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# Genetic Regulation of Secondary Metabolism and Its Association with Pharmacological Traits in Honeysuckle (*Lonicera japonica*)

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**Abstract** This study mainly introduces several important metabolic pathways such as phenolic acids, flavonoids, iridoids and volatile terpenoids, discusses some key structural genes in these pathways, summarizes the key influences played by several core transcription factors in regulating these metabolic networks, and analyzes genomic, transcriptomic and metabolomic data together. It was observed that the metabolic flow varies under different tissues, developmental stages, and environmental conditions. This further explained the accumulation mode of metabolites and their relationship with pharmacological effects. The application prospects of MAS, CRISPR/Cas gene editing, and synthetic biology in the quality improvement and targeted enhancement of active components of honeysuckle were also discussed. This study aims to establish a relatively complete “metabolism-gene-trait” association framework, providing a reference for the rapid breeding of honeysuckle and more accurate pharmacological prediction in the future.

**Keywords** Honeysuckle (*Lonicera japonica*); Secondary metabolism; Transcriptional regulation; Multi-omics integration; Pharmacological traits

## 1 Introduction

*Lonicera japonica* Thunb., also known as honeysuckle, is a medicinal plant that has been used in traditional Chinese medicine for a long time. Its dried flower buds (Flos Lonicerae) have been used for heat-clearing, detoxifying, anti-inflammatory, antibacterial and antiviral purposes as early as over 1 500 years ago. Modern pharmacology has further demonstrated that the effects of honeysuckle are not limited to traditional Chinese medicine. Nowadays, it is also made into health food, health supplements and cosmetics. Studies have shown that it has multiple biological activities such as anti-inflammation, anti-oxidation, immune regulation and anti-tumor, and is an important source for the integration of traditional Chinese and Western medicine and the development of new drugs (Zheng et al., 2022; Zheng and Wang, 2024).

Honeysuckle contains many types of secondary metabolites, mainly including chlorogenic acids, flavonoids, iridoids and volatile components, etc. (Ma et al., 2024). Among them, chlorogenic acids have antioxidant, antibacterial, antiviral, anti-inflammatory and hypoglycemic effects, and are important indicators for evaluating the quality of honeysuckle (Zeng et al., 2017; Wang et al., 2020). Flavonoids (such as rutin, quercetin, luteolin, etc.) not only have anti-inflammatory, anti-tumor and cardiovascular protective effects, but also play a role in the treatment of chronic diseases such as diabetes and liver injury (Muro et al., 2025). Iridoids (such as loganin) have also attracted attention due to their anti-inflammatory and anti-shock effects. The volatile components and polysaccharides in honeysuckle also exhibit antibacterial and immunomodulatory potential (Yang et al., 2023). The content and composition of these secondary metabolites will directly affect the medicinal efficacy of honeysuckle and also relate to its application value in the medical and health fields.

This study mainly analyzed the genetic regulatory mechanisms of secondary metabolites in honeysuckle, with a focus on the synthetic pathways and regulatory methods of chlorogenic acid, flavonoids, iridoids and volatile components, and explored their relationships with pharmacological traits. Through multi-omics integration, key genes, transcription factors and some epigenetic modifications involved in regulation were identified. This study aims to provide a theoretical basis and practical reference for the improvement of the medicinal quality of honeysuckle, molecular breeding and new drug development.

## 2 Secondary Metabolic Pathways in Honeysuckle

### 2.1 Biosynthetic pathways of phenolic acids

The phenolic acids in honeysuckle (such as chlorogenic acid) are an important source of its medicinal effects. Phenolic acids are mainly produced through the phenylpropane pathway. This pathway starts with phenylalanine and then passes through the catalysis of key enzymes such as PAL, C4H, and 4CL in sequence, eventually forming a variety of phenolic acid substances. Transcriptomic and metabolomic studies have found that genes like *4CL* vary greatly in expression among different tissues and developmental stages, and these expression changes are closely related to phenolic acid content (Wang et al., 2020; Wang et al., 2023). In addition, environmental conditions such as nitrogen supply, light exposure, and salt stress can also affect the expression of related genes, thereby altering the accumulation of phenolic acids (Cao et al., 2025). Some epigenetic regulations, such as the methylation of H3K4 and H3K9, have also been proven to be related to the synthesis of phenolic acids like chlorogenic acid.

