNA sequencing
GA	gallic acid
EA	ellagic acid
TCM	traditional Chinese medicine
DHD/SDH	3-dehydroquinate dehydratase/shikimate dehydrogenase
DHQ	3-dehydroquinate
DHS	dehydroshikimate
SA	shikimic acid
MeJA	methyl jasmonate
ABA	abscisic acid
IAA	indole-3-acetic acid

Declarations

Acknowledgements

None

Authors' contribution

L.X.A: Formal Analysis, Data Curation, Investigation, Methodology, Software, Validation, Writing-Original Draft.
.Z.Y.L: investigation. T.Y.G: investigation. N.N.F: investigation. W.B.H: Supervision. F.H.L: Supervision.
H.J.C: Supervision. F.D.Y: Supervision, Validation. S.Z.L: Supervision, Validation. N.Z.H: Conceptualization, Data curation, Funding acquisition, Methodology, Writing-Review and Editing.

Funding

This work was supported by the Zhejiang Public Welfare Fund Program (No. LTGN24H280001) and Zhejiang Provincial Natural Science Foundation of China (No. LD25H280001).

Data availability

Sequencing data from this study have been deposited at the National Center for Biotechnology Information. The genome sequence data are available under NCBI BioProject number PRJNA666516 (*Rubus chingii* Hu raw sequence reads, <https://www.ncbi.nlm.nih.gov/sra/PRJNA666516>). We have uploaded the gene annotation, functional annotation and repeat sequence to the GDR database in time (Sook et al., 2004). The detailed links are as follows: the accession number was tfGDR1051, the access link is https://www.rosaceae.org/publication_datasets and the genome page below has links to various pages was <https://www.rosaceae.org/Analysis/11326199>. **Declarations**

Ethics approval and consent to participate

The authors declare that all methods were carried out in accordance with relevant guidelines and regulations. These plant materials do not include any wild species at risk of extinction. No specific permits are required for sample collection in this study. We comply with relevant institutional, national, and international guidelines and legislation for plant study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Conflict of interest

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

References

1. Yu G, Luo Z, Wang W, et al. *Rubus chingii* Hu: A review of the phytochemistry and pharmacology[J]. *Frontiers in Pharmacology*. 2019;10:799.
2. Jia-Yun S, Si-Qi W, Kao-Hua LI, U, et al. *Rubus chingii* Hu: An overview of botany, traditional uses, phytochemistry, and pharmacology[J]. *Chin J Nat Med*. 2020;18(6):401–16.

3. Zhang L, Wang Y, Mei Q et al. Composition and antioxidant and antidiabetic activities of different polyphenol fractions from the fruits of *Rubus chingii* Hu[J]. 2022.
4. Sun P, Zhang GY, Xiang P, et al. Expression and regulation of the shikimic acid pathway gene DHD/SDH in tea plant (*Camellia sinensis*)[J]. *Chin J Appl Environ Biol*. 2018;24:322–7.
5. Muir RM, Ibáñez AM, Uratsu SL, et al. Mechanism of gallic acid biosynthesis in bacteria (*Escherichia coli*) and walnut (*Juglans regia*)[J]. *Plant Mol Biol*. 2011;75:555–65.
6. Feng LJ, Yin YL, Zhao XQ, et al. Research progress on metabolism and health function of gallic acid in pomegranate[J]. *J Fruit Sci*. 2014;31(4):710–6.
7. Saijo R. Pathway of gallic acid biosynthesis and its esterification with catechins in young tea shoots[J]. *Agric Biol Chem*. 1983;47(3):455–60.
8. Bontpart T, Marlin T, Vialet S, et al. Two shikimate dehydrogenases, VvSDH3 and VvSDH4, are involved in gallic acid biosynthesis in grapevine[J]. *J Exp Bot*. 2016;67(11):3537–50.
9. Habashi R, Hacham Y, Dhakarey R, et al. Elucidating the role of shikimate dehydrogenase in controlling the production of anthocyanins and hydrolysable tannins in the outer peels of pomegranate[J]. *BMC Plant Biol*. 2019;19:1–15.
10. Singh SA, Christendat D. Structure of Arabidopsis dehydroquinase dehydratase-shikimate dehydrogenase and implications for metabolic channeling in the shikimate pathway[J]. *Biochemistry*. 2006;45(25):7787–96.
11. Bischoff M, Schaller A, Bieri F, et al. Molecular characterization of tomato 3-dehydroquinase dehydratase-shikimate: NADP oxidoreductase[J]. *Plant Physiol*. 2001;125(4):1891–900.
12. Ding L, Hofius D, Hajirezaei MR, et al. Functional analysis of the essential bifunctional tobacco enzyme 3-dehydroquinase dehydratase/shikimate dehydrogenase in transgenic tobacco plants[J]. *J Exp Bot*. 2007;58(8):2053–67.
13. Huang K, Li M, Liu Y, et al. Functional analysis of 3-dehydroquinase dehydratase/shikimate dehydrogenases involved in shikimate pathway in *Camellia sinensis*[J]. *Front Plant Sci*. 2019;10:1268.
14. Guo J, Carrington Y, Alber A, et al. Molecular characterization of quinate and shikimate metabolism in *Populus trichocarpa*[J]. *J Biol Chem*. 2014;289(34):23846–58.
15. Michel G, Roszak AW, Sauvé V, et al. Structures of shikimate dehydrogenase AroE and its paralog YdiB: a common structural framework for different activities[J]. *J Biol Chem*. 2003;278(21):19463–72.
16. Peek J, Lee J, Hu S, et al. Structural and mechanistic analysis of a novel class of shikimate dehydrogenases: evidence for a conserved catalytic mechanism in the shikimate dehydrogenase family[J]. *Biochemistry*. 2011;50(40):8616–27.
17. Wang L, Lei T, Han G, et al. The chromosome-scale reference genome of *Rubus chingii* Hu provides insight into the biosynthetic pathway of hydrolyzable tannins[J]. *Plant J*. 2021;107(5):1466–77.
18. Chen C, Chen H, Zhang Y, et al. TBtools: an integrative toolkit developed for interactive analyses of big biological data[J]. *Mol Plant*. 2020;13(8):1194–202.
19. Zishu X, Yi D, Hui L et al. Analysis of WAK Genes in Nine Cruciferous Species with a Focus on *Brassica napus* L. [J]. *International journal of molecular sciences*, 2023, 24(17) : 13601.
20. Wang Y, Tang H, DeBarry JD, et al. MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity[J]. *Nucleic Acids Res*. 2012;40(7):e49–49.
21. Zhao Y, Duan B, Liu Y, et al. Identification and characterization of the LDAP family revealed GhLDAP2_Dt enhances drought tolerance in cotton[J]. *Front Plant Sci*. 2023;14:1167761.

22. Xu J, Zhang H, ** W. Genome-wide identification, characterization, and expression profiles of auxin responsive GH3 gene family in *Salvia miltiorrhiza* involved in MeJA treatment[J]. *J Plant Biochem Biotechnol*. 2022;31(1):85–97.
23. Liang Xianli Z, Liqing K, Liping, et al. Cloning and functional analysis of the chalcone synthase gene *GhCHS1* in colored cotton. *J Agricultural Biotechnol*. 2019;27(6):993–1002.
24. Wang X, Yuan B, Zhu N, et al. Identification and functional analysis of the promoter of a leucoanthocyanidin reductase gene from *Gossypium hirsutum*[J]. *Mol Biotechnol*. 2023;65(4):645–54.
25. Yu Z, Zhao C, Zhang M, et al. Analysis of Polysaccharide Content and Biosynthesis Pathway in Different Tissues of *Nootropic*[J]. *Chin J Trop Subtropical Bot*. 2021;29(6):669–77.
26. Wu Y, Zhang C, Yang H, et al. Selection and validation of candidate reference genes for gene expression analysis by RT-qPCR in *Rubus*[J]. *Int J Mol Sci*. 2021;22(19):10533.
27. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2 – $\Delta\Delta CT$ method[J]. *Methods*. 2001;25(4):402–8.
28. National Pharmacopoeia Commission. *Pharmacopoeia of the People's Republic of China*, 2020 edition, Part I [M]. Beijing: China Medical Science and Technology; 2020.
29. Feng L, Jiao Q, Yin Y, et al. Cloning and sequence analysis of *DHQ/SDH* gene from pomegranate peel. *Jiangsu Agric Sci*. 2017;45(1):26–9.
30. Li G, He Y, Qu N, et al. Alternative splicing participates in the molecular response of tea plant to glyphosate stress. *J Tea Sci*. 2024;65(1):11–9.

Figures

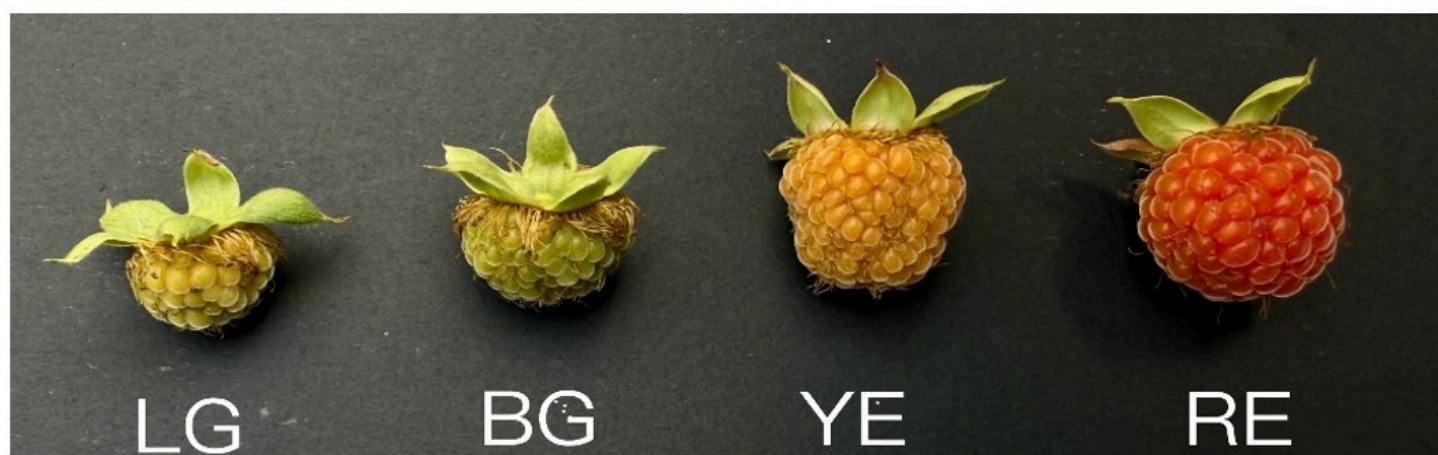


Figure 1

Fruits of *Rubus chingii* at different developmental stages.