### 2.2 Flavonoid biosynthesis and structural diversification

Flavonoids are another important type of secondary metabolites in honeysuckle, possessing various activities such as anti-inflammatory and antioxidant properties. The synthesis of flavonoids also comes from the phenylpropane pathway, and then goes through multiple enzymatic steps such as CHS, CHI, and F3H to form different flavonoid structures. Studies have shown that some R2R3-MYB type transcription factors (such as LjaMYB12 and LjaMYB305) are very crucial in regulating flavonoid synthesis. They can directly activate the expression of structural genes such as *4CL* and *CHS*, and jointly regulate the accumulation of flavonoids with environmental signals such as light (Qi et al., 2019). In addition, modifying enzymes such as UDP-glycosyltransferase are involved in the glycosylation of flavonoids, thereby increasing the structural diversity of flavonoids (Cai et al., 2022). The types and contents of flavonoids vary significantly among different tissues and developmental stages, with the highest contents in flowers and flower buds (Wang et al., 2023).

### 2.3 Iridoid and volatile terpenoid biosynthetic pathways

The glycosides (such as iridoids glycosides and monoterpene glycosides) and volatile terpenoids (such as geraniol and α -terpineol) in honeysuckle determine its aroma and are also related to some pharmacological effects. Iridoids glycosides are mainly precursors provided by the MVA and MEP pathways, among which the expression level of the key enzyme G10H is very high in flowers. G10H converts geranol to 10-hydroxygeraniol, which is the rate-limiting step in the synthesis of iridoids glycosides (Yang et al., 2019). The synthesis of volatile terpenoids involves different branches such as monoterpenoids, sesquiterpenoids and triterpenoids, and its synthesis process is jointly regulated by multiple enzymes (such as α -terpineol synthase, farnesyl pyrophosphate synthase, etc.) and transcription factors (such as MYB, WRKY, etc.). The terpene content varies greatly among different varieties, different developmental stages and different tissues. In addition, ultraviolet rays and light exposure can also induce the expression of related enzymes, thereby promoting the accumulation of terpenoids and glycosides (Fang et al., 2019).

## 3 Genetic Basis of Secondary Metabolism

### 3.1 Key structural genes encoding enzymes in major metabolic pathways

The medicinal value of honeysuckle mainly comes from its rich secondary metabolites, such as chlorogenic acid, luteolin, flavonoids, triterpene saponins and iridoids, etc. Many key genes in related synthetic pathways have been identified. Genes such as *PAL*, *C4H*, *4CL*, *HCT*, *C3'H*, *F3'H*, *CHS*, *CHI*, and *FNSII* in the phenylpropane pathway are the most important batch of genes in the process of chlorogenic acid and flavonoid generation (Zhang et al., 2022). The expression of these genes changes with the development of flowers, and this change directly affects the content of active ingredients (Cai et al., 2022). In the synthesis of triterpene saponins, gene clusters such as *OAS* and *UGT73* play important roles in the biosynthesis of oleanolic acid and related saponins. In the synthetic pathway of iridoids, *G10H* is the key gene that was first cloned and its function verified in honeysuckle, demonstrating its great significance in iridoid synthesis (Zhang et al., 2025). In addition, enzymes such as CYP73A(C4H) and CYP98A(C3'H) in the CYP450 family also play important roles in the synthesis of phenolic compounds such as chlorogenic acid, and different subtypes are closely related to the content of active substances

(Qi et al., 2017). Some modifying enzymes, such as UDP-glycosyltransferase, fatty acid synthesis-related enzymes and terpene synthases (TPS family), are also involved in the modification and accumulation of multiple secondary metabolites. The transcriptome results indicated that the expression of these structural genes varied significantly in different tissues and at different growth stages, and was highly correlated with the distribution of medicinal components (Wang et al., 2020).