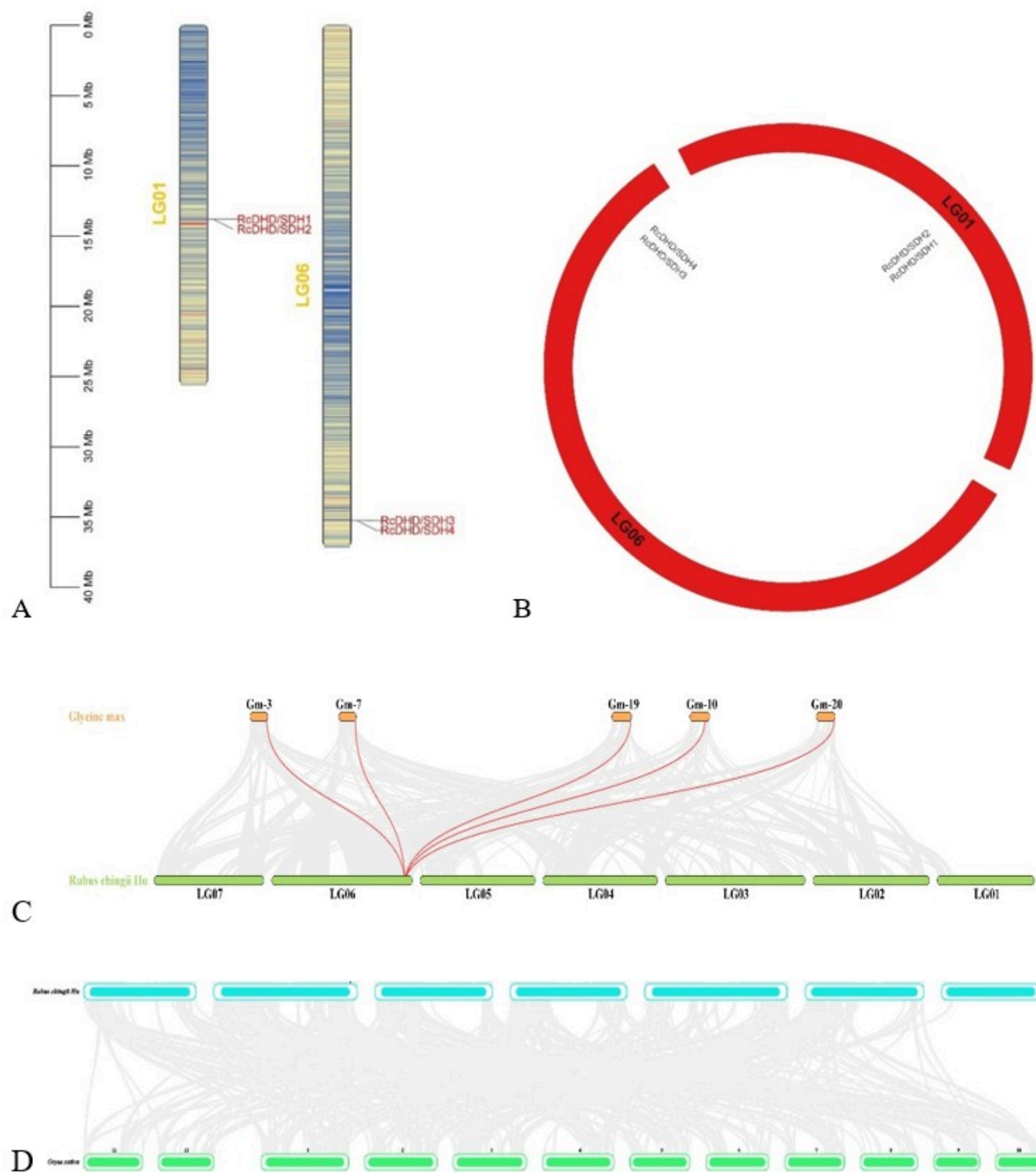


Figure 2

Chromosomal location and syntenic analyses of *RcDHD/SDH* in *R. chingii*

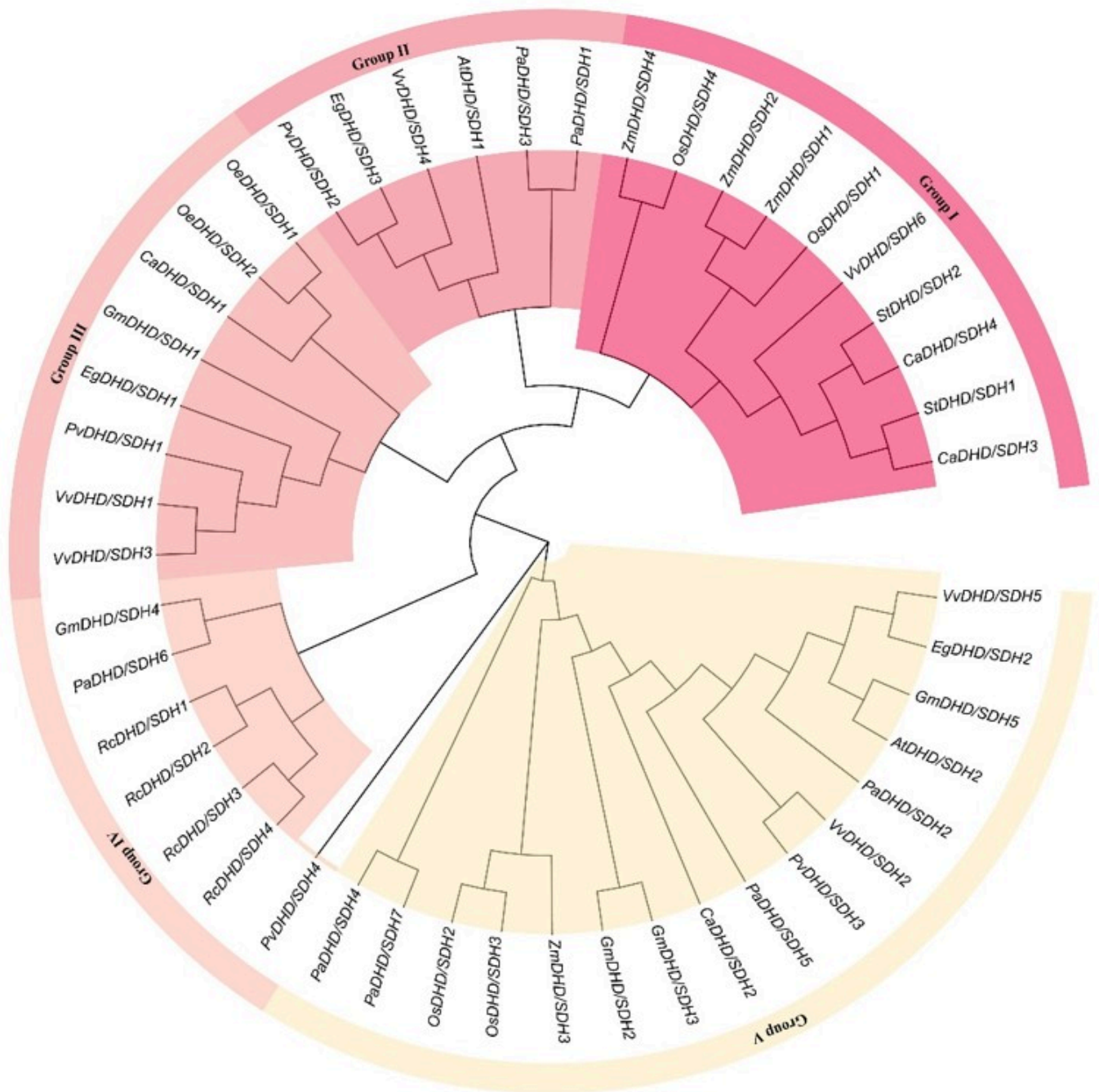


Figure 3

Phylogenetic tree of DHD/SDH family members in *R. chingii*, *A. thaliana*, *Glycine