### 3.2 Transcription factors regulating secondary metabolism (MYB, bHLH, AP2/ERF, WRKY)

Multiple families of transcription factors play a core role in the regulation of secondary metabolism in honeysuckle. In the MYB family, LjaMYB305 can directly bind to and activate the promoters of Lja4CL2 and LjaCHS3, thereby promoting flavonoid production. Furthermore, it can also be co-regulated with LjaHY5 related to optical signals (Fang et al., 2025). Other MYB members, such as MYB114 and MYB12, are also significantly positively correlated with the accumulation of substances like flavonoids and luteolin. The bHLH family usually forms the MBW complex together with MYB, further finely regulating flavonoid metabolism (Wang et al., 2019). Some members of the AP2/ERF family also affect the synthesis of chlorogenic acid and flavonoids, and are involved in the regulation of flower development. In the WRKY family, LjWRKY50 can regulate the jasmonic acid synthesis gene *LjAOS*, affecting the opening time of flower buds and the accumulation of active substances. In addition, families such as NAC and bZIP are also related to processes such as antioxidation, aging, and hormone signaling, and are interrelated with multiple secondary metabolic pathways (Wang et al., 2019).

### 3.3 Regulatory modules and gene families associated with compound accumulation

The secondary metabolic regulatory network of honeysuckle is jointly constructed by multiple regulatory modules and gene families. Among them, the MYB-BHLH-WD40 (MBW) complex is the most classic module for regulating flavonoid synthesis, and the synergistic effect of MYB and HY5 can significantly enhance the expression of structural genes and promote the accumulation of related metabolites (Wang et al., 2019). At the gene family level, the TPS family has approximately 70 members and shows high diversity in terpene synthesis and stress response (Wang et al., 2025). The CYP450 family consists of 151 members and is widely involved in multiple metabolic pathways such as chlorogenic acid, flavonoids, and iridoids. It is a key node in the entire network (Qi et al., 2017). Furthermore, miRNA regulates metabolic flow and compound accumulation by targeting structural genes and transcription factors (Liu et al., 2017). Post-translational modifications (such as ubiquitination and succinylation) also affect the activities of key enzymes, thereby further altering the metabolic pathways of active ingredients (Wang et al., 2021).

## 4 Multi-Omics Insights into Metabolic Regulation

### 4.1 Transcriptomic profiling of different tissues, developmental stages, and stress conditions

Transcriptome sequencing revealed that different tissues of honeysuckle (such as flowers, leaves, stems, and buds) showed significant differences in the expression of secondary metabolism-related genes. For instance, chlorogenic acid-related genes are highly expressed in leaves and stems; Flavonoid synthesis genes are more active in flowers and stems. Iridoids-related genes are more frequently found in leaves and terminal buds. These differences also reflect the distinct characteristics of the medicinal components of each tissue (Wang et al., 2023). During the development of flowers, the genes responsible for the synthesis of carotenoids and anthocyanins will also be dynamically adjusted along with the change of flower color, gradually changing from green to white and then to yellow (Figure 1) (Xia et al., 2021; Xue et al., 2020). Furthermore, low temperature and salt stress can also significantly upregulate some secondary metabolic pathway genes, such as the phenylpropane pathway and the carotenoid pathway, thereby promoting stress resistance and the accumulation of active ingredients (Zhang et al., 2022).

### 4.2 Metabolomics for identifying core metabolic signatures and biochemical flux

In metabolomics studies, whether by targeted or non-targeted methods, the main metabolites of different tissues and different developmental stages of honeysuckle were identified, including 28 flavonoids, 35 phenolic acids and 43 iridoids (Wang et al., 2023). These types of metabolites vary greatly among different tissues. In the early stage of flower development, anthocyanins and chlorophyll are mainly accumulated, while in the later stage, a large amount of carotenoids are accumulated, such as α -carotene, γ -carotene and zeaxanthin, etc. These changes directly affect flower color (Xia et al., 2021; Xue et al., 2020). Salt stress can also affect metabolic pathways such as phenylpropane, terpenoids and glycolysis. Among them, low salt stress can also increase the content of some medicinal components. There are also differences in the accumulation of phenylpropane metabolites in flower buds at different harvest periods, and these differences can be used for the quality assessment of medicinal materials and the optimization of harvest periods.