max*, *Z. mays* and *V. vinifera*

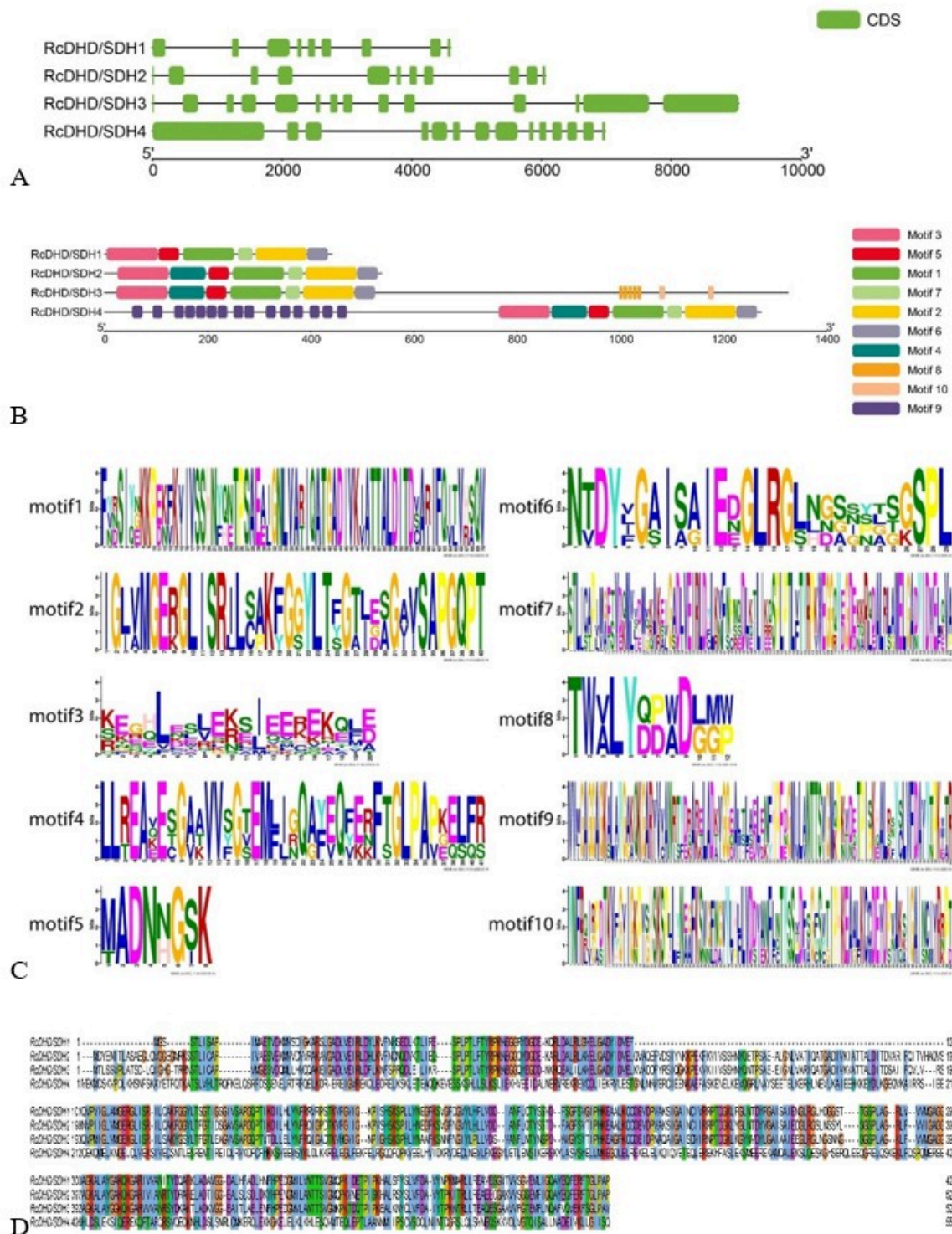


Figure 4

Gene structure **A** **conserved motifs** **B** **motif logo** **C** **and protein sequence alignment** **D** **of RcDHD/SDH family members in *R. chingii***

RcDHD/SDH1

RcDHD/SDH2

RcDHD/SDH3

RcDHD/SDH4

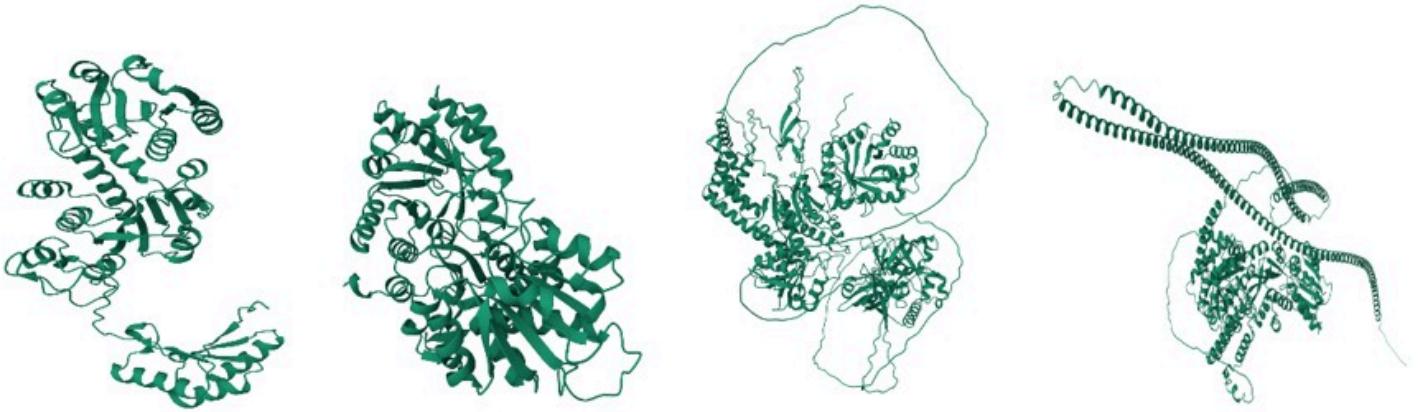


Figure 5

Analysis of tertiary structure of RcDHD/SDH family proteins in *R. chingii*

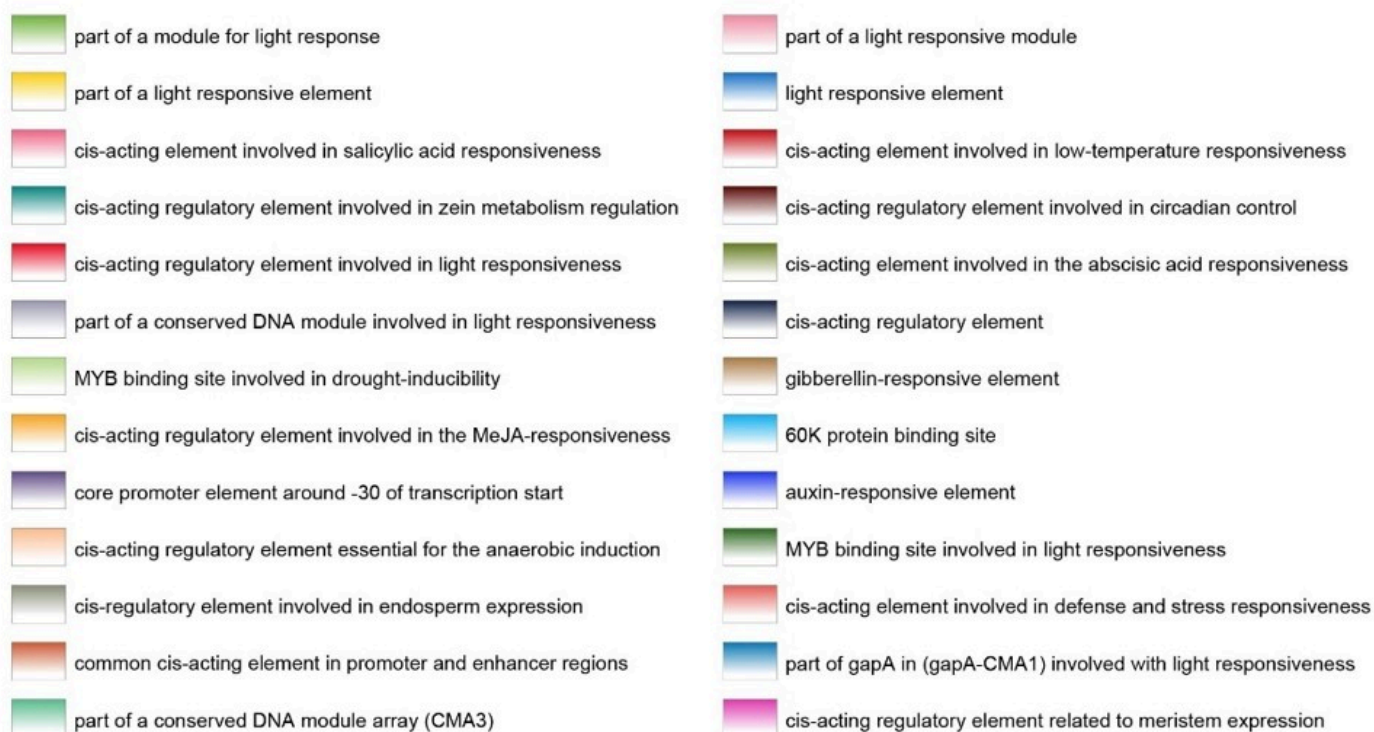
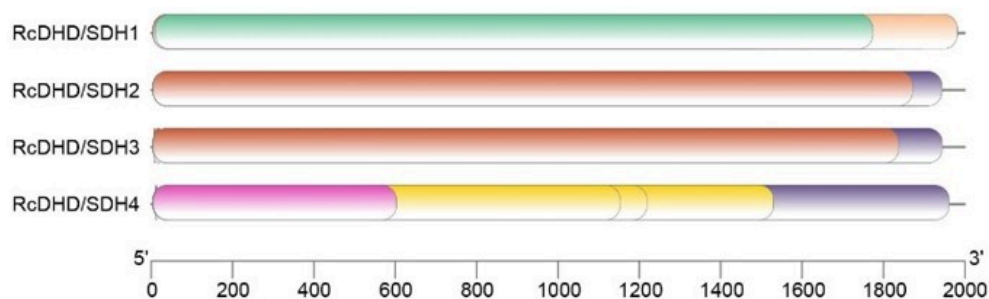