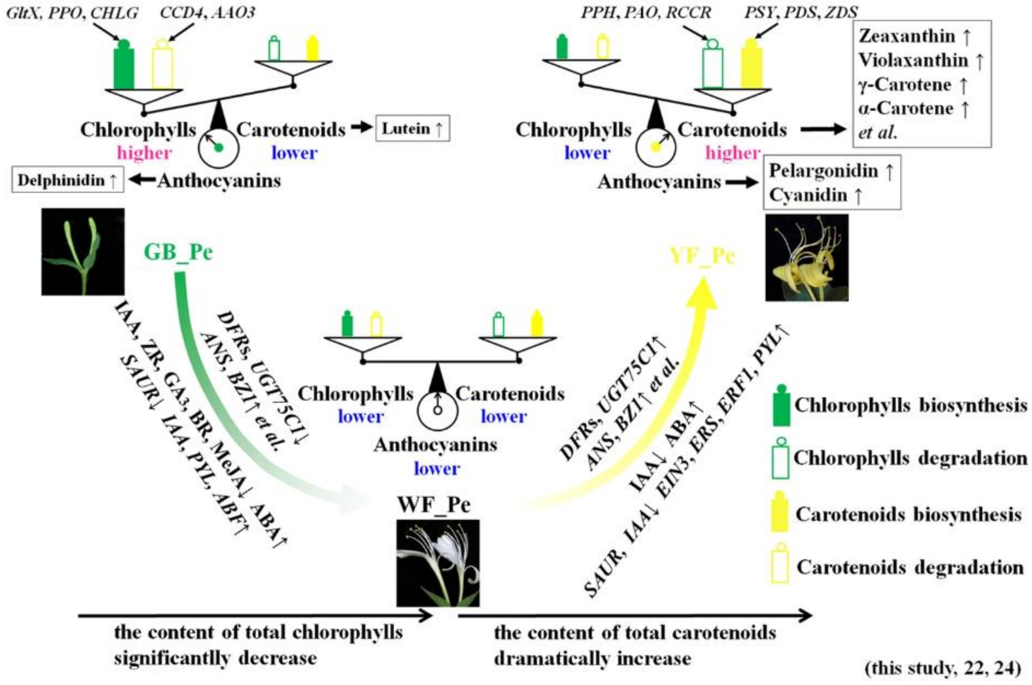

Figure 1 Schematic of changes in the regulatory genes and metabolites in petal color- transition in *L. japonica* (Adopted from Xia et al., 2021)

### 4.3 Integration of genomics, transcriptomics, and metabolomics for pathway reconstruction

After integrating genomic, transcriptomic and metabolomic data, researchers have been able to systematically reconstruct the secondary metabolic pathways of honeysuckle. Chromosome-level genomic assembly has revealed the expansion of gene families related to multiple metabolic pathways, such as carotenoid, phenylpropane and terpene pathways, and has identified the corresponding expression modules. WGCNA analysis further identified gene modules highly correlated with metabolite accumulation (Pu et al., 2020). Multi-omics joint analysis also indicated that the glycolytic - phenylpropane pathway was more active in the early stage of flower development, promoting flavonoid accumulation. In the late stage of flower development, the tricarboxylic acid cycle - terpenoid backbone synthesis pathway is further strengthened, thereby promoting terpene generation (Yang et al., 2019). In addition, the synergistic regulation between multiple transcription factors (such as MYB, bHLH, AP2/ERF) and key enzyme genes is at the core of the changes in the entire secondary metabolic network (Xiao et al., 2021; Tan et al., 2025). These integrated analyses provide an important theoretical basis for the molecular breeding and quality improvement of the medicinal components of honeysuckle.

## 5 Environmental and Developmental Influences on Metabolite Accumulation

### 5.1 Effects of light, temperature, soil nutrients, and geographic environments

The secondary metabolites in honeysuckle are regulated by various environmental factors. Lighting is a very important factor. Strong light can promote the synthesis of flavonoids and phenolic acids, and up-regulate the expression of related genes such as *4CL* and *CHS*, thereby increasing the flavonoid content (Fang et al., 2019). On the contrary, if shading treatment is carried out, the content of chlorogenic acid (CGA) will significantly decrease, and the expression of genes related to CGA biosynthesis will also decline. The expression level of the optical signal factor HY5 is positively correlated with the content of CGA. Geographical conditions such as temperature, precipitation and altitude can also affect the active components of honeysuckle. Honeysuckle from different origins has significant differences in the contents of components such as chlorogenic acid and apigenin glycoside, indicating that a suitable growth environment can promote the accumulation of special metabolites (Cheng et al., 2024). In addition, the type of nitrogen source in the soil can also affect the metabolic flow direction. Supplying nitrate nitrogen ($NO_3^-$) will allow more carbon to flow towards phenolic synthesis, thereby increasing the content of flavonoids and chlorogenic acid. Ammonium nitrogen ($NH_4^+$) is more used in the synthesis of amino acids and lignin (Figure 2) (Cao et al., 2025). Specific light qualities such as blue light can also enhance some active metabolites and antioxidant capacity in callus.