Figure 6

Analysis of cis-acting elements in the promoter region of *RcDHD/SDH* gene

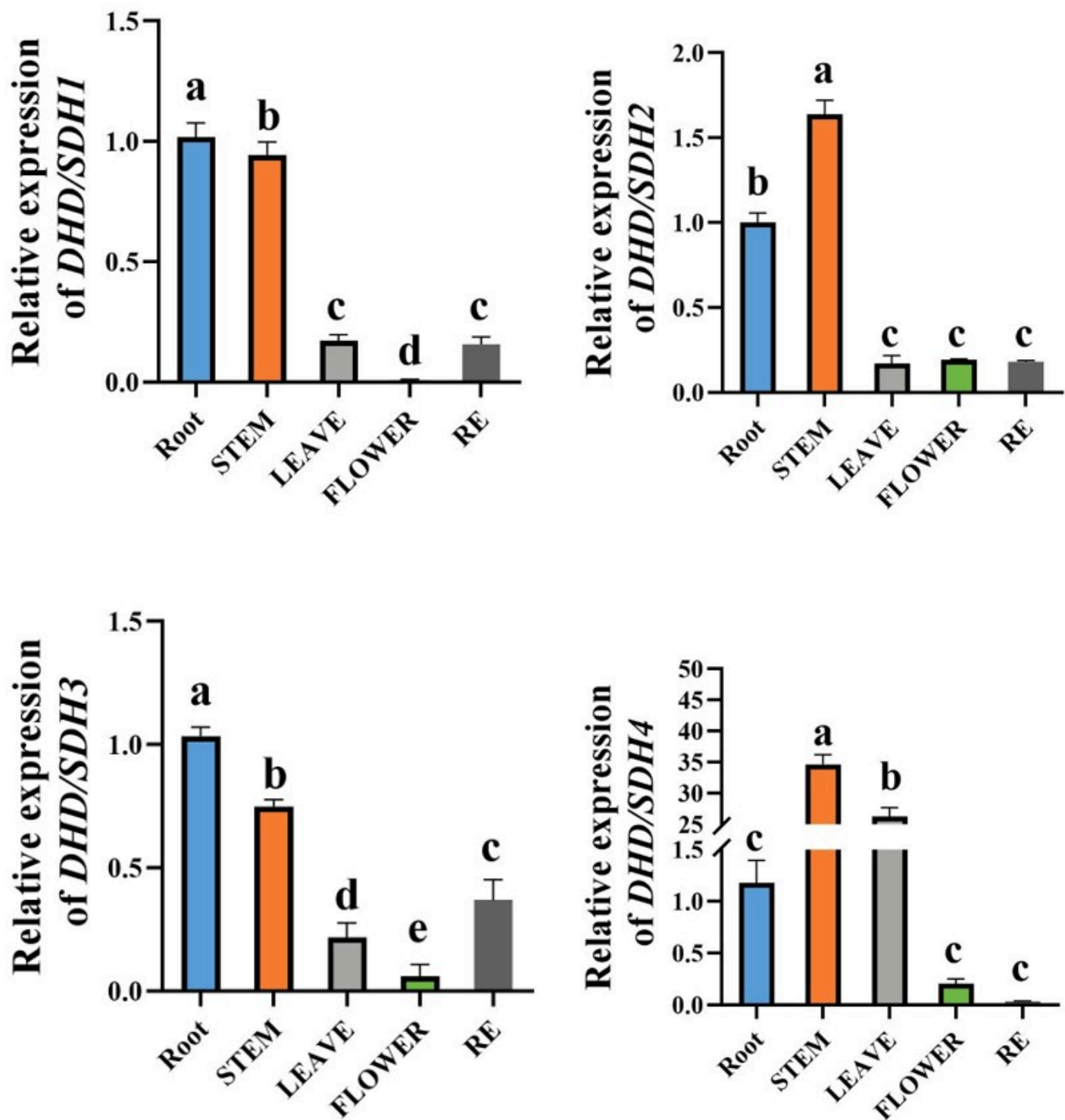


Figure 7

Tissue specific expression analysis of *RcDHD/SDH* gene family in *R. chingi*

Note: Ro=Root. St=Stem. Le=Leaf. Fl=Flower. Re=Red Fruit. Different letters indicate significant difference at 0.05 level. The same as following.

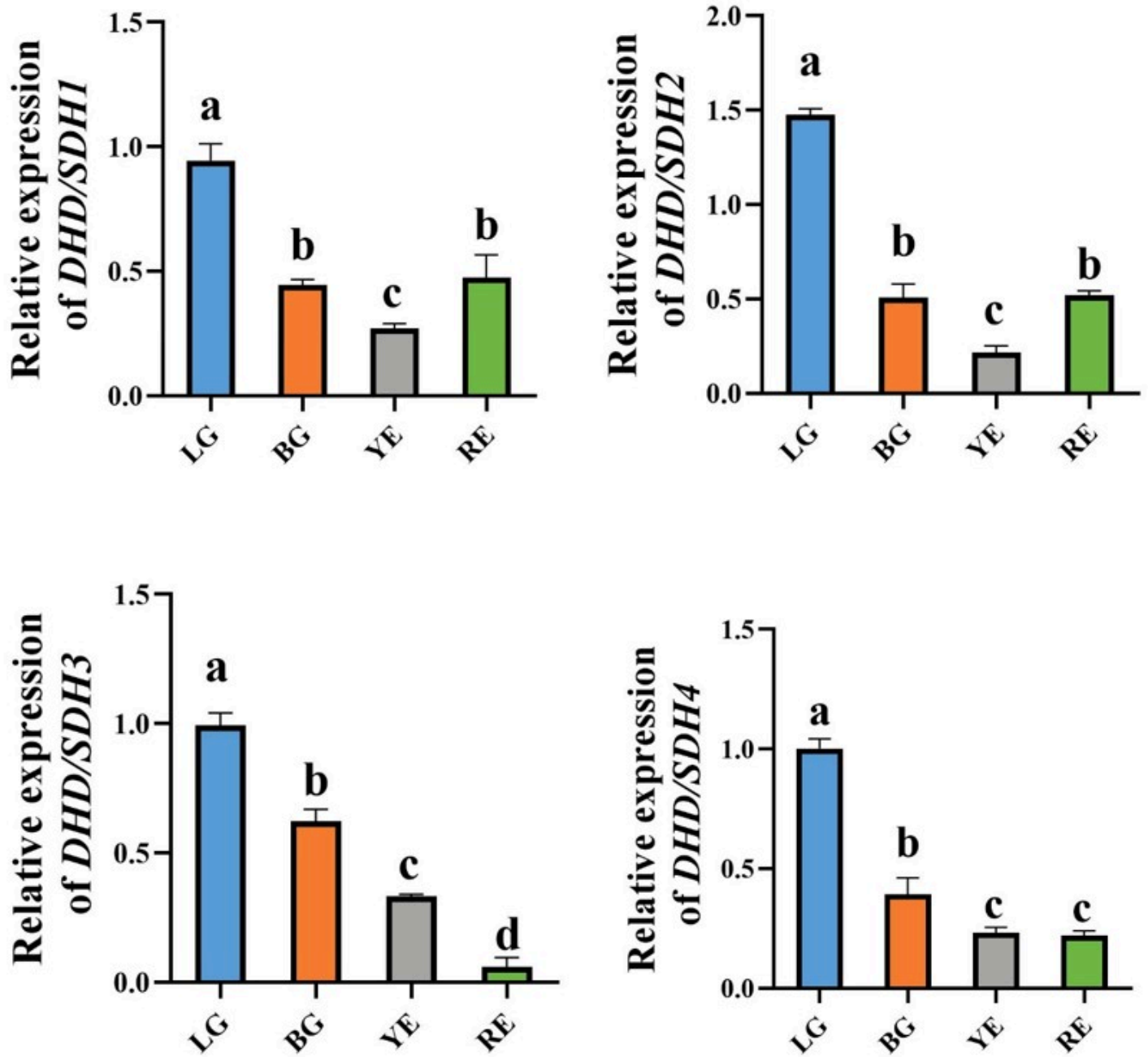


Figure 8

Expression analysis of *RcDHD/SDH* gene family at fruit developmental stages in *R. chingii*

Note: LG: Little green fruit. BG: Big green fruit. YE: Yellow fruit. RE: Red fruit.