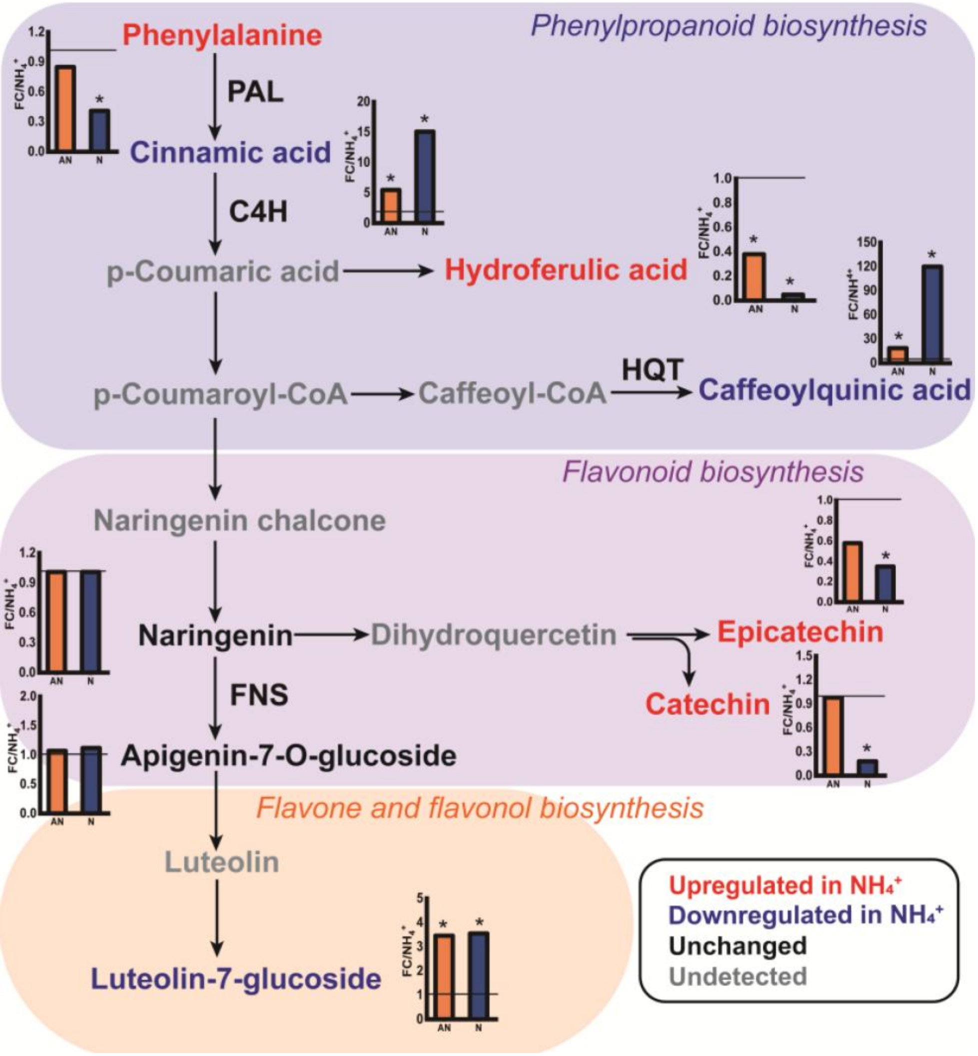

Figure 2 Expression pattern of metabolic profiles and genes involved in phenolic metabolism (Adopted from Cao et al., 2025)
Image caption: Red coloring reflects significantly ($p < 0.05$) increased metabolites under sole $NH_4^+$ condition compared to mixed N and sole $NO_3^-$ condition, while blue indicates the significantly decreased ones. Metabolites in black were present in the pathways but were not significantly changed, and those in grey were absent from our metabolic profile. The green letter denotes the relative key enzymes involved in the pathway. The column plot shows the relative value of each metabolite in treatments AN and N compared to treatment A. Asterisks indicate values determined to be significantly different ($p < 0.05$) between treatment AN, N and A. PAL, phenylalanine ammonia-lyase; C4H, cinnamate 4-hydroxylase; HQT, hydroxycinnamoyl–CoA quinate transferase; FNS, flavone synthase (Adopted from Cao et al., 2025)