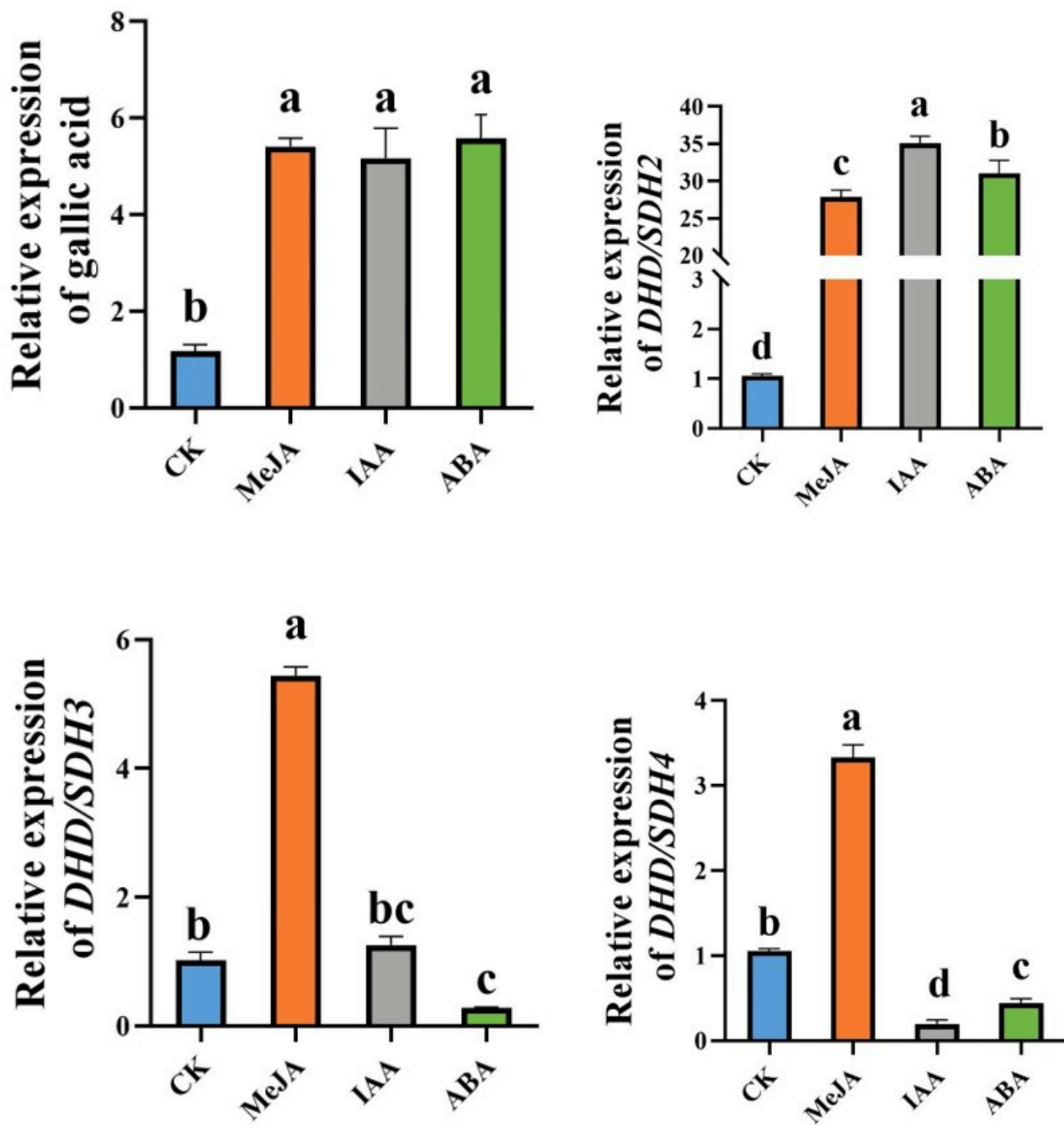


Figure 9

Expression levels of *RcDHD/SDH* family under MeJA/IAA/ABA induction treatment in *R. chingii*

Note: CK=Control. MeJA/IAA/ABA=Treatment.

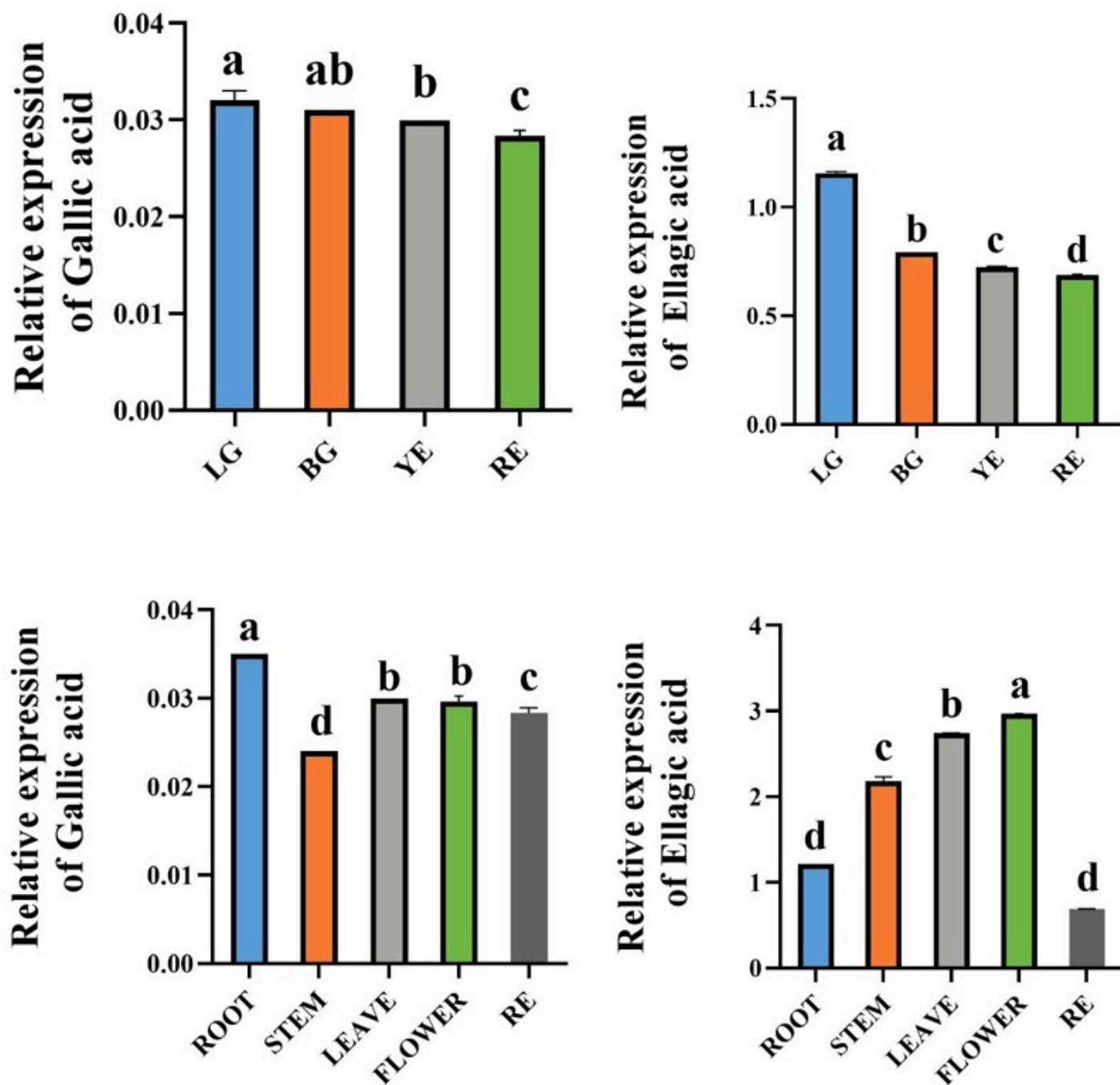


Figure 10

Gallic Acid and Ellagic Acid Contents in Different Tissues and Fruit Developmental Stages of *Rubus chingii*

Note: LG: Small green fruit. BG: Big green fruit. YE: Yellow fruit. RE: Red fruit.