### 5.2 Developmental regulation during bud, flower, and leaf maturation

The content of secondary metabolites in honeysuckle will also change significantly at different developmental stages. The bud stage (such as green buds and white buds) is usually the period when phenolic acids and flavonoids accumulate the most. As the flowers gradually bloom, components such as chlorogenic acid and luteolin will gradually decrease (Ran et al., 2023). Analysis based on transcriptome and metabolome revealed that in the early stage of flower development, glucose metabolism and the phenylpropane pathway are more active, leading to a large accumulation of phenolic substances. In the later stage, the terpene synthesis pathway becomes stronger (Yang et al., 2019). Post-translational modifications of proteins (such as ubiquitination and succinylation) are more common in the early stage of flower development and are closely related to the accumulation of simple phenylpropanoid substances (Wang et al., 2021). The distribution of metabolites varies among different tissues. The contents of phenolic acids, flavonoids and iridoids in flowers and flower buds are usually higher than those in leaves (Wang et al., 2023).

### 5.3 Hormonal regulation (JA, SA, ABA) on secondary metabolite biosynthesis

Plant hormones also play an important role in the regulation of secondary metabolism in honeysuckle. Jasmonic acid (JA), salicylic acid (SA), and abscisic acid (ABA) can all be involved in regulating the synthesis of these metabolites. JA and SA can up-regulate the expression of key enzyme genes such as *PAL*, *CHS*, and *C4H*, thereby promoting the accumulation of chlorogenic acid and flavonoids (Lv et al., 2021). ABA can affect the production of petal pigments (such as carotenoids and anthocyanins) and the expression of related genes, thereby altering flower color and the content of active ingredients. Studies have found that differentially expressed genes in the hormone signaling pathway occur in large quantities during flower development and pigment accumulation, indicating that hormone regulation, together with flower development and environmental signals, jointly affects the secondary metabolic network of honeysuckle (Xia et al., 2021).

## 6 Pharmacological Traits Linked to Secondary Metabolism

### 6.1 Anti-inflammatory, antiviral, antioxidant, and antibacterial activities

The flowers, leaves and stems of honeysuckle all contain a variety of active secondary metabolites, including flavonoids, phenolic acids, triterpene saponins and volatile oils, etc. These components together constitute its powerful anti-inflammatory, antiviral, antioxidant and antibacterial capabilities. Studies have shown that honeysuckle extract can reduce the expression of inflammatory factors (such as TNF-α, IL-6, IL-1β) in the body and regulate immune-related pathways, such as IL-17, PI3K-AKT and AGE/RAGE, thereby exerting anti-inflammatory and immune-regulating effects (Xu et al., 2023). Flavonoids (such as luteolin, quercetin, kaempferol) and phenolic acids (such as chlorogenic acid) are the main antioxidant and antibacterial components, which can eliminate free radicals, inhibit bacterial growth, and protect cell structure (Feng et al., 2023; Liu et al., 2023). In addition, some components such as verbascoside and kaempferol-7-O-glucoside have shown antiviral potential, even against the novel coronavirus (Tong et al., 2025).

### 6.2 Metabolite-driven medicinal quality assessment and standardization

The medicinal quality of honeysuckle is closely related to the types and contents of these secondary metabolites. Multi-omics studies have shown that the contents of the main active components (such as chlorogenic acid, hyperoside, luteolin-7-O-glucoside, etc.) of honeysuckle with different tissues, different developmental stages and different flower colors vary significantly, and this is directly related to pharmacological activities such as antioxidation and antibacterial (Wang et al., 2023). Physicochemical indicators such as color difference, total phenols, and total flavonoids can be used to directly evaluate the quality of medicinal materials, while metabolite fingerprinting and multivariate statistical analysis (such as PLS-DA) can provide molecular basis for the standardization and traceability of medicinal materials (Liu et al., 2023). Environmental stress (such as salt threats) and processing methods can also affect the accumulation of these secondary metabolites, thereby altering the medicinal effects of honeysuckle.

### 6.3 Molecular correlation between metabolite profiles and pharmacological potency

Through multi-omics integration and network pharmacological analysis, researchers have found a clear molecular connection between the secondary metabolites of honeysuckle and their pharmacological effects. Some components (such as flavonoids, phenolic acids and triterpene saponins) are positively correlated with anti-inflammatory, antioxidant and antibacterial activities, and they usually exert their effects by regulating key signaling pathways such as AKT, NF-κB and IL-17 (Li et al., 2019; Liu et al., 2024). Gene expression regulation can also affect the accumulation of these components. For example, the R2R3-MYB transcription factor

LjaMYB305 can regulate flavonoid synthesis, thereby affecting pharmacological activity (Yang et al., 2019; Fang et al., 2025). The construction of these metabolite-pharmacological activity networks provides strong theoretical support for explaining the pharmacological basis of honeysuckle and conducting molecular breeding.

## 7 Case Study: Genetic Analysis of Chlorogenic Acid Accumulation in Honeysuckle

### 7.1 Study background and selection of high- and low-content germplasms

Chlorogenic acid (CGA) is the most typical and important phenolic acid active component in honeysuckle. Its quantity will directly affect the medicinal quality of honeysuckle and also influence its market price. The CGA content of honeysuckle varies greatly among different varieties, cultivation methods and developmental stages. For instance, the CGA content of tetraploid honeysuckle is generally higher than that of diploid honeysuckle at all growth stages. In addition, the accumulation patterns of CGA in tissues such as flower buds, leaves, and stems are also different (Wang et al., 2020). Therefore, if the CGA content is systematically collected from different materials and determined, representative germplasms with high or low content can be found, providing a basis for subsequent genetic mechanism research and molecular breeding.

### 7.2 Identification of key genes and transcription factors via multi-omics

Multi-omics studies (transcriptomics, metabolomics and genomics) have identified the key enzyme genes involved in CGA synthesis and their regulatory networks. The main structural genes include *PAL*, *C4H*, *HCT*, *HQT* and *C3H*, etc. (Qi et al., 2017). The expression levels of *HQT* and *HCT* are closely related to the content of CGA. Overexpression of these genes can significantly increase the accumulation of CGA (Zhang et al., 2017). In addition, C3H and C4H in the CYP450 family also play important roles in the biosynthesis of CGA (Qi et al., 2017). In terms of transcriptional regulation, transcription factors such as MYB, bZIP, and WRKY are also involved in regulating these structural genes. For example, LjbZIP8 can bind to the G-box element on the PAL2 promoter, thereby inhibiting the expression of PAL2 and affecting the content of CGA. In addition, DNA methylation can also affect the binding ability of transcription factors, and thus indirectly change the synthetic level of CGA (Zha et al., 2017).

### 7.3 Key findings: regulatory mechanism, candidate markers, and breeding implications

Studies have shown that the accumulation of CGA is jointly controlled by multiple genes and multiple regulatory levels. It is related not only to the expression level of structural genes themselves, but also to transcription factors and epigenetic regulation (Yao et al., 2021). The expression levels of genes such as *HQT*, *HCT* and *C3H* can be used as molecular markers to determine whether honeysuckle is a germplasm with a high content of CGA, facilitating molecular breeding (Qi et al., 2017). The functional differences among transcription factors such as MYB and bZIP also provide a genetic basis for the changes in CGA content (Zha et al., 2017). The research combining multiple omics has provided theoretical basis and technical tools for the breeding of honeysuckle germplasm with high CGA content and high quality. In the future, through molecular marker-assisted selection (MAS) and genetic engineering methods, it is expected to cultivate new varieties with higher CGA and better medicinal value, promoting their application in traditional Chinese medicine and functional foods (Zhang et al., 2017).

## 8 Molecular Breeding and Biotechnology for Quality Improvement

### 8.1 Marker-assisted selection (MAS) for high-metabolite lines

Marker-assisted selection (MAS) can help quickly screen out strains with high secondary metabolite content by taking advantage of the linkage relationship between molecular markers and target traits. For instance, through population structure analysis and molecular experiments, researchers have identified the key gene *LjWRKY50* that affects the flower bud duration of honeysuckle and developed the corresponding dCAPS molecular marker, which provides a practical tool for molecular breeding (Li et al., 2025). Meanwhile, by comparing different genotypes and combining with metabolome data, a database of “genotype - phenotype - metabolome” can be established to provide support for precision breeding and functional product development (Wang et al., 2020). With the help of MAS, it is possible to more efficiently select and cultivate superior materials with higher contents of active ingredients such as chlorogenic acid and flavonoids, thereby enhancing their medicinal value.

### 8.2 CRISPR/Cas genome editing for pathway gene modification

The CRISPR/Cas gene editing system is widely used to regulate secondary metabolic pathways in plants due to its precision and efficiency. Although its application in honeysuckle is still limited, in other medicinal plants, CRISPR/Cas9 has successfully achieved the editing of key enzyme genes or transcription factors and significantly increased the content of certain phenolic acids or flavonoids (Devi et al., 2023). With the gradual improvement of the genetic transformation system of honeysuckle, in the future, the key genes in its synthetic pathways such as chlorogenic acid and saponins can be precisely edited, thereby achieving customized improvements in quality and efficacy.

### 8.3 Synthetic biology and metabolic engineering strategies

Synthetic biology and metabolic engineering can achieve efficient production of target secondary metabolites by reconstructing or optimizing metabolic pathways. After comparative studies on the genomes and transcriptomes of honeysuckle and its related species, key enzyme genes involved in the synthesis of chlorogenic acid, saponins, etc. have been identified, such as OAS and UGT73, providing clear targets for metabolic engineering (Yin et al., 2023). In the future, these key genes can be cloned and introduced into microbial systems (such as Escherichia coli) or plant cell factories for large-scale production of medicinal active ingredients. Furthermore, the combination of systems metabolic engineering with omics data, synthetic modules and evolutionary engineering can further optimize metabolic flow and improve the yield and quality of products.

## 9 Challenges and Future Perspectives

### 9.1 Genetic complexity and redundancy in multi-branch metabolic pathways

The secondary metabolites such as phenolic acids, flavonoids and terpenoids in honeysuckle are jointly completed by multiple branch metabolic pathways. There are a large number of gene families involved in these pathways, and amplification, functional duplication and differentiation are common. The number of gene families responsible for secondary metabolism in the honeysuckle genome has significantly increased. The synthesis of the same metabolite is often regulated by multiple homologous genes together. Therefore, the distribution of metabolic flux and product accumulation will become very dynamic and complex. Furthermore, the redundancy of these gene families themselves, coupled with their expression differences in different tissues and periods, makes the functional research of individual genes and their application in breeding rather difficult. Diploid and tetraploid honeysuckle also show differences in key gene expression and product accumulation, which further increases the complexity of the genetic regulatory network.

### 9.2 Integration of multi-omics under diverse environmental conditions

The secondary metabolism of honeysuckle is also influenced by various factors such as the developmental stage, environmental stress (such as salt stress and low temperature), and hormone signals. Multi-omics studies (genome, transcriptome, proteome, metabolome, miRNA) have revealed that metabolic networks undergo dynamic changes at different developmental stages and under different environmental conditions. However, how to effectively integrate these multi-omics data across different genotypes and environments, and accurately identify the main regulatory factors and their modes of action, remains a current challenge. For example, in materials of different varieties or from different origins, the expression patterns of miRNA and transcription factors are often different, and their effects on secondary metabolism have not been fully understood. In addition, how environmental factors affect the accumulation of metabolites and the interaction between these effects and genetic background also require more in-depth research.

### 9.3 Prospects for precision breeding and pharmacological trait prediction models

With the acquisition of high-quality genomes, the establishment of SNP fingerprint maps, and the exploration of more functional genes, new opportunities have emerged for the molecular breeding and pharmacological trait prediction of honeysuckle. In the future, through the integration of multi-omics data, the development of molecular markers, and the construction of pharmacological trait prediction models, it is expected to achieve precise breeding of varieties with highly active components and further improve the efficacy of drugs. However, at present, the aspects such as gene function verification, genetic transformation system, germplasm resource bank and mutant bank are still not perfect. Furthermore, how to truly transform multi-omics information into operational breeding strategies and achieve targeted improvement of complex pharmacological traits remains a key issue that needs to be urgently broken through in the future.

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## Